Rapid aneuploidy detection in prenatal diagnosis
The clinical use of Multiplex Ligation-dependent Probe Amplification

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Aan mijn dierbaren

CONTENTS

Chapter 1

General introduction

Prenatal diagnosis using karyotyping is routinely offered to all pregnant women in developed countries who have an increased risk of carrying a child with a chromosomal abnormality. The aim of prenatal diagnosis is to determine the presence or absence of chromosomal abnormalities to allow parents an informed choice on the course of pregnancy[1]. Prenatal diagnosis is ultimately a patient's choice. Prenatal diagnosis starts with counseling of the patient; explaining the intervention, the chromosomal abnormalities that can be detected and the consequences of these abnormalities. In case an abnormality is detected, prenatal diagnosis implies decision making on the continuation of pregnancy, timely medical treatment and emotional and psychological care.

REFERRAL INDICATIONS

Prenatal diagnosis is offered to pregnant women with a higher than reference risk for fetal chromosomal abnormalities. The assessment of risk for fetal chromosomal abnormalities is based on several risk indicators; a family history of chromosomal abnormalities, the presence of ultrasound abnormalities, advanced maternal age, and an increased risk following prenatal screening tests.

Presence of a parental chromosomal abnormality or a previous pregnancy with a chromosomal abnormality leads to an increased risk of chromosomal abnormalities in (the subsequent) pregnancy[2]. For example, if one of the parents is a carrier of balanced translocation between chromosome 13 and 14, the risk on having offspring with a trisomy 13 is 1%[3].

Single and especially multiple fetal abnormalities seen on ultrasound scan are associated with the presence of chromosomal abnormalities[4, 5]. These ultrasound abnormalities can be detected early in pregnancy. Since its introduction in 1990, first trimester nuchal translucency (NT) measurement has been implemented as a screening test for fetal chromosomal abnormalities. NT thickness is increased in fetus with trisomies 13, 18, and 21, and it is also associated with cardiac defects[6] and genetic syndromes[7]. The 20-week anomaly scan was initially developed for the detection of neural tube defects but is now part of the national prenatal screening programme and carried out to detect or rule out the presence of structural abnormalities. Occasionally, soft markers are identified, e.g. echogenic bowel, mild ventriculomegaly, and echogenic focus in the heart. These soft markers may be related to fetal chromosomal abnormalities[8]. Subsequently, advanced ultrasound screening is done at a prenatal diagnostic centre. If the findings are confirmed, invasive prenatal diagnosis is offered[9].

The most common indications for prenatal diagnosis are 1) advanced maternal age, 2) an increased risk of Down syndrome following prenatal screening, and 3) abnormalities detected at ultrasound scan. For these indications, Down syndrome is the most commonly detected abnormality.

Advanced maternal age is defined either as a maternal age of 35 years or 36 years during the 18th gestational week. In the Netherlands, the most common indication is advanced maternal age (66%)[10, 11]. Women of at least 36 years of age in the 18th gestational week are eligible for prenatal diagnosis in the Netherlands. The risk of carrying a child with Down syndrome at term increases from 1: 940 at 30 years of age to 1:353 at 35 years of age and 1:85 at 40 years of age. The combined risk for other common chromosomal abnormalities (Patau syndrome, Edward syndrome and sex chromosomal abnormalities) is also age-dependent, rising from 1:384 (30 years), to 1:178 (35 years), to 1:62 (40 years). On balance, when amniocentesis is performed, 43.5% of the chromosomal abnormalities detected are Down syndrome (trisomy 21), 10.3% are Edward syndrome (trisomy 18), 1.6% are Patau syndrome (trisomy 13) and 13% are sex chromosomal abnormalities, 15.4% are balanced structural rearrangements, 9.6% are mosaics, and the final 6.6% consist of unbalanced structural rearrangements, marker chromosomes or polyploidies[2].

Thirty years ago, prenatal screening using maternal serum markers became available to estimate the risk of carrying a child with Down syndrome. First trimester screening based on maternal age, serum markers, and nuchal translucency measurement is regarded upon as an effective screening test with a detection rate of 75.9-90.0% and a 3.3-5.0% false positive rate[12-16]. If an increased risk of carrying a baby with Down syndrome is present, prenatal diagnosis is offered. In the Netherlands, this is the second most common referral indication for prenatal diagnosis (10%). A cut-off risk level of 1 in 200 at the time of testing, comparable with a risk of 1 in 280 at term[17], is used in the Netherlands[18]. The number of pregnant women participating in prenatal screening increased due to a change in government policy in 2003, making screening for Down's syndrome available to all pregnant women, regardless their age. In 2006, 45,000 tests were performed[18], leading to an uptake of approximately 27%.

Psychological indicators are not formally part of the selection criteria for prenatal diagnosis. However, in clinical practice, parental distress or anxiety is considered an admissible criterium to undergo invasive prenatal diagnosis. It is used in 1% as a reason to undergo invasive prenatal diagnosis in our country. In the Netherlands, for all the above mentioned indications, except parental anxiety invasive prenatal diagnosis is fully covered by the insurance companies.

PRENATAL DIAGNOSIS

If at least one of the above mentioned risk indicators is present, prenatal diagnostic care is offered and parents can decide to undergo an invasive diagnostic test; i.e. prenatal diagnosis. Prenatal diagnosis is performed on amniotic fluid cells obtained by amniocentesis or chorionic villi obtained by chorionic villus sampling (CVS).

Amniocentesis is the most commonly used invasive prenatal diagnostic procedure worldwide[19] and is performed in one in 30 pregnancies in the Netherlands[10]. Amniocentesis usually is performed at 15 to 20 weeks' gestational age. Amniocentesis (figure 1) has a miscarriage risk of 0.06-1.4%[20-22]. The amniotic fluid cells are cultured for karyotyping and the result is known in 2-3 weeks. Once a chromosomal abnormality has been detected and parents decide to terminate the pregnancy, delivery is induced.

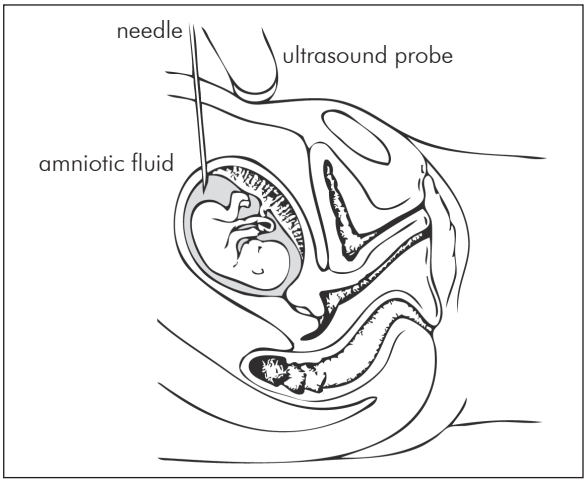


Figure 1: Amniocentesis: amniotic fluid cells are aspirated transabdominally using ultrasound guidance.

CVS (figure 2) usually is done at 10 to 13 weeks of pregnancy either transabdominally or transcervically. CVS has a miscarriage risk of 1.3%-2%[21, 22]. Specimens yielded are cultured which takes 8-10days to give a result. Once a chromosomal abnormality has been detected a dilatation and evacuation can be performed to terminate pregnancy.

Karyotyping

After withdrawal of fetal material either by amniocentesis or CVS, karyotyping (figure 3) is performed. It has been used for almost 50 years to determine if fetal chromosomal aberrations are present. It is a robust technique that is able to detect a range of numerical and structural chromosomal abnormalities with high accuracy (99.4-99.9%)[23-25]. Karyotyping requires culture of fetal cells in order to obtain cells at the metaphase stage. The cells may be grown

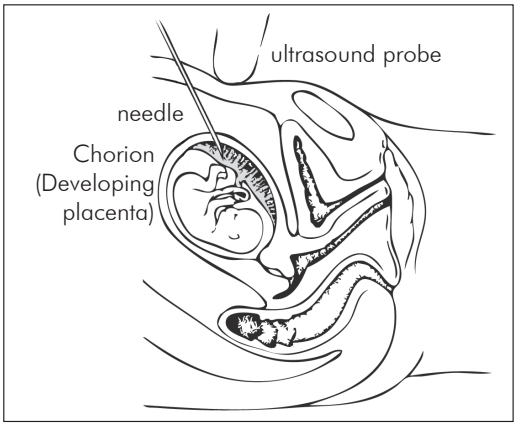


Figure 2: Chorionic villus sampling: chorionoc villi are aspirated transabdominally or transcervically using ultrasound guidance.

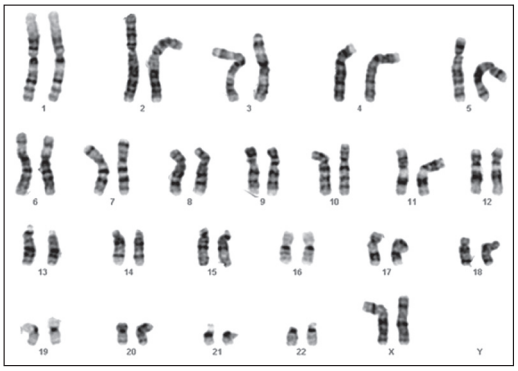


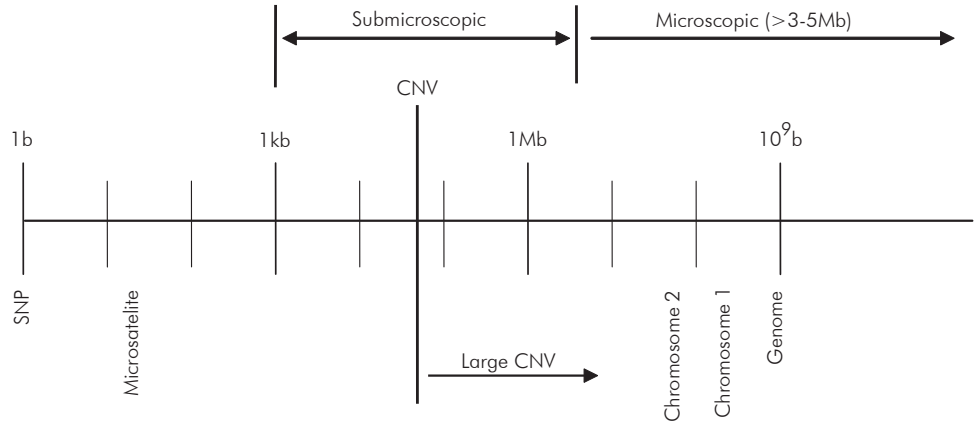
Figure 3: Normal female karyotype

in tissue flask (the flask method), in which the cells have to be enzymatically removed prior to harvest or with an in situ method, in which cells are analysed without subculture. Failure to culture the fetal cells obtained occurs in a small number of cases; the average rate reported in the UK in 1999 was 0.3% of cases[26]. In the last five years, the failure rate for the culture of amniocytes was less than 0.01% in the Netherlands. On average 10 metaphases from 10 different colonies are examined and analysed[27].

Cell culture takes on average 10 to 14 days before slides are stained for chromosomal banding. Parents have to wait two to three weeks for the test results, which generally leads to parental anxiety[28]. Karyotyping is considered time consuming and labour-intensive, both leading to high costs.

Karyotyping is able to detect any microscopic chromosomal abnormality of 3 to 5 Mega base (Figure 4)[29], including chromosomal abnormalities with unclear or mild clinical relevance. The latter findings may cause difficult counselling issues, patient anxiety, and emotional dilemmas concerning the continuation of pregnancy in situations in which the outcome is uncertain or the phenotype predicted to be relatively mild[30].

Figure 4: Arrangement and size of submicroscopic and microscopic abnormalities. Mb= Megabase, b= base, Kb= kilobase. SNP= single nucleotide polymorphism, CNV= copy number variant



RAPID ANEUPLOIDY DETECTION

Due to technical progress in molecular biology, new molecular techniques have become available which have also been applied in prenatal diagnosis. These techniques, commonly referred to as rapid aneuploidy detection (RAD) techniques, do not need cultured cells and are therefore able to deliver quick results. Currently, there are three RAD techniques; fluorescent in situ hybridisation (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA). These techniques share several characteristics. They only use a small part of a chromosome and are able to detect only a few chromosomes within one test. Moreover, these tests are designed to detect only the most common fetal chromosomal abnormalities; i.e. aneuploidies of chromosomes 13, 18, 21, X and Y. RAD is therefore a targeted test on chromosomes 13, 18, 21, X and Y which also implies that other chromosomal abnormalities will remain undetected. Compared to karyotyping, several advantages of RAD have been put forward; the shortening of the waiting time, the procedure is considerably less labour intensive since cultured cells are avoided, the test requires less amniotic fluid and it is suitable for high throughput testing. These factors all add to the assumed higher efficiency of RAD compared to karyotyping. Below we discuss the three RAD techniques in more detail.

Fluorescent in situ hybridisation (FISH)

FISH is a type of hybridization that uses a labelled complementary DNA or RNA strand (i.e., probe) to localize a specific DNA or RNA sequence in a portion or section of tissue in the interphase nucleus. The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away. Then, the probe that is labelled with fluorescent-labelled bases is localized and quantified in the tissue using fluorescence microscopy. FISH is a powerful general technique and has also become an integral part of a comprehensive cytogenetic evaluation of structurally abnormal chromosomes, mosaicism and marker chromosomes in prenatal diagnosis[31]. A variety of probe types can be employed to detect chromosome rearrangements and aneuploidy. For RAD, probes are used for chromosomes 13, 18, 21, X and Y only. Although FISH produces results in 1 to 2 days, the process still is still labour intensive requiring much expertise.

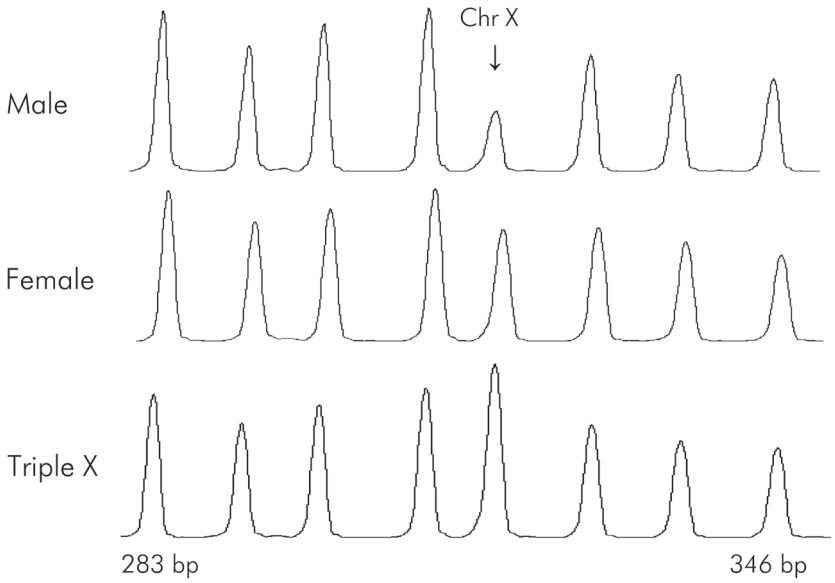
Quantitative fluorescent polymerase chain reaction (QF-PCR)

In QF-PCR, highly polymorphic short tandem repeats (STRs) on chromosome 13, 18, 21, X and Y are amplified using fluorescence primers and PCR in a multiplex assay, followed by the automated analysis of fluorescence intensity of the alleles in a genetic analyser[32]. Generally, a minimum of 3-4 STRs for each chromosome tested is used to reduce the number of uninformative results. In normal cases at least two informative marker results for each investigated chromosome consistent with a normal diallelic (heterozygous) pattern with two peaks in a 1:1 ratio are required, a monoallelic (homozygous) pattern with one peak being uninformative. In trisomic cases three alleles are evident by three peaks in a 1:1:1 ratio (triallelic trisomy pattern) or two alleles in 2:1 or 1:2 ratios (diallelic trisomy pattern). Peak height, peak area or both can be used to calculate allele ratios. QF-PCR can be performed at highly automated protocols. At the start of our clinical study, no commercially available kits were available and non-informativeness of the polymorphic markers occurred regularly.

Multiplex Ligation-dependent Probe Amplification (MLPA)

The third RAD technique is Multiplex Ligation-dependent Probe Amplification (MLPA, MRC Holland). The commercially available kit SALSA P095 is designed to detect trisomies 13, 18, 21, X and Y. More than 40 loci per multiplex can be tested in one reaction. For each genomic target, a set of 2 probes is designed to hybridize immediately adjacent to each other on the same target strand. Once hybridized, the two probes are joined by a ligase and the probe can then be amplified by PCR. All ligated probes have identical end sequences, permitting simultaneous PCR amplification by only one primer pair (a universal primer). The different length products are separated on an automated capillary sequencer. The relative

Figure 5: Detection of chromosome X (Chr X): the ratio for the male sample, containing one X chromosome, is 0.5; the ratio for the normal female sample, containing two X chromosomes, is 1.0; the ratio for the Triple X sample, containing three X chromosomes, is 1.5.



quantity of each of the PCR products is proportional to the number of copies of target sequence. Results are given as allele copy numbers as compared to normal controls: a ratio of about 1 is obtained if both alleles are present, a ratio of about 0.5 when one allele is absent and a ratio of about 1.5 if one allele is duplicated (figure 5). MLPA is not expected to detect low grade chromosomal mosaicism[33, 34].

In 2003, a preclinical study of Slater et al. showed MLPA to be robust in detecting aneuploidies of chromosome 13, 18, 21 and non-mosaic sex chromosome abnormalities using amniotic fluid: highly automated protocols provided a test result within a few days[33]. This preclinical study, however, did not reveal if the favourable performance of MLPA could also be achieved in routine clinical care, nor the impact of MLPA on patient's health related quality of life, patient's and physician's preferences and its costs-effectiveness.

THE CLINICAL PROBLEM

In prenatal diagnosis, there is neither agreement on the specific chromosomal abnormalities that should and should not be detected, nor on the degree of certainty required for a result to be negative or positive. Initially, only karyotyping was available and its 'broad' detection capacity and its high diagnostic accuracy made karyotyping to be accepted as gold standard.

Nowadays, due to technical progress, other prenatal diagnostic tests have become available next to karyotyping. The decision problem which test to use, and under what circumstances, indirectly raises the question what to test for in prenatal diagnosis. Which test strategy is considered optimal, depends on evaluative data from comparative clinical studies, with support from psychological, and decision analytic studies.

In this thesis, MLPA is our RAD technique of choice, since a preclinical study showed MLPA to be a good test with high diagnostic accuracy at highly automated protocols[33, 35]. At study onset the commercially available SALSA P095 kit had been validated on amniotic fluid in the eight genetic centres in the Netherlands in contrast to other RAD tests. MLPA by design cannot detect chromosomal abnormalities other than aneuploidies of chromosome 13, 18, 21, X and Y. Therefore, we evaluated if the diagnostic accuracy of MLPA was non-inferior to karyotyping when applied in a routine prenatal diagnosis setting and we assessed patient outcomes as well as the preferences of pregnant women and physicians for various tests and test strategies. We also estimated cost-effectiveness of MPLA compared to standard karyotyping.

STUDY AIM

The aim of the study was to assess the diagnostic accuracy, impact on patient's quality of life and preferences, and cost-effectiveness of MLPA in comparison to karyotyping as the reference diagnostic test, in clinical practice for women undergoing amniocentesis on behalf of their age, increased Down syndrome risk following first trimester prenatal screening, or parental anxiety. Should MLPA be implemented in prenatal diagnostic care and if yes, what is its optimal test strategy?

The specific research questions were:

Is diagnostic accuracy of MLPA to detect trisomies 13, 18, 21 and sex chromosomal aneuploidies in routine clinical practice comparable (non-inferior) to karyotyping?

Do anxiety and quality of life differ between a combined strategy (MLPA followed by karyotyping) and karyotyping? And if MLPA has comparable diagnostic accuracy, is quality of life influenced by offering individual choice between standalone karyotyping and standalone RAD?

Is MLPA cost effective compared to karyotyping, taking into account short term and long-term effects?

Which test and which test characteristics do patients value most? Which type of test is preferred by physicians involved in prenatal diagnosis?

Since karyotyping and MLPA have different detection capacities, which chromosomal abnormalities should be detected in prenatal diagnosis according to experts?

OUTLINE OF THE THESIS

Part 1: Clinical evaluation

Chapter 2 describes the diagnostic accuracy and failure rate of MLPA compared to karyotyping as reference test on 4585 amniotic fluid samples. Undetected chromosomal abnormalities are described (research question 1).

In Chapter 3 we analyse different aspects of health related quality of life, using validated questionnaires between women who receive both MLPA as well as a karyotype result and women who only receive karyotyping results (research question 2).

In Chapter 4 we assess the motives and reasons to choose either karyotyping or RAD and evaluate different aspects of health related quality of life, using validated questionnaires of women who are offered individual choice between standalone RAD and karyotyping (research question 2).

In Chapter 5 we present a detailed cost-effectiveness analysis. The analysis includes short term costs, i.e. time frame from amniocentesis until the decision to continue or terminate pregnancy, and long term costs, i.e. time from decision to continue or terminate pregnancy (research question 3).

Part 2: Patients 'and physicians' preferences

Chapter 6 describes the differences in preferences for prenatal testing between physicians (obstetricians, clinical geneticists, clinical cytogeneticists, midwives, general practitioners) involved in prenatal diagnosis and pregnant women undergoing amniocentesis (research question 4).

In Chapter 7 we investigate patient's preferences for karyotyping or RAD and assess the value women place on test specific characteristics by using discrete choice experimentation. (research question 4)

In Chapter 8 we present the consensus and dissensus opinions of an expert panel of professionals on broad versus targeted testing by evaluating which chromosomal abnormalities should be detected and which should not be detected (research question 5).

Part 3: General discussion, conclusion and summary

In Chapter 9 we discuss the results and give clinical implications and implications for future research. Finally our conclusions are postulated.

In Chapter 10 and 11 we summarize the results presented in this thesis in English and Dutch.

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PART I

CLINICAL EVALUATION

Chapter 2

Comparison of Multiplex Ligation-dependent Probe Amplification and Karyotyping in Prenatal Diagnosis

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ABSTRACT

Objective

To estimate whether Multiplex Ligation-dependent Probe Amplification (MLPA), a molecular technique used for detecting the most common chromosomal aneuploidies, is comparable to karyotyping for the detection of aneuploidies of chromosomes X, Y, 13, 18 and 21 in routine clinical practice and to estimate the costs differences of both techniques.

Methods

In this prospective nationwide cohort study, we consecutively included 4585 women who had an amniocentesis on behalf of their age, increased risk following prenatal screening or maternal anxiety. Amniotic fluid samples were tested independently with both MLPA and karyotyping. The primary outcome was diagnostic accuracy of MLPA to detect aneuploidies of chromosomes X, Y, 13, 18 and 21. Secondary outcome measures were turnaround time and costs. A sample size was calculated using a critical noninferiority margin of 0.002, therefore at least 4497 paired test results were needed (one-sided alpha 0.05, power 0.90).

Results

Diagnostic accuracy of MLPA was 1.0 (95 % confidence interval 0.99 to 1.0), sensitivity was 100% (95% confidence interval 0.96-1.0) and specificity was 100% (95% confidence interval 0.999-1.0). Diagnostic accuracy of MLPA was statistically similar (noninferior) to that of karyotyping ($P<0.001$). In 75 cases MLPA failed (1.6%); karyotyping failed once (0.02%). Compared with karyotyping, MLPA shortened the waiting time with 14.5 days ($P<0.001$, 95% confidence interval 14.3-14.6), and cost less (-47%, $P<0.001$).

Conclusions

In routine clinical practice, diagnostic accuracy of MLPA for detection of trisomies X, Y, 13, 18, and 21 is comparable to that of karyotyping and it reduces waiting time at lower costs.

INTRODUCTION

Prenatal diagnosis is routinely offered to all pregnant women in developed countries who have an increased risk of carrying a child with a chromosomal abnormality. Amniocentesis is the most commonly used invasive prenatal diagnostic procedure worldwide and is performed in one in 30 pregnancies in developed countries[1,2].

Karyotyping detects fetal chromosomal abnormalities in amniotic fluid cells[3,4]. It is a robust technique and detects a range of numerical and structural chromosomal abnormalities with high accuracy (99.4-99.9%)[3,5,6]. However, due to the required fetal cell culture, karyotyping is time consuming and labor-intensive leading to high costs. The detection capacity of karyotyping may be perceived as a disadvantage as it detects chromosomal abnormalities with unclear or mild clinical relevance. The latter can cause patient anxiety, emotional dilemmas concerning the continuation of pregnancy in situations in which the outcome is uncertain or the phenotype predicted to be relatively mild[7].

In the last decade new molecular techniques have become available for rapid aneuploidy detection of the most common chromosome abnormalities (aneuploidies of chromosomes X, Y, 13, 18 and 21). Multiplex Ligation-dependent Probe Amplification (MLPA) is a rapid high-throughput technique shown to be robust in a preclinical setting[8,9]. MLPA avoids the detection of abnormalities with unclear clinical relevance.

If under standard clinical conditions MLPA can accurately and rapidly detect aneuploidies of chromosomes X, Y, 13, 18 and 21, it would be a suitable test for routine diagnostic application in prenatal diagnosis. Therefore, we conducted a nationwide prospective study in which we compared MLPA with karyotyping in routine clinical practice and evaluated the cost differences of both techniques. We hypothesized that MLPA has equivalent diagnostic accuracy in detecting aneuploidies of chromosomes 21, 13, 18, X and Y at lower costs.

MATERIALS AND METHODS

The M.A.K.E. (MLPA And Karyotyping, an Evaluation) study was a prospective multicentre diagnostic cohort study, comparing MLPA on amniotic fluid in a routine clinical setting with karyotyping (10). All eight Dutch prenatal diagnostic centers and their affiliated hospitals participated. The Institutional Review Boards approved the study and all participating women gave written informed consent.

We consecutively included pregnant women from March 2007 to October 2008. Pregnant women were eligible for study participation if they had a singleton pregnancy and chose

to undergo amniocentesis for advanced maternal age (36 years or older), increased risk of Down syndrome following prenatal screening or parental anxiety. We excluded women with other indications for amniocentesis since they have an increased risk of chromosomal abnormalities other than the most common aneuploidies which MLPA cannot detect and karyotyping is mandatory; ultrasound abnormalities including a nuchal translucency measurement of 3.5 mm, a parental chromosomal abnormality, or a previous child with a chromosomal abnormality.

In all centers experienced maternal fetal medicine specialists performed amniocentesis following national guidelines[11]. Samples were included if the aspirated volume was at least 14 ml, leaving sufficient amniotic fluid available for MLPA analysis. No extra amniotic fluid was withdrawn in favor of the study.

For the MLPA procedure, DNA was isolated from 1 to 8 ml uncultured amniotic fluid samples, depending on the total amount of amniotic fluid received. We used a commercially available kit, the SALSA MLPA P095 (MRC Holland, the Netherlands). For each genomic target, a set of 2 probes is designed to hybridize immediately adjacent to each other on the same target strand. Both probes consist of a short target sequence and a universal polymerase chain reaction (PCR) primer-binding site. One of the probes contains a stuffer sequence with a unique length and sequence. Following hybridization, each pair of adjacent probes is joined by a ligation reaction. Next, PCR is performed using a fluorescent-labeled primer pair, which ensures that the relative yield of each of the PCR products is proportional to the amount of each of the target sequences. The different length products are separated on an automated capillary sequencer. The size and peak areas for each probe are quantified and analyzed by data analyzing software (GeneMarker, SoftGenetics, LLC, State College, PA, USA or Genescan and Genemapper version 3.7/4.0, Applied Biosystems, CA, USA) (8). Relative probe signals are calculated and compared with samples of normal male and female sex. In chromosomally normal samples, the relative probe signal is expected to be 1 for all probes. A normal value is defined as a relative probe signal between 0.7 and 1.3. A relative probe value of <0.7 indicates a monosomy, whereas a relative probe value of >1.3 indicates a trisomy. MLPA is not expected to detect low grade chromosomal mosaicism[9,12].

Technicians had a molecular genetics or a cytogenetics background; all were trained in the execution of MLPA prior to the study onset. MLPA was performed in duplicate, provided that at least 2 ml of amniotic fluid was available. MLPA results were conclusive if the results of both results matched. If one or either results were inconclusive and sufficient DNA was available, the MLPA reaction was repeated. If the results still disagreed after the repetition, MLPA failed. Technicians carrying out MLPA were blinded to karyotyping results and vice

versa. However, if MLPA detected an aneuploidy the head of the laboratory could initiate the earliest possible harvesting of cell culture.

We allowed a phase 1 (median time 6 months) in which test results were not reported to patients and centers could train extra personnel for sample identification, tracking and accurate reporting of test results. In phase 2 conclusive MLPA results were reported to pregnant women as a provisional result, awaiting the definite karyotype result. Patients were also informed if MLPA failed. For karyotyping, fetal cells were cultured and spread on slides, which were stained for chromosomal banding. Routinely, metaphases for 10 colonies were investigated. All centers followed national quality guidelines but minor differences in the amount of cell colonies cultured, staining and reporting of the results were allowed[13].

The primary outcome variable was diagnostic accuracy for detecting aneuploidies of chromosomes 21, 13, 18, X and Y. We quantified the other chromosomal abnormalities that were not detected by MLPA and recorded reasons for failed test results. Turnaround time for test results was measured on laboratory level (time span between carrying out the amniocentesis and authorization of test result) and, in phase 2, on patient level (time span between amniocentesis and the result given to the patient).

Mean cost differences between MLPA and karyotyping as standalone strategies were evaluated according to international guidelines[14,15]. Costs per strategy were calculated as the sum of resource use between amniocentesis and the decision to continue or terminate pregnancy, using individual data from the case record forms and direct observations in three centers, multiplied by resource unit prices, covering for personnel costs, equipment, consumables, additional costs in case of chromosomal abnormality, and overhead costs. Costs were calculated in Euros and then converted into U.S. dollars (€1.00 = U.S. $1.37).

Sample size was estimated to demonstrate noninferiority of the index test (MLPA) to karyotyping. During a pre-trial meeting, experts in prenatal diagnosis, clinical epidemiology and statistics agreed on a critical noninferiority margin of 0.002. At least 4497 paired test results were needed (one-sided alpha 0.05, power 0.90), to reject the null hypothesis that MLPA is inferior to karyotyping. We calculated diagnostic accuracy by dividing the sum of the true positive and true negative results by the total number of participants. Sensitivity and specificity were calculated by standard formulas for binominal proportions; 95 percent confidence intervals were calculated by the Wilson interval method[16,17]. Failed results were expressed in absolute numbers and percentages. To identify patient, procedural and centre-specific characteristics associated with failure rate, we performed backward-selection logistic regression analysis. Differences in costs were tested with Student's t-tests (SPSS version 16.0). Differences in turnaround time for test results were compared with a Kruskal-Wallis followed by the post hoc Dunn's test.

RESULTS

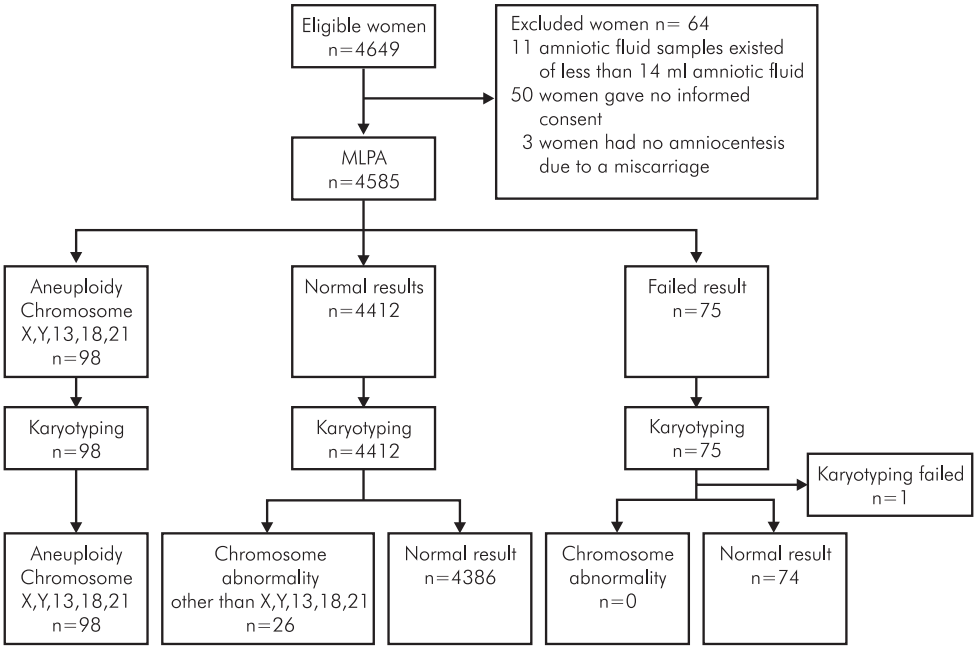
In total 4648 women were eligible and 64 (1.4%) were excluded; 4585 amniotic fluid samples were tested with both MLPA and karyotyping (figure 1). The laboratory results of 280 women were published before (18). Patient and procedural characteristics are listed in table 1 and 2. In 4484/4585 samples (97.8%) MLPA and karyotyping were concordant, showing normal results in 4386/4585 (95.7%) and aneuploidy in 98/4585 (2.1%) (table 3). Discordant results were found in 26/4585 (0.6%) samples, representing an abnormal

Table 1. Demographic Characteristics of the Studied Cohort. n=4585

Demographic characteristic	Number	%
Median Age	38.1*	29.0†
Indication		
Advanced maternal age	3463	75.6%
Increased risk following prenatal screening	1074	23.4%
Anxiety	47	1.0%
Median Gravidity	2*	13†
Median Parity	1*	8 †
Median Gestational age (weeks +days)	16 + 1*	14+6-17+4 †

† range

Figure 1: Enrolment of patients undergoing amniocentesis in the M.A.K.E.study according to STARD guidelines



karyotype not detected by MLPA (table 4 and table 5). Diagnostic accuracy of MLPA was 1.0 (95% confidence interval (CI) 0.99 to 1.0) with a sensitivity of 100% (95% CI 0.96-1.0) and a specificity of 100% (95% CI 0.999 to 1.0). Therefore, we rejected the null hypothesis that MLPA is inferior to karyotyping (P< 0.001).

In 75 cases (1.6%) the MLPA test result failed. Karyotyping failed in one of these 75 cases (0.02%). The failure rate of MLPA was 2.4% in the first four months of the study, thereafter decreasing to 1.5% in the last 11 months. Variables significantly associated with increasing failure rate were: contaminated amniotic fluid (odds ratio (OR) 5.29 95% CI 2.4 to 11.6) and

Table 2. Procedural Characteristics of the 4585 studied amniocentesis.

Procedural characteristic	Description	Number	%
Amniotic fluid (ml)		20*	10†
Color of amniotic fluid	Clear/yellow	4465	97.4%
	Red/Brown/Turbid/Green	118	2.6%
Attempts of amniocentesis	1 attempt	4506	98.3%
	>1 attempt	79	1.7%
Needle size	20 Gauche	2620	57.1%
	22 Gauche	1942	42.4%
	Other (18 Gauche,19 Gauche)	14	0.3%
	Unknown	9	0.2%
Transplacental approach	Yes	477	10.4%
	No	4053	88.4%
	Unknown	55	1.2%
Operator technique of amniocentesis	Single operator no continuous US**	1131	24.7%
	Single operator with continuous US***	1152	25.1%
	Dual operator with continuous US****	2271	49.5%
	Unknown	31	0.7%
Cell pellet color	White	3923	85.6%
	Trace of blood	381	8.3%
	Red/Brown/ Turbid/Green	256	5.6%
	Unknown	25	0.5%
Amniotic fluid for MLPA (ml)		4*	9†

*median, † ranges

**Single operator technique without continuous US: obstetrician makes the ultrasound (US), selects the needle insertion site, inserts the needle under direct ultrasound guidance, and aspirates the amniotic fluid thereby keeping the needle in a fixed position. During the aspiration of 20 ml amniotic fluid the needle will not be visualized in utero. Directly following the removal of the needle, the obstetrician makes an ultrasound.

***Single operator technique with continuous US: obstetrician makes the ultrasound, selects needle insertion site, inserts the needle with ultrasound monitoring and aspirates the amniotic fluid with continuous ultrasound guidance.

****Dual operator technique with continuous US: a (physician-)sonographer performs and maintains ultrasound guidance while the obstetrician inserts the needle and withdraws the fluid.

Table 3. Concordant test (n= 4484) results of MLPA and Karyotyping in the study.

MLPA results	n	Karyotype results	n
Normal female/male	4386	46,XX or 46,XY	4386
Abnormal (total)	98	Abnormal (total)	98
trisomy 21	69	47,XX,+21 / 47,XY,+21	68
		mos 47,XY,+21[8]/46,XY[3]	1
trisomy 18	15	47,XX,+18 / 47,XY,+18	15
trisomy 13	1	47,XX,+13	1
XXY	5	47,XXY	5
XYY	1	47,XYY	1
XXX	2	47,XXX	2
mosaic Trisomy 21 and mosaic Turner	1	45,X[13]/47,XX,+21[11]	1
mosaic Turner	1	mos 45,X[9]/46,XX [17]	1
mosaic Klinefelter	1	47,XXY[4]/46,XY[8]	1
structural chromosome X aberration suspected	2	45,X[6]/46,X,psu idic(X)(p21)[7]	1
		46,X,i(X)(q10)	1
Total MLPA results	4484	Total karyotype results	4484

Table 4. Discordant and failed results of MLPA and Karyotyping in the study.

MLPA	n	Karyotyping	n
Normal male/female	26	Abnormal (total)	26
		mosaicism	3
		supernumerary marker chromosome	4
		structural inherited balanced chromosome aberration	14
		structural de novo apparently balanced chromosome aberration	4
		structural de novo unbalanced chromosome aberration	2
Failed	75	46,XX or 46,XY	74
		Failed	1

contaminated cell pellet (OR 3.39 95% CI 1.98 to 5.81). Variables significantly associated with a lower risk on failure were: time from start of study participation (per month OR 0.95 95% CI 0.90 to 0.99) and milliliters amniotic fluid available for MLPA (per ml OR 0.78 95% CI 0.69 to 0.88). Compared with dual operator technique, the single operator technique with (OR 0.22 95% CI 0.1 to 0.48) and without (OR 0.28 95% CI 0.14 to 0.55) continuous ultrasound control was significantly associated with a lower risk of failure.

We performed 1223 MLPA reactions in phase 1 (median time for phase 1 was 6 months) and 3362 in phase 2. Median laboratory turnaround time for MLPA was 6 days (interquartile range (IQR) 4 to 8) in phase 1, 3 days (IQR 2 to 7) in phase 2 and 17 days (IQR 15 to 20) for karyotyping (figure 2) (medians phase 1 vs phase 2 vs karyotyping: $P<0.001$; medians phase 1 vs phase 2: $P<0.001$; medians phase 1 vs karyotyping: $P<0.001$; and medians phase 2 vs karyotyping: $P<0.001$; all Kruskal-Wallis test followed by Dunn's test).

Figure 2: Laboratory turnaround time for multiplex ligationdependent probe amplification (MLPA) in phase 1 (MLPA result not reported to clients) and phase 2 (MLPA result reported to clients) and for karyotyping.

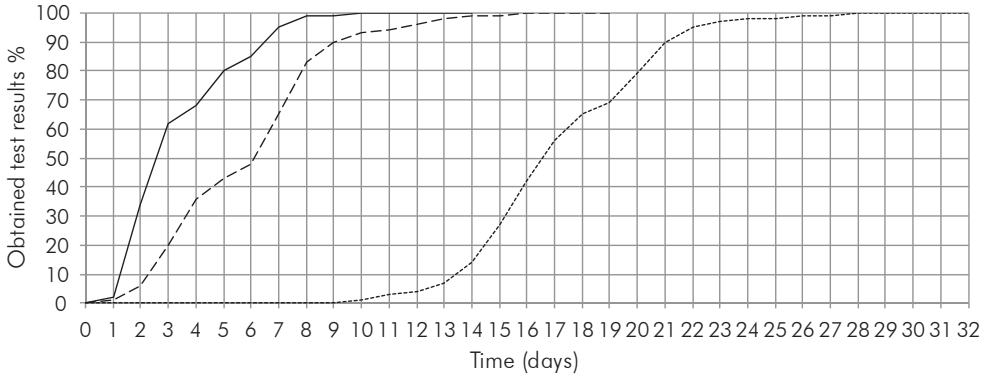


Table 5. Total of chromosomal abnormalities detected with karyotyping and not detected with MLPA out of 4585 amniocentesis; arranged in order of clinical consequences

No clinical consequences for the current pregnancy (17)
45,XY,der(13;14)(q10;q10)[10]mat
45,XY,der(13;14)(q10;q10)mat
45,XY,der(13;22)(q10;q10)pat
46,X,inv(Y)(p11.2q11.221)pat
46,XX,inv(11)(q21q23)pat
46,XX,inv(17)(p?11.2p?13.3)pat
46,XX,inv(5)(p14p15.1)pat
46,XX,t(11;22)(q23;q11.2)pat
46,XX,t(4;21)(q26;q21)pat
46,XX,t(5;16)(q35;p12)pat
46,XY,inv(9)(p24q22.1)pat
46,XY,t(13;14)(p21.1;q27)pat
46,XY,t(9;13)(q31;q12)pat
46,X,inv(Y)(p11.1q11.2)pat
47,XY,+mar.ish psu idic(15)(q11.2)(289D12+,SNRPN-,446P9-)mt
mos 47,XX,+mar[7].ish rob(?;?)(p10;p10)(wcp14+,wcp15+)15q11.2(SNRPN-,D15S10-)[7]/46,XX[10]dn
mos 47,XY,+20[2]/46,XY[16]
Uncertain clinical consequences for the current pregnancy (6)
46,XX,t(4;11)(q31?1;p1?3)dn
46,XX,t(11;13)(q21;q14)dn
46,XX,t(11;22)(q23;q11.2)dn
46,XY,t(6;9)(p22;13)dn
mos 45,X[6]/46,XX[11] confirmed postpartum 45,X[3]/46,XX[32]
Mos 47,XX,der (17)(p11.1q11.1)[10]/46,XX[12]
Severe clinical consequences for the current pregnancy (3)
46 XY,del(7)(p?15p2?2)
46,XX,del(18)(p11.21)[15]/46,XX,dup(18)(p11.21p11.32)[13]
47,XX,+mar.ish del(9)(q1?3)(wcp9+)9p24.3(GS-43-N6+)dn

Median time between amniocentesis and informing pregnant women was 3 days (IQR 3 to 7) for MLPA and 18 days (IQR 16 to 21) for karyotyping. Mean time reduction of MLPA compared with karyotyping was 13.8 days (P<0.001, 95% CI 13.7 to 14.0) and 14.5 days (P<0.001, 95%CI 14.3 to 14.6), on laboratory and patient level respectively.
Costs for MLPA were $472. Costs for karyotyping were $915. Mean cost reduction per sample was $433 (95% CI $416 to $449; - 47%) in favor of MLPA (P<0.001).

DISCUSSION

In this nationwide prospective cohort study including more than 4500 women, we demonstrated that diagnostic accuracy of MLPA to detect aneuploidies of chromosomes 21, 13, 18, X and Y is comparable to karyotyping and MLPA is less costly than karyotyping. Our large study under standard practice conditions confirms and extends the findings of recent preclinical studies on MLPA[19,20]. Compared with other techniques for rapid aneuploidy detection, diagnostic accuracy of MLPA is similar to quantitative fluorescent polymerase chain reaction (QF-PCR) (0.99-1.0) and fluorescence in situ hybridization (FISH) (0.99-1.0) with comparable failure rates of 0.1%-3.7% for QF-PCR and 0.0%-4.9% for FISH (21-26). However, few of these results were obtained under practice conditions. Compared with QF-PCR, MLPA is relatively sensitive to DNA quality and does not detect maternal cell contamination in female samples or female triploidies. MLPA can detect 40 genomic targets in one reaction and avoids the problem of noninformativeness of the polymorphic markers that may occur with QF-PCR. Compared with FISH, MLPA and QF-PCR are both more suitable for high-throughput testing at lower costs[22]. Therefore, QF-PCR and MLPA represent the preferred techniques for routine prenatal diagnosis. FISH, however, is preferred if chromosomal mosaicism is suspected, as detection levels of 5% can be achieved[23].

Our study showed lower costs of MLPA compared to karyotyping; however, similar to studies on QF-PCR and FISH, considerable variation among laboratories exists, mainly caused by differences in sample throughput and logistics[22]. Further research is warranted to determine the additional costs accrued by life time costs of chromosomal abnormalities.
The failure rate of 1.6%, similar to previous studies[12,19,20], is a concern. In a standalone policy, failure implies repeating the amniocentesis with its inherent risks. It is likely that the true failure rate in a standalone policy is lower. Firstly, there was a 38% reduction of the failure rate (from 2.4 % to 1.5%) between early and later experience with the test. Secondly, the study protocol prioritized karyotyping, which requires 12 ml of amniotic fluid. In a standalone policy, more amniotic fluid is available for MLPA and the failure rate will fall. Thirdly, a further

decrease of failure may occur when a lower number of bloody samples can be achieved. The American College of Obstetricians and Gynecologists recommends continuously visualizing the needle for this purpose[27]. From our study results and the available evidence, we recommend using the single operator technique with continuous ultrasound control. Furthermore, there are two options to manage macroscopically blood-stained samples; one is to detect the proportion of fetal hemoglobin (HbF) versus adult hemoglobin and perform MLPA if the HbF level is ≥ 85% of the total hemoglobin[20], or to omit MLPA and perform karyotyping on these samples. Finally, in a standalone policy, we recommend short-term storage of AF cells to allow karyotyping should MLPA fail and subsequent storage of DNA to allow follow-up molecular diagnostics without repeated amniocentesis should ultrasound examination show an abnormality.

The main argument against replacing karyotyping by rapid aneuploidy detection is that some clinically severe chromosomal abnormalities will remain undetected. Of the 26 chromosomal abnormalities (out of 4585; 0.6%) which MLPA could not detect, 17 were without clinical consequences for the current pregnancy (see table 5). Of these, 14 were inherited balanced rearrangements, which may lead to future unbalanced rearrangements. Six of the remaining nine abnormalities were chromosomal abnormalities with uncertain clinical consequences. If detected, this type of abnormality leads to difficult counseling issues and emotional dilemmas[7]. It is questionable whether their detection is in the best interest of the parents as it may lead to an unwarranted termination of pregnancy[1,28]. The last three chromosomal abnormalities were of serious clinical significance (see table 5); this overall residual risk of 0.07% confirms findings by others[1,21]. In our study, with knowledge of the karyotype, standard follow-up ultrasound examination showed abnormalities in one out of three. Hence, when using standalone MLPA combined with ultrasound examination, two chromosomal abnormality of serious clinical significance remain undetected. In total, three of the 26 pregnancies were terminated (one of uncertain clinical consequence, two of serious clinical significance) and 23 were continued. Therefore, in our sample of 4585 pregnancies, the added knowledge from karyotyping leads to three extra terminations of pregnancy.

The provision of rapid, unambiguous and low cost results is an incentive to implement MLPA. Successful implementation also requires the support of pregnant women[29]. So far two studies show that pregnant women prefer rapid aneuploidy detection over karyotyping[22,30]. A Swedish study showed that 70% of women offered an actual choice preferred rapid testing over karyotyping[31]. At the public health level these studies suggest that rapid testing is the preferred strategy. If one adheres to individual choice, one could argue that the decision to either obtain as much cytogenetic information as possible versus a rapid specific result is most appropriately made by individuals who will bear the responsibility of raising the child.

In this era of rapid developments in prenatal diagnosis, the debate on what to test for remains essential. At present, the use of micro arrays, which can detect even more chromosomal abnormalities than karyotyping, is being studied[32]. Within a few years, non-invasive diagnosis of fetal chromosomal abnormalities in maternal blood may be available[33], excluding the procedure-related miscarriage risk. Even with these new developments, the debate on targeted or whole genome testing remains in force. The widespread introduction of molecular tests changes the scope of prenatal diagnosis and should encourage the development of strategies that tailor the type of diagnostic test offered to the risk identified. Future studies should focus on the application of tailor-made strategies, including the views of pregnant women and possible barriers that hamper successful implementation of new prenatal test strategies. For now, the use of MLPA in prenatal diagnosis appears a prudent strategy.

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Chapter 3

The impact of rapid aneuploidy detection (RAD) in addition to karyotyping versus karyotyping on maternal quality of life

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ABSTRACT

Objective

To assess the impact of rapid aneuploidy detection (RAD) combined with fetal karyotyping versus karyotyping only on maternal anxiety and health related quality of life.

Methods

Women choosing to undergo amniocentesis were selected into group 1 i.e., receiving a karyotype result only (n=132) or to group 2 i.e., receiving both the result of RAD and karyotyping (n=181).

Results

There were no systematic differences in time of RAD combined with karyotyping versus karyotyping only in terms of anxiety (P=0.91), generic physical health (P=0.76, P=0.46), generic mental health (P=0.52, P=0.72), personal perceived control (P=0.91), and stress (P=0.13). RAD combined with karyotyping reduced anxiety and stress two weeks earlier compared to karyotyping only.

Conclusion

RAD as add-on to karyotyping reduces anxiety and stress in the short term, but it does not influence overall anxiety, stress, personal perceived control, and generic mental and physical health when compared to a karyotype only strategy.

INTRODUCTION

Amniocentesis is the most frequently used invasive prenatal diagnostic procedure in Western countries and is performed in about one in 30 pregnancies[1,2]. Advanced maternal age and/or increased risk following first trimester prenatal screening for Down syndrome are the most common indications for invasive testing.

Karyotyping is considered the gold standard for the detection of fetal chromosomal abnormalities in amniotic fluid cells[3,4]. The reporting time may take up to three weeks, due to the required cell culture to obtain dividing cells. This waiting period is considered stressful and emotionally burdening to the parents[5-7].

In the last decade new molecular techniques have become available for rapid aneuploidy detection (RAD) of the most common aneuploidies; aneuploidies of chromosomes X, Y, 13, 18 and 21. A major advantage of RAD is a highly accurate result available within a few days. Potentially this should reduce parental stress and anxiety. Multiplex Ligation-dependent Probe Amplification (MLPA) is one of these techniques and was shown to be robust[8,9].

As part of the nationwide MLPA And Karyotyping, an Evaluation (M.A.K.E.) study in which we compared MLPA head to head with karyotyping, we assessed the impact of MLPA as add-on to karyotyping on anxiety, mental and physical health, personal perceived control and stress during prenatal diagnosis. The aim of this comparative study was to assess the impact of MLPA as add-on to karyotyping on the experienced anxiety, stress and Health Related Quality of Life (HRQoL) of the prospective parents during the testing process. Our research question was: Do women who undergo amniocentesis experience less anxiety and better HRQoL when MLPA as add-on test is carried out prior to karyotyping when compared to a karyotyping only strategy?

METHODS

Study design

This study was designed as a prospective cohort study. The aim was to compare the level of anxiety and HRQoL of women receiving a karyotype result (group 1) with women receiving both an MLPA and karyotype result (group 2). The study was carried out alongside the clinical M.A.K.E. study. The M.A.K.E. study has been described before in detail[10.] For the HRQoL study, we aimed at an inclusion of 175 women per group. Assuming an incomplete response rate of 30%, this would leave 123 women per group for analysis. Group 1 was recruited from December 2006 to August 2007 in three prenatal clinics (Leiden University Medical Center, Leiden; Academic Medical Center, Amsterdam; Onze Lieve Vrouwe Gasthuis, Amsterdam)

before the introduction of MLPA. Group 2 was recruited following the laboratory validation of MLPA and took place from April 2007 to December in 2007 in five hospitals (Leiden University Medical Center, Leiden; Erasmus University Medical Center, Rotterdam; Radboud University Medical Center, Nijmegen; Rijnstate hospital, Arnhem; St. Elisabeth hospital, Tilburg).
In group 1, fetal karyotype was disclosed as soon as the result was available, usually within 16-21 days. In group 2, participants received the MLPA results within 3-4 days and karyotyping results as soon as they were available.
Women attending the department of prenatal care, who chose to have amniocentesis and considered eligible for study participation, were invited to participate. After a woman consented to participation, she received the set of surveys with prepaid envelopes. The surveys were to be filled in at home, according to the following scheme: one before amniocentesis was done (Q1), the other surveys at respectively 2 (Q2), 14 (Q4), 23 (Q5) and 63 days (Q6) after amniocentesis (table 2). For group 2 an additional survey was given at 5 days (Q3) after amniocentesis to measure anxiety after the MLPA result had been disclosed. Patient characteristics were collected in survey Q1 (table 2).

Procedures

Amniocentesis was carried out by trained obstetricians following national guidelines[11]. Four genetic laboratories performed all diagnostic tests (Leiden University Medical Center, Academic University Medical Center, Erasmus University Medical Center, and Radboud University Medical Center). We used the MLPA P095 kit (MRC Holland, Amsterdam) for the detection of aneuploidies of chromosome X, Y, 13, 18 and 21. The principle of MLPA has been described before[8]. Karyotyping was carried out according to national guidelines[12].

Study population

The target population consisted of all pregnant women who chose to have amniocentesis for advanced maternal age (36 years or older), increased risk following first trimester screening for Down syndrome (risk > 1: 200)[13] or parental anxiety for having a child with a chromosomal abnormality. Other inclusion criteria were a singleton pregnancy, sufficient command of the Dutch language, and age at least 18 years. Women were excluded from the analysis if they had received an abnormal test result or in case of a miscarriage. The Institutional Review Board approved of the study and written informed consent was judged not to be necessary.

Outcome measures

Spielberger State-Trait Axiety Inventory (STAI)

The aim of the STAI is to quantify the level of anxiety experienced at any point in time (State, s-anxiety), while allowing for the inherent anxiety (Trait, t-anxiety) normally felt by the subject[14].

The State and Trait subscales, with 20 items each in a 4-point response format, range from 20-80, with higher scores indicating greater anxiety. We used the validated Dutch version of the STAI[15]. The Trait scale was only asked at Q1, as this generally reflects the woman's inherent anxious personality which should not change significantly in different situations. The State subscale was included in all surveys, as we expected fluctuations in State anxiety in response to various situations.

Medical Outcome Study Short Form (SF-36)

The SF-36 questionnaire is a self-administered health survey used to assess physical and mental health concerning the past 4 weeks. It comprises of 36 questions summarized in the following eight domain scores: physical functioning, role functioning due to physical and emotional problems, social functioning, bodily pain, mental health, vitality, and general health perceptions. The eight domain scores are summarized in two summary scales: the physical component summary scale (PCS) and the mental component summary scale (MCS), which are derived using weighted averages of the individual domain scores. The PCS and MCS scores range from 0-100 (100 indicating optimal score). We used the validated Dutch version of the SF-36 (mean Cronbach's alpha 0.84)16. We included the SF-36 in surveys Q1 and Q6, since the questionnaire focuses on the physical and mental health during the preceding 4 weeks.

Personal Perceived Control (PPC)

The PPC was introduced in 1997 to evaluate the process of genetic counseling[17]. The aim of the PPC is to measure an individual's belief that they have the resources necessary to respond to an event in a way that will decrease its negative effect[18]. The quality of counseling can influence the feeling of control and thereby influence the patient's experienced control and stress. We used the Dutch PPC as a one-dimensional instrument with a possible score ranging from 0 to 2, with higher scores indicating more perceived control[19]. The PPC was included in all surveys to assess the woman's control during the complete prenatal diagnostic process.

Impact of Event Scale (IES)

The aim of the 15-item IES is to measure the impact of a named stressor[20]. The scale distinguishes the components intrusion and avoidance in a 4-point response format with a possible total score ranging from 0 to 75. Higher scores indicate higher stress levels. We used the validated Dutch version of the IES[21]. The stressor varied according to the process of care: in Q2 and Q4 it was amniocentesis, in Q3 the MLPA result, in Q5 the karyotyping result, and in Q6 the complete prenatal diagnosis process.

Missing data

Participants with two or more missing surveys (Q1-Q6) were regarded as incomplete responders and excluded from analysis. Missing data were imputed if one survey was missing using REML procedures of linear mixed models (LMM, SPSS 16.0). In case less than 50% of the responses to a questionnaire were missing, data were imputed using mean imputation; otherwise the questionnaire was regarded missing.

Statistical analyses

Descriptive summaries of patient characteristics and quality of life measurements included means and standard deviations (SD) for normally distributed variables and medians with ranges for other variables. Demographic characteristics of group 1 and 2 were compared by independent t test and X^2 test, or Mann Whitney U, if appropriate. We compared demographic characteristics as well as mean baseline quality of life scores between women completing all questionnaires and those who did not in order to test for selection bias. We used repeated measurement analysis (SPSS 16.0, linear mixed models, unstructured covariance) on transformed State, PPC (log transformed), and IES ($\sqrt{IES+0.5}$ transformed) scores to evaluate longitudinal differences between the groups with adjustment for differences in baseline characteristics. Differences in State, PPC and IES between the groups at the separate time points were assessed with Mann Whitney U. Linear regression analysis was used to analyze difference in the eight domains of the SF-36, and PCS and MCS scores, adjusted for differences in baseline characteristics. A P-value of <0.05 (two sided) was considered statistically significant in all analyses.

RESULTS

Eligible for study participation in group 1 and 2 were 175 and 225 consecutive women respectively, of whom 165 (94.3%) and 209 (92.3%) agreed to participate. The most commonly cited reasons to decline participation were disinterest and lack of time (figure 1). Seven women in group 1 and six women in group 2 were excluded after MLPA or karyotyping disclosed a chromosomal abnormality (figure 1). One woman in group 2 was excluded due to ruptured membranes after the amniocentesis and subsequent termination of pregnancy.

Table 1 shows demographic characteristics of both groups and for complete and incomplete responders. A total of 313 (83.7%) women completed all or all but one questionnaires. 132 out of 158 (83.5%) women in group 1 and 181 out of 203 (89.2%) women in group 2. In group 1, the incomplete responders had a significant lower Trait anxiety (P= 0.002) (table 1). In group 2, the complete responders were significantly more often of western origin (P=

Figure 1: Flowchart study

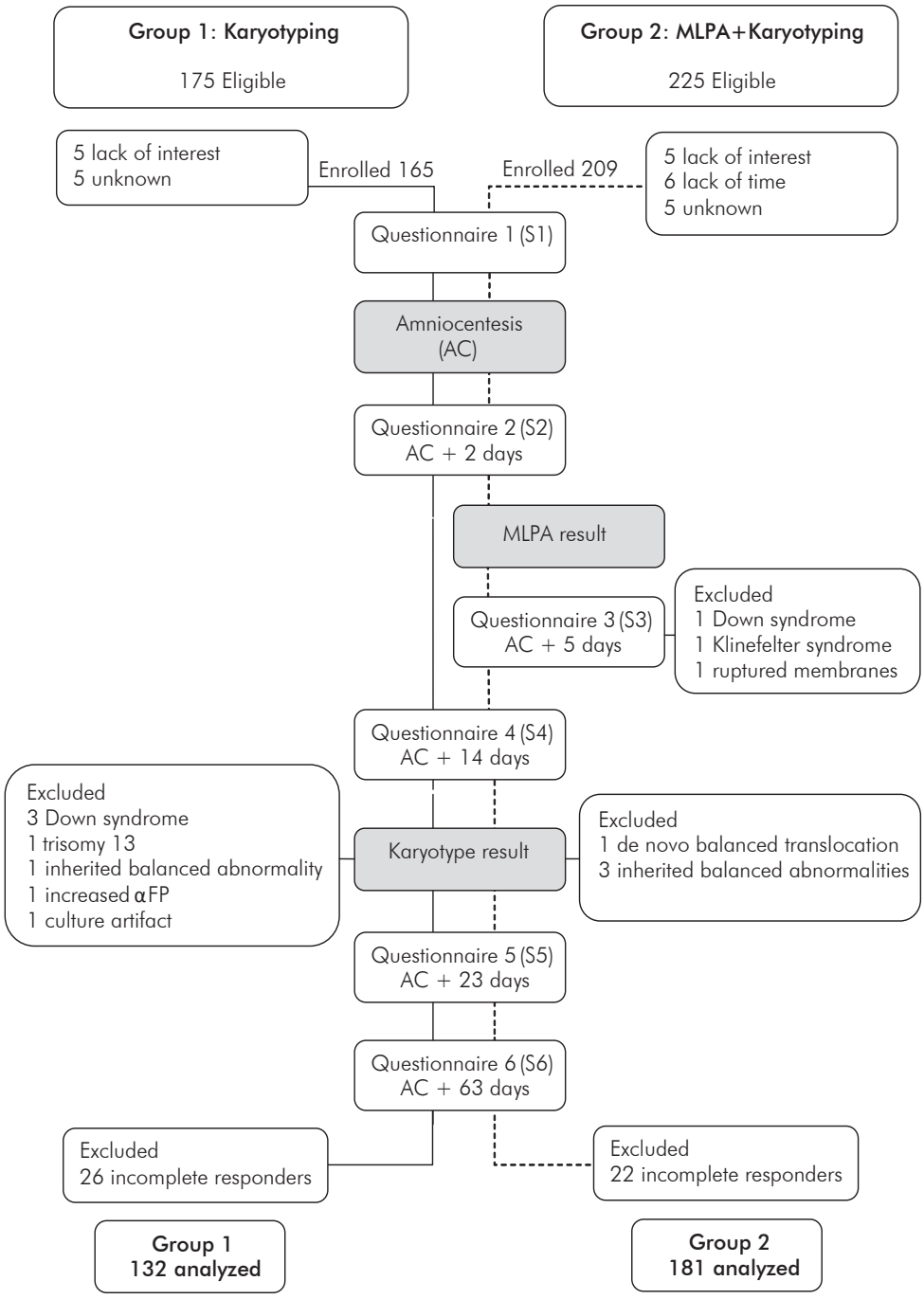

Table 1: Patient characteristics and baseline scores of complete and incomplete responders of group 1 (karyotyping) and group 2 (MLPA and karyotyping).

	Group 1 (Karyotyping)		Within group 1
	complete	incomplete	
Number	139	26*	
Chromosomal abnormality	7 (5.0%)	1 (3.8%)	
Analysed	132		
Mean age (SD)	38.3 (2.69)	38.5 (3.09)	p 0.77
Mean parity (SD)	0.9 (1.00)	1.0 (0.70)	p 0.47
Mean gravidity (SD)	1.6 (1.40)	1.8 (1.10)	p 0.49
Indication			p 0.38
maternal age	112 (84.8%)	21 (80.0%)	
prenatal screening	16 (12.1%)	3 (11.5%)	
anxiety	4 (3.0%)	1 (3.8%)	
Highest educational level			p 0.65
Lower vocational, lower secondary school	17 (12.9%)	4 (15.4%)	
Intermediate and higher vocational, higher secondary	33 (25.0%)	5 (19.2%)	
College/University	82 (62.1%)	16 (61.5%)	
Western?	118 (89.4%)	19 (73.1%)	p 0.17
Religion			p 0.41
atheist	76 (57.6%)	16 (61.5%)	
catholic	37 (28.0%)	6 (23.1%)	
protestant	9 (6.8%)	0 (0%)	
other	10 (7.6%)	3 (11.5%)	
Previous PND?	25 (18.9%)	8 (30.8%)	p 0.11
Median state at S1 (IQR)	35.0 (30-42)	36.5 (31-47)	P 0.35
Median trait at S1 (IQR)	32.0 (26-38)	37.0 (32-42)	p 0.002
Median PCS at S1 (IQR)	52.7 (48-57)	54.6 (49-56)	p 0.39
Median MCS at S1 (IQR)	48.9 (41-53)	48.1(33-52)	p 0.22
Median PPC at S1 (IQR)	1.3 (0.9-1.6)	1.3 (1.0-1.7)	p 0.39

*one participant returned questionnaire B and D, therefore baseline characteristics are unknown

0.03) (table 1). Complete responders in group 2 had a significantly lower educational level than women in group 1 (P= 0.02), but otherwise the groups did not differ (table 1).

Quality of life outcome

State-anxiety

There were no systematic longitudinal differences in anxiety between the groups (P=0.91, table 3). Pattern of anxiety differed significantly over time, with a decrease of anxiety in both groups after obtaining a test result (figure 2). Cross-sectionally, anxiety scores between the groups differed significantly at 2, 14, and 23 days (P=0.001, P= 0.02, P= 0.009, respectively Supplementary Table S1) following amniocentesis, with alternating dominance of test technique (figure 2). Women with high State and Trait scores prior to amniocentesis reported high anxiety scores in the subsequent questionnaires (P<0.001, table 3); demographic factors did not

Group 2 (MLPA + Karyotyping)		Within group 2	Complete group 1 vs 2
complete	incomplete		
187	22		
6 (3.2%)	1 (4.5%)		
181			
37.9 (2.69)	37.3 (3.20)	p 0.45	p 0.16
1.1 (1.08)	1.0 (0.98)	p 0.68	p 0.15
1.8 (1.57)	2.0 (1.43)	p 0.51	p 0.26
		p 0.41	p 0.63
157 (86.7%)	20 (90.9%)		
21 (11.6%)	2 (9.1%)		
3 (1.7%)	0 (0%)		
		p 0.13	p 0.02
29 (16.0%)	4 (18.2%)		
69 (38.1%)	6 (27.3%)		
83 (45.9%)	12 (54.5%)		
168 (92.8%)	17 (77.3%)	p 0.03	p 0.29
		p 0.27	p 0.16
90 (49.7%)	8 (36.4%)		
58 (32.0%)	9 (40.9%)		
24 (13.3%)	2 (9.1%)		
9 (5.0%)	3 (13.6%)		
44 (24.3%)	3 (13.6%)	p 0.26	p 0.26
36.0 (30-47)	41 (31-48)	p 0.55	p 0.33
32.0 (27-39)	31.0 (25-42)	p 0.71	p 0.45
53.0 (45-57)	53.0 (45-57)	p 0.33	p 0.76
49.3 (42-53)	51.0 (42-55)	p 0.79	p 0.52
1.4 (0.8-1.7)	1.3 (1.1-1.6)	p 0.99	p 0.90

affect anxiety scores. Interaction effects between anxiety levels and group over time differed significantly on day 2, 14 and 23 between group 1 and 2 (P= 0.007; P<0.001; P=0.01 respectively, table 3).

SF-36

There were no significant differences in mean PCS and MCS scores, nor in the eight domain scores between the groups before and at 63 days after amniocentesis (PCS: P= 0.76; P= 0.46; MCS: P= 0.52; P=0.72; table 4). The changes in PCS and MCS, adjusted for education and western origin, did not differ significantly between the groups (PCS: beta -0.17 95% CI -2.11 to 1.77 p 0.86; MCS: beta -0.34 95% CI -2.33 to 1.65 p 0.74).

PPC

There were no systematic longitudinal differences between the groups (P=0.91, Supplemental

Table 2: Description of questionnaires and its contents in time

Questionnaire	Point in time	Content	Group
Questionnaire 1	Before amniocentesis	Demographic characteristics, STAI, PPC, MOS SF-36	1 and 2
Questionnaire 2	Amniocentesis + 2 days	STAI, PPC, IES	1 and 2
Questionnaire 3	Amniocentesis + 5 days	STAI, PPC, IES	2
Questionnaire 4	Amniocentesis + 14 days	STAI, PPC, IES	1 and 2
Questionnaire 5	Amniocentesis + 23 days	STAI, PPC, IES	1 and 2
Questionnaire 6	Amniocentesis + 63 days	STAI, IES, PPC, MOS SF-36	1 and 2

Figure 2: Median scores with interquartile ranges (IQR) of 132 women receiving karyotyping and 181 women receiving MLPA and karyotyping.
Significant differences in cross-sectional analysis are marked with the P-value

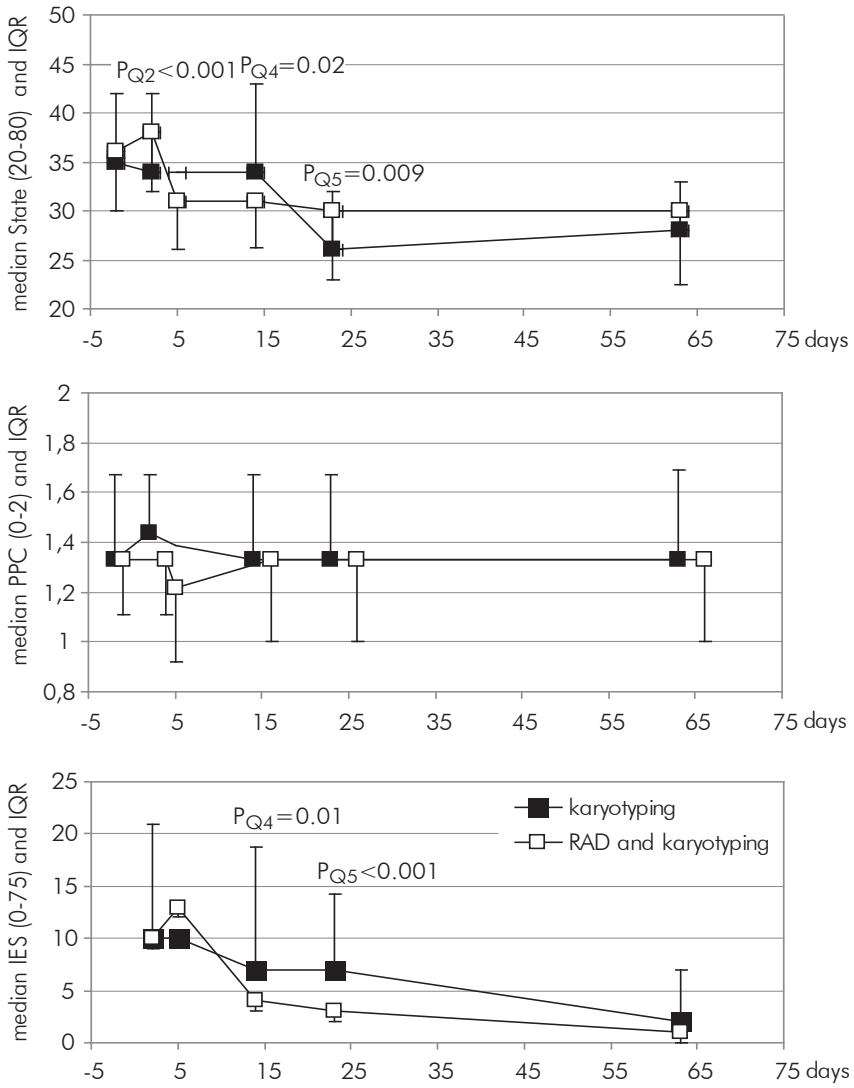

Table 3: Repeated measurement analysis. Linear mixed models on log transformed State scores.

Parameter	Estimate	95% CI	P value
Intercept	1.82	1.59 to 2.05	**<0.001**
AC + 2 days	0.29	0.25 to 0.33	**<0.001**
AC + 5 days	0.06	0.02 to 0.09	**0.001**
AC + 14 days	0.10	0.06 to 0.13	**<0.001**
AC +23 days	0.01	-0.02 to 0.03	0.66
AC +63 days	ref	.	.
Group 1	-0.002	-0.05 to 0.05	0.91
Group 2	ref	.	.
Interaction AC + 2 days *group 1*	-0.09	-0.15 to -0.02	**0.007**
Interaction AC + 14 days* group 1*	0.11	0.06 to 0.17	**<0.001**
Interaction AC +23 days* group 1*	-0.05	-0.09 to -0.01	**0.01**
Interaction AC +63 days*group1*	ref	.	.
Baseline log State	0.33	0.26 to 0.40	**<0.001**
Baseline Trait	0.01	0.01to 0.01	**<0.001**
Lower vocational, lower secondary school	-0.04	-0.10 to 0.01	0.11
Intermediate and higher vocational, higher secondary	0.004	-0.04 to 0.04	0.85
College/University	ref	.	.
Non western origin	0.009	-0.05to 0.07	0.79
Western origin	ref	.	.

-2 loglikelyhood= -658,02, * reference: interaction time*group2, AC= amniocentesis; group 1= karyotyping; group 2= MLPA + karyotyping. Bold values indicate p-values of $P < 0.05$

file S2). Time had a significant effect on PPC levels before and 2 days after amniocentesis ($P<0.001$) with higher perceived control in both groups before obtaining a test result. Compared to a university education, lower and middle education was associated with a lower PPC score. Cross-sectionally, there were no significant differences between the groups (figure 2).

IES

There was no systematic difference in stress level between the groups ($P=0.13$; supplementary Supplemental file S3). Pattern of stress, however, differed significantly over time, with a decrease of stress in both groups after obtaining a test result (figure 2). Cross-sectionally, group 1 had significantly higher stress scores at 14 and 23 days after amniocentesis compared to group 2 ($P= 0.01$, $P<0.001$ respectively; figure 2). Interaction effects between IES scores and group over time differed significantly on day 14 and 23 following amniocentesis ($P<0.001$; $P<0.001$).

DISCUSSION

Overall, our study showed no systematic gain in quality of life, personal perceived control and stress by RAD combined with karyotyping compared to karyotyping only.

The impact of providing a RAD result, followed by later reporting of the full karyotype consisted of three components. Firstly, providing a rapid test result induced significantly higher levels of anxiety at 2 days following amniocentesis. Secondly, after disclosure of the MLPA result, anxiety decreased significantly. Thirdly, the karyotype only group had significant lower anxiety scores after disclosure of the karyotype result. We also found that the reduction of anxiety after disclosure of MLPA in the combined strategy approximately equals the reduction of anxiety after disclosure of the karyotype result in the karyotyping only strategy. On balance, a combined strategy accelerates the reduction of anxiety and stress with two weeks compared to a karyotyping only strategy.

The strength of our study is the prospective study design with high response rate of the consecutively included women and the broad perspective evaluating various aspects of psychological health. The limitation of this study design is its non-randomized design. The various outcome measures had to be adjusted for differences between patient characteristics at baseline and between complete and incomplete responders. We cannot rule out over- or under adjustment. Furthermore, it is unclear if the patterns of anxiety, stress, PPC and HRQoL for normal results also apply to women with fetal chromosomal abnormalities.

The temporary reduction of anxiety after receiving the MLPA result is consistent with previous studies[22-25] on QF-PCR and FISH. We found a rise in anxiety just before the disclosure of the RAD test result. This increase in anxiety may be related to the novelty of introducing RAD. It is more likely represent anxiety related with a prospective but uncertain outcome[26]. A similar pattern of increasing anxiety was seen in the ARIA trial before disclosure of the karyotype[22]. In our study we did not measure anxiety at that point. If the anxiety peak truly exists, a strategy can be developed to decrease this peak. During the counseling process the pattern of anxiety can be discussed and this knowledge might be helpful to cope better with the anxiety. We found lower anxiety scores in the karyotype only group after disclosure of the karyotype compared to the combined group. A similar pattern was observed in the ARIA trial[22], but other studies do not show this difference[23,25]. We speculate that the lower anxiety scores in the karyotype only group can be explained by the 'relief' effect in learning that the test result was normal[27,28], while the RAD group already expected a normal result, since the RAD result was normal. Alternatively, it is also possible that the combined strategy induces anxiety temporarily. Issuing a result twice may induce anxiety by fixing the parent's attention twice on a possible genetic problem, hereby reducing the feeling of a carefree pregnancy. In the long term, however, no differences in anxiety were present. This finding is in agreement with other studies[23,25]. Compared to the similar studies previously mentioned[22,23,25], the average anxiety levels in our groups were remarkably lower. We speculate that this difference is mainly caused by differences in referral indication, which was mostly advanced maternal

age in our study rather than increased risk following first trimester prenatal screening in the other studies[22,25]. However, in our analysis indication had no significant effect on anxiety, stress, personal perceived control and HRQoL (data not shown). Another explanation could be that Dutch women experience less anxiety during prenatal diagnosis, because of cross-cultural differences or the organization of Dutch obstetrical care. The decentralized obstetrical care with independent midwives, the Dutch physiological approach of pregnancy by maternal health care providers, and the national prenatal screening program may all lead to increased personal perceived control, possibly leading to decreased anxiety levels. Previous similar studies did not measure PPC and thus this cannot be compared. Our groups reported equal personal perceived control; thereby supporting the notion that differences in anxiety and stress were not the result of differences in personal control.

The major differences in anxiety and stress on the short term apparently did not translate into long term effects on overall mental and physical health. Previous studies on mental health in pregnancies showed stable MCS scores during first and second trimester[29,30]. Our results suggest that the RAD plus karyotype strategy has no adverse long term effects on HRQoL and, in addition, that normal results reassure and improve mental HRQoL. The reduction in physical health during the test process is in agreement with two studies that reported on the relationship between RAD and HRQoL[23,25] and is associated with increasing gestational age[28,29]. Assuming that a 5 point difference on a scale from 0-100 is clinically relevant[25,31], the size of effect of rapid testing on anxiety and stress reduction by issuing a rapid result is clinically relevant. However, the clinically relevant difference between the groups was not observed in the days following RAD disclosure. Therefore, we conclude that the reduction in anxiety and stress by providing an MLPA result next to karyotyping is clinically relevant on the short term, but on the long term there is no psychological benefit from the add-on strategy.

Recently, a debate emerged in European countries whether RAD should replace karyotyping in prenatal diagnosis for advanced maternal age, increased risk following first trimester prenatal screening and maternal anxiety; i.e. substitution rather than a combined strategy as in our study. The substitution would lead to substantial cost savings, with a risk of 0.06 % of failure to detect a chromosomal abnormality likely to have serious clinical significance which can be detected with karyotyping[1,23]. We feel that the decision to either obtain as much cytogenetic information as possible versus a rapid but specific result of the most common chromosomal abnormalities is most appropriately made by individuals who will bear the responsibility of raising the child. Future studies should assess the impact of offering an individualized choice on HRQoL.

CONCLUSION

Although many women and caregivers regard stress and anxiety as important side effects of prenatal diagnosis, the low STAI and IES scores indicate that most women can cope with the situation. RAD as add-on to karyotyping reduces anxiety and stress in the short term by providing a rapid result. Overall, there were minor psychological benefits of the combined strategy compared to karyotyping only. Therefore, from the psychological point of view RAD as add-on is of limited value to women having amniocentesis for relatively low risk indications.

Supplementary Table S1: Cross-sectional analysis of median differences in State, PPC, IES, SF-36 in time.

Questionnaire	Time	Median group 1	Median group 2	P value
State	Before AC	35.0	36.0	0.33
	AC + 2 days	34.0	38.0	0.001
	AC + 14 days	34.0	32.0	0.02
	AC +23 days	26.0	30.0	0.009
	AC +63 days	28.0	30.0	0.42
PPC	Before AC	1.33	1.33	0.98
	AC + 2 days	1.44	1.33	0.41
	AC + 14 days	1.33	1.33	0.16
	AC +23 days	1.33	1.33	0.27
	AC +63 days	1.33	1.33	0.46
IES	AC + 2 days	10.0	10.0	0.69
	AC + 14 days	7.0	4.0	0.01
	AC +23 days	7.0	3.0	<0.001
	AC +63 days	2.0	1.0	0.22
SF-36 PCS	Before AC	52.7	53.0	0.76
	AC +63 days	47.7	47.3	0.46
SF 36 MCS	Before AC	48.9	49.3	0.52
	AC +63 days	54.4	54.4	0.72

AC= amniocentesis; group 1= karyotyping, group 2= MLPA + karyotyping
PPC= personal perceived control; IES= Impact of Event Scale; SF-36 PCS= physical component score, SF-36 MCS= mental component score

Supplemental file S2: Repeated measurement analysis. Linear mixed models on log transformed personal perceived control scores.

Parameter	Estimate	95% CI	P value
Intercept	0.76	0.71 to 0.82	<0.001
Before AC	0.11	0.07 to 0.17	<0.001
AC + 2 days	0.11	0.07 to 0.15	<0.001
AC + 5 days	0.03	-0.01 to 0.07	0.11
AC + 14 days	0.03	-0.01 to 0.07	0.15
AC +23 days	-0.01	-0.04 to 0.02	0.49
AC +63 days	ref	.	.
Group 1	0.00	-0.07 to 0.08	0.91
Group 2	ref	.	.
Interaction before AC *group 1*	-0.04	-0.11 to 0.03	0.24
Interaction AC + 2 days *group 1*	-0.01	-0.07 to 0.06	0.84
Interaction AC + 14 days* group 1*	0.04	-0.02 to 0.10	0.17
Interaction AC +23 days* group 1*	0.01	-0.04 to 0.06	0.81
Interaction AC +63 days*group1*	ref		
Lower vocational, lower secondary school	-0.12	-0.18 to 0.06	<0.001
Intermediate and higher vocational, higher secondary	-0.07	-0.11 to -0.02	<0.001
College/University	ref	.	.
Non western origin	-0.03	-0.11 to 0.04	0.38
Western origin	ref	.	.

-2 loglikelyhood= -819.38
* reference: interaction time*group2
AC= amniocentesis, group 1= karyotyping, group 2= MLPA+karyotyping.

Supplemental file S3: Repeated measurement analysis. Linear Mixed Models on root+0.5 transformed Impact of Event scale scores.

Parameter	Estimate	95% CI	P value
Intercept	1,79	1.58 – 1.99	<0.001
AC + 2 days	1.70	1.44 – 1.96	<0.001
AC + 5 days	1.65	1.42 – 1.89	<0.001
AC + 14 days	0.57	0.35 – 0.78	<0.001
AC +23 days	0.36	0.16 – 0.57	<0.001
AC +63 days	ref	.	.
Group 1	0.25	-0.07 – 0.57	0.13
Group 2	ref	.	.
Interaction AC + 2 days *group 1*	-0.23	-0.60 – 0.14	0.22
Interaction AC + 14 days* group 1*	0.57	0.24 – 0.91	0.001
Interaction AC +23 days* group 1*	0.57	0.25 – 0.88	<0.001
Interaction AC +63 days*group1*	ref	.	.
Lower vocational, lower secondary school	0.39	-0.03 – 0.82	0.07
Intermediate and higher vocational, higher secondary	0.07	-0.25 – 0.40	0.67
College/University	ref	.	.
Non western origin	-0.03	-0.57 – 0,51	0.91
Western origin	ref	.	.

-2 loglikelyhood= 4297.65
* reference: interaction time*group2
AC= amniocentesis, group 1= karyotyping, group 2= MLPA+karyotyping.

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Chapter 4

Individualized choice in prenatal diagnosis: the impact of karyotyping and standalone rapid aneuploidy detection on quality of life

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Prenatal Diagnosis Accepted

ABSTRACT

Objective

To assess the reasons and perceptions of women who are offered a choice between karyotyping and standalone rapid aneuploidy detection (RAD) and to compare the impact of both tests on anxiety and health related quality of life.

Methods

In this prospective comparative study, women undergoing amniocentesis on behalf of their age or for an increased Down syndrome risk were offered a choice between karyotyping (group1, n=68) and standalone RAD (group 2, n=61). Follow-up was 9 weeks post amniocentesis.

Results

The most commonly cited reason for choosing karyotyping was obtaining as much information as possible, while for choosing standalone RAD it was the short waiting time. Prenatal screening (OR 7.09), no knowledge of karyotyping (OR 4.2) and an intermediate perceived risk for chromosomal abnormalities (OR 3.6) were associated with choosing standalone RAD. There were no systematic differences in time of karyotyping and standalone RAD in terms of anxiety (P= 0.11), generic physical and mental health (P=0.94, 0.52; P=0.66, P=0.07), personal perceived control (P=0.69), and stress (P=0.66).

Conclusion

Offering a choice between karyotyping and standalone RAD does not influence anxiety, stress, personal perceived control, or generic health. Individual choice in prenatal diagnosis meets individual needs and thereby could reduce anxiety and stress.

INTRODUCTION

In the last five years, a debate emerged in European countries whether rapid aneuploidy detection (RAD) should replace karyotyping in prenatal diagnosis for advanced maternal age and increased Down syndrome risk following first trimester prenatal screening (PNS). Opponents' main argument is that substitution of karyotyping by RAD would lead to an increase of live births of children with undetected chromosomal abnormalities with severe clinical consequences in women undergoing an invasive diagnostic test. Proponents' main argument is that the substitution of karyotyping would lead to a shortening of the stressful waiting time for parents, more straightforward prenatal and genetic counseling, and substantial cost savings, while the risk of missing a chromosomal abnormality with serious consequences is small[1,2]. At this point in time, prenatal centers decide individually whether or not to implement RAD as standalone test.

Thus far, clinical studies have mainly focused on diagnostic accuracy of RAD tests compared to karyotyping[3-5] but little is known about testing behavior and women's perceptions associated with targeted versus broad testing.

To strengthen the debate, we aimed to evaluate quality of life and testing behavior of women when they are offered a real choice between standalone RAD and karyotyping. The aim of this comparative study was 1) to find out which test is preferred by women who are offered a real choice between karyotyping and RAD, and to elicit the reasons for their choice and 2) to assess the impact of the chosen test on the experienced anxiety, stress and Health Related Quality of Life (HRQoL).

METHODS

Study design

This study was designed as a prospective cohort study. We compared the level of anxiety and HRQoL of women undergoing amniocentesis. Group 1 received a karyotype result; group 2 received a RAD result (i.e. Multiplex Ligation-dependent Probe Amplification, MRC Holland), detecting aneuploidies of chromosomes X, Y, 13, 18 and 21. In group 1, fetal karyotype was disclosed as soon as the result was available, usually within 16-21 days. In group 2, participants received the RAD results as soon as the result was available, usually within 3 to 4 days. The study was carried out as final part of the clinical MLPA And Karyotyping, an Evaluation (M.A.K.E.) study. The M.A.K.E. study has been described before in detail[5,6].

Study population

Women were recruited consecutively in six prenatal clinics (Leiden University Medical Centre, Leiden; Bronovo Hospital, The Hague; Radboud University Medical Centre, Nijmegen; Rijnstate hospital, Arnhem; St. Elisabeth Hospital, Tilburg; Jeroen Bosch Hospital, 's Hertogenbosch) after the results of the M.A.K.E. study showed comparable diagnostic accuracy of MLPA and karyotyping[6]. The population consisted of all pregnant women who chose to have amniocentesis for advanced maternal age (36 years or older) or increased Down syndrome risk following first trimester screening (risk > 1: 200 at the time of testing)[7]. Other inclusion criteria were a singleton pregnancy, sufficient command of the Dutch language, and age at least 18 years. Women were excluded from the analysis if they had received an abnormal test result or in case of a miscarriage. The Institutional Review Board approved of the study and all participants gave informed consent. Women attending the department of prenatal care, who chose to have amniocentesis and considered eligible for study participation, were invited to participate. It was routine policy to offer a choice between standalone RAD and karyotyping to women who opted for amniocentesis, even if they did not want to participate in this HrQoL study. The prenatal counselor explained the tests using both oral and written information. Women received the set of surveys with prepaid envelopes and completed the surveys at home, according to the following scheme: one before amniocentesis was done (Q1), the other surveys at respectively 2 (Q2), 5 (Q3), 14 (Q4), 23 (Q5) and 63 days (Q6) after amniocentesis (table 2). Patient characteristics and reasons and perceptions to choose karyotyping or RAD were collected once in survey Q1 (table 1).

Outcome measures

Reasons and perceptions

In Q1, we asked if women had some knowledge of both tests, whether the counselor gave them information on both tests and whether women had tried to obtain extra information. Patients indicated the most important reason for selecting the specific test, stated if they still supported their test of choice, and their risk perceptions (high, neither low nor high, and low risk) for carrying a fetus with a chromosomal abnormality.

Spielberger State-Trait Axiety Inventory (STAI)

The aim of the STAI is to quantify anxiety levels experienced at any point in time (State, s-anxiety), while allowing for the inherent anxiety (Trait, t-anxiety) normally felt by the subject[8]. The State and Trait subscales, with 20 items in a 4-point response format, range from 20-80, with higher scores indicating greater anxiety. A State score of 42 and more represents pathological anxiety[9]. We used the validated Dutch version of the STAI[10]. The Trait scale was asked at Q1, as this reflects the woman's inherent anxiety which should not

change in different situations. The State subscale was included in all surveys, as we expected fluctuations in State anxiety in response to various situations.

Medical Outcome Study Short Form (SF-36)

The SF-36 questionnaire is a self-administered health survey assessing physical and mental health concerning the past 4 weeks. It comprises of 36 questions summarized in eight domain scores: physical functioning, role functioning due to physical and emotional problems, social functioning, bodily pain, mental health, vitality, and general health perceptions. The eight domain scores are summarized in two summary scales: the physical component summary scale (PCS) and the mental component summary scale (MCS), which are derived using weighted averages of the individual domain scores. The PCS and MCS scores range from 0-100 (100 indicating optimal health score). We used the validated Dutch version of the SF-36 in Q1 and Q6, see table 1[11].

Personal Perceived Control (PPC)

The PPC measures an individual's belief that they have the resources necessary to respond to an event in a way that will decrease its negative effect[12,13]. The quality of counseling can influence the feeling of control and thereby influence the patient's experienced control and stress. We used the Dutch PPC ranging from 0 to 2, with higher scores indicating more perceived control and included it in all surveys (table 1)[14].

Impact of Event Scale (IES)

The aim of the 15-item IES is to measure the impact of a named stressor[15]. The scale distinguishes the components intrusion and avoidance in a 4-point response format with a total score ranging from 0 to 75. Higher scores indicate higher stress levels. We used the validated Dutch version of the IES[16]. The stressor varied according to the process of care.

Statistical analyses

We aimed to include 45 women per group in the analysis (difference log transformed State 0.15 on Q3; SD 0.25). Adjusted for refusal and drop-out, we aimed to include 60 women per group. Descriptive summaries of patient characteristics and quality of life measurements included means and standard deviations (SD) for normally distributed variables and medians with ranges for other variables. Demographic characteristics of group 1 and 2 were compared by independent t test and X^2 test, or Mann Whitney U, if appropriate. We compared demographic characteristics as well as mean baseline quality of life scores between women completing all questionnaires and those who did not in order to test for selection bias. The reasons and perceptions to choose either karyotyping or MLPA are displayed in percentages. To identify patient characteristics associated with the chosen test,

we performed backward-selection logistic regression analysis (forward-selection logistic regression produced similar results). We used repeated measurement analysis (SPSS 16.0, linear mixed models, unstructured covariance) on log transformed State, PPC, and IES scores to evaluate longitudinal differences between the groups with adjustment for differences in baseline characteristics. Differences in State, PPC and IES between the groups at the separate time points were assessed with Mann Whitney U. Linear regression analysis was used to analyze differences in the eight domains of the SF-36, and PCS and MCS scores, adjusted for differences in baseline characteristics. A P-value of <0.05 (two sided) was considered statistically significant in all analyses. Participants with two or more missing surveys (Q1-Q6) were regarded as incomplete responders and excluded from analysis. Missing data were imputed if one survey was missing using REML procedure of linear mixed models (LMM, SPSS 16.0). In case less than 50% of the responses to a questionnaire were missing, data were imputed using mean imputation; otherwise the questionnaire was regarded missing.

RESULTS

In group 1 and 2, 87 and 74 women agreed to participate. One woman in group 1 and two women in group 2 were excluded after MLPA or karyotyping disclosed a chromosomal abnormality (figure 1).

Table 2 shows demographic characteristics of both groups. In group 1 and 2, 68 (85%) and 61 (85%) women completed all or all but one questionnaire, respectively. The indication of complete responders in group 1 vs. group 2 was significantly more often maternal age ($P<0.01$) with significantly lower State scores ($P<0.01$) and lower perceived control than women in group 1 ($P= 0.05$), otherwise the groups did not differ (table 2). In group 1, the incomplete responders had a significantly higher State anxiety ($P= 0.05$) (table 1). In group 2, the complete responders were significantly more often of western origin ($P= 0.04$) with significantly lower perceived control scores ($P<0.01$) (table 2).

Table 1: Description of questionnaires and its contents in time.

Questionnaire	Point in time	Content	Group
Questionnaire 1	Before amniocentesis	Demographic characteristics, motivation to choose RAD or karyotyping, STAI, PPC, MOS SF-36	1 and 2
Questionnaire 2	Amniocentesis + 2 days	STAI, PPC, IES	1 and 2
Questionnaire 3	Amniocentesis + 5 days	STAI, PPC, IES	1 and 2
Questionnaire 4	Amniocentesis + 14 days	STAI, PPC, IES	1 and 2
Questionnaire 5	Amniocentesis + 23 days	STAI, PPC, IES	1 and 2
Questionnaire 6	Amniocentesis + 63 days	STAI, IES, PPC, MOS SF-36	1 and 2

STAI= State Trait and Anxiety Inventory, PPC= Personal Perceived Control, MOS SF-36= Medical Outcome Study Short Form, IES= Impact of Event Scale

Figure 1: Flowchart of the study.

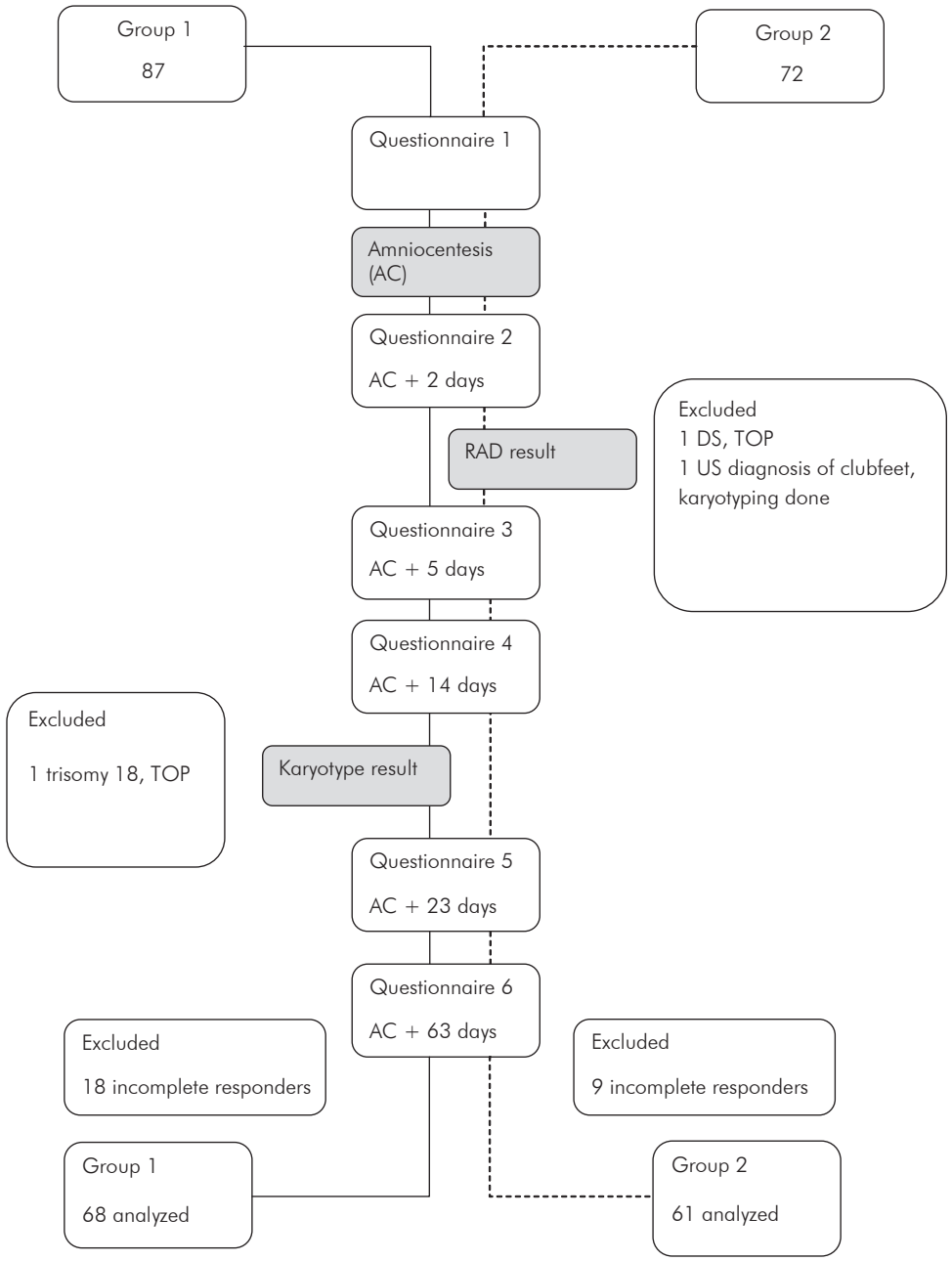


Table 2: Patient characteristics and baseline scores of complete and incomplete responders of group 1 (karyotyping) and group 2 (RAD).

	Group 1 (Karyotyping)		Within group 1
	complete	incomplete	
Number	69	18	
Chromosomal abnormality	1	0	
Analysed	68		
Median age (IQR)	38.3 (35-43)	37.9 (37-40)	p 0.48
Median parity (IQR)	1.0 (0-2)	1.0 (0-2)	p 0.86
Median gravidity (IQR)	1.0 (1-2)	1.0 (1-2)	p 0.36
Indication			p 0.79
maternal age	63	17	
Prenatal screening	5	1	
Highest educational level			p 0.17
Lower vocational, lower secondary school	10	6	
Intermediate and higher vocational, higher secondary	14	2	
College/University	44	10	
Western	66	18	p 0.46
Religion			0.69
Atheist	30	7	
Religious	38	11	
Previous PND	13	2	p 0.42
Median state at S1 (IQR)	34.0 (26-46)	44.5 (31-53)	p 0.05
Median trait at S1 (IQR)	30.0 (27-36)	36.0 (29-38)	p 0.07
Median PCS at S1 (IQR)	55.2 (48-58)	53.2 (47-57)	p 0.30
Median MCS at S1 (IQR)	50.3 (47-54)	48.6 (46-54)	p 0.65
Median PPC at S1 (IQR)	1.3 (1.1-1.8)	1.2 (0.9-1.5)	p 0.15

Reasons and perceptions to choose RAD or karyotyping

In group 1 and 2, 88% versus 72% had knowledge of karyotyping and 35% versus 26% of RAD; 88% versus 87% of women were informed on both tests and 31% versus 32% had tried to find complementary information. The dominant reason to choose karyotyping was: to obtain as much information as possible (54.4%), less uncertainty (32.4%), previous experience with the test (7.4%), the historical use of the test (4.4%), and advised by my obstetrician (1.5%). For group 2 the dominant reason to choose RAD was: the short waiting time (47.5%), less anxiety (18%), the clear consequences of the test (14.8%), the detection of the most common chromosomal abnormalities (13.1%), and recommended by my obstetrician or midwife (6.6%). For group 1 and 2, 86.8% vs. 80.0% were confident of their choice.

Patient characteristics significantly associated with choosing RAD were: referral indication PNS (vs. advanced maternal age) (OR 7.09 95% CI 1.82 to 27.65), no knowledge of karyotyping (vs. knowledge) (OR 4.2 95% CI 1.3 to 14.3) and a neither low nor high perceived risk for fetal chromosomal abnormalities (vs. a low perceived risk) (OR 3.6 95% CI 1.12 to 11.55). Age, knowledge of RAD (vs. no knowledge), baseline State and Trait scores

Group 2 (RAD)		Within group 2	Complete group 1 vs 2
complete	incomplete		
63	9		
2	0		
61			
37.9 (32-41)	38.6 (37-39)	p 0.36	p 0.06
1.0 (0-2)	1.0 (0-2)	p 0.93	p 0.63
1.0 (1-2)	2 (0-3)	p 0.53	p 0.80
		p 0.38	**p <0.01**
46	8		
15	1		
		p 0.27	p 0.20
11	3		
20	4		
30	2		
58	6	p < 0.01	p 0.56
		0.34	p 0.46
22	2		
38	7		
7	3	p 0.08	p 0.23
42.0 (32-54)	33 (25-48)	p 0.13	**p < 0.01**
32.0 (27-38)	29 (27-34)	p 0.18	p 0.20
54.6 (48- 59)	54.3 (48-57)	p 0.71	p 0.94
49.5 (44-54)	54.4 (50-57)	p 0.08	p 0.66
1.3 (1-1.6)	1.7 (1.4-1.9)	**p < 0.01**	p 0.05

and a high perceived risk for a chromosomal abnormality (vs. a low risk) did not influence the test choice.

Quality of life outcome

State-anxiety

There were no systematic longitudinal differences in anxiety between the groups (P=0.11, table 3). Pattern of anxiety differed significantly over time, with a decrease of anxiety in both groups after obtaining a test result with alternating dominance of test technique (figure 2). Women with high State and Trait scores at Q1 reported high anxiety scores in the subsequent questionnaires (P<0.01, table 3); women with the indication advanced maternal age had significantly higher State scores (P=0.02), other demographic factors did not affect anxiety scores. Interaction effects between anxiety levels and group over time differed significantly on day 2, 5, and 23 (P< 0.01; P<0.01; P=0.05 respectively, table 3). From Q1 to Q6 the following percentages of group 1 vs. 2 suffered pathological anxiety; 31% vs. 46%, 18% vs. 43%, 12% vs. 2%, 7% vs. 8%, 6% vs. 5% and 4% vs. 2%.

Table 3: Repeated measurement analysis: Linear mixed models on log transformed ([a]) State, ([b]) PPC, (c) IES scores. AC= amniocentesis, group 1= karyotyping; group 2= RAD, AMA=advanced maternal age. Bold values indicate p-values of $P < 0.05$.

Parameter	Estimate	95% CI	P value
State score[a]	1.82	1.47 – 2.16	0.00
Intercept			
Group 1	0.07	-0.02 – 0.15	0.11
Group 2	ref	.	
AC + 2 days	0.42	0.33 – 0.51	0.00
AC + 5 days	0.00	-0.07 – 0.06	0.95
AC + 14 days	0.07	0.00 – 0.14	0.06
AC +23 days	0.06	0.00 – 0.12	0.04
AC +63 days	ref	.	
Interaction AC + 2 days *group 1*	-0.23	-0.35 - -0.11	0.00
Interaction AC + 5 days* group 1*	0.14	0.05 – 0.23	0.00
Interaction AC + 14 days* group 1*	0.08	-0.02 – 0.17	0.12
Interaction AC +23 days* group 1*	-0.08	-0.16 – 0.00	0.05
Interaction AC +63 days*group 1*	ref	.	
Baseline log State	0.28	0.17 – 0.38	0.00
Baseline Trait	0.01	0.01 – 0.01	0.00
Indication AMA	0.09	0.02 – 0.17	0.02
Indication other	ref	.	
Western	-0.03	-0.17-0.10	0.63
Non western	ref	.	
PPC score[b]			
Intercept	0.70	0.58- 0.81	0.00
Before AC	0.10	0.01-0.2	0.04
AC + 2 days	0.10	0.01-0.19	0.03
AC + 5 days	-0.06	-0.16-0.04	0.22
AC + 14 days	0.01	-0.07-0.09	0.85
AC +23 days	-0.02	-0.09-0.04	0.47
AC +63 days	ref	.	
Group 1	0.03	-0.11 – 0.16	0.69
Group 2	ref	.	
Interaction before AC *group 1*	0.06	-0.07-0.19	0.38
Interaction AC + 2 days* group 1*	0.03	-0.09-0.15	0.58
Interaction AC + 5 days* group 1*	0.19	0.05-0.32	0.01
Interaction AC +14 days* group 1*	0.08	-0.03-0.19	0.17
Interaction AC +23 days*group 1*	0.03	-0.06-0.12	0.51
Interaction AC +63 days*group 1*	ref	.	
Indication AMA	-0.04	-0.12-0.05	0.39
Indication other	ref	.	
Western	0.05	-0.11-0.25	0.53
Non western	ref	.	
IES score[c]			
Intercept	1.21	0.79-1.64	0.00
AC + 2 days	1.54	1.25-1.83	0.00
AC + 5 days	1.80	1.51-2.08	0.00
AC + 14 days	0.40	0.10-0.71	0.01
AC +23 days	-0.04	-0.28-0.20	0.74
AC +63 days	ref	.	

Table 3: (Cont)

Parameter	Estimate	95% CI	P value
Group 1	-0.09	-0.47-0.30	0.66
Group 2	ref	.	
Interaction AC + 2 days* group 1*	-0.18	-0.58-0.21	0.36
Interaction AC + 5 days* group 1*	-0.93	-1.31- -0.54	0.00
Interaction AC +14 days* group 1*	0.27	-0.14-0.68	0.19
Interaction AC +23 days*group 1*	0.48	0.16-0.81	0.00
Interaction AC +63 days*group 1*	ref	.	
Indication AMA	-0.43	-0.85- -0.02	0.04
Indication other	ref	.	
Western	-0.39	-1.13-0.36	0.31
Non western	ref	.	

-2 log likelihood State = -170.99; -2 log likelihood PPC= -208.01; -2 log likelihood IES= 1415.29. * reference: interaction time*group2

SF-36

There were no significant differences in mean PCS and MCS scores between the groups before and at 63 days after amniocentesis (PCS: P= 0.94; P= 0.52; MCS: P= 0.66; P=0.07). Both groups showed a lower PCS score at Q6 compared to Q1, while for the MCS both groups showed a higher score at Q6. The changes in PCS and MCS, adjusted for indication and western origin did not differ significantly between the groups (PCS: beta 0.05 95% CI -1.33 to 2.15 p 0.64; MCS: beta -0.20 95% CI -3.57 to 0.02 p 0.06).

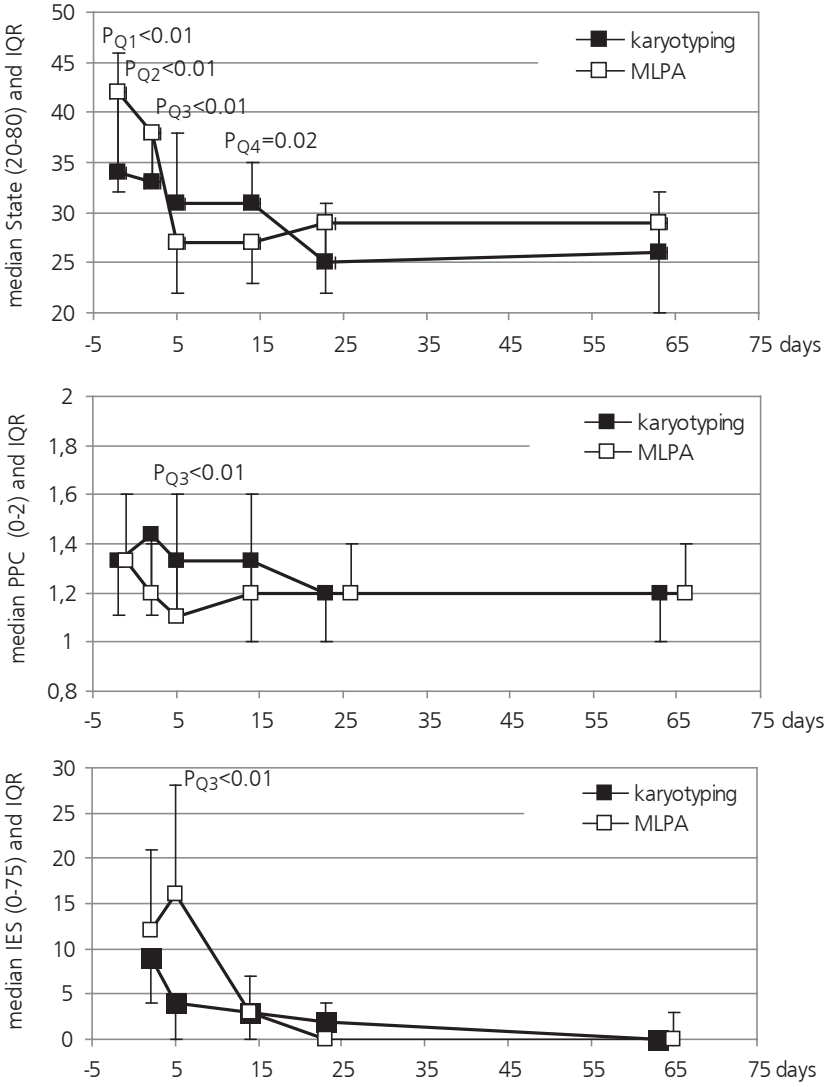
PPC

There were no systematic longitudinal differences between the groups (P=0.69, Table 3). Time had a significant effect on PPC levels before and 2 days after amniocentesis (P=0.04 and P= 0.03) with higher perceived control in both groups before obtaining a test result. Demographic characteristics did not influence the PPC score. Interaction effects between PPC scores and group over time differed significantly on day 5 (P< 0.01; table 3).

IES

There was no systematic difference in stress level between the groups (P=0.66; Table 3). Interaction effects between IES scores and group over time differed significantly on day 5 and 23 following amniocentesis (P<0.01; P<0.01; Table 3).

Figure 2: Median scores with interquartile ranges (IQR) of women receiving karyotyping and women receiving RAD (MLPA). Significant differences in cross-sectional analysis are marked with the P-value.



DISCUSSION

This study assessed both the reasons to choose standalone RAD or karyotyping and the difference in health-related quality of life. Overall, women had a clear individual preference for targeted or broad testing. Despite individual differences, our study showed no systematic differences in time of standalone RAD versus karyotyping in terms of anxiety, general physical

and mental health, personal perceived control and stress. Offering a choice does not lead to increased anxiety levels or reduced health-related quality of life.

The strength of our study is the prospective design with sufficient power. Most studies are limited to psychological health; we also evaluated the reasons and perceptions for the test choice. In contrast to previous literature[17-19], we offered women a real choice between RAD and karyotyping. Therefore, we cannot compare our results to other studies. One study mimicked a standalone policy by treating the RAD result as final diagnosis[20]. However, karyotyping was still performed but the result was only given in case of an additional chromosomal abnormalities. The course of anxiety in this study was similar to our results, although the average anxiety levels in our groups were remarkably lower. We observed this difference in a previous study, most likely caused by cross-cultural differences or the organization of Dutch obstetrical care[17].

Offering a choice makes a randomized controlled trial impossible. We had to adjust various outcome measures for differences between patient characteristics at baseline and between complete and incomplete responders. We cannot rule out over- or under-adjustment. A confounder may be the quality of prenatal counseling. Although this should be nondirective, five women stated that the dominant reason to choose RAD (4) or karyotyping (1) was the caregiver's advice. Some counselors may implicitly hint at their views, or explicitly impose their own views upon counselees[21,22]. This study shows patterns of anxiety, stress, PPC and HRQoL for normal results. For women with fetal chromosomal abnormalities patterns may differ.

We found an association with prenatal screening, no knowledge of karyotyping and an intermediate perceived risk and the choice for standalone RAD. An unfavorable outcome of prenatal screening and an intermediate perceived risk leads to an urgent need for a definite diagnosis[23]. It may be logical that prenatal screening for Down syndrome is followed by the choice for diagnostic test focused on Down syndrome and therefore RAD. Knowledge of karyotyping was associated with not choosing RAD. We assume that this is the expose effect, i.e. people tend to choose karyotyping because they are familiar with it.

Women in the prenatal screening group had higher State scores. The unfavorable outcome of PNS likely leads to increased anxiety[9]. State scores in group 2 are remarkably higher before amniocentesis and just before the RAD result. This result cannot be explained by a difference in personal anxiety (Trait). Expecting a result within a few days may lead to increased anxiety and stress scores. In both groups anxiety scores decreased significantly after disclosure of the test result. This was also seen in pathological anxiety; women in group 1 and 2 had pathological anxiety of respectively 33% and 50% before disclosure of the result, decreasing to 6% and 2% after disclosure. The reduction of anxiety after test disclosure in group 1 was higher than the reduction of anxiety after disclosure of the karyotype result

in the karyotyping strategy. We speculate that (pathologically) anxious women are quickly reassured by RAD and therefore may benefit most of RAD tests.

The stress scores (IES) on day 5 for group 2 were higher, which can be explained by the limited amount of time leading to a bigger impact of the named stressor, i.e. the RAD result. With respect to the low IES scores during the testing process, both groups can handle the stress. The PPC scores were stable in both groups, thus the quality of the counseling process did not influence anxiety.

The differences in anxiety on the short term did not translate into long term effects on overall mental and physical health (SF-36). Our results suggest that the RAD vs. karyotyping strategy has no adverse long term effects on quality of life and, in addition, that normal results reassure and improve mental quality of life. The reduction in physical health during the test process is in agreement with two studies that reported on the relationship between RAD and HRQoL[19,20] and is associated with increasing gestational age[24,25].

Assuming that a 5 point difference on a scale from 0-100 is clinically relevant[20,26] the effect size of a rapid test on anxiety is clinically relevant and leads to higher scores before RAD disclosure and lower scores after RAD disclosure compared to women who chose karyotyping.

We conclude that anxiety and HRQoL following standalone RAD or karyotyping do not differ systematically. Therefore, from the psychological point of view, offering an individualized choice in prenatal diagnosis seems an appropriate strategy and encourages the development of strategies that tailor the type of diagnostic test.

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Chapter 5

Economic evaluation of Multiplex Ligation-dependent Probe Amplification and karyotyping in prenatal diagnosis: results from the M.A.K.E. study

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ABSTRACT

Objective

To assess the cost-effectiveness of Multiplex Ligation dependent Probe Amplification (MLPA) compared to karyotyping.

Methods

A cost-minimization analysis alongside a nationwide prospective clinical study of 4585 women undergoing amniocentesis on behalf of their age (≥36 years), an increased risk following first trimester prenatal screening or parental anxiety.

Results

Diagnostic accuracy of MLPA was comparable to karyotyping (1.0 95% CI: 0.999 to 1.0). Health-related quality of life did not differ between the strategies (summary physical health: mean difference 0.31, p=0.82; summary mental health: mean difference 1.91, p=0.22). Short term costs were lower for MLPA: mean difference € 315.68 (bootstrap 95% CI: € 315.63 to € 315.74; - 44.4%). The long term costs were slightly higher for MLPA: mean difference € 76.42 (bootstrap 95% CI: 71.32 to 81.52; +8.6%). Total costs were on average € 240.13 (bootstrap 95% CI: 235.02 to 245.23;-14.9%) lower in favor of MLPA. Cost differences were sensitive to proportion of terminated pregnancies, sample throughput, individual choice and performance of tests in one laboratory, but not to failure rate or the exclusion of polluted samples.

Conclusion

From an economic perspective, MLPA is the preferred prenatal diagnostic strategy in women who undergo amniocentesis on behalf of their age, following prenatal screening or parental anxiety.

INTRODUCTION

In many countries, prenatal diagnosis by chorionic villus sampling or amniocentesis is routinely offered to pregnant women who have an increased risk of carrying a child with a chromosomal abnormality. Amniocentesis is the most commonly used invasive prenatal diagnostic procedure in Western countries and is performed in about one in 30 pregnancies[1,2]. Karyotyping is considered the reference test to detect fetal genetic abnormalities in amniotic fluid cells with considerable accuracy[3,4]. However, it is labour intensive and the costs are high. Furthermore, obtaining results takes 2-3 weeks and the extensive detection capacity of karyotyping can be perceived as a disadvantage due to the detection of abnormalities with unclear or mild clinical relevance, causing difficult counselling issues, patient anxiety, emotional dilemmas concerning the continuation of pregnancy and, albeit rare, unwarranted pregnancy terminations[1,5,6].

Due to these disadvantages, karyotyping as routine test has been challenged for relatively low risk indications. In 2003, a molecular PCR-based technique, MLPA (multiplex ligation-dependent probe amplification) became available to detect fetal aneuploidies in amniotic fluid cells[7]. Following the results of preclinical laboratory studies, MLPA has been proposed as a promising alternative for the detection of the most common chromosomal aneuploidies, i.e. trisomy 13, 18, 21 and sex chromosome aneuploidies. Compared to karyotyping, MLPA has several potential advantages; the waiting time for test results is reduced with simultaneous reduction of anxiety, the preceding prenatal counselling process can focus on the most common chromosomal aneuploidies, and the technique is considerably less labour-intensive and more suitable for high-throughput testing, thereby exploiting economies of scale.

Nowadays, much effort has been put into priority setting based on a trade-off of costs and health gains. From an economic perspective, the preferred prenatal diagnostic strategy is the one that overall yields favourable health gains relative to associated cost differences[8].

In order to compare the MLPA and karyotyping strategies in terms of diagnostic accuracy, health-related quality of life and cost-effectiveness, we initiated a prospective diagnostic study comparing MLPA with karyotyping in routine clinical practice; the MLPA And Karyotyping, an Evaluation (M.A.K.E.) study (ISRCTN47252164)[9]. If MLPA has comparable diagnostic accuracy and is able to reduce maternal anxiety and costs in routine clinical practice, MLPA could present a suitable substitute for karyotyping. Our research question was: what are the costs and effects of MLPA compared to karyotyping when applied to the indications advanced maternal age, increased risk following prenatal screening and anxiety?

METHODS

Clinical study

The clinical M.A.K.E. study was set up as a prospective nationwide cohort study enrolling 4,585 consecutive women undergoing amniocentesis for advanced maternal age (≥36 years), increased risk following prenatal screening or anxiety. Other referral indications were excluded (e.g. ultrasound abnormalities) since these are associated with an increased risk of a chromosomal abnormality other than trisomies 13, 18, 21 and sex chromosome abnormalities. Details of the study design have been published elsewhere[9,10]. In summary, after obtaining informed consent, amniocentesis was carried out by specifically trained obstetricians. All amniotic fluid samples were tested with both MLPA and karyotyping, allowing a pair wise comparison of MLPA and the reference test; karyotyping. Sample size was estimated to demonstrate non-inferiority (i.e. comparable diagnostic accuracy) of MLPA to karyotyping. During a pre-trial meeting, experts in prenatal diagnosis, clinical epidemiology and statistics agreed on a critical non-inferiority margin of 0.002. At least 4,497 paired test results were needed (one-sided alpha 0.05, power 0.90) to be able to reject the null hypothesis that MLPA is inferior to karyotyping.

MLPA

DNA was isolated from 1 to 8 ml uncultured amniotic fluid samples, depending on the total amount of amniotic fluid received. MLPA samples were analysed with the commercially available SALSA MLPA P095 kit (MRC Holland, the Netherlands). For each genomic target, a set of 2 probes is designed to hybridize immediately adjacent to each other on the same target strand. Both probes consist of a short target sequence and a universal polymerase chain reaction (PCR) primer-binding site. One of the probes contains a stuffer sequence with a unique length and sequence. Following hybridization, each pair of adjacent probes is joined by a ligation reaction. Next, PCR is performed using a fluorescent-labelled primer pair, which ensures that the relative yield of each of the PCR products is proportional to the amount of each of the target sequences. The different length products are separated on an automated capillary sequencer. The size and peak areas for each probe are quantified and analyzed by data analyzing software (GeneMarker, SoftGenetics, LLC, State College, PA, USA or Genescan and Genemapper version 3.7/4.0, Applied Biosystems, CA, USA)[7]. Relative probe signals are calculated and compared with samples of normal male and female sex. In chromosomally normal samples, the relative probe signal is expected to be 1 for all probes. A normal value is defined as a relative probe signal between 0.7 and 1.3. A relative probe value of <0.7 indicates a monosomy, whereas a relative probe value of >1.3

indicates a trisomy. Technicians had a molecular genetics or a cytogenetics background; all were trained in the execution of MLPA prior to the study onset. MLPA was performed in duplicate, provided that at least 2 ml of amniotic fluid was available. All eight genetic centres have different sample throughput, depending on the amount of patients in their referring prenatal diagnostic centres.

Karyotyping

Fetal cells were cultured and karyotyped after banding. Routinely, metaphases of at least 10 colonies were investigated. All centres followed national quality guidelines but minor differences in the number of cell colonies cultured, chromosome banding and reporting of the results were allowed[11].

Economic analysis framework

The economic analysis was performed from the societal perspective which means that all significant costs and health effects both in the short and long term should be considered, regardless of who experiences the costs or the health gains[8]. The economic evaluation was initially designed as a cost-effectiveness analysis (CEA)[8], with incremental costs per case of Down's syndrome missed by MLPA. In case of comparable diagnostic accuracy for the detection of Down syndrome, a cost-utility analysis (CUA) was considered the appropriate economic framework, calculating the difference in costs in relation to differences in health-related quality of life[8]. If differences in quality of life between the strategies were also absent, a cost-minimisation analysis (CMA) was carried out. CMA implies that the preferred strategy from the societal perspective is the one with lowest costs, since health effects are equal[8]. We did not include a do-nothing strategy, since the target population is eligible for karyotyping.

Health-related quality of life

Alongside the clinical M.A.K.E. study we assessed health-related quality of life (HRQoL) in two groups: group 1 having karyotyping and group 2 having MLPA. Included were women with the indications maternal age, increased risk following the findings of prenatal screening for Down syndrome and parental anxiety. We used the MOS SF-36 health survey, measuring overall mental and overall physical health. Mental and physical health were measured before amniocentesis and at day 63 following amniocentesis, since the SF-36 focuses on health status during the preceding 4 weeks. Overall mental and physical health were calculated according to accepted scoring algorithms[12].

Costs

We distinguished the costs of the MLPA and karyotyping strategies in two components; short and long term costs. The first component, the short term costs, comprises all societal costs

that occur between amniocentesis and parents' decision to terminate or continue pregnancy. These costs consist of the costs of the diagnostic tests and other costs. The second component consists of the long term costs, i.e. all societal costs that occur between parents' decision to terminate or continue pregnancy and lifetime costs. Although it is controversial whether the costs associated with chromosomal abnormalities should be included in this type of analysis[13-15], we decided to display the impact of missed chromosomal abnormalities on long term costs.

Short term and long term costs were further distinguished in direct medical costs (i.e. laboratory costs, additional in-hospital medical costs during follow-up), direct non-medical costs (patient expenses e.g. patient time and travel costs) and indirect costs (societal costs due to absence from work)[8].

The main outcome parameters were the difference in short term costs between the MLPA and TKT strategies, the long term cost difference, and the overall cost difference.

Short term costs

The short term costs consisted of the costs associated with performing MLPA and/or karyotyping and other costs related to the testing process. Direct medical costs of performed tests were calculated as actual volumes of resource use multiplied by the costs per unit of resource. Number and type of tests performed were recorded in the clinical record form or obtained by observation or questionnaire. We used direct observations and measurements of working time, materials, and depreciation costs of equipment to quantify resource use associated with MLPA. Costs per units were obtained from a university hospital's budgetary and accounting system and were subsequently applied to the resource use observed in a small and large centre. The costs per units reflected the costs of staff, materials, equipment, housing and departmental and hospital overheads.

The other short term costs consisted of additional diagnostic tests, costs of genetic counselling in case a chromosomal abnormality was detected, and travel costs. Use of additional diagnostic tests was recorded in the case record form. In case of a chromosomal abnormality, we assumed parents visited the hospital twice for genetic counselling (50% of cases by performed by gynaecologists (trisomies 13, 18 and 21) and 50% seen by clinical geneticists and social workers (all other chromosomal abnormalities)). Travel costs per client were based on the average travel distance to hospitals[16]. The unit costs of direct non-medical costs were based on Dutch guidelines[16,17]. Short-term indirect costs did not occur.

Study specific costs as well as costs not associated with diagnostic test performance (prenatal test counselling, amniocentesis, ultrasound, sample transport, procedure related miscarriages) were expected to be independent of the type of diagnostic test, and were therefore excluded

from analysis. Given the time horizon no discount rate is used. When necessary, costs were updated to the 2007 price level by using the Dutch Consumer Price Index[18].

Long term costs

The long term costs were defined as the costs associated chromosomal abnormalities and consisted of 1) incremental costs for a child having a chromosomal abnormality and 2) costs for parents of the affected child. We first categorised chromosomal abnormalities according to clinical relevance: severe consequences and other chromosomal abnormalities leading to severe fetal morbidity or mortality (category I; includes trisomies 13, 18, and 21); uncertain consequences (including sex chromosomal abnormalities) and de novo balanced chromosomal abnormalities which can lead to 6% mental retardation and/or congenital abnormalities[19] (category II); and no consequences including inherited chromosomal abnormalities and chromosomal abnormalities of known clinical irrelevance (category III). For category I chromosomal abnormalities, we used an incremental lifetime cost of € 200.000 per child[20-22], a weighted average of the costs of trisomies 13, 18 and 21 adjusted for the average costs per child. The costs of a category II abnormality were estimated to be 6% of the costs of a child with category I abnormality. Category III abnormalities were considered not to induce extra costs.

Productivity loss due to absence from work in case of a chromosomal abnormality was estimated according to the friction cost method[16]. In case the pregnancy was terminated, both parents were considered to have a sick leave; on average 6 weeks for mothers and 2 weeks' leave for partners. If the parents decided to continue the pregnancy in case of a severe chromosomal abnormality, the productivity loss exceeded the friction period (22 weeks) and no extra costs beyond the friction period were included. Assuming pregnant women to have on average one child, working 26 hours/week and aged of 25 to 44 years, the productivity loss is € 33.60 per hour lost[18]. Assuming the partner to be male, aged 25 to 44 years old and working fulltime, his productivity loss is on average € 40.86 per hour lost[18].

Sensitivity analysis

We used a sensitivity analysis to test the robustness of the cost differences. We varied the major assumptions underlying the cost-effectiveness model for the following parameters: 1) proportion of failed MLPA results, according to the 5th and 95th percentiles of the observed failure rate; 2) only samples with clear amniotic fluid are analysed with MLPA, all other samples with karyotyping; 3) the proportion of terminated pregnancy in case of category I chromosomal abnormalities varies from 70% to 80%); 4) women are allowed individual choice; 50% of women opts for MLPA and 50% for karyotyping[23]; 5) Sample throughput based on a small centre (n=286) and a large centre (n=1153); 6) One nationwide MLPA

laboratory; 7) All samples are analysed with both MLPA and karyotyping. Parameters 1 and 5 are subject to different laboratory practices. Parameter 3 might relate to societal trends, counselling style or the counsellor's medical specialty[24]. Parameters 2, 4, 6, and 7 might change following changes in prenatal diagnostic protocols or guidelines.

Statistical analysis

Data were recorded and analysed by using statistical software (SPSS version 16.0; SPSS, Chicago, Illinois). Observed data described with descriptive statistical measures; medians with range, or mean differences with 95% confidence intervals (CIs). Due to skewness of cost data, the 95% CI of the mean short term, long term and overall cost differences between the strategies were obtained with the nonparametric bootstrap method, based on10,000 bootstrap samples[25]. *P* value less than .05 (two-tailed) was considered statistically significant.

RESULTS

Patients and test results

Between March 2007 and October 2008 we included 4585 consecutively pregnant women. Patient and procedural characteristics are displayed in Table 1.

Outcomes: diagnostic accuracy and quality of life

In 4484/4585 samples (97.8%) MLPA and karyotyping were concordant, showing normal results in 4387/4585 (95.7%) and aneuploidy in 98/4585 (2.1%). Discordant results were

Table 1. Baseline and Procedural characteristics

Median age (years) (5th to 95th %)	38.1 (31.8 to 42.4)
Indication (%)	
Advanced maternal age	3464 (75.6)
Increased risk following prenatal screening	1074 (23.4)
Anxiety	47 (1.0)
Median gravidity (5th to 95th %)	2 (1 to 5)
Median parity (5th to 95th %)	1 (0 to 3)
Median gestational age (weeks +days) (5th to 95th %)	16 +1 (14+6 to 17+4)
Withdrawn amniotic fluid (median) (5th to 95th %)	20 ml (16.0 to 20.0)
Colour of amniotic fluid	
Clear/yellow	4467 (97.4%)
Red/Brown/ Turbid/Green	118 (2.6%)
Cell pellet colour	
White	3923 (85.6%)
Trace of blood	381 (8.3%)
Red/Brown/Green, Yellow, Turbid	281 (6.1%)
Amniotic fluid for MLPA (median) (5th to 95th%)	4 ml (2.0 to 8.0)

found in 26/4585 (0.6%) samples, representing an abnormal karyotype undetected by MLPA. All aneuploidies of chromosomes 13, 18, 21 and non-mosaic X and Y were also detected by MLPA. MLPA, by design, could not detect three severe chromosomal abnormalities other than trisomies 13, 18, 21. Diagnostic accuracy of MLPA was comparable (non-inferior) to karyotyping (1.0 95% CI: 0.999 to 1.0). Sensitivity and specificity for the detection of trisomies 13, 18, 21, X and Y were 100% (95%CI: 96% to 100%) and 100% (95%CI: 99.9% to 100%), respectively.[10] There were neither statistical nor clinically relevant differences in HRQoL. Summary physical and mental health scores between people receiving a karyotype or MLPA did not differ (mean difference 0.31; 95% CI -3.06 to 2.44 p 0.82 and mean difference 1.91 95% CI -1.15 to 4.99 p 0.22, respectively) (see supplemental file Table 1). Therefore we considered cost minimisation analysis the appropriate framework.

Supplemental file Table 1: 9 weeks change score in generic health using the SF-36

	Mean change score compared with baseline		p	95% CI
	TKT	MLPA		
PCS	-5.14	-5.8	0.63	-2.14 to 3.51
MCS	5.16	3.11	0.23	-1.31 to 5.41
PCS*	0.04	0.35	0.82	-3.06 to 2.44
MCS*	3.12	1.21	0.22	-1.15 to 4.99

*Corrected for education, indication, religion, and offering a choice between the tests.
PCS= Physical Component Score; MCS= Mental Component Score

Short term costs

The costs of the MLPA test performed in duplicate were € 344.60 per sample (65% direct and 35% overhead costs) while the costs of karyotyping was € 668.00 per sample (74% direct and 26% overhead costs).

Table 2 details the volumes of resource use, unit costs per resource and the total short term costs. In the MLPA strategy, 173 subsequent karyotyping were performed because MLPA failed (n=75) or because MLPA showed a chromosomal abnormality and inheritance patterns needed to be examined (n=98). Repeat amniocentesis did not occur. MLPA was repeated in 1.6% (5^{th} to 95^{th} percentile: 1.3% to 2.1%) due to an inconclusive result. Five subsequent FISH analyses were done; three because MLPA showed a deletion on a single probe and the laboratory wished to exclude a sub-microscopic deletion, and two for a mosaic chromosome pattern (combined mosaic pattern of Turner and Down syndrome and a mosaic pattern for Turner syndrome and a normal female cell line). In 22 cases in the MLPA strategy and 34 cases in the karyotyping strategy, advanced ultrasound examination was required to exclude other severe congenital abnormalities (e.g. cardiac abnormalities) in the presence of the chromosomal abnormality to support the decision to continue or terminate the pregnancy.

In the karyotyping strategy, 11 subsequent FISH analysis were performed for various reasons; additional information on the grade of mosaicism (mosaic pattern Turner and Down syndrome, mosaic pattern of Turner syndrome)(n=2), for marker chromosomes (n=4), *de novo* unbalanced chromosomal abnormalities (n=2), a chromosomal abnormality which appeared to be a normal variant (n=2), and for a mosaic pattern of male and female karyotype which was determined to be a culture artefact (n=1). In the latter case biochemical investigation on amniotic fluid was also carried out to determine the testosterone/FSH ratio and karyotyping was repeated in a postnatal sample. In 24 cases parental karyotyping was performed to address the origin of the chromosomal abnormality (inherited or *de novo*). To assess the consequences of the *de novo* interstitial deletion, MLPA on subtelomeres and a genomic micro array was carried out. One karyotype failed due to contaminated amniotic fluid (blood and clots). Repeat amniocentesis was offered but the prospective parents declined. The median short term costs per sample, i.e. from amniocentesis until the decision to continue or terminate pregnancy, were € 344.60 (range 344.60 to 3,216.08) for the MLPA strategy and € 668.00 (range 668.00 to 4,669.48) for the karyotyping strategy. The short term costs of the MLPA strategy were on average € 315.68 (bootstrap 95% CI: € 315.63 to € 315.74) lower than the karyotyping strategy (- 44.4%).

Table 2. Short term costs: Resource use and costs between amniocentesis and the decision to continue or terminate pregnancy.

Resource use	MLPA strategy (n=4585) No	% of total costs MLPA	karyotyping strategy (n=4585) No	% of total costs karyotyping	Unit costs Euro
***Direct costs in the hospital**					
Primary procedure					
MLPA	4585	87.3%	0	0.0%	€ 344.60
Karyotyping	0	0.0%	4585	93.98%	€ 668.00
Additional diagnostic tests					
Karyotyping	173	6.4%	0	0.0%	€ 668.00
FISH	5	0.22%	11	0.27%	€ 809.00
Additional diagnostic tests in case of CA					
Parental karyotyping	0	0.0%	44	0.90%	€ 668.00
DNA and/or biochemical investigation	0	0.0%	3	0.09%	€ 934.00
Ultrasound examination (type II)	22	0.79%	34	0.68%	€ 653.00
Outpatients visit in case of CA					
Consult gynaecologist (2 visits)	35	0.48%	35	0.26%	€ 246.00
Consult clinical geneticist and social worker (2 visits)	63	4.82%	89	3.78%	€ 1385.00
***Direct medical costs outside the hospital**					
Travel costs in case of CA (2 visits to hospital)	98	0.05%	124	0.04%	€ 9.48
Total short term costs		**€ 394.93**		**€ 710.65**	

CA= chromosomal abnormality

Table 3: Long term costs: Resource use and costs after the decision to terminate or continue pregnancy

Long term consequences	MLPA strategy (n=4585)	% of total costs	karyotyping strategy (n=4585)	% of total costs	Unit costs
Terminated pregnancies for detected CA					
Total termination of pregnancy	76	2.2%	79	2.5%	€ 1,314.00
Clinically severe CA (T21/13/18 and other)	72		74		-
Clinically uncertain CA (X/Y and other)	4		5		-
Travel costs	76	0.01%	79	0.01%	€ 4.74
Productivity loss for terminated pregnancies with CA	76	11.2%	79	12.9%	€ 6,730.38
Continued pregnancies for detected CA					
Clinically severe CA (T21/13/18 and other)	13	56.8%	14	68.1%	€ 200,000.00-
Clinically uncertain CA (X/Y and other)	9	2.4%	14	4.1%	€ 12,000,-
Clinically not relevant CA (other)	0	0.0%	17	0.0%	–
Confirmation of prenatal cytogenetic result after birth	0	0.0%	2	0.04%	€ 739.72
Productivity loss for continued pregnancies with severe CA	13	9.6%	14	11.6%	€ 33,940.00
Productivity loss for continued pregnancies with uncertain CA	9	0.4%	14	0.7%	€ 2,036.40
Productivity loss for continued pregnancies with not relevant CA	0	0.0%	17	0.0%	–
Costs for undetected chromosomal abnormalities					
Other clinically severe CA	3	13.1%	0	0.0%	€ 200,000,00
Other clinically uncertain CA	6	1.6%	0	0.0%	€ 12,000.00
Other clinically not relevant CA	17	0.0%	0	0.0%	–
Productivity loss for undetected severe CA	3	2.4%	0	0.0%	€ 36,861.00
Productivity loss for undetected uncertain CA	6	0.3%	0	0.0%	€ 2,211.66
Productivity loss for undetected not relevant CA	17	0.0%	0	0.0%	€ 0,00
Total long term costs		**€ 997.85**		**€ 896.19**	

CA= chromosomal abnormality

Long term and total costs

Table 3 displays the main volumes of resource use, the unit costs per resource use and the long term costs following the decision to continue or terminate pregnancy. Seventy-six pregnancies in the MLPA strategy (72 clinically severe; 4 clinically uncertain; 0 clinically not relevant) and 79 pregnancies in the karyotyping strategy (74 clinically severe; 5 clinically uncertain; 0 clinically not relevant) were terminated. In two pregnancies, postnatal karyotyping was carried out to confirm the prenatal diagnosis (mosaic marker chromosome and mosaic Turner).

The median long term costs per sample, i.e. from the decision to continue or terminate pregnancy onwards, were € 0.00 (range 0 to 233,940.00) for the MLPA strategy and € 0.00 (range 0 to 237,000.08) for the karyotyping strategy (table 2). The long term costs of

Table 4. Sensitivity analysis. Impact of parameters varied on short term, long term and total MLPA costs per sample (Euros, %) compared to baseline; and impact on the total (short term and long term) cost difference of MLPA – karyotyping

Parameter varied	Short term costs of MLPA strategy per sample (€ (% change))*	Long term costs of MLPA strategy per sample (€ (% change))*
Baseline strategy	394.93 (n.a.)	997.85 (n.a.)
Failure rate		
1.31%	392.75 (-0.6%)	997.85 (–)
2.05%	397.70 (+0.7%)	997.85 (–)
Only samples with clear amniotic fluid analysed with MLPA	401.44 (+1.7%)	997.85 (–)
% TOP if severe CA		
80%	394.93 (–)	1195.18 (+20.8%)
70%	394.93 (–)	1638.32 (+64.2%)
Women are allowed individual choice	552.18 (+39.8%)	971.65 (-2.8%)
Sample throughput		
286 samples/year	500.55 (+26.7%)	997.85 (–)
1153 samples/ year	374.94 (-5.1%)	997.85 (–)
One nationwide MLPA laboratory	294.68 (-29.6%)	997.85 (–)
All samples analysed with MLPA and karyotyping	660.85 (+167.3%)	896.19 (-10.1%)

CA=chromosomal abnormality; TOP = termination of pregnancy. * change compared to baseline ** change compared to karyotyping strategy. A positive change implies a reduction of the cost difference.

the MLPA strategy were on average € 76.42 higher compared to the karyotyping strategy (bootstrap 95% CI: 71.32 to 81.52; +8.6%).

The total costs, including both short and long term costs, were median € 344.60 (range 344.60 to 237.000,08) for the MLPA strategy and € 668.00 (range 668.00 to € 238,956.48) for the karyotyping strategy. The total cost difference was € 240.13 (bootstrap 95% CI: 235.02 to 245.23) in favour of MLPA (cost reduction: -14.9%).

Sensitivity analysis

Table 4 displays the results of the sensitivity analyses. Total MLPA costs were sensitive to the following parameters: the proportion of women deciding to terminate pregnancy, women allowed individual choice, the level of sample throughput, and performing both MLPA and karyotyping. Except for the combined MLPA and karyotyping strategy, the total costs difference remained in favour of MLPA.

DISCUSSION

In this study we evaluated the cost-effectiveness of two prenatal diagnostic test strategies; MLPA and karyotyping. Diagnostic accuracy of MLPA was comparable (non-inferior) to karyotyping

Total cost of MLPA strategy (€ (% change))*	Total cost difference MLPA vs. karyotyping per sample ((€ (% change))**
n.a.	-214.06 (-13.3%)
-2.19 (-0.2%)	-216.20 (-0.5%)
+2.77 (+0.2%)	-211.29 (+1.3%)
+6.50 (+0.5%)	-207.55 (-3.0%)
+197.33 (+14.2%)	-213.59 (-0.5%)
+640.48 (+48.7%)	-214.06 (–)
+131.05 (+9.4%)	- 83.00 (+61.2%)
+105.62 (+7.6%)	-108.60 (+ 49.3%)
- 19.99 (-1.4%)	-234.05 (- 0.5%)
-100.25 (-7.20%)	-314.31 (-46.83%)
+599.19 (+43.02)	+345.10 (+61.2%)

and health-related quality of life was equal between strategies. For the complete testing process, the MLPA strategy leads to a 14.9% cost reduction per amniotic fluid sample for women with relatively low risk indications (-44.4% on the short term, and +8.6% on the long term).

Our study has several limitations. Firstly, we used the outcome data of the nationwide, prospective M.A.K.E.study which prioritised karyotyping, since at least 12 ml of amniotic fluid was required. The failure rate of MLPA (1.6%) may be lower when MLPA is applied as standalone technique since MLPA requires at least 1-2ml. Sensitivity analysis however showed that variations in failure rate had little impact on the overall cost difference. Secondly, we were unable to measure quality of life for women who decided to continue or terminate pregnancy in case of a chromosomal abnormality and in parents with rare prenatally undetected fetal chromosomal abnormalities. However, since diagnostic accuracy was high and comparable, we can speculate that the decision to continue or terminate pregnancy in case of a chromosomal abnormality is the same, regardless of the diagnostic test used. The three severe chromosomal abnormalities undetected by MLPA may result in a decrease in quality of life at the individual level but not in differences at the group level. Thirdly, we did not adjust the costs associated with pre-test counselling. We expect that targeted testing reduces complex counselling issues and is therefore less costly. Taking this into account, the cost reduction of MLPA compared to karyotyping may be even larger than we estimated.

Compared to other RAD techniques, MLPA and quantitative fluorescent polymerase chain reaction (QF-PCR) are both suitable techniques for high-throughput testing at lower costs compared to fluorescent *in situ* hybridization (FISH)[26]. A cost analysis of QF-PCR and FISH (2003) revealed that both tests are sensitive to sample throughput and staff skill-mix[26]. Grimshaw reported for a laboratory with a throughput of 1000 samples per annum, that karyotyping is the most expensive test to perform, with FISH and Q-PCR calculated to incur approximately half the direct test costs of karyotyping[26]. However, these studies did not include long term costs. Due to differences in methodology, a full comparison with our study is impossible.

Costs differences were insensitive to variations in failure rate, or the use of MLPA on contaminated amniotic fluid samples. However, the costs of MLPA proved sensitive to the proportion of terminated pregnancies and therefore to societal trends, but this is unlikely to affect the overall cost difference. Furthermore, the costs of the MLPA strategy were sensitive to sample throughput as well as the concentration of MLPA analyses in one nationwide centre. This shows that the costs and cost differences depend on the way care is organised. Since the impact of concentration on costs was larger than the impact of higher throughput, we recommend the use of one (or several) nationwide MLPA laboratories. Our study also shows that a combined strategy of MLPA followed by karyotyping is rather inefficient. Costs are considerably increased without any gain in diagnostic accuracy or health-related quality of life compared to the karyotyping only strategy.

The provision of a rapid, unambiguous and a low cost result is an incentive to implement MLPA. Successful implementation, however, also requires the support of pregnant women. If one supports individualised choice for principle or other reasons[27], one could argue that the decision to either obtain as much cytogenetic information as possible versus a rapid but specific result on the most common chromosomal abnormalities is most appropriately made by individuals who will bear the responsibility for raising the child. Our study shows that allowing individualized choice –assuming that 50% chooses karyotyping and 50% chooses MLPA[23]– also has large impact on costs, reducing the cost difference of € 240 per sample to € 83 per sample). While individual choice as strategy is less efficient than a uniform strategy in which every patient would receive MLPA, the overall cost reduction is still in favour of MLPA over the current karyotyping strategy. One could argue that offering a choice between the tests meets most individual needs and wishes, and thereby might outweigh the cost difference. In a discrete choice experimentation[28], women valued the comprehensive information of karyotyping at £ 791 and the simple and quick information of a Down only test at £ 690. This supports our idea that the option to choose may outweigh the previously mentioned efficiency loss of € 240 per sample to € 83 per sample.

In summary, MLPA is able to detect trisomies 13, 18, 21, X and Y with comparable diagnostic accuracy and without adverse effect on quality of life at considerably lower costs for the complete testing process. We conclude that MLPA is the preferred strategy and recommend substitution of karyotyping for MLPA for relatively low risk indications. Future research should be done to evaluate which RAD technique delivers best 'value for money', to estimate the cost-effectiveness of this RAD technique on chorionic villus biopsy, and to evaluate the most advantageous organisation for the optimal RAD technique.

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PART II

PATIENTS' AND PHYSICIANS' PREFERENCES

Chapter 6

Karyotyping or rapid aneuploidy detection in prenatal diagnosis? The different views of users and providers of prenatal care

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ABSTRACT

Developments in prenatal diagnosis raise the question which test strategy should be implemented. However, preferences of women and caregivers are underexposed. This study investigates what kind of prenatal test pregnant women and caregivers prefer and if differences between the groups exist, using self-report questionnaires. Women preferred either karyotyping (50%) or rapid aneuploidy detection (43%). Caregivers opted for the latter (78%). A test targeted on Down syndrome was the least preferred in both groups. We recommend the use of individualised choice for genetic test in prenatal diagnosis, overcoming the existing differences in preferences between women and caregivers.

INTRODUCTION

In developed countries, prenatal diagnosis is routinely offered to all women who are considered to be at increased risk for chromosomal abnormalities. In invasive prenatal diagnosis, karyotyping on amniocytes is considered the gold standard. Karyotyping can detect a range of numerical and structural chromosomal abnormalities with considerable accuracy and reliability[1]. Karyotyping is labour intensive, the costs are high and parents have to wait two to three weeks for the test result. Furthermore, karyotyping can detect chromosomal abnormalities with unclear or mild clinical relevance which can cause patient anxiety and emotional dilemmas concerning the continuation of pregnancy.

In the last decade, new techniques (e.g. fluorescent in situ hybridisation, quantitative polymerase chain reaction, multiplex ligation-dependent probe amplification) have become available in prenatal diagnosis. These techniques, often referred to as rapid aneuploidy detection (RAD) can detect aneuploidies of chromosome 21, 13, 18, X and Y within 1-4 days[2]. Various studies showed high diagnostic accuracy of RAD for the detection of these aneuploidies[2], which account for more than 80% of the clinically relevant chromosomal abnormalities. Besides, the costs are low and rare abnormalities with unclear or mild consequences are not detected.

In recent years, a debate emerged in European countries whether RAD should replace karyotyping in prenatal diagnosis for the following indications; advanced maternal age, increased risk following prenatal screening and maternal anxiety. In the Netherlands, a decision on the use of RAD as sole diagnostic tool has not been reached yet. Although the views of stakeholders should be incorporated in medical decision-making, little effort has been put into exploring the preferences of pregnant women and caregivers. So far, two studies have been published on this topic[3,4]. One study, with small sample size, showed that pregnant women preferred rapid aneuploidy detection as long as the test result was known six days prior to the karyotype result[4]. The other study, with fairly simple design, showed that pregnant women and caregivers in the UK opted for rapid aneuploidy detection[3]. These two studies give us insufficient evidence of clear consensus in favour of one of the strategies.

Considering the low evidence on this topic and important health care dilemmas, we aimed to determine the preferences of pregnant women and caregivers on this topic in the Dutch health care system. Our research questions were: Which basic type of prenatal test do pregnant women and caregivers prefer and, second, do the preferences of these two stakeholders differ? To answer these questions, we collected preferences on three hypothetical tests through a self-report questionnaire, which focused on the key factors in the current debate among caregivers.

METHODS

As part of the ongoing Dutch nationwide M.A.K.E. (MLPA And Karyotyping, an Evaluation) study (ISRCTN 47252164) in which the technical and clinical performance of multiplex ligation-dependent probe amplification (MLPA) versus karyotyping in prenatal diagnosis is compared, we developed a self-report questionnaire to determine pregnant women's and caregivers' preferences for three hypothetical, yet close-to-reality, test strategies in prenatal diagnosis (see appendix). We described three different tests (A, B and C), each characterized by three test characteristics; 1) detection capacity, 2) the comprehensibility of the result in case of a detected abnormality, and 3) the waiting time for the test result. We focused on these three characteristics because these are the key factors in the debate among caregivers and essential to women scheduled for prenatal diagnosis. Test A is described as a test where Down's syndrome is the single aneuploidy tested for, with clear consequences in case of a detected aneuploidy and a waiting time of 4 days. Test B is described as a test detecting the most common aneuploidies (trisomy 21, 13, 18 and sex chromosomal abnormalities); with almost always clear consequences in case of a detected chromosomal aneuploidy and a waiting time of 4 days. Test C, resembling traditional karyotyping, is described as a test where any chromosomal abnormality can be detected, with often-clear consequences in case of a detected chromosomal abnormality and a waiting time of 21 days. We aimed at neutral wording of the probability to have an unequivocal result, rather than the probability to have an uncertain or difficult to interpret result.

The target patient population consisted of 150 pregnant women undergoing amniocentesis in one of four Dutch hospitals: Onze Lieve Vrouwe Gasthuis and Academic Medical Centre in Amsterdam, St. Elisabeth Hospital in Tilburg and Leiden University Medical Centre in Leiden. Eligible for participation were women with sufficient command of the Dutch language. Women received a questionnaire with a prepaid return envelope when they attended the hospital for consultation prior to amniocentesis. Participating women were asked to complete the questionnaire at home two days after amniocentesis, when the karyotype result was still unknown. At this stage procedure related anxiety had been eliminated, while the choice between the tests as presented in the survey still mimicked a realistic choice.

The target professional population consisted of 140 caregivers involved in prenatal diagnosis, i.e. obstetricians, midwives, clinical geneticists, cytogeneticists and general practitioners. These caregivers were randomly selected from the eight Dutch centres performing Prenatal Diagnosis.

Both pregnant women and caregivers were invited to rank the three tests in order of preference. The Institutional Review Board approved the study and decided that informed consent was redundant.

We used the χ^2 test to calculate differences in preferences between referral indications as well as the various groups of caregivers.

RESULTS

From January 2007 until July 2007 150 consecutive pregnant women received a questionnaire. Seventy-five percent (113/150) responded to the survey. Median age of the responders was 38 years (29 to 44 years); median parity was 1.0 (0 to 7). The non-responders had comparable characteristics; median age was 38 years (33 to 41) and median parity was 1.0 (0 to 2). In 84.1% (95/113) of the responders the indication for amniocentesis was advanced maternal age (AMA), in 11.5% (13/113) it was increased risk following prenatal screening and in 2.7% (3/113) maternal anxiety was the indication. Almost all responding pregnant women expressed a preference either for test C resembling karyotyping (50.4%) or test B, the rapid technique detecting the most common aneuploidies (43.4%). A minority of women chose the test detecting Down's syndrome only (table 1). The dominant preference did not differ between the various indication groups (χ^2 5.21 p=0.771).

In June 2007, 140 caregivers received an identical questionnaire and prepaid return envelope. Of the caregivers (70% female; median age 44 years, 26 to 63 years), 55% (77/140) returned the questionnaire. Non-responders had a similar gender distribution

Table 1. Ranking order of three prenatal diagnostic tests.

Test A represents a rapid test, detecting aneuploidy of chromosome 21 (Down syndrome)

Test B represents a rapid test, detecting aneuploidies of chromosome 21, 13, 18, X and Y

Test C represents traditional karyotyping, detecting a range of numerical and structural chromosome abnormalities of all chromosomes

	Ranking order test A, B and C						
	A-B-C	A-C-B	B-A-C	B-C-A	C-A-B	C-B-A	Total
Pregnant women	**5**	**2**	**13**	**35**	**0**	**56**	**111***
- Advanced maternal age	4	2	9	32	0	48	**95**
- Risk following prenatal screening	1	0	4	2	0	6	**13**
- Parental anxiety	0	0	0	1	0	2	**3**
Caregivers	**7**	**1**	**48**	**12**	**3**	**5**	**76****
- Obstetricians and gynaecologists	3	1	15	2	3	1	**25**
- Midwives and general practitioners	4	0	26	6	0	1	**37**
- Clinical (cyto)geneticists	0	0	7	4	0	3	**15****

*2 pregnant women only stated their 1st preference

** the answer of 1 clinical geneticist is excluded

(64% female). The majority of the caregivers preferred test B (77.9%) while the remaining preferences for test A and C were equally divided (10.4%) (table 1). The answer of one clinical geneticist (1.3%) who proposed a non-existing combination of test B and C was excluded. The dominant preference did not differ between the various groups of caregivers (χ^2 7.28 p=0.122).

DISCUSSION

The aim of this study was to address which type of prenatal diagnostic test is preferred by pregnant women and caregivers and to investigate whether the preferences of these two stakeholders differ. The study showed a considerable difference in preferences amongst pregnant women. Half of our respondents opted for a test providing extensive information with considerable waiting time, while the other half preferred RAD, a rapid test providing specific information on the most common aneuploidies. In contrast to pregnant women, the majority of caregivers favour the rapid aneuploidy test, which can detect the most common aneuploidies. The caregivers studied represent all professional stakeholders in the field of prenatal diagnosis in our country. Hence, the fact that they largely agree seems promising and offers ample opportunity to reach consensus and equity in the provision of prenatal diagnostic care. On balance, there is a remarkable difference in what women want and caregivers prefer.

A number of limitations of this study are recognized. Firstly, in absence of extensive debriefing, we can only speculate why women and caregivers choose Test A, B or C. A discrete choice experiment of sufficient sample size may reveal the background of women's preference for either an extensive yet slow test or a selective and rapid test in more detail. Secondly, the generalizability of our results to other European countries can be a concern, because the majority of women chose to undergo amniocentesis on behalf of their age. In the Netherlands advanced maternal age still is the major indication for invasive prenatal diagnosis, while the National UK Screening Committee recommends not offering prenatal diagnosis for an age-related risk alone[5]. We did not include costs as test characteristic, since in the Dutch health care systems costs are usually fully covered by the insurance companies. However, in countries where co-payment is required or all costs are born by the pregnant woman; cost considerations may influence the preferred test. Another concern relates to the response rate of caregivers: with a response rate of 55%, selection bias cannot be ruled out.

Compared to the previous preference studies, the strength of our study is that we included women actually undergoing amniocentesis and we provided information on the tests

using the three main test characteristics[3,4]. Furthermore, this was a prospective study of a consecutive cohort, with a high (75%) response rate. In addition, we invited pregnant women and caregivers who live and work in different areas of the Netherlands, excluding possible regional differences.

In 2004, the UK National Screening Committee[5] recommended the use of RAD as a sole diagnostic tool (i.e. QF-PCR) to all women of increased risk of Down's syndrome (with a nuchal translucency scan <3.0 mm). Although the preceding HTA report showed consensus for women and caregivers in favour of RAD[3], the subsequent use of RAD as standalone test was not adopted in all centres. At this moment, we do not have insight in the reasons why the nationwide implementation was not successful. In the Netherlands, the perspective seems even less favourable: there is no clear consensus between caregivers and pregnant women. Although it is generally acknowledged that patient's preferences should play a role in the process of medical decision-making, caregivers and policymakers may enforce a uniform policy leading to the implementation of RAD, which is preferred by caregivers and seems less expensive. Assuming the differences to be truly representative for women's preferences, a uniform policy should be avoided, because it will not meet the choice of 50% of the consumers.

To bridge the gap between caregivers and pregnant women, several solutions come to hand. One is that centres supply either karyotyping or RAD and women can choose which centre they attend. Another option is that centres offer both tests and women are allowed to choose, with or without additional payment. In line of our study results, an individualised choice in prenatal diagnosis should be offered, with or without economic incentives.

When offering individualised choice, one should realise that the prenatal counselling process is of the highest importance. It is well known that counsellors should provide adequate nondirective counselling service, taking into account both a patient individualised risk assessment and her preferences. However, several studies suggest that counsellors frequently deviate from nondirectiveness: their attitudes and preferences can influence women's decision-making[6]. Especially in case of individualised choice, the difference in preferences between women and caregivers could jeopardise the non-directive counselling process. Alerting counsellors to the discrepancy between client's and their own preference will help to improve the process of informed and autonomous decision-making.

In this new era with rapid developments in genetic testing and growing societal individualisation, a uniform policy seems out of date. The provision of prenatal genetic testing should be based upon individualized choice since the choice may have far reaching consequences. Future research and policymaking should focus on the implementation of available diagnostic tests and on the optimisation of the prenatal counselling process adjusted to the techniques.

CONCLUSION

This study shows that the preferences for prenatal tests differ greatly among pregnant women. Caregivers mostly opt for RAD detecting the most common chromosomal aneuploidies. The divergent preferences amongst women and the difference between women and caregivers make us plea for individualised choice, provided that nondirective counselling is available.

Questionnaire

We describe three hypothetical prenatal tests, named Test A, Test B and Test C. The three tests have different test characteristics, which are described in the text boxes below. For all three tests, amniocentesis has to be carried out. There is a risk of one in 250 for a miscarriage with amniocentesis.

Firstly, read the description of the three hypothetical tests. Secondly, rank the tests in order of your preference.

Test A

Characteristic 1: The test will determine whether or not your baby has **Down syndrome** (trisomy 21).

Characteristic 2: In case of a detected Down's syndrome, the doctor can provide **clear information** on the consequences of the abnormality.

Characteristic 3: You have to wait **4 days** for the test result.

Test B

Characteristic 1: The test will determine whether or not your baby has **one of the most common** chromosomal abnormalities, including Down syndrome (trisomy 21). These chromosomal abnormalities account for 80% of all chromosome aberrations identified prenatally.

Characteristic 2: In case of a detected chromosomal abnormality, the doctor can provide **in most cases** clear information on the consequences of the abnormality.

Characteristic 3: You have to wait **4 days** for the test result.

Test C

Characteristic 1: The test will determine whether or not your baby has a chromosomal abnormality by testing **all chromosomes**, including Down syndrome (trisomy 21).

Characteristic 2: In case of a detected chromosomal abnormality, the doctor can **often** provide you clear information on the consequences of the abnormality. Sometimes however, the exact consequences for the development of your child can't be provided.

Characteristic 3: You have to wait **21 days** for the test result.

Question 1: Which of the described tests do you prefer most?

☐ Test A ☐ Test B ☐ Test C

Question 2: Which of the described tests do you prefer second best?

☐ Test A ☐ Test B ☐ Test C

Question 3: Which of the described tests do you prefer least?

☐ Test A ☐ Test B ☐ Test C

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Chapter 7

Patients' preferences for rapid aneuploidy detection or karyotyping in prenatal diagnosis

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ABSTRACT

Objective

To determine the pregnant women's preference for rapid aneuploidy detection (RAD) or karyotyping and which test attributes influence their preference most.

Methods

We designed a discrete choice experiment, in which pregnant women had to choose between prenatal test profiles that differed in five treatment attributes: detection capacity, anxiety, waiting time, failure rate and consequences of detected chromosomal abnormalities. We assessed preference for i) a RAD test for the detection of aneuploidies of chromosome 21, 18, and 13, ii) a RAD test for aneuploidies of chromosome 13, 18, 21, X, and Y, and iii) karyotyping. Test specific attributes that influence women's preference were estimated.

Results

In total 103/118 (87%) women participated. Women placed most value on the detection of chromosomal abnormalities with severe consequences for their child ($P<0.01$). The failure rate of the test, the waiting time for test results, and the experienced anxiety influenced women's preferences significantly ($P <0.05$). For the currently available tests, women prefer karyotyping to RAD ($P <0.01$) in a setting where the detected chromosomal abnormalities have severe consequences for their child. However, in a setting where karyotyping detects chromosomal abnormalities with uncertain or no clinical consequences for their child, RAD tests are preferred ($P <0.01$).

Conclusion

While anxiety and waiting time have some effect on women's preferences, the potential clinical consequences of the detected chromosomal abnormality and failure rate influenced test choice most. Since RAD and karyotyping both detect the most common chromosomal abnormalities with severe consequences, both tests are appropriate for prenatal diagnosis.

INTRODUCTION

In the last decade, a debate emerged in Europe whether rapid aneuploidy detection (RAD) should replace traditional karyotyping in prenatal diagnosis for relatively low risk indications such as advanced maternal age, increased risk following first trimester prenatal screening and maternal anxiety[1,2]. Opponents argue that substitution of karyotyping by RAD leads to undetected chromosomal abnormalities with severe clinical consequences while having a procedure related miscarriage risk. Proponents' main argument is that substitution of karyotyping leads to a shortening of the stressful waiting time for parents, efficient prenatal counselling, substantial cost savings, and a negligible risk of missing a chromosomal abnormality with clinical significance[3,4].

While it is generally acknowledged that patient preferences should be incorporated into medical decision making[5], and that many decisions need to be individualized, especially when they involve choices between possible outcomes that may be viewed differently by different patients[6], only few studies have assessed pregnant women's preferences for RAD or karyotyping[7,8]. In order to support policy-making for prenatal testing, it is important to know what pregnant women want and which test features are important to them.

We assessed women's preferences for RAD, both with or without detection of sex chromosome aneuploidies, and karyotyping. We used a so-called discrete choice experimentation (DCE) to determine the preferred diagnostic test strategy of women who considered having amniocentesis, and to evaluate which test characteristics they valued most. We also analyzed if differences in preferences existed based on the prenatal test women chose in real life.

METHODS

This study was conducted alongside the clinical MLPA And Karyotyping, an Evaluation (M.A.K.E.) study, a cohort study comparing MLPA and karyotyping on 4586 amniotic fluid samples[9,10]. The M.A.K.E. study has been described in detail elsewhere[9,10]. The diagnostic accuracy of MLPA was comparable to karyotyping as reference test.

From October 2008 to October 2009, women were invited attending a tertiary hospital (Erasmus Medical Centre, Rotterdam) to obtain information on prenatal testing. We selected this group because they represent the users of prenatal diagnostic tests and at that point they had not decided which test to choose. Women without sufficient command of the Dutch language were excluded. There were no other exclusion criteria. The prenatal counsellor provided nondirective oral and written information on prenatal screening and diagnosis.

Referral indication, gravidity, parity, ethnicity, previous prenatal testing and education were recorded. This study was approved by the medical review ethics committee of the Onze Lieve Vrouwe Gasthuis, Amsterdam (reference number 06032).

DCEs are increasingly being used as a means to elicit patient preferences[11-13]. DCEs assume that a given healthcare intervention or treatment (e.g. screening programme) can be fully described by its characteristics ('attributes'; e.g. test duration) and that any woman's preference for an intervention or treatment are determined by the variants of the attributes ('levels'; e.g. 2, 4 and 6 days)[14]. The attributes and levels should be identified beforehand as potentially important determinants for the choice of an intervention or treatment[15,16]. In a DCE, each individual is presented a series of two hypothetical interventions in which the levels are varied. Next, individuals are invited to tick the preferred option. In the analysis, the series of choices made are linked to the differences in levels between options in order to obtain the relative weight assigned to that level or attribute.

We distinguished three test strategies; RAD with and without the possibility to detect sex chromosome abnormalities (RAD+XY and RAD-XY respectively) and karyotyping (TKT). Both RAD tests are able to detect aneuploidies of chromosomes 13, 18 and 21. Although RAD can also detect sex chromosome aneuploidies using appropriate probes or primer sets, some centres do not test for sex chromosome anomalies. Karyotyping detects both numerical and structural chromosomal abnormalities of at least 5 Megabases. The study was designed as one set of choices comparing RAD+XY and TKT, and one set comparing RAD-XY and TKT. Each choice set consisted of two juxtaposed vignettes; one vignette depicted a RAD+XY or RAD-XY strategy, the other displayed a TKT strategy. Each vignette consisted of an equal and fixed number of attributes. The levels within each attribute varied between the test strategies. To identify the relevant attributes, we conducted a literature review and a pilot survey among 150 women who underwent amniocentesis. The survey contained 12 questions on detection capacity, consequences, waiting time, failure rate, anxiety, false positive and false negative test results, including a ranking exercise. The five most important attributes were detection capacity, consequences, anxiety, failure rate and waiting time. We did not include costs or co-payment as attribute, since these costs are fully covered in the Dutch healthcare system and therefore do not affect choices. The specific levels for each test attribute covered the range of possible test outcomes for each of the three strategies (RAD-XY, RAD+XY and TKT) based on literature review. The attribute levels are shown in Table 1, where detection capacity is incorporated in the test strategy.

We adopted a so-called labelled design (i.e. each strategy was labelled RAD-XY, RAD+XY or TKT) because the levels of several attributes differed according to the test strategy. Such an approach is considered to increase realism and validity of our results[17,18]. The full factorial

Table 1: Alternatives, attributes and the alternative specific levels.

Characteristics	Alternatives and levels		
	RAD-XY	RAD+XY	TKT
Waiting time (days)	4	4	14
	7	7	21
Anxiety	No	No	No
	Little	Little	Little
	Quite a lot	Quite a lot	Quite a lot
	A great deal	A great deal	A great deal
Failure rate	3/1000	3/1000	3/1000
	10/1000	10/1000	
	20/1000	20/1000	
	30/1000	30/1000	
Consequences	Severe	Severe	Severe
		Mild	Mild
			Uncertain
			No

RAD-XY= test detecting trisomies 21, 18, 13
RAD+XY= test detecting trisomies 21,18, 13, X,Y
TKT= traditional karyotyping

design, combining all possible attributes with all possible levels, resulted in 1024 possible RAD-XY versus TKT comparisons and 2048 possible RAD+XY versus TKT comparisons. Since it is not feasible to present a single individual with all these combinations, we applied a so-called fractional factorial design consisting of 16 RAD-XY versus TKT and 16 RAD+XY versus TKT comparisons. This design, which was 92% efficient[18], is the most efficient design possible under the restrictions imposed on the design. Further details about the DCE design are available on request.

Each of the 32 comparisons was presented as two juxtaposed vignettes, one being a RAD+XY or RAD-XY strategy, the other being a TKT strategy. Each comparison was displayed graphically and with text to present the numbers in a balanced manner[19]. Vignettes, study materials and procedures were pilot-tested in 15 pregnant women and optimized before study onset. Figure 1 displays one of the vignettes (see page 118).

The DCE survey containing the 32 comparisons was devised as a booklet. First, we provided general information on prenatal testing, prenatal diagnosis and chromosomal abnormalities. Secondly, we outlined the study aim and explained the vignettes. A legend chart of all used symbols and colours was provided to facilitate the choices. Thirdly, women were invited to evaluate the 32 comparisons and tick the preferred option. Finally, women's socio-demographic characteristics were collected. Women who agreed to participate received the survey after the prenatal counselling visit, filled in the survey at home and returned the survey by prepaid envelope. No remuneration was done.

The choices between the three strategies were analyzed by multinomial logit regression models with test specific parameters using SAS version 9.1, (SAS Institute Inc., Cary, NC, USA). We assumed that there was no linear relationship between the different levels of the characteristics. We estimated the following models:

V RAD-XY= β0+ βref wait4 + β1wait7+ βref anxietygreatdeal+ β2anxietyquite+ β3anxietylittle+ β4anxietyno + βref failure3+ β5failure10+ β6failure20+ β7failure30

V RAD+XY= β8+ βref wait4+ β9wait7+ βref anxietygreatdeal+ β10anxietyquite+ β11anxietylittle+ β12anxietyno+ βref failure3+ β13failure10+ β14failure20+ β15failure30+ βrefsevereconsequence+ β16mildconsequence

V TKT= βref det cap+ βref wait14+ β17wait21+ βref anxietygreatdeal+ β18anxietyquite+ β19anxietylittle+ β20anxietyno + βrefsevereconsequence+ β21mildconsequence + β22uncertainconsequence+ β23noconsequence

$V_{\text{base case TKT}} = 0$ (i.e. base case is karyotyping with a waiting time of 14 days, experiencing no anxiety, and detecting chromosomal abnormalities with severe consequences)

V represents the preference score for each strategy on an interval scale, relative to V_{TKT} as base case. I.e., the higher the V score, the stronger the preference for that strategy, but the absolute value of V has no direct interpretation[14]. The constants β_0 and β_8 are alternative specific constants that indicate the general attitude of women towards RAD-XY and RAD+XY compared to karyotyping. The β-coefficients represent the preference weights associated with the levels of the respective attributes ($\beta_{1,9,17}$ are the preference weights associated with the attribute waiting time, $\beta_{2\text{-}4,\ 10\text{-}12,\ 18\text{-}20}$ with anxiety, $\beta_{5\text{-}7,13\text{-}15}$ with failure rate, and $\beta_{16,21\text{-}23}$ with respect to consequences of the chromosomal abnormality for the unborn child). As in any regression, the sign of the β-coefficients indicates whether the alternative specific level has a positive or negative effect on V compared to the reference level. The magnitude of the β-coefficients indicates the relative importance of the specific level compared with the reference level for that attribute (see Table 3). A two-sided p-value ≤ 0.05 was considered statistically significant.

We calculated the relative preference (V) for currently available tests by choosing the most realistic level of each attribute by using the results of the clinical M.A.K.E. study[9]; i.e., the relative preference (V) of a currently available test was equal to the sum of the coefficient weights of its attribute levels. For RAD-XY, we included the constant coefficient (β0), waiting

time of 7 days (β1), quite a lot of anxiety (β2), and a failure rate of 10/1000 (β5). For RAD+XY, we included the constant coefficient (β8), waiting time of 7 days (β9), a lot anxiety (β10), and a failure rate of 10/1000 (β13), detecting chromosomal abnormalities with severe consequences (βref). For TKT, we included a waiting time of 21 days (β17), a great deal of anxiety (βref), and detecting chromosomal abnormalities with severe consequences (βref). Wald chi-squared test assessed the differences in preferences per currently available test.
A priori we expected all attributes to be important and we expected positive effects of a short waiting time, a low failure rate, and no anxiety. Subgroup analysis was done for women who chose to have amniocentesis versus the women who opted for prenatal screening, no prenatal testing, chorionic villus sampling, or did not decide yet).

RESULTS

A total of 103/118 (87.3%) women returned the survey. Often cited reasons to decline participation were no interest, survey is difficult to fill in, and lack of time. Table 2 shows respondent's characteristics. Two women who only filled in part of the survey were excluded from analysis.

Table 2: Respondents' characteristics

	median	IQR
Age (years)	37	37-39
Gravidity	1	1-2.75
Parity	1	0-1
	amount	%
Indication PND		
maternal age	95	94.1%
anxiety	5	5%
prenatal screening	1	1%
Western-European ethnicity	94	93.1%
Education		
lower vocational	2	2
lower secondary	18	17.8 %
intermediate and higher vocational, higher secondary	41	40.6 %
college/university	40	39.6 %
PND in previous pregnancy	14	17.3%
PNS in previous pregnancy	30	37.0%
Definitive choice		
no testing	2	2%
prenatal screening	30	29.7%
chorionic villus sampling	12	11.9%
Amniocentesis	52	51.5%
I have not decided yet	5	5%

n=103, 2 missing, IQR= interquartile range, PND= prenatal diagnosis, PNS= prenatal screening

Table 3 shows the β-coefficients associated with the levels of each strategy. All β-coefficients had p-values <0.05, i.e. consequences for the unborn child, failure rate, detection capacity, waiting time and anxiety all significantly attributed to women's preferences. The signs of all significant coefficients of the attributes were as we expected except for waiting time for RAD-XY. The negative signs of failure rate (β5-7, β13-15) and consequences for the unborn child (β16, β21-23) indicate that women prefer a test with the lowest failure rate and a test that is able to detect chromosomal abnormalities with severe consequences for the unborn child. For TKT, a short waiting time was preferred over a longer waiting time. In contrast,

Table 3: Coefficients of the different tests and attributes

Attributes and levels		β	Coeff	CI	P-value
Detection capacity	RAD-XY with severe consequences	β0	-1.78	-2.21 to -1.36	<.01
	RAD+XY	β8	-1.43	-1.85 to -1.02	<.01
	TKT with fail 3/1000		ref		
Waiting time	4 days, RAD-XY		ref		
	7 days, RAD-XY	β1	0.23	0 to 0.47	0.05
	4 days, RAD+XY		ref		
	7 days, RAD+XY	β9	0.07	-0.18 to 0.31	0.60
	14 days, TKT		ref		
	21 days, TKT	β17	-0.17	-0.35 to 0	0.05
Anxiety	great deal, RAD-XY		ref		
	quite a lot, RAD-XY	β2	-0.05	-0.38 to 0.27	0.75
	little, RAD-XY	β3	-0.16	-0.47 to 0.16	0.33
	no, RAD-XY	β4	-0.12	-0.44 to 0.2	0.46
	great deal, RAD+XY		ref		
	quite a lot, RAD+XY	β10	0.14	-0.18 to 0.47	0.40
	little, RAD+XY	β11	0.40	0.03 to 0.76	0.03
	no, RAD+XY	β12	0.36	0.01 to 0.70	0.04
	great deal, TKT		ref		
	quite a lot, TKT	β18	0.46	0.21 to 0.70	0.00
	little, TKT	β19	0.33	0.06 to 0.59	0.01
	no, TKT	β20	0.34	0.10 to 0.59	0.01
Failure rate	3/1000, RAD-XY		ref		
	10/1000, RAD-XY	β5	-0.13	-0.45 to 0.20	0.45
	20/1000, RAD-XY	β6	-0.21	-0.53 to 0.11	0.20
	30/1000, RAD-XY	β7	-0.33	-0.66 to -0.01	0.05
	3/1000, RAD+XY		ref		
	10/1000, RAD+XY	β13	-0.14	-0.45 to 0.16	0.36
	20/1000, RAD+XY	β14	-0.84	-1.21 to -0.47	<0.01
	30/1000, RAD+XY	β15	-1.03	-1.38 to -0.68	<0.01
Consequences	severe, RAD+XY		ref		
	mild, RAD+XY	β16	-0.73	-0.98 to -0.47	<0.01
	severe, TKT		ref		
	mild, TKT	β21	-1.72	-2.01 to -1.44	<0.01
	uncertain, TKT	β22	-2.50	-2.78 to -2.22	<0.01
	no, TKT	β23	-2.52	-2.80 to -2.24	<0.01

for RAD-XY and RAD+XY women expressed a positive attitude toward a waiting time of 7 days compared to 4 days (β=0.23, p=0.05; β=0.07, p=0.60). Furthermore, women valued less anxiety positively for RAD+XY and TKT. In contrast, for RAD-XY, women valued less anxiety negatively (β2-4 <0), but coefficients were small and not significant. Detection capacity, failure rate and consequences had β's of high magnitude, with highest for TKT,

Table 4: Subgroup analysis of women choosing amniocentesis versus women who opt for prenatal screening, no testing, chorionic villus sampling

Attributes and levels		β AC	95% CI	β no AC	95% CI	P-value
Detection capacity	RAD-XY with severe consequences	-2.51	-3.22 to -1.8	-1.39	-1.96 to -0.81	0.02
	RAD+XY	-1.86	-2.51 to -1.21	-1.15	-1.71 to -0.59	0.10
	TKT with failure 3/1000	ref		ref		
Waiting time	4 days, RAD-XY	ref		ref		
	7 days, RAD-XY	0.21	-0.16 to 0.58	0.25	-0.06 to 0.57	0.86
	4 days, RAD+XY	ref		ref		
	7 days, RAD+XY	0.00	-0.4 to 0.4	0.12	-0.22 to 0.45	0.67
	14 days, TKT	ref		ref		
	21 days, TKT	-0.12	-0.43 to 0.18	-0.26	-0.49 to -0.02	0.50
Anxiety	no, RAD-XY	-0.09	-0.57 to 0.39	-0.02	-0.47 to 0.44	0.82
	little, RAD-XY	-0.32	-0.81 to 0.17	-0.07	-0.5 to 0.36	0.45
	quite a lot, RAD-XY	-0.25	-0.74 to 0.25	-0.05	-0.49 to 0.39	0.56
	great deal, RAD-XY	ref		ref		
	no, RAD+XY	-0.11	-0.59 to 0.36	0.37	-0.09 to 0.82	0.15
	little, RAD+XY	-0.02	-0.58 to 0.54	0.67	0.17 to 1.18	0.07
	quite a lot, RAD+XY	0.02	-0.54 to 0.58	0.54	0.08 to 1.01	0.16
	great deal, RAD+XY	ref		ref		
	no, TKT	0.15	-0.26 to 0.55	0.66	0.33 to 0.99	0.06
	little, TKT	0.23	-0.27 to 0.73	0.41	0.07 to 0.74	0.57
	quite a lot, TKT	0.06	-0.35 to 0.47	0.51	0.18 to 0.84	0.09
	great deal, TKT	ref		ref		
Failure rate	3/1000, RAD-XY	ref		ref		
	10/1000, RAD-XY	-0.11	-0.59 to 0.37	-0.14	-0.59 to 0.31	0.93
	20/1000, RAD-XY	-0.35	-0.85 to 0.15	-0.15	-0.59 to 0.30	0.54
	30/1000, RAD-XY	-0.38	-0.88 to 0.13	-0.34	-0.79 to 0.11	0.91
	3/1000, RAD+XY	ref		ref		
	10/1000, RAD+XY	-0.09	-0.53 to 0.35	-0.19	-0.63 to 0.25	0.77
	20/1000, RAD+XY	-1.07	-1.67 to -0.48	-0.80	-1.29 to -0.30	0.48
	30/1000, RAD+XY	-1.28	-1.86 to -0.7	-0.95	-1.41 to -0.48	0.38
Consequences	severe, RAD+XY	ref		ref		
	mild, RAD+XY	-1.13	-1.54 to -0.73	-0.49	-0.83 to -0.15	0.02
	severe, TKT	ref		ref		
	mild, TKT	-2.49	-3.01 to -1.96	-1.27	-1.64 to -0.91	<0.01
	uncertain, TKT	-3.22	-3.74 to -2.7	-2.12	-2.47 to -1.76	<0.01
	no, TKT	-3.26	-3.78 to -2.75	-2.12	-2.47 to -1.76	<0.01

AC= amniocentesis group, No AC= rest (prenatal screening, no testing, chorionic villus sampling or not decided yet)

where women valued the consequences of the detected chromosomal abnormality other than severe significantly more negatively (β= -2.52; β= -2.50; β= -1.72).

When the results of the M.A.K.E. study (9) are entered into the models, the relative preference (V) score for each test strategy can be estimated: $V_{RAD\text{-}XY}$ = -1.73, V_{RAD+XY}= -1.37 and V_{TKT}= -0.17. These results show that compared to the base case TKT (i.e. detection capacity 23, waiting time 14 days, no anxiety, failure 3/1000, severe consequences), women value the three strategies negatively. Calculating the difference in preference for the currently available, women have no significant preference for either RAD-XY or RAD+XY (p= 0.14). Karyotyping is preferred over RAD-XY and RAD+XY as long as it detects chromosomal abnormalities with severe consequences for the unborn child (p <0.01 and p <0.01 respectively). When karyotyping detects chromosomal abnormalities with mild consequences for the unborn child, no test is preferred (RAD-XY vs. TKT p =0.98; RAD+XY vs. TKT p= 0.11). When TKT detects chromosomal abnormalities with uncertain or no clinically relevant consequences, both RAD tests are preferred (p <0.01).

Table 4 shows the preferences weights when women who chose to undergo amniocentesis (n=52) are distinguished from women who did not (n=49). The only characteristic that was valued significantly different at all levels was the consequence of the detected chromosomal abnormality (table 4). Women undergoing amniocentesis valued the consequences of the detected chromosomal abnormality significantly more.

DISCUSSION

Our study demonstrates that women place more value on the consequences of a chromosomal abnormality, detection capacity, and the test's failure rate while anxiety and waiting time are valued less. Especially consequences of the detected chromosomal abnormalities for the unborn child have considerable impact on preferences.

Women prefer karyotyping over RAD as long as the karyotype detects chromosomal abnormalities with severe consequences for the unborn child. If karyotyping detects chromosomal abnormalities with uncertain or no clinically relevant consequences, RAD is preferred. Women have no preference if chromosomal abnormalities are detected with mild consequences. The fact that women base their preference mainly on the outcome of a test may be interpreted as of little to help to current practice in which pregnant women have to choose a test in advance. However, these results do indicate the importance of risk selection before undergoing prenatal testing and prenatal counselling. Pregnant women should be informed clearly on the detection capacity of prenatal tests and the consequences of detected

chromosomal abnormalities taking into account the personalized risk. In that way physicians can assist pregnant women and their partners with choosing between the available tests.

Women who actually chose to have an amniocentesis placed more value on the characteristic consequences compared to women who chose not to undergo amniocentesis. Likely, the former are women who prefer clear results in everyday life, while the group choosing no test or prenatal screening can cope with uncertainties.

Only few studies on preferences for RAD and karyotyping in prenatal diagnosis have been published. Two studies have been published on this topic and show no clear preference for RAD or karyotyping[7,8]. Compared to our study, both studies used a simplified design not representative for the complexity of real life decision-making in prenatal diagnosis. The simplified DCE, only including 49 women of which 10 were actually undergoing amniocentesis[7], showed that women preferred a broad detection capacity and a short waiting time corresponding to our results. Grimshaw *et al*[8] showed that of the 141 women who had been nonrandomly allocated to receive RAD and karyotyping, 67% chose RAD before amniocentesis, while 52% chose RAD after all test results were known. These results show that preferences can differ between individuals[6,20] and preferences can change with circumstances[21]. In Stockholm, women are offered a real choice between QF-PCR and karyotyping and it has been reported that the majority (70%) opts for RAD[22]. Although, this study does give insight in the motivations of choices, it is the only study reporting on women's actual decisions.

The labelled design with realistic scenarios adds to the validity of the results. Women placed value on all included attributes and except for waiting time for RAD, the signs of the coefficients were as we expected except for waiting time. Apparently, a waiting time of four days is too short for most women and the optimum time lies between 4 and 7 days. Furthermore, our prospective DCE included many women consecutively. Despite the complex design, the questionnaire was feasible given the high response rate of 87%. The design included many parameters, which might have led to loss of power with inability to detect all significant betas. However, an interim analysis of the survey results of 60 respondents showed that the coefficients of the betas were similar to the results reported here.

None of the currently available tests meets the preference of women. Since RAD-XY, RAD+XY and TKT are all capable of detecting the most common severe chromosomal abnormalities (i.e. trisomies 13, 18 and 21), all tests are appropriate to offer in prenatal diagnosis and women should be allowed to make decisions in the context of their own priorities and life circumstances.

At this point, microarray comparative hybridization, detecting more and smaller chromosomal abnormalities than karyotyping, is presented as having the potential to become the primary

prenatal diagnostic laboratory procedure[23]. In accordance with our results, microarray settles with the preferences for maximal detection, but also detects more uncertain or clinically irrelevant abnormalities, the most negatively valued characteristic of this study. From here we envisage two options: either we develop a new test that meets all preferences of women, or we offer an individual choice to women, taking into considerations her individual expectations and needs. The only currently available realistic option to meet most needs is offering an individual choice, so that the decision to either obtain as much cytogenetic information as possible versus a rapid but specific result on the most common chromosomal abnormalities is most appropriately made by individuals who will bear the responsibility of raising the child.

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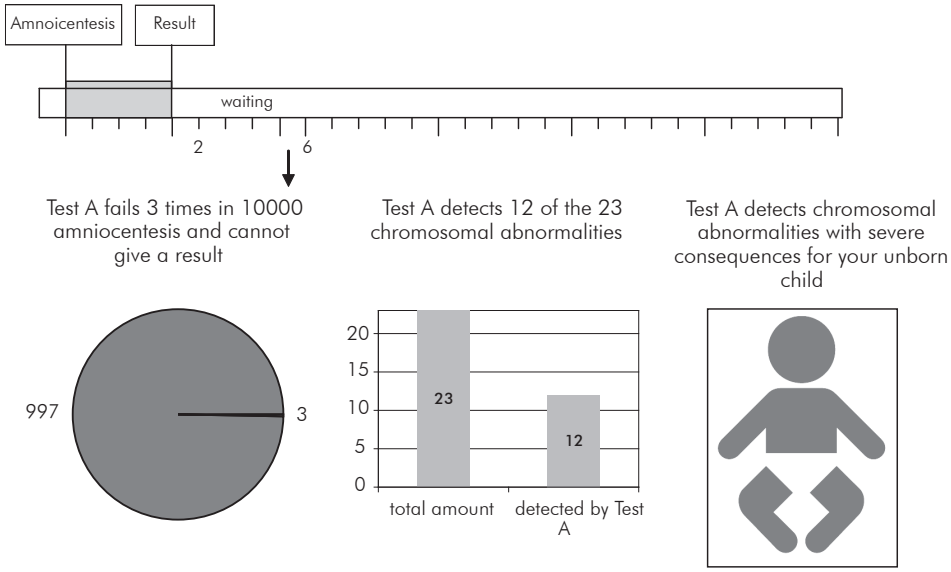
Figure 1: Example of one of the 32 choice sets

Test A

Choice 1

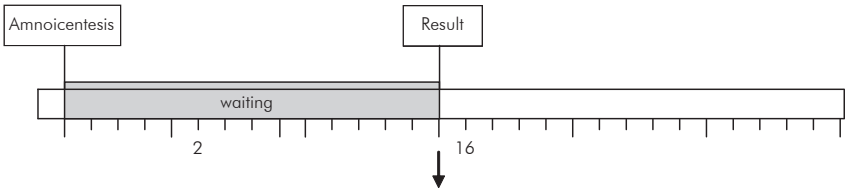


118

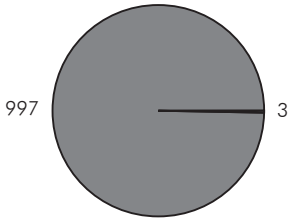
The waiting time for the test result	► 4 days 7 days 14 days 21 days
Your anxiety during the waiting time for the test result	► no little quite a lot a great deal
The number of chromosomal abnormalities detected by the test	► 12 15 23
The failure rate of the test per 1000 amniocentesis	► 3/1000 10/1000 20/1000 30/1000
The consequences of the chromosomal abnormality, detected by the test	► severe mild uncertain no

Test B

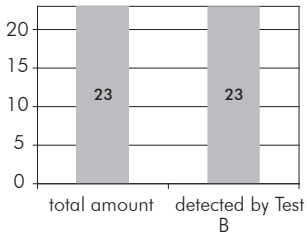
Choice 1



Test B fails 3 times in 1000 amniocentesis and cannot give a result



Test B detects 23 of the 23 chromosomal abnormalities



Test B detects chromosomal abnormalities with severe consequences for your unborn child

The waiting time for the test result	► 4 days 7 days 14 days 21 days
Your anxiety during the waiting time for the test result	► no little quite a lot a great deal
The number of chromosomal abnormalities detected by the test	► 12 15 23
The failure rate of the test per 1000 amniocentesis	► 3/1000 10/1000 20/1000 30/1000
The consequences of the chromosomal abnormality, detected by the test	► severe mild uncertain no

Chapter 8

Aiming at multidisciplinary consensus: What should be detected in Prenatal Diagnosis?

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Accepted in Prenatal Diagnosis

ABSTRACT

Objective

To determine expert-consensus on which chromosomal abnormalities should and should not be detected in prenatal diagnosis, and for which abnormalities disagreement remains after structured discussion.

Methods

An expert panel of 24 prenatal experts (8 clinical cytogeneticists, 8 clinical geneticists and 8 obstetricians) rated 15 chromosomal abnormalities sampled from a nationwide study on rapid aneuploidy detection. In two individual anonymous rating rounds and one group meeting, participants rated pro or against detection and stated their main argument. The 15 chromosomal abnormalities were described in detail by a stylized vignette containing an obstetrical history, the indication for prenatal diagnosis and the range of possible outcomes of the chromosomal abnormality. Consensus was defined to be present if at least 80% of the experts agreed.

Results

Consensus was reached in 12 of 15 cases. In 10 cases, there was agreement pro detection and in two cases experts agreed against detection. At the end of the 3rd round, dissensus remained on three abnormalities.

Conclusion

Experts largely agreed on detecting chromosomal abnormalities with severe consequences and against detection in case of irrelevant clinical consequences. For chromosomal abnormalities with mild or uncertain outcomes, dissensus remained. None of the currently available tests corresponds to these demands.

INTRODUCTION

In prenatal diagnosis, conventional karyotyping is considered the gold standard to detect fetal chromosomal abnormalities. New molecular techniques emerged which challenged routine practice based on karyotyping. Many evaluation studies have focused on diagnostic accuracy of these new techniques, either at the laboratory or at the clinical level. These studies, however, are ineffective if 1) outcomes undisputedly relate to different domains (e.g. ethical, emotional and medical aspects), 2) multiple stakeholders are relevant with potentially unreconciled interests (e.g. government, patients, doctors, ethical and religious representatives, lay people), 3) new techniques develop too rapidly to allow a long term comprehensive analysis.

Availability of rapid aneuploidy detection (RAD) tests allows an accurate result on trisomies 13, 18, 21 and numerical sex chromosome aberrations to be obtained within a few days[1,2] and at considerably lower costs compared to karyotyping[2,3]. RAD -by design- cannot detect other chromosomal abnormalities than trisomies 13, 18, 21 and numerical sex chromosome aberrations, leading to an estimated undetected chromosomal abnormality with severe consequences for the unborn child in 1: 1000 to 1:1659 invasive tests[4-6]. A profound debate has emerged on which test should be offered: should it be targeted and quick at low costs with a small risk of missing rare severe chromosomal abnormalities, or should the test detect as much as possible with higher costs and longer waiting time, and accepting the detection of chromosomal abnormalities with no or unclear clinical consequences? The latter provoke difficult counseling issues and emotional parental dilemmas to continue or terminate pregnancy. So far, formal evaluations and decision-support techniques have failed to arrive at an agreed set on which chromosomal abnormalities should be detected and which not.

This paper describes an alternative approach ('nominal group technique') to arrive at a consensus judgment on which chromosomal abnormalities experts want to detect in prenatal diagnosis for women with advanced maternal age, increased risk following prenatal screening or parental anxiety. A formalized expert meeting was set up, with participation of a nationwide sample of prenatal diagnosis experts, representing current policymakers. We aimed to reach consensus on which chromosomal abnormalities should be detected, and which not, regardless the test. Secondly, we explored the key arguments underlying the remaining cases of dissent.

METHODS

Study design

The nominal group technique, also known as expert panel, is a consensus method and was developed in the United States in the 1960s. It is used when unanimity of opinion cannot be achieved due to divergent outcomes on multiple domains or conflicts of primary interests and a lack of remuneration on consensus. The nominal group technique gathers information from relevant experts, using a highly structured face-to-face meeting with or without a preceding individual survey. In the former case the individual round serves as anonymous input for the face-to-face meeting. The final goal of the nominal technique is to assess the initial agreement, to resolve disagreement by structured information exchange, to assess final agreement, and to redefine remaining issues of dissensus in terms of principal arguments at stake[7]. In health care the method has been applied to examine the appropriateness of clinical interventions, where straightforward application of guidelines was insufficient[8].

Our expert study consisted of three rounds. In the first round we asked for anonymous individual judgment regarding the desirability to detect 15 chromosomal abnormalities, presented in stylized fashion ('vignettes', see below). In the second round, again anonymous individual judgment was aimed at, now after feedback of the total group's responses. In the third round, individual judgments were no longer anonymous; group consensus was aimed at after extensive group considerations and remaining dissensus was explored.

Participants

We purposely selected 24 leading experts from three professional stakeholder groups; clinical geneticists (8), clinical cytogeneticists (8) and obstetricians (8) from all prenatal clinics in the Netherlands, based on their experience and publications in the area. The participants were informed on the study aim, the amount and type of work expected, the study deadlines, and the lack of monetary compensation. The experts were guaranteed that no individual data on their personal opinion or stated arguments would be presented in any public report or would be made available otherwise.

Clinical Study

From February 2007 until July 2008, we performed a prospective study comparing the diagnostic accuracy of Multiplex Ligation-dependent Probe Amplification (MLPA), a molecular technique that detects trisomies 13, 18, 21 and numerical sex chromosome abnormalities, with karyotyping on 4585 amniotic fluid samples[2,9]. In 4484/4585 samples (97.8%) MLPA and karyotyping were concordant, showing normal results in 4387/4585 (95.7%) and

Table 1. Selection of chromosomal abnormalities (vignettes) for the expert study drawn from the data of the clinical MLPA and Karyotyping, an Evaluation (M.A.K.E.) study

chromosomal abnormalities in M.A.K.E.study	n	vignettes
Trisomy 21	69	1
trisomy 18	15	1
trisomy 13	1	1
sex chromosomal abnormalities	12	2
mosaic trisomy 21 and mosaic Turner	1	0
structural inherited balanced chromosome aberration	14	1
structural de novo apparently balanced chromosome aberration	4	1
supernumerary marker chromosome	3	3
mosaicism	3	2
structural de novo unbalanced chromosome aberration	2	2

aneuploidy in 98/4585 (2.1%). Discordant results were found in 26/4585 (0.6%) samples, representing in all cases an abnormal karyotype yet undetected by MLPA (supplemental table 1). The failure rate was 1.6% for MLPA and 0.02% for karyotyping. The cases in which a chromosomal abnormality was detected are used in the current expert study.

Vignettes

A research team of four people (EMB, EB, GB, JvL) described chromosomal abnormalities in a stylized fashion ('vignette', see table 2) and facilitated the meeting (figure 1). Vignettes have been used in a variety of other studies to communicate relevant specific info to a panel of voters with a specific task[10,11]; this technique of data reduction and effective communication is common in decision science. We included all chromosomal abnormalities which both tests can detect, as well as the abnormalities that RAD cannot disclose. Per group of comparable chromosomal abnormalities (e.g. inherited apparently balanced chromosomal abnormalities), we randomly drew one case from this group, otherwise we described each chromosomal abnormality separately (table 1). In the vignette we used the unchanged patient background data from the M.A.K.E. study. The consequences of the chromosomal abnormality were described by using the full range of outcomes as described in literature. Amendments to the vignettes were allowed during the course of the process, as experts sometimes could dispose of information yet to become available in the public domain. An independent clinical geneticist helped with describing and, when necessary, adjusting the cases.

Consensus rule

Consensus was stated to be reached when at least 80% of the experts agreed on whether chromosomal abnormalities should or should not be detected, otherwise dissensus existed. For all cases, we used a multiple choice response mode with four options; strongly PRO

detection, probably PRO detection, probably AGAINST detection, strongly AGAINST detection. The adjectives 'probably or strongly' were regarded equal to calculate consensus.

Study procedures

The questionnaire for round 1 was piloted among four gynecological researchers. For round 1 and 2, we used web-based provider of survey software (QuestionPro, Seattle USA). Responses were collected through computer generated reports. One reminder was sent three weeks after the initial sending of the survey invitation. The study took place from December 2008 to February 2009.

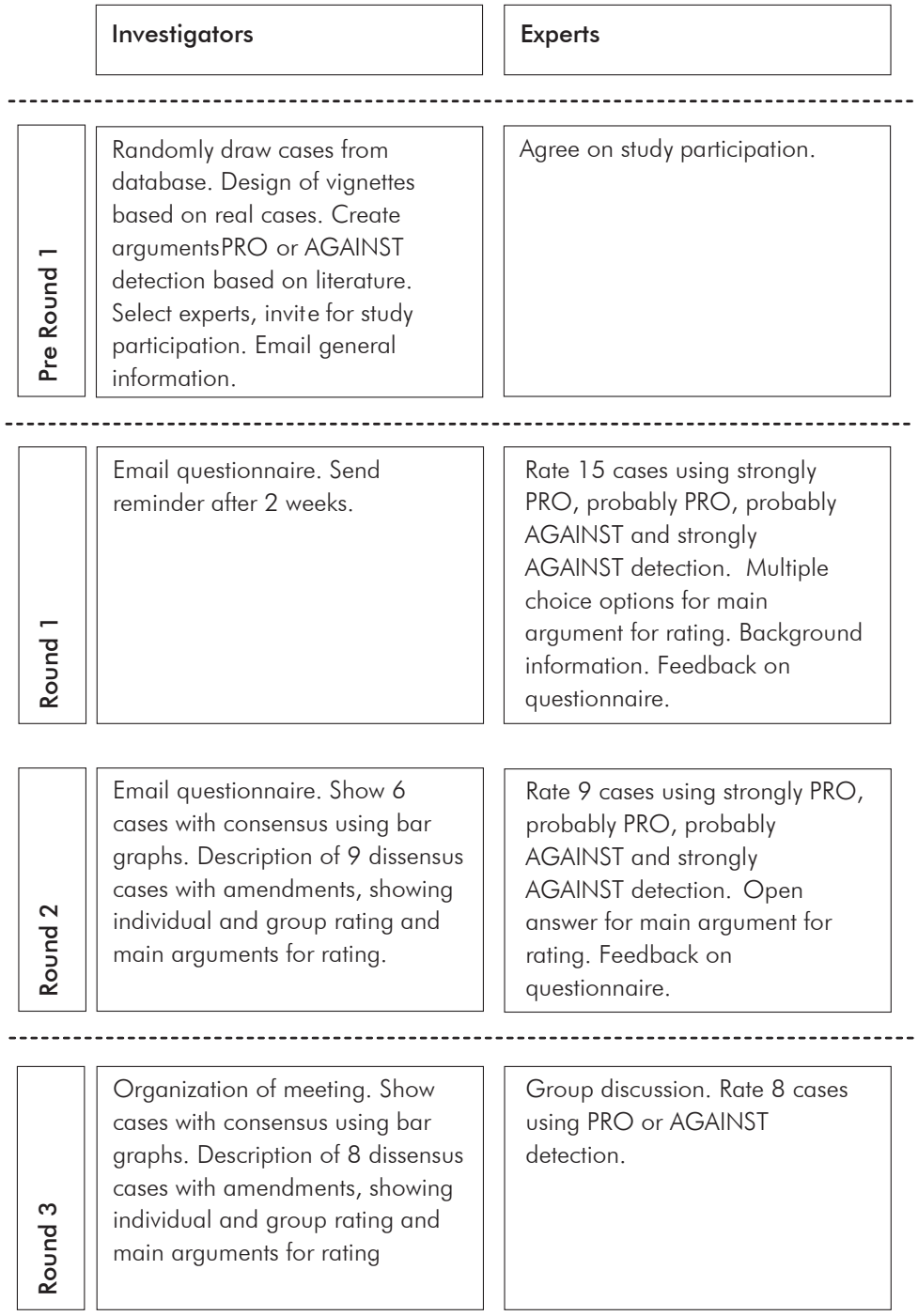
Round 1: Individual session

Here we collected the answers anonymously and the experts were unaware of the results of other participating experts. We gathered participants' socio-demographical data. We presented fifteen 15 vignettes describing the chromosomal abnormalities (table 1, figure 1 and supplemental figure 1). For each vignette, respondents answered whether the chromosomal abnormality should be detected in prenatal diagnosis for the indication advanced maternal age or increased risk following prenatal screening. We used a multiple choice response mode with four options; strongly PRO detection, probably PRO detection, probably AGAINST detection, strongly AGAINST detection. Next we calculated consensus. Participants also had to state the most important reason(s) why they thought the chromosomal abnormality should or should not be detected by using a multiple choice set including an open answer. Participants were allowed to explain their answers in detail or add further remarks, which were used to optimize the vignettes in round 2 with the help of the independent clinical geneticist.

Round 2: Individual session

In 2nd round, participants were informed on the consensus cases of round 1 and on the adjustments made in the remaining dissensus cases. For each dissensus case, feedback was given by displaying the individual expert's judgment compared to the overall group's judgment using bar graphs as well as the most frequently cited reasons pro and against detection. Next, experts were invited to reconsider their answers from round 1, but it was emphasized that conformation to the group view was unnecessary[11]. Again they were asked to give their opinion: strongly PRO detection, probably PRO detection, probably AGAINST detection, strongly AGAINST detection, of the case presented. The same consensus rule was applied.

Figure 1: Design of the study



Round 3: Plenary session

Round 3 consisted of a plenary two hour meeting of the experts. We first summarized the results of consensus cases in round 1 and 2. Next, for the dissensus cases, we showed the expert's judgment in round 1 and 2 using bar graphs with the most frequently stated reasons. Participants had time to read the vignette and evaluate the feedback from the previous rounds. An open discussion followed to exchange different views, to share knowledge, and to make sure that all experts voted with identical information of the cases. Next, each expert voted openly PRO or AGAINST detection. Figure 1 shows a flowchart of the study.

Analysis

Results were reported as absolute numbers and percentages. Agreement was established after each round according to the above mentioned consensus rule. In case consensus was established in round 1 or 2, the case was excluded from further rounds. We calculated the percentage of agreement and multi-rater kappa (adapted from Fleiss, http://justusrandolph.net/kappa/) between rounds for the complete group and per professional group to investigate a potential role for professional background. Although no absolute definitions are available, we used the following interpretation of ranges of the multi-rater kappa: within the range 0.21 to 0.40 agreement was judged as fair, from 0.41 to 0.60 as moderate, from 0.61 to 0.80 as good and from 0.81 to 1.00 as very good[12].

RESULTS

All 24 invited experts agreed to participate (11 male, 13 female). Participants were on average 47 years old and were employed for on average 13 years in the field of prenatal diagnosis in academic hospitals. Twenty-one of the 24 respondents participated in all three rounds (87.5%); one clinical geneticist, one cytogeneticist and one obstetrician were not available for round 3.

In the 1st round consensus was reached for six of the fifteen cases. Experts voted that these cases should be detected in prenatal diagnosis (table 2). For the dissensus cases, most cited reasons PRO and AGAINST detection are given in table 3. Overall agreement for all participants in round one was moderate (overall agreement 70%, free-marginal kappa 0.40).

In the 2nd round, three amendments were made; 1) we added that mosaic Turner syndrome is associated with an increased risk of congenital heart abnormalities, 2) we added for the case with an inherited marker chromosome that the mother (who had the same chromosomal abnormality) had no clinical consequences 3) we added that learning difficulties (not

Table 2. Example of the representation of a chromosomal abnormality (vignette of case 1)

Case 1	
Age and parity	43 year old G3P0
Obstetrical history	2 miscarriages
Indication Prenatal Diagnosis	Maternal age and increased risk for Down syndrome 1:50
Procedure	Uncomplicated amniocentesis at gestational age 16+1 weeks
MLPA	Male, trisomy 21
Karyotype	47,XY+21
Chromosome abnormality fetus	Down syndrome; 47,XY+21
Consequences of the chromosomal abnormality for the fetus	Down syndrome leads to a mild or severe mental handicap, and specific physical features. There is an increased risk of congenital abnormalities and physical disabilities. Physical and mental development is delayed. There is considerable interpersonal variance.
Consequences of the chromosomal abnormality for a future pregnancy	The additional risk of a child with a chromosomal abnormality in a future pregnancy is 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

otherwise specified) in children with mosaic trisomy 20 were observed. Of the nine cases, consensus was reached only in one case, indicating that normal variants should not be detected in prenatal diagnosis (table 3). Most cited reasons PRO and AGAINST detection did not change between round 1 and 2 (table 4), except for the Triple X case, where the most cited reasons pro detection was the possible severe consequences for offspring. Experts changed their opinion between round 1 and 2 from PRO or AGAINST detection on average 1.5 times per 9 cases (min 0 changes, max 4 changes). Overall agreement between participants in round two was poor (overall agreement 58%, free-marginal kappa 0.15).

The 3rd round took two hours. We presented the eight remaining dissensus cases. Six adjustments were made during this group discussion, mostly on the consequences for the unborn child; 1) for the Klinefelter syndrome case, a 15% risk of psychological difficulties was added, 2) For the Triple X case, normal fertility was accepted 3) for the Robertsonian translocation, a 1% risk for the unborn child of having offspring with trisomy 13 was added 4) for the mosaic Turner syndrome case, participants agreed that the grade of mosaicism was not related to the consequences, 5 and 6) for the inherited and mosaic marker chromosome, participants agreed that a 5% risk for the unborn child on uniparental disomy was present and -if present- this leads to severe consequences. After ample consideration of the vignettes and group discussion, again participants voted by PRO or AGAINST detection. Consensus was reached in 5 out of 8 cases (table 3). Experts changed their opinion between round 2 and 3 from PRO and AGAINST detection on average 2.4 times per 8 cases (min 0 changes, max 4 changes). Overall, agreement was moderate (overall agreement 77%, free-marginal kappa 0.53). Obstetricians voted more frequently against detection compared to the other

Table 3: Rating scores and reached consensus on 15 cases per round

Case		Round 1 N=24			
nr	description	++	+	-	- -
1	Trisomy 21	22	2	0	0
2	Trisomy 13	19	4	1	0
3	Trisomy 18	20	3	1	0
4	Klinefelter syndrome	7	7	9	1
5	Triple X	4	3	13	4
6	Normal variant	2	3	8	11
7	Robertsonian translocation	12	6	5	1
8	Mosaic Turner syndrome	7	8	8	1
9	Inherited marker chromosome 15	1	5	15	3
10	De novo marker chromosome 9	18	4	2	0
11	De novo deletion chromosome 7	20	2	2	0
12	Mosaic unbalanced rearrangement of chromosome 18	18	4	2	0
13	Mosaic trisomy 20	4	5	14	1
14	Mosaic marker chromosome 7	9	9	5	1
15	De novo balanced translocation of chromosome 11 and 13	9	9	5	1

Round 1 and 2: ++ = Strongly pro detection; + = Probably pro detection; - = Probably against detection; -- = Strongly against detection
Round 3: + = Pro detection; - = Against detection

Table 4: Stated reasons for dissensus cases PRO or AGAINST detection in round 1.

Most cited reason PRO detection	Cases
Provide informed decision-making to parents, so they can decide whether to continue or terminate pregnancy	Klinefelter syndrome Triple X syndrome Inherited marker chromosome 15 Mosaic trisomy 20 Mosaic marker chromosome 7 De novo balanced translocation of chromosome 11 and 13
The chromosomal abnormality can lead to severe consequences for offspring	Normal variant Robertsonian translocation (inherited)
Early support may lead to improved clinical outcome	Mosaic Turner syndrome
Most cited reason AGAINST detection	**Cases**
The chromosomal abnormality does not lead to severe morbidity	Klinefelter syndrome Mosaic Turner syndrome Mosaic trisomy 20 Mosaic marker De novo balanced translocation of chromosome 11 and 13
The chromosomal abnormality does not lead to an abnormal phenotype	Triple X syndrome Normal variant Robertsonian translocation (inherited) Inherited marker chromosome 15

	Round 2 N=24			Round 3 N=21		Consensus
++	+	-	--	+	-	
						Yes, PRO
						Yes, PRO
						Yes, PRO
6	9	8	1	15	6	No
2	3	15	4	2	19	Yes, AGAINST
2	2	7	13			Yes, AGAINST
15	3	4	2	14	7	No
12	7	3	2	17	4	Yes, PRO
6	3	13	2	21	0	Yes, PRO
						Yes, PRO
						Yes, PRO
						Yes, PRO
4	7	11	2	1	20	Yes, AGAINST
7	7	8	2	21	0	Yes, PRO
11	7	5	1	16	5	No

experts. Table 5 shows agreement between the different professional groups per round. Agreement varied from poor to moderate between rounds. In the 2nd round agreement was lowest; in particular with low agreement among clinical geneticists.

DISCUSSION

This study systematically elicited the views of prenatal experts on which chromosomal abnormalities should be detected bearing in mind the emerging possibilities of targeted and broad prenatal tests. Experts agreed on 12 of 15 chromosomal abnormalities; in 10 cases they agreed that they should be detected, and in 2 cases that they should not be detected. At the end of the 3rd round, a majority favored detection in the remaining three cases, but a significant minority disagreed. Overall, experts agreed on the detection of chromosomal abnormalities with severe consequences and on not detecting chromosomal abnormalities with no or minor consequences.

Immediate consensus was reached in round one for the chromosomal abnormalities with severe, untreatable consequences for the unborn child. Apparently, there is greater agreement at the extremes; the professional experts preferred to detect all chromosomal abnormalities with severe consequences, and they also agreed not to detect chromosomal abnormalities without clinical consequences. However, for the chromosomal abnormalities

Table 5: Differences in multi-rater kappa and agreement per professional group between the three rounds

Experts	Round 1		Round 2		Round 3	
	kappa	% of overall agreement	kappa	% of overall agreement	kappa	% of overall agreement
Obstetricians	0.43	71%	0.25	62%	0.55	77%
Clinical cytogeneticists	0.39	69%	0.19	60%	0.52	76%
Clinical geneticists	0.41	71%	-0.04	48%	0.57	79%

with uncertain or mild consequences, consensus was not reached. Even in round three in which all respondents had identical information, opinions still differed markedly. Most likely, these differences are based on disagreement in principle. Some experts value being informed as the most important asset, while for others clinical consequences are decisive.

Despite the high consensus rate, change of opinion between rounds was common, with poor to moderate agreement across rounds. The judgments also differed by expert group; obstetricians rated more frequently AGAINST detection and changed their opinion more often than the other expert groups. The caregiver's role in the counseling process might be an explanation for this difference, or the amount of education and experience with the conditions. While obstetricians inform patients on possibilities in prenatal diagnosis before a definite diagnosis is made, geneticists are mainly involved when a chromosomal abnormality has been detected.

We made several observations during the group meeting. Firstly, we noticed that experts judged the cases from a doctor-patient perspective rather than from a public health perspective. Apparently by using vignettes, experts judge the case as their own patient, which is reassuring from a validity point of view. Furthermore, in the 3rd round two experts were responsible for most of the adjustments. The group did not judge these two experts to dominate or otherwise unduly influence opinion; rather the reverse, there comments were appreciated and their additions regarded as correct and justified. We cannot explain why these experts did not provide this feedback in the first two anonymous rounds. This would probably have led to earlier consensus. However, the adaptation of vignette information at this stage should not be regarded a bias, it increased the validity of the judgments and consensus verdicts. Finally, in the 3rd round we reached consensus in more cases than we expected based on results in previous rounds. While we in retrospect believe this convergence was for the greater part explained by the information exchange and group deliberation, we cannot exclude that group pressure may have played a role.

This prospective, multidisciplinary study with high participation rate gives insight into which chromosomal abnormalities should and should not be detected according to experts and

provides important information on the experts' motives. The execution of a group meeting improved knowledge transfer and it allowed the exchange of opinions and an open discussion. During the group meeting it was remarkable that experts spent much time on deliberations on knowledge of facts, but little effort was spent to explain their principles and to persuade others to vote pro or against detection.

While our multidisciplinary experts were all clinicians practicing prenatal diagnosis in academic hospitals, clearly more stakeholders are involved outside of this study design: obstetricians working in non-university training and non-training centers, general practitioners and midwives. The expected level of expertise and experience will be less, but the effect on opinion and consensus is difficult to predict[13]. Evidently client's opinion should be incorporated[14], although the vignettes used in this study are not suitable for this matter. Future research should focus on eliciting the views of these stakeholders.

In view of the consensus verdicts, one may consider the most appropriate test. On the one hand, the expert group recommends detecting all chromosomal abnormalities with severe consequences for the child. RAD as it is currently designed will not meet the case since it detects only the most common chromosomal abnormalities with severe consequences (trisomies 13, 18, 21), but karyotyping does to a large degree. On the other hand, experts want to avoid the detection of chromosomal abnormalities without clinical consequences. While RAD is capable of that to a large degree, karyotyping certainly is not. Hence, a test that meets all criteria is currently unavailable. From here we envisage three directions; 1) Effort should be put into the development of a test that is more in agreement with the expert's recommendations. However, it is likely that each test has its benefits and disadvantages and it is likely that each test has its limitations. 2) A uniform test is offered, meeting most needs of the experts, in this case karyotyping. 3) An individual choice is offered allowing a tailor-made strategy according to a patient's risk and preferences. Or, within the same scope, agreements can be made on which test results are reported to clients and which not. For instance, in the currently performed microarray study by Wapner et al abnormalities smaller than 1 Mb are not reported to patients[15]. However, future research should assess whether it is feasible in clinical practice to implement this strategy and if parents and caregivers are able to make a shared informed decision on the abnormalities that should be detected.

We hypothesize that if the most experienced stakeholders (i.e. experts) disagree on what should be detected in prenatal diagnosis, the implementation of a uniform nationwide policy is outdated. Tailor-made strategies, incorporating patient's risk and demands, can overcome this problem. Patients, in consultation with their doctor, decide which test meets their risk and demands most. Future studies should focus on the provision of clear prenatal counseling in

order to allow informed decision-making and on the availability of an up-to-date genetic database on rare chromosomal abnormalities.

Concluding, experts agree that chromosomal abnormalities with severe consequences should be detected and not to disclose abnormalities without clinical consequences. Experts disagree on abnormalities with uncertain clinical consequences. These differences are based on disagreement in principle; some experts value 'being informed' as an extremely valuable asset, while for others the clinical consequences are decisive. None of the currently available tests corresponds to the experts' demands. While karyotyping is able to detect chromosomal abnormalities without clinical consequences, RAD is unable to disclose all chromosomal abnormalities with severe consequences.

Supplemental table 1: Total of chromosomal abnormalities detected with karyotyping and not detected with MLPA out of 4585 amniocentesis; arranged in order of clinical consequences

No clinical consequences for the current pregnancy (17)
45,XY,der(13;14)(q10;q10)[10]mat
45,XY,der(13;14)(q10;q10)mat
45,XY,der(13;22)(q10;q10)pat
46,X,inv(Y)(p11.2q11.221)pat
46,XX,inv(11)(q21q23)pat
46,XX,inv(17)(p?11.2p?13.3)pat
46,XX,inv(5)(p14p15.1)pat
46,XX,t(11;22)(q23;q11.2)pat
46,XX,t(4;21)(q26;q21)pat
46,XX,t(5;16)(q35;p12)pat
46,XY,inv(9)(p24q22.1)pat
46,XY,t(13;14)(p21.1;q27)pat
46,XY,t(9;13)(q31;q12)pat
46,X,inv(Y)(p11.1q11.2)pat
47,XY,+mar.ish psu idic(15)(q11.2)(289D12+,SNRPN-,446P9-)mat
mos 47,XX,+mar[7].ish rob(?;?)(p10;p10)(wcp14+,wcp15+)15q11.2(SNRPN-,D15S10-)[7]/46,XX[10]dn
mos 47,XY,+20[2]/46,XY[16]
Uncertain clinical consequences for the current pregnancy (6)
46,XX,t(4;11)(q31?1;p1?3)dn
46,XX,t(11;13)(q21;q14)dn
46,XX,t(11;22)(q23;q11.2)dn
46,XY,t(6;9)(p22;13)dn
mos 45,X[6]/46,XX[11] confirmed postpartum 45,X[3]/46,XX[32]
Mos 47,XX,der (17)(p11.1q11.1)[10]/46,XX[12]
Severe clinical consequences for the current pregnancy (3)
46 XY,del(7)(p?15p2?2)
46,XX,del(18)(p11.21)[15]/46,XX,dup(18)(p11.21p11.32)[13]
47,XX,+mar.ish del(9)(q1?3)(wcp9+)9p24.3(GS-43-N6+)dn

Supplemental figure 1: Description of the 15 cases

Case 1

Age and parity	43 year old G3P0
Obstetrical history	2 miscarriages
Indication Prenatal Diagnosis	Maternal age and increased risk for Down syndrome 1:50
Procedure	Uncomplicated amniocentesis at gestational age 16+1 weeks
MLPA	Male, trisomy 21
Karyotype	47,XY,+21
Chromosome abnormality fetus	Down syndrome; 47,XY,+21
Consequences of the chromosomal abnormality for the fetus	Down syndrome leads to a mild or severe mental handicap, and specific physical features. There is an increased risk of congenital abnormalities and physical disabilities. Physical and mental development is delayed. There is considerable interpersonal variance.
Consequences of the chromosomal abnormality for a future pregnancy	The additional risk of a child with a chromosomal abnormality in a future pregnancy is 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 2

Age and parity	39 year old G1P0
Obstetrical history	-
Indication Prenatal Diagnosis	Maternal age and increased risk for Down syndrome 1:82
Procedure	Uncomplicated amniocentesis at gestational age 16+2 weeks
MLPA	Male, trisomy 13
Karyotype	47,XY,+13
Chromosome abnormality fetus	Patau syndrome; 47,XY,+13
Consequences of the chromosomal abnormality for the fetus	Patau syndrome leads to severe intellectual disabilities, and specific physical features. Due to the presence of several life-threatening medical problems, intrauterine death is common. 75% of the infants with trisomy 13 die within their first 2 months of life, and 95% of the infants die within 1 year.
Consequences of the chromosomal abnormality for a future pregnancy	The additional risk of a child with a chromosomal abnormality in a future pregnancy is 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 3

Age and parity	38 year old G4P1
Obstetrical history	Normal delivery, 2 miscarriages
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 15+5 weeks
MLPA	Male, trisomy 18
Karyotype	47,XY,+18
Chromosome abnormality fetus	Edwards syndrome; 47,XY,+18
Consequences of the chromosomal abnormality for the fetus	Edwards syndrome leads to severe intellectual disabilities, and specific physical features. Due to the presence of several life-threatening medical problems, intrauterine death is common. 95% of infants with trisomy 18 die within their first 6 months.
Consequences of the chromosomal abnormality for a future pregnancy	The additional risk of a child with a chromosomal abnormality in a future pregnancy is 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 4

Age and parity	37 year old G3P1
Obstetrical history	1 miscarriage, 1 normal delivery
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 16+6 weeks
MLPA	Male, two X chromosomes, one Y chromosome
Karyotype	47,XXY
Chromosome abnormality fetus	Klinefelter syndrome; 47,XXY
Consequences of the chromosomal abnormality for the fetus	In general, men with Klinefelter syndrome have few noticeable symptoms and normal IQ scores, however verbal IQ scores lower than performance IQ. In some men physical features are present; small testes, a tall posture and in 30% reversible gynecomastia. The most common symptom is infertility.
Consequences of the chromosomal abnormality for a future pregnancy	The additional risk of a child with a chromosomal abnormality in a future pregnancy is less than 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 5

Age and parity	36 year old G1P0
Obstetrical history	-
Indication Prenatal Diagnosis	Maternal age and increased risk for Down syndrome 1:120
Procedure	Uncomplicated amniocentesis at gestational age 16+6 weeks
MLPA	Female, three X chromosomes
Karyotype	47,XXX
Chromosome abnormality fetus	Triple X syndrome; 47,XXX
Consequences of the chromosomal abnormality for the fetus	Women with triple X syndrome have normal intelligence, without physical complaints and no specific physical features although they are often tall. Developmental delay in motor and verbal skills is possible. IQ scores are within the normal range, but are 10-20 points less compared to siblings.
Consequences of the chromosomal abnormality for a future pregnancy	The additional risk of a child with a chromosomal abnormality in a future pregnancy is less than 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 6

Age and parity	36 year old G1P0
Obstetrical history	-
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 16+4 weeks
MLPA	Normal female
Karyotype	46,XX,der(22)t(Y;22)(q12;p12)[10]pat
Chromosome abnormality fetus	Normal variant; 46,XX,der(22)t(Y;22)(q12;p12)pat
Consequences of the chromosomal abnormality for the fetus	None
Consequences of the chromosomal abnormality for a future pregnancy	None
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 7

Age and parity	35 year old G1P0
Obstetrical history	-
Indication Prenatal Diagnosis	Increased risk for Down syndrome 1:110
Procedure	Uncomplicated amniocentesis at gestational age 16+1 weeks
MLPA	Normal male
Karyotype	45,XY,der(13;22)(q10;q10)
Chromosome abnormality fetus	Balanced inherited Robertsonian translocation; 45,XY,der(13;22)(q10;q10)pat
Consequences of the chromosomal abnormality for the fetus	The probability on congenital abnormalities is considerably low. Offspring of the infant can inherit the abnormality in an unbalanced manner, which usually leads to miscarriage, but it can also lead to a severely handicapped child.
Consequences of the chromosomal abnormality for a future pregnancy	The probability of a child with trisomy 13 in a future pregnancy is 1%. There is an increased risk on miscarriages. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	Parents and siblings of the father may also have the chromosomal abnormality and are offered karyotyping.

Case 8

Age and parity	40 year old G4P1
Obstetrical history	1 normal delivery, 2 miscarriages
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 16+4 weeks
MLPA	Normal female
Karyotype	mos 45,X[6]/46,XX[11]
Chromosome abnormality fetus	Mosaicism of a normal female karyotype in 11 cell clones and monosomy X in 6 cell clones, mosaic Turner syndrome; mos 45,X[6]/46,XX[11]
Consequences of the chromosomal abnormality for the fetus	84 % of women with this chromosomal abnormality have no specific physical features (Hsu). In some cases fertility problems exist and specific physical features (16%); e.g. short stature, no or little secondary sex characteristics . Of these women 10-34% will get pregnant (Birkebaek 2002). In several case reports a slightly increased risk on chromosomal abnormalities in their offspring is reported.
Consequences of the chromosomal abnormality for a future pregnancy	The additional of a child with a chromosomal abnormality in a future pregnancy is less than 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 9

Age and parity	34 year old G2P1
Obstetrical history	Normal delivery
Indication Prenatal Diagnosis	increased risk for Down syndrome 1:150
Procedure	Uncomplicated amniocentesis at gestational age 16+0 weeks
MLPA	Normal male
Karyotype	47,XY+ mar(15)(q11.2)
Chromosome abnormality fetus	Inherited marker chromosome 15; 47,XY+mar.ish psu idic(15)(q11.2)(289D12+,SNRPN-,446P9-)mat
Consequences of the chromosomal abnormality for the fetus	There is a low risk on congenital abnormalities in this pregnancy. For offspring of the infant, there is an increased risk on this chromosomal abnormality.
Consequences of the chromosomal abnormality for a future pregnancy	There is an increased probability on another infant with an inherited marker chromosome. However, the risk figure is unknown. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	Parents and siblings of mother may also have the chromosomal abnormality and are offered karyotyping.

Case 10

Age and parity	43 year old G3P2
Obstetrical history	2 normal deliveries
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 16+2 weeks
MLPA	Normal female
Karyotype	47,XX,+mar (9)(p)
Chromosome abnormality fetus	De novo marker of chromosome 9; 47,XX,+mar (9)(p) dn
Consequences of the chromosomal abnormality for the fetus	The infant probably has mental disabilities with or without congenital abnormalities. Most common abnormalities are; heart defects; and abnormalities of kidneys, brain, skull, eyes, ears, and extremities. Due to the presence of several life-threatening medical problems, most infants die in utero. Only one case report showed one infant to be alive at 4 months that was severely handicapped.
Consequences of the chromosomal abnormality for a future pregnancy	Likely, there is no recurrence risk. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 11

Age and parity	38 year old G2P0
Obstetrical history	1 miscarriage
Indication Prenatal Diagnosis	Maternal age and increased NT
Procedure	Uncomplicated amniocentesis at gestational age 16+0 weeks
MLPA	Normal male
Karyotype	46,XY,del(7)(p?15p2?2)
Chromosome abnormality fetus	De novo interstitial deletion of chromosome 7; 46,XY,del(7)(p?15p2?2)dn
Consequences of the chromosomal abnormality for the fetus	Likely, the infant has mental disabilities with or without congenital abnormalities. More than 60% of these cases have specific physical disabilities.
Consequences of the chromosomal abnormality for a future pregnancy	There is a recurrence risk of 0.3%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 12

Age and parity	31 year old G2P1
Obstetrical history	1 normal delivery
Indication Prenatal Diagnosis	increased risk for Down syndrome 1:61
Procedure	Uncomplicated amniocentesis at gestational age 15+6 weeks
MLPA	Normal female
Karyotype	mos 46,XX,del(18)(p11.21)[15]/ 46,XX,dup(18)(p11.21p11.32)[13]
Chromosome abnormality fetus	Mosaicism of an unbalanced structural abnormality of chromosome 18. In 15 cell clones a deletion is present and in 13 a duplication is present. mos 46,XX,del(18)(p11.21)[15]/ 46,XX,dup(18)(p11.21p11.32)[13]
Consequences of the chromosomal abnormality for the fetus	Likely, the infant has mental disabilities with or without congenital abnormalities. The disabilities may vary per individual and are not predictable.
Consequences of the chromosomal abnormality for a future pregnancy	There is a recurrence risk of less than 1%. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 13

Age and parity	36 year old G4P2
Obstetrical history	2 normal deliveries, 1 miscarriage
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 16+3 weeks
MLPA	Normal male
Karyotype	mos 47,XY,+20[2]/46,XY[16]
Chromosome abnormality fetus	Mosaicism of a normal male karyotype in 16 cell clones and an additional chromosome 20 in 2 cell clones; mos 47,XY,+20[2]/46,XY[16]
Consequences of the chromosomal abnormality for the fetus	Of individuals with this chromosomal abnormality 90%-93% have normal intelligence and no physical disabilities or specific features. However, scoliosis, hypotonia and 'dropping' shoulders cannot be excluded. Learning problems despite normal IQ have been reported.
Consequences of the chromosomal abnormality for a future pregnancy	There is no recurrence risk. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 14

Age and parity	36 year old G2P1
Obstetrical history	2 miscarriages
Indication Prenatal Diagnosis	Maternal age
Procedure	Uncomplicated amniocentesis at gestational age 16+3 weeks
MLPA	Normal female
Karyotype	mos 47,XX+mar[7]/46,XX[10]
Chromosome abnormality fetus	Mosaicism of a normal female karyotype in 10 cell clones and an additional marker chromosome without coding DNA in 7 cell clones; mos47,XX+mar[7]/46,XX[10].ish(acro)(p10)(wcp14+,wcp15+)15q11.2(PWS/AS-)dn
Consequences of the chromosomal abnormality for the fetus	There is a 5% risk on mental retardation or congenital abnormalities.
Consequences of the chromosomal abnormality for a future pregnancy	Likely, there is no recurrence risk. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

Case 15

Age and parity	36 year old G3P2
Obstetrical history	2 normal deliveries
Indication Prenatal Diagnosis	Maternal age and increased risk for Down syndrome 1:116
Procedure	Uncomplicated amniocentesis at gestational age 15+1 weeks
MLPA	Normal female
Karyotype	46,XX,t(11;13)(q21;q14)
Chromosome abnormality fetus	De novo balanced structural abnormality; 46,XX,t(11;13)(q21;q14)dn
Consequences of the chromosomal abnormality for the fetus	There is a 10% probability of mental retardation or congenital abnormalities. Normal findings at the advanced ultrasound reduce the probability to 5%.
Consequences of the chromosomal abnormality for a future pregnancy	Likely, there is no recurrence risk. Prenatal diagnosis is offered in a next pregnancy.
Consequences of the chromosomal abnormality for relatives of the parents	None

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PART III

GENERAL DISCUSSION, CONCLUSION AND SUMMARY

Chapter 9

General discussion

Prenatal diagnosis is routinely offered to all pregnant women in developed countries who have an increased risk of carrying a child with a chromosomal abnormality. Karyotyping is considered the standard method in prenatal cytogenetic diagnosis. The test is capable of detecting a range of numerical and structural chromosomal abnormalities with high accuracy (99.4-99.9%)[1-3] within two to three weeks (see introduction).

Since 1990 new competitive techniques have become available due to rapid technological developments in molecular genetics. These techniques, i.e. FISH, QF-PCR and MLPA commonly referred to as rapid aneuploidy detection (RAD), are able to detect the most common aneuploidies within a few days through highly automated protocols at lower costs. The studies evaluating diagnostic accuracy of RAD techniques were seldom performed under clinical practice conditions. Information on health-related quality of life and cost-effectiveness were absent. Moreover, little evidence existed on patient and physicians preferences regarding the choice between karyotyping and RAD. Little was known about the patient's and professional's opinions on which chromosomal abnormalities should be detected or never missed.

Despite considerable gaps in knowledge, in our study we chose MLPA as our RAD technique of choice (2005), since a preclinical study showed MLPA to be robust in detecting aneuploidies of chromosomes 13, 18, 21 and non-mosaic sex chromosome abnormalities using amniotic fluid[4]. For MLPA a commercially available kit (SALSA P095) was available and validated on amniotic fluid in the eight genetic centres in the Netherlands.

The aim of the study and this thesis was to resolve most of these knowledge gaps for MLPA as RAD test. For that purpose, several empirical studies were conducted between 2006 and 2009. The final objective was, by combining new and existing information, to arrive at an assumed best practice proposal, which might include standard combined testing of MLPA and karyotyping, substitution of karyotyping by MLPA, or the addition of MLPA to the current test process (serial testing).

The MLPA And Karyotyping, an Evaluation (M.A.K.E.) study

We performed the nationwide M.A.K.E. study between March 2007 and October 2008. All eight Dutch prenatal diagnostic centres and their affiliated hospitals participated. In the M.A.K.E. study we tested 4585 consecutive amniotic fluid samples with both MLPA and karyotyping, allowing a pair wise comparison of test results. Diagnostic accuracy of MLPA was similar (non-inferior) to that of karyotyping for the detection of trisomies 13, 18, 21 and sex chromosomal aneuploidies. In 75 cases MLPA failed (1.6%); karyotyping failed once (0.02%). Compared with karyotyping, MLPA shortened the waiting time with 14.5 days ($P<0.001$, 95% confidence interval: 14.3-14.6).

For our first quality of life study, we compared anxiety, personal perceived control, stress and health related quality of life of 132 women receiving a karyotype result and 181 women receiving MLPA and karyotyping. The combined strategy reduced anxiety and stress two weeks earlier compared to karyotyping only, but it did not influence overall anxiety, stress, personal perceived control, and generic mental and physical health, when compared to a karyotype only strategy. For our second study, we compared anxiety, personal perceived control, stress and health related quality of life of 68 women receiving standalone karyotyping and 61 women receiving standalone MLPA after they were offered individual choice between the two tests. Furthermore, we explored the reasons and perceptions to choose between standalone MLPA versus fetal karyotyping. Overall, women had a clear individual preference for targeted or broad testing. Despite individual differences in choices and motives, our study showed no systematic differences in health-related quality of life and anxiety over time.

The cost-minimization analysis showed that a standalone MLPA strategy reduced costs compared to karyotyping with € 240.13 or 14.9% per sample. This cost reduction is mainly related to the lower cost of the MLPA test; part of the MLPA cost reduction is offset by costs induced by missed cases. Costs were sensitive to the likelihood of termination of pregnancy, sample throughput, whether or not individual choice is offered, and centralised care.

In our preference study, comparing standalone MLPA and karyotyping, we found that women's preferences were highly divergent: 50% opted for karyotyping and 43% for MLPA. Our discrete choice experimentation showed that the potential clinical consequences of the detected chromosomal abnormality and failure rate had large impact on the preferred test, while the effect of anxiety and waiting time on women's preferences was modest. We also found a striking heterogeneity in caregiver's preferences. A preference study among 77 caregivers in prenatal diagnosis (i.e. obstetricians, midwives, clinical geneticists, clinical cytogeneticists and general practitioners), showed that most caregivers preferred MLPA over karyotyping.

Our expert panel - which judged in detail 15 test cases - consisted of 8 clinical cytogeneticists, 8 clinical geneticists and 8 obstetricians. They were invited to rate pro or con the detection of 15 chromosomal abnormalities using a nominal group technique; these experts largely agreed on the detection with severe consequences and against the detection of chromosomal abnormalities with irrelevant clinical consequences. For chromosomal abnormalities with mild or uncertain outcomes, however, dissensus remained.

MLPA in international perspective

While we conducted our study, several larger retrospective and prospective monocentre cohort studies on MLPA emerged and provided further evidence on the quick, high quality and low cost results of RAD. These studies showed a sensitivity of MLPA of 96%-100%, a specificity of 99.8%-100% and failure rate of 1.7%-4.5% using amniotic fluid cells and chorionic villi[5-10]. The aggregate results of these monocentre studies and our multicentre study showed an overall sensitivity of 99.8% and overall specificity of 100% for the detection of trisomies 13, 18, 21, X and Y.

MLPA compared to other RAD techniques

Sound methodological studies that directly compare the various RAD techniques simultaneously have not been performed, and may be difficult to carry out for practical (e.g. available amniotic fluid) and financial constraints. In contrast, many studies have been performed on diagnostic accuracy of QF-PCR and FISH specifically. These studies show a sensitivity of 62.5%-100% and specificity of 99.9-100% for QF-PCR[11-19] and a sensitivity of 80-100% and specificity of 95%-100% for FISH[20-29], with the karyotype result as reference. If one excludes all studies before the commercial kits became available, sensitivity of QF-PCR improves to 98.8%-100%. The sensitivity of FISH is 99.3% if we only include studies from 2001.

Although no direct comparison of RAD techniques is available, these data suggest that diagnostic accuracy of the three RAD techniques is similar. We cannot conclude which RAD technique is best, since required comparative studies on quality of life and costs are still absent. Compared with FISH, MLPA and QF-PCR seem both more suitable for high-throughput testing at lower costs[30] (Chapter 5). FISH, however, is preferred if chromosomal mosaicism is suspected, as detection levels of 5% or more can be achieved[31]; in context of this thesis this feature is not relevant as no ready signs exists for mosaicism. Compared with QF-PCR, MLPA is relatively sensitive to DNA quality and is unable to detect maternal cell contamination in female samples or female triploidies. MLPA can detect 45 genomic targets in one reaction and avoids the problem of non-informativeness of the polymorphic markers that may occur with QF-PCR[4,32].

All RAD techniques are able to detect sex chromosome aneuploidies using appropriate probes or primer sets. Despite increasing knowledge on the consequences of sex chromosomal abnormalities in the last decades, the knowledge of the consequences of various chromosomal abnormalities are still limited. Currently, each laboratory decides whether or not X and Y probes are included. For specific features, e.g. cystic hygroma or hydrops, cardiac abnormalities or NT>4mm which are suggestive of Turner syndrome, X probes are added on request. While it is feasible to develop other compositions of probes,

we are not aware of studies on this topic. Furthermore, non-disclosure following karyotyping is also possibility to adjust the detection capacity.

Apart from test performance, it is important to be informed on the patient's and physician's preferences regarding the detection capacity of a test. Essentially, this is a normative question, where some regard the judgement to be confined to the political process of decision-making. We tried to reveal the preference of relevant stakeholders.

Preferences of patients and physicians

In our study, pregnant women least preferred a Down only test compared to a RAD test detecting the most common chromosomal abnormalities and karyotyping (Chapter 6). This suggests that the additional diagnostic information on the presence of sex chromosome abnormalities is regarded important. In our discrete choice experimentation, however, women had no overt preference for a RAD test with or without X and Y probes (Chapter 7). Results from our expert study suggest that experts did not agree whether Klinefelter syndrome (47,XXY) should or should not be detected in prenatal diagnosis, but they agreed that Turner syndrome (45,X) should be detected. In summary, there is no clear answer whether the probes for sex chromosomes should or should not be included in the test.

Two studies of alternating quality reported on patient preferences for RAD and karyotyping. Both showed a preference for RAD over karyotyping[30,33]. The design of both studies was rather simplified and did not give insight into the motivations why women chose for RAD or karyotyping. Our discrete choice experimentation showed that women value the potential clinical consequences of detected chromosomal abnormality highest. This is supported by previous research which showed that patients in clinical practice prefer clear consequences with major impact for their child over uncertain or mild consequences[34]. These studies suggest that clear results are favoured over complete results. The fact that pregnant women in our study based their preference mainly on the consequence of the test result is understandable. In our expert study, we noticed that the prenatal experts also wanted as much information as possible on the clinical outcomes before voting pro or con detection. One study assessed caregivers' preference for RAD and karyotyping, with 57% of the obstetricians and 71% of the midwives choosing RAD[30]. These results are comparable to our preference study which showed that 78% of the caregivers (mostly midwives and gynaecologists) opted for RAD (Chapter 6).

Alternative diagnostic strategies

In theory, five prenatal diagnostic strategies can be distinguished; standalone karyotyping (strategy 1), standalone RAD (strategy 2), RAD with subsequent karyotyping in case of a negative RAD result (strategy 3), RAD with subsequent karyotyping in case of a positive RAD result (strategy 4) and combination of RAD and karyotyping (strategy 5). First, we will discuss

the five strategies and their implications and eventually arrive at the optimal test protocol and discuss individual choice for patients.

Strategy 1: Standalone karyotyping

This strategy represents the current practice in prenatal diagnosis. As stated before, karyotyping detects a range of numerical and structural chromosomal abnormalities with high accuracy. There were no differences in quality of life compared to strategy 4, RAD followed by karyotyping in case of a positive RAD test result. The associated costs of karyotyping are substantially higher compared to other test strategies, but they are accepted as part of current practice.

Strategy 2: Standalone RAD

The accuracy, low cost and quick results of RAD make RAD a suitable routine test for prenatal diagnosis. However, if standalone RAD replaces karyotyping, some severe chromosomal abnormalities will remain undetected since RAD cannot detect by design other chromosomal abnormalities than trisomies 13, 18, 21, X and Y. Published audits of chromosome abnormalities found by karyotyping at prenatal diagnosis have consistently shown a prevalence of between 0.07% and 0.1% for clinically significant abnormalities that would not be detected by RAD (consisting of the above mentioned probes) in samples from pregnancies without fetal ultrasound abnormalities[35-38]. In a more cautiously-interpreted audit, approximately 1 in 100 samples referred with a Down syndrome risk to be subjected to QF-PCR only would have an undetected autosomal chromosome abnormality. Thirty-three percent of these would have a substantial risk of serious phenotypic consequences, equivalent to a prevalence of 0.33%[39]. A disadvantage of standalone RAD is that genetic inheritance of the common aneuploidies, e.g. trisomy 21 and 18 will not be sorted out. The diagnostic accuracy of standalone RAD for the common aneuploidies is comparable to karyotyping. The costs of a standalone RAD strategy must be lower than the strategy RAD with subsequent karyotyping in case of a positive RAD result (strategy 4), but the difference in average costs per sample is minimal. Evidence on quality of life in a RAD only strategy is lacking. We feel that the limited cost reduction of strategy 2 compared to strategy 4 does not outweigh the additional knowledge of the subsequent karyotype in case of a positive RAD result, which can be of great importance for genetic counselling.

Strategy 3: RAD with subsequent karyotyping in case of a negative RAD result

Although this strategy exists in theory, it is of little value in clinical practice. The advantage of this strategy is that the chromosomal abnormalities missed by RAD are detected with karyotyping. Therefore, the diagnostic accuracy of this strategy must be equal to standalone

karyotyping (strategy 1). The disadvantages of this strategy, however, are considerable. Firstly, the additional knowledge of the karyotype in case of a positive RAD test results is still lacking. Secondly, the prior likelihood of a chromosomal abnormality is low; therefore this strategy almost amounts to strategy 5. The costs are much higher than strategy 1, without any additional gain.

Strategy 4: RAD with subsequent karyotyping in case of a positive RAD result

In this strategy, RAD is, like in strategy 3, used as a diagnostic technique. However, here subsequent karyotyping will be carried out if RAD detects a chromosomal abnormality to detect genetic inheritance. This strategy is similar to strategy 2. Rare chromosomal abnormalities with clinical significance remain undetected with an estimated prevalence of 0.07-0.33% at time of testing[35-39]. In contrast to strategy 2, the advantage is that karyotype reveals the genetic inheritance pattern.

No studies measured differences in patients' quality of life after being allocated to either RAD or karyotyping alone. One study mimicked a standalone policy by treating the RAD result as final diagnosis[40]. However, as karyotyping was still performed and the result was only disclosed in case of an additional chromosomal abnormality, the crucial difference of substitution was not reflected in this design.

When strategy 4 is applied, costs will significantly decrease compared to strategy 1[30] (Chapter 5) because the costs of the RAD test are lower than the costs of karyotyping and karyotyping is avoided for the majority of pregnant women with a RAD negative test result. A fair comparison requires that the long term effects of undetected chromosomal abnormalities should be incorporated in the RAD strategy, reflecting the comparable but still imperfect diagnostic accuracy. While this effect may be small due to the low probabilities, many studies regard the inclusion of the costs associated with a chromosomal abnormality in this type of analysis as controversial[41]. In our study, we nevertheless achieved a crude estimate of these long term costs based on extrapolations from scarce literature[42], and concluded that overall RAD was still significantly less costly compared to karyotyping (Chapter 5).

Strategy 5: Combining RAD and karyotyping

In this strategy, all women receive RAD first and, regardless of the RAD test result, subsequent karyotyping is initiated. Since both tests are always performed, the overall detection capacity of this strategy is equal to that of standalone karyotyping. The main advantage is that a quick first test result for the common abnormalities covered by RAD becomes available which may reduce parent's anxiety. Several studies have assessed the course of anxiety when the combined strategy RAD and karyotyping is compared to a karyotyping only strategy [25, 30, 40, 43] (Chapter 3). These studies showed that maternal anxiety was sharply reduced during

after receiving the RAD result, but anxiety slightly increased after receiving karyotyping results in the combined group[30, 40, 43]. Compared to the karyotyping only strategy, quality of life was therefore not influenced by the combined strategy with quick first results. Furthermore, the preference study shows that the valuation assigned to shortened waiting time is modest compared to other test characteristics (Chapter 7). The main disadvantage of this strategy is that the costs are increased considerably[30] (Chapter 5) since all RAD tests are repeated also for the women with negative RAD test results. We conclude that strategy 5 produces no gain over strategy 1.

If all pros and cons of the five strategies are weighed, only strategy 1, strategy 2 and strategy 4, remain as acceptable alternatives. However, in clinical practice, karyotyping is always performed after a positive RAD test to exclude rare cases of genetic inheritance. Therefore, we only describe strategy 1 and 4. Table 1 summarizes the differences in detected chromosomal abnormalities and costs between these strategies.

The clinical M.A.K.E. study shows that three clinically significant chromosomal abnormalities would remain undetected by MLPA, but the total costs are considerably less. From an efficiency point of view, strategy 4 is the optimal test protocol, unless society is prepared to spend over € 364,000 incrementally to detect one extra chromosomal abnormality with clinically significant consequence. Although society may nevertheless adopt that point of

Table 1: Comparison of standalone karyotyping (strategy 1) and RAD with subsequent karyotype in case of a positive RAD test result (strategy 4).*

	Standalone karyotyping (strategy 1)	RAD with subsequent karyotype in case of a positive RAD result (strategy 4)
Detected trisomies 13, 18, 21	85	85
Detected aneuploidies of X and Y	13	13
Undetected abnormalities with clinically significant consequences	0	3
Undetected abnormalities with uncertain clinical consequences	0	6
Undetected abnormalities with no clinical consequences	0	17
Total costs	€ 7,368,086.52	€ 6,275,310.77
Average total costs per detected trisomy	€ 86,683.37	€ 73,827.19
Incremental costs per undetected abnormality with clinically significant consequence	€ -	€ 364,258.84
Incremental costs per undetected abnormality with clinically or uncertain significant consequence	€ -	€ 121,419.61
Incremental costs per any undetected abnormality	€ -	€ 42,029.87

* Based on clinical and cost data from the M.A.K.E. study

view, we have to realise that the selection of women before undergoing prenatal diagnosis is currently not optimal. The amount of chromosomal abnormalities and their associated costs are also subject to the prenatal indications applied and risk selection process and criteria.
Prenatal screening tests i.e. nuchal translucency measurement and maternal serum screening improve the detection rate for Down syndrome and are cost-effective[44]. In 2007, the average screening uptake in the Netherlands was only 27%. In our country, co-payment is required for women below 36 years of age who enter prenatal screening. Abolishment of copayments could lead to a higher uptake. Furthermore, in the Netherlands, advanced maternal age (at least 36 years of age in the 18th gestational week) still is the most important referral indication for prenatal diagnosis (59%)[45]. Approximately 20% of women older than 36 years choose to have prenatal diagnosis. Maternal age, however, is a -less effective- risk selection criterium. It should be noted that the prior risk of an abnormality in our study is about 1/50. An increase in the uptake of prenatal screening could increase the prior risk as well as the relative optimality of strategy 4.

Room for individual choice?

In current practice, women are entitled to accept or decline participation in prenatal screening, in prenatal diagnosis, and to decide on the termination of pregnancy. If they decide to undergo prenatal diagnosis, they have to choose for amniocentesis or chorionic villus biopsy. However, choice is restricted; women cannot choose the test they want, since no other accepted test than karyotyping is available yet. However, if one is prepared to accept strategy 4 as the optimal test protocol, the question rises how that strategy should be offered. If we implement strategy 4 as uniform strategy, all pregnant women who consent to prenatal diagnostics undergo MLPA first and receive karyotyping if the MLPA test result is positive. Alternatively, one may offer free choice to women who consent to prenatal diagnostics; some women prefer definite results over probabilities and therefore may prefer a prenatal diagnostic instead of a risk estimate.
One normative question has to be addressed at this point; Are pregnant women free to decide on the testing procedure, i.e. on (a) the screening test that is being used, on (b) whether prenatal diagnostic test(s) is (are) offered standalone or serially, and on (c) on the contents of the test? If the answer generally is yes, we have to accept inefficiencies associated with free choice, e.g. inefficiencies due to logistics and sample throughput at a smaller scale. Allowing individualised choice is less efficient than offering women strategy 4 as a uniform policy because women are also allowed to choose the least efficient strategy (Chapter 6).
When the economic perspective dominates, one may stick to the current RAD yield as societal acceptable; the gain from karyotyping by detecting relevant abnormalities at a price

of 350.000 euro each may not imply a change in the course of pregnancy. Therefore it is important to discuss the possibilities of individual choice.

Two perspectives on individual choice exist. The first perspective is that individualised decision making is allowed for reasons of principle or ethics, regardless of efficiency, quality of care, quality of life or other considerations. Whether or not to participate in screening programmes or women's prerogative to decide on the continuation or termination of pregnancy belongs to this perspective. According to the second perspective, it is the patient who is designated to decide which strategy is best when there are fundamental differences in outcomes associated with these strategies. Both perspectives lend arguments in favour of individual choice. Choices can, however, be validly limited by economic constraints. Kassirer has distinguished seven cases of fundamental differences in which patient preferences could be decisive[46]. Several of these cases also apply to the optimal prenatal diagnostic strategy. These are the following: 1) there are major differences in the kinds of possible outcomes (e.g. Down syndrome versus balanced translocation); 2) one of the choices can result in a small risk of a grave outcome (e.g. de novo unbalanced abnormality); 3) a patient attaches unusual importance to certain possible outcomes (e.g. broad detection capacity); 4) the patient is highly adverse to taking risks (e.g. broad detection capacity); and 5) the patient has to trade off long term and short term outcomes (long term consequences associated with an undetected chromosomal abnormality). In our quality of life study (chapter 4), women were offered a choice between MLPA and karyotyping. The majority reported that they were convinced they had made the correct choice. There were no systematic differences over time between standalone RAD versus karyotyping in terms of anxiety, general physical and mental health, perceived personal control and stress. When the outcomes of this study are compared with the same outcome parameters of women who were allocated to karyotyping or a combined strategy (chapter 3), the decrease in anxiety and stress two days following amniocentesis is sharper in the choice group. This suggests that individual choice might have a favourable impact on anxiety. Similar studies have not been carried out yet. While our results suggest that HRQOL is not compromised when women are offered individual choice, it is conceivable that HRQOL might also improve. When women are offered individual choice and women base their choice on individual considerations and needs.

Taking into account the specific field of prenatal diagnosis and the results from the quality of life and preferences studies, we support individual choice. Our primary argument is that such a decision is of lifelong interest to the family, rather than a short term decision for the pregnant woman only, and also we believe non-health arguments prevail. Such a decision -

within reasonable economic constraints - is most appropriately made by individuals who will bear the responsibility of raising the child.

Policymaking in the Netherlands

Following the results from the M.A.K.E. study, a nationwide debate has emerged on the implementation of RAD. Despite several meetings of the Working Party of Prenatal Diagnosis and Fetal Therapy, no agreement was reached. Although it seems that experts prefer to offer a nationwide uniform policy in the Netherlands, the lack of consensus has contributed to regional differences; individual choice is offered in Nijmegen, a combined strategy is performed in Maastricht, while the other centres still use the karyotype. In other European countries, no uniform policy is formed (personal communication Bui, Voligno, Rieneri); some centres offer a choice, while others perform a combined strategy. In the UK, the UK National Screening committee recommended the use of standalone RAD. However, a nationwide implementation did not follow. Even in the participating hospitals 12.5% of women received the combined strategy of RAD and karyotyping[47]. Overall, looking at our fellow European countries, reveals that uniform policymaking in prenatal diagnosis is difficult to reach and in practice to achieve. Elaborating on the patient and physician preference studies, it is unlikely that all stakeholders on the short term will agree on one strategy. From here we envisage four options;

1 Policymakers decide which test generally meets most needs and this test is offered nationwide. A major disadvantage of this strategy is that women's preferences cannot play a role.
2 Each centre decides which test(s) is (are) offered. A major disadvantage of this strategy is that clients may not be informed on the differences between centres or clients have to travel for the test they want. Besides, arrangements on financial compensation for the laboratories should be made.
3 Shared decision-making is offered. Clinicians and clients discuss the benefits and disadvantages of prenatal testing and find mutual agreement on the test(s) to be performed. They can decide to perform karyotyping, MLPA or other molecular tests on e.g. cystic fibrosis.
4 Future studies on new techniques are awaited before a uniform policy is formed.

Implementation of RAD in clinical practice

The identification of RAD as optimal test strategy as well as the opportunity of individualised choice implies that two alternative prenatal diagnostic strategies will co-exist. This has profound consequences for clinical practice. Firstly, the failure rate of RAD is a burden leading to a longer waiting time and possible repeat amniocentesis. Our study showed that

failed RAD tests are mainly associated with contaminated samples. Therefore, obstetricians should try to obtain uncontaminated samples by e.g. avoiding a placental approach and using continuous ultrasound control[48] (Chapter 2). We propose that still 10 to 20 millilitres of amniotic fluid should be withdrawn for short-term storage of remaining material to allow karyotyping should RAD fail and subsequent storage of DNA to allow follow-up molecular diagnostics and avoid repeated amniocentesis should ultrasound examination show an abnormality.

Secondly, clinical cytogeneticists and molecular geneticists are recommended to cooperate closely. However, this is not only the case in prenatal diagnosis, since the role of molecular applications rise rapidly in all fields of medicine. Substitution of karyotyping will lead to a decrease in cytogenetic procedures and increase in molecular tests.

Thirdly, partial substitution of karyotyping will also change the role of clinical geneticists in prenatal diagnosis, since they will often be the clinical professional involved when babies are born with undetected chromosomal abnormalities. Furthermore, they have to decide which test should be applied after RAD has been performed on the stored material, choosing from a range of molecular applications when second trimester ultrasound detects abnormalities.

To achieve successful implementation of individual choice, the following requirements must be met;

1 Both tests should be offered.
2 Clear, reliable and complete information on test options should be provided, describing the consequences, benefits and disadvantages of each test.
3 Clinicians should be willing to offer both tests and provide nondirective prenatal counselling. An open discussion on benefits and disadvantages of tests should follow including clients' risks and preferences.
   When these three requirements are satisfied, shared decision making is feasible[49].
4 Effort should be put into educating the public on the possibilities, the advantages and disadvantages of prenatal tests, and the variety of consequences of chromosomal abnormalities. In that way, the future users of prenatal diagnosis are informed and hopefully public awareness and acceptance is created.

Future techniques

At this point, microarray genomic analysis (MA) and non-invasive prenatal diagnosis are the most promising future techniques. It is remarkable that the current debate focuses on targeted testing, while the future techniques are likely to detect more than the current broad technique which is karyotyping.

At this moment studies are carried out on the diagnostic accuracy, appropriate construction of prenatal devices, and costs of MA in prenatal diagnosis, which allows the detection of multiple microscopic and submicroscopic deletions and duplications in a single simultaneous assay, without the detection of balanced rearrangements[50]. MA is presented as having the potential to become the primary prenatal diagnostic laboratory procedure. First of all diagnostic accuracy (clinical validity) needs to be established. Currently, four genetic laboratories in the USA perform a prospective study funded by the US National Institute of Child Health and Development comparing MA and karyotyping on consecutive prenatal samples[51]. The results of this study are expected in 2013. Two aspects of the MA technique are challenging. Firstly, by extending the detection capacity to hundreds of discrete genomic loci for DNA copy number gains and losses, it is likely to lead to the identification of findings without known clinical interpretation. Many of these will be benign variants which have the potential to be misinterpreted as clinically significant abnormalities. This will result in the need for complex genetic counselling, additional costly testing, and perhaps unwarranted pregnancy termination. Besides, it is unclear who should decide which content is suitable for a MA in prenatal diagnosis; should it be based on whole-genome analysis, or should it be targeted or can parents decide themselves what they want to know? The ethical debate about genetic selection is likely to intensify over the next years. Again, data on costs, quality of life, procedural aspects and preferences should be assessed before offering the technique in prenatal diagnosis.

Another promising technique is non-invasive prenatal diagnosis (NIPD). In the near future, it is expected that NIPD using fetal cells from maternal blood will be available in the first trimester of pregnancy, avoiding the procedure related miscarriage risk of amniocentesis and chorionic villus sampling[52, 53]. Again diagnostic accuracy (clinical validity) needs to be established. Apart from evident major improvements, it is feared that the relative ease of the test will lead to an increase in testing, selective abortion, testing for minor abnormalities and non-medical traits as well[54]. At this point, the technique focuses on the detection of Down syndrome, but in the future expansion of its detection capacity is possible. The above mentioned debate on genetic selection therefore also applies to NIPD as well. Future research should assess if this test complies with women's demands and if NIPD will replace prenatal screening and invasive diagnostic tests.

Future research

In this thesis, the clinical use of RAD is established for women undergoing amniocentesis for advanced maternal age, increased Down syndrome risk following prenatal screening or parental anxiety. These results cannot be extrapolated to chorionic villus biopsy. One

preclinical study assessed 100% diagnostic accuracy to detect euploidies and non-mosaic aneuploidies in 152 samples[7]. A clinical evaluation study should be performed to evaluate its performance in clinical practice. Future research may also be valuable in optimizing the RAD technique itself and improving sampling techniques, thereby improving the success rate. As stated above, new techniques are within reach. Clinical validity studies are needed to establish the performance of these techniques, i.e. microarray and non-invasive prenatal diagnosis. Above all ethical dilemmas need to be resolved; should late onset disease be detected and how should we ensure the 'right not to know' of the future child? Does the high uptake of NIPD lead to an increase in selective abortion because it can be done early in pregnancy?

A very important aspect of offering tailor-made strategies is the quality of prenatal counselling. Future research should assess whether patients are informed properly on the pros and cons of the tests and if it is feasible in clinical practice to make a shared informed decision. Web based decision aids may be helpful to prospective parents to better understand the possibilities in prenatal diagnosis, the disadvantages of each test, and to clinicians to see which test or process characteristics parents value high and low. A valuable tool for prenatal counsellors would be a clearly structured document with training to support uniform counselling since, especially in the Netherlands, prenatal counselling is provided by many professionals and it should include all aspects of prenatal testing. It is a challenge to develop a tool with multiple stakeholders involved (parents, obstetricians, midwives, geneticists, ethicists). Especially with rapidly developing techniques with a different scope, informed shared decision-making is an incentive for high quality care.

The changing scope in prenatal diagnosis from cytogenetic karyotyping to a variety of molecular tests may lead to major policy changes. It is expected that in the nearby future, women in the US will have a microarray analysis instead of karyotyping. Currently, microarrays are more often used for ultrasound abnormalities in the Netherlands. The goal of prenatal diagnosis is the equal provision of information so the parents can make an informed decision. The widespread introduction of molecular tests changes the scope of prenatal diagnosis and should encourage the development of strategies that tailor the type of diagnostic test offered to the risk identified.

Concluding recommendations

The field of prenatal diagnosis is rapidly changing. With the introduction of karyotyping in 1966, development of prenatal screening in the 1980s and introduction of RAD tests in 1990s, and forthcoming techniques within the next ten years intensify the need for clarity on what to test for in prenatal diagnosis. In this thesis we show that MLPA followed by

karyotyping when the MLPA test result is positive, is a suitable strategy for prenatal diagnosis. Women's quality of life is not considerably affected by applying a combined or karyotyping only strategy. If we offer women a choice between the tests, anxiety and stress reduction is higher compared to a group not being able to choose. Women's preferences differ greatly based on their own considerations and attitude. Customized prenatal diagnosis incorporating patients' risk and preferences seems feasible. The results presented in this thesis, together with studies on other RAD techniques and patient preferences provide convincing evidence to support this view. Constructive collaboration among obstetricians, cytogeneticists, molecular geneticists and clinical geneticists is essential to achieve successful implement RAD and to incorporate shared decision-making. To facilitate this process, guidelines for the Dutch Society of Obstetricians and Gynaecologists (NVOG) will be needed, together with patient material and education. It is our intention to provide these requirements.

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Chapter 10

Summary

OBJECTIVE OF THIS THESIS

Prenatal diagnosis is routinely offered to all pregnant women in developed countries who have an increased risk of carrying a child with a chromosomal abnormality. The aim of prenatal diagnosis is to provide information on the presence and nature of chromosomal abnormalities, in order to allow parents an informed choice on the course of pregnancy. This may imply a decision to continue or terminate pregnancy, but it also implies the arrangement of treatment planning and psychological preparation. The selection of women with a higher than average risk for fetal chromosomal abnormalities is based on several factors: maternal age, family history, prenatal screening tests and ultrasound abnormalities. In the Netherlands, the main indications for prenatal diagnosis are advanced maternal age and an increased Down syndrome risk following first trimester prenatal screening. Prenatal diagnosis for these indications is for the most part focussed on detecting Down syndrome. Since almost 50 years, karyotyping is the diagnostic test used to detect fetal chromosomal abnormalities. Karyotyping is highly accurate. It requires cultured cells at metaphase stage, leading to a waiting time of two to three weeks. It is labour-intense leading to high costs. Karyotyping can detect any structural and numerical microscopic chromosomal abnormality, which may lead to chromosomal abnormalities with no, mild, or unclear clinical consequences. The latter findings lead to difficult counselling issues and emotional dilemmas for parents who have to decide to continue or terminate pregnancy.

In 2003, MLPA became available, detecting the most common chromosomal abnormalities, i.e. trisomies 13, 18, 21, and non-mosaic sex chromosomal abnormalities on uncultured amniocytes. Preclinical studies showed high sensitivity and specificity and presented the technique as promising and possibly able to replace karyotyping. However, no large clinical comparative studies were available to support these claims. Besides, the replacement of karyotyping is not only based on test performance. Patient preferences and quality of life, physicians' preferences and costs should be incorporated into this medical decision. Therefore, we performed the M.A.K.E. (MLPA And Karyotyping, an Evaluation) study, a nationwide study comparing MLPA and karyotyping in routine prenatal diagnosis.

Patients

Pregnant women were included in the M.A.K.E. study when they chose to undergo amniocentesis for advanced maternal age (36 years or older), increased risk of Down syndrome following prenatal screening or parental anxiety. We excluded women with other indications for amniocentesis since they have an increased risk of chromosomal abnormalities other than the most common aneuploidies which MLPA cannot detect and karyotyping is

mandatory; ultrasound abnormalities including a nuchal translucency measurement of 3.5 mm, a parental chromosomal abnormality, or a previous child with a chromosomal abnormality.

Interventions

For the MLPA procedure, DNA was isolated from 1 to 8 ml uncultured amniotic fluid samples, depending on the total amount of amniotic fluid received. We used a commercially available kit, the SALSA MLPA P095 (MRC Holland, the Netherlands). For each genomic target, a set of 2 probes is designed to hybridize immediately adjacent to each other on the same target strand. Both probes consist of a short target sequence and a universal polymerase chain reaction (PCR) primer-binding site. One of the probes contains a stuffer sequence with a unique length and sequence. Following hybridization, each pair of adjacent probes is joined by a ligation reaction. Next, PCR is performed using a fluorescent-labeled primer pair, which ensures that the relative yield of each of the PCR products is proportional to the amount of each of the target sequences. The different length products are separated on an automated capillary sequencer. The size and peak areas for each probe are quantified and analyzed by data analyzing software (GeneMarker, SoftGenetics, LLC, State College, PA, USA or Genescan and Genemapper version 3.7/4.0, Applied Biosystems, CA, USA). Relative probe signals are calculated and compared with samples of normal male and female sex. In chromosomally normal samples, the relative probe signal is expected to be 1 for all probes. A normal value is defined as a relative probe signal between 0.7 and 1.3. A relative probe value of <0.7 indicates a monosomy, whereas a relative probe value of >1.3 indicates a trisomy. MLPA is not expected to detect low grade chromosomal mosaicism.

For karyotyping, fetal cells were cultured and spread on slides, which were stained for chromosomal banding. Routinely, metaphases for 10 colonies were investigated. All centers followed national quality guidelines but minor differences in the amount of cell colonies cultured, staining and reporting of the results were allowed.

OUTCOMES OF THE M.A.K.E. STUDY

Diagnostic accuracy (Chapter 2)

In a national comparative cohort study, 4585 amniotic fluid samples were included of women who had an amniocentesis on behalf of their age (75.6%), increased risk following prenatal screening (23.4%)or maternal anxiety (1%). Amniotic fluid samples were tested independently with both MLPA and karyotyping. In 4484/4585 samples (97.8%) MLPA and karyotyping were concordant, showing normal results in 4386/4585 (95.7%) and aneuploidy in 98/4585 (2.1%). Discordant results were found in 26/4585 (0.6%) samples, representing an abnormal karyotype not detected by MLPA. Diagnostic accuracy of MLPA was 1.0 (95 % confidence interval 0.99 to 1.0), sensitivity was 100% (95% confidence interval 0.96-1.0) and specificity was 100% (95% confidence interval 0.999-1.0). Diagnostic accuracy of MLPA was statistically similar (noninferior) to that of karyotyping ($P<0.001$). In 75 cases MLPA failed (1.6%); karyotyping failed once (0.02%). Compared with karyotyping, MLPA shortened the waiting time with 14.5 days ($P<0.001$, 95% confidence interval 14.3-14.6).

Quality of life (Chapter 3 and 4)

First, we assessed anxiety, personal perceives control, stress and health related quality of life by validated questionnaires between women receiving a karyotype result only (n=132) and women receiving both the result of MLPA and karyotyping (n=181). There were no systematic differences in time of the combined strategy versus karyotyping only in terms of anxiety ($P=0.91$), generic physical health ($P=0.76$, $P=0.46$), generic mental health ($P=0.52$, $P=0.72$), personal perceived control ($P=0.91$), and stress ($P=0.13$). The combined strategy reduced anxiety and stress two weeks earlier compared to karyotyping only. In general MLPA as add-on to karyotyping reduces anxiety and stress in the short term, but it does not influence overall anxiety, stress, personal perceived control, and generic mental and physical health when compared to a karyotype only strategy

Secondly, we assessed the impact of standalone MLPA and karyotyping on anxiety, personal perceives control, stress and health related quality of life by validated questionnaires. Furthermore, we explored the reasons and perceptions to choose between standalone MLPA versus fetal karyotyping. Women were offered a choice between karyotyping (n=68) and standalone MLPA (n=61). The most commonly cited reason for choosing karyotyping was obtaining as much information as possible, while for standalone MLPA it was the short waiting time. Prenatal screening (OR 7.09), no knowledge of karyotyping (OR 4.2) and an intermediate perceived risk for chromosomal abnormalities (OR 3.6) were associated with choosing standalone MLPA. There were no systematic differences in time of karyotyping and

standalone MLPA in terms of anxiety (P= 0.11), generic physical and mental health (P=0.94, 0.52; P=0.66, P=0.07), personal perceived control (P=0.69), and stress (P=0.66). In general, offering a choice between karyotyping and standalone RAD does not influence anxiety, stress, personal perceived control, and generic health.

Costs (Chapter 5)

This chapter describes the costs of MLPA compared to karyotyping for women who choose to undergo amniocentesis on behalf of their age (≥36 years), an increased risk following first trimester prenatal screening or parental anxiety. Clinical data and the use of medical resources were based on the clinical M.A.K.E. study. The cost-minimisation analysis showed that MLPA reduced costs in the short term (time from amniocentesis until the decision to continue or terminate pregnancy) with € 315.68 (95% CI: € 315.63 to € 315.74; -44.4%) per sample compared to karyotyping. In the long term (time from the decision to continue or terminate pregnancy), MLPA increased cost € 76.42 (95%CI: 71.32 to 81.52; +8.6%) per sample. Overall, MLPA reduced costs with € 240.13 (bootstrap 95%CI: 235.02 to 245.23;-14.9%) per sample. Costs were sensitive to the likelihood of termination of pregnancy, sample throughput and centralised care. From a societal economic perspective, MLPA is the preferred strategy in women who undergo amniocentesis on behalf of their age, following prenatal screening or parental anxiety.

Patient preferences (Chapter 6 and 7)

We assessed patient preferences in two chapters.

In chapter 5, we assessed patient preferences for three test strategies (a test detecting trisomy 21, a test detecting trisomies 13, 18, 21, X and Y, and a test with comparable detection capacity as karyotyping). Detection capacity, the comprehensibility of the result in case of a detected abnormality, and the waiting time for the test result were described. We invited 150 pregnant women undergoing amniocentesis on behalf of their age (84.1%), increased risk following prenatal screening (11.5%) and maternal anxiety (2.7%). Seventy-five percent (113/150) responded to the survey. They expressed a preference for the test resembling karyotyping (50.4%) or the rapid technique detecting the most common aneuploidies (43.4%). A minority of women chose the test detecting Down's syndrome only.

Secondly (Chapter 7) we described patient preferences and determined which test attributes influence their preference most by using a discrete choice experiment. Pregnant women who visited the hospital for prenatal counselling were invited to participate. In total 103/118 (87%) women participated. Women placed most value on the detection of chromosomal abnormalities with severe consequences for their child (P<0.01). The failure rate of the test, the waiting time for test results, and the experienced anxiety influenced women's preferences

significantly (P <0.05). For the currently available tests, women prefer karyotyping to RAD (P <0.01) in a setting where the detected chromosomal abnormalities have severe consequences for their child. However, in a setting where karyotyping detects chromosomal abnormalities with uncertain or no clinical consequences for their child, RAD tests are preferred (P <0.01). While anxiety and waiting time have some effect on women's preferences, the potential clinical consequences of the detected chromosomal abnormality and failure rate influenced test choice most. Since RAD and karyotyping both detect the most common chromosomal abnormalities with severe consequences, both tests are appropriate for prenatal diagnosis.

Expert opinion (Chapter 6 and 8)

In two chapters we discuss the professionals' opinion on karyotyping and MLPA. In chapter 6, we assessed caregivers' preferences for three test strategies (a test detecting trisomy 21, a test detecting trisomies 13, 18, 21, X and Y, and a test with comparable detection capacity as karyotyping). Detection capacity, the comprehensibility of the result in case of a detected abnormality, and the waiting time for the test result were described. We invited 140 caregivers involved in prenatal diagnosis, i.e. obstetricians, midwives, clinical geneticists, clinical cytogeneticists and general practitioners to participate. Of the caregivers (70% female; median age 44 years, 26 to 63 years), 55% (77/140) returned the questionnaire. The majority of the caregivers preferred a test detecting the most common chromosomal abnormalities (77.9%) while the remaining preferences for the other tests were equally divided (10.4%).

In chapter 8, we determined expert consensus on which chromosomal abnormalities should and should not be detected in prenatal diagnosis, and for which abnormalities disagreement remains after structured discussion. An expert panel of 24 prenatal experts (8 clinical cytogeneticists, 8 clinical geneticists and 8 obstetricians) rated pro or against detection for 15 chromosomal abnormalities in two individual anonymous rating rounds and one group meeting. Consensus was defined to be present if at least 80% of the experts agreed. Consensus was reached in 12 of 15 cases. In 10 cases, there was agreement pro detection and in two cases experts agreed against detection. At the end of the 3rd round, dissensus remained on three abnormalities. Experts largely agreed on detecting chromosomal abnormalities with severe consequences and against detection in case of irrelevant clinical consequences. For chromosomal abnormalities with mild or uncertain outcomes, dissensus remained. None of the currently available tests (MLPA and karyotyping) corresponds to these demands, except if non-disclosure for karyotyping is applied for chromosomal abnormalities with uncertain consequences.

CONCLUSIONS

In chapter 9 the findings of this thesis are discussed, clinical implications are given and future research recommendations are made. The results of this thesis show that MLPA is a highly accurate test for the detection of trisomies 13, 18, 21, X and Y and is noninferior to karyotyping with comparable patient quality of life and significant cost reduction.

Based on decision analytic considerations and our study results, two strategies can be applied: 1) karyotyping only or 2) RAD with subsequent karyotype in case of a positive RAD result. The latter leads to substantial cost reduction compared to a karyotyping strategy without considerable loss in information. If economics decide, the gain from karyotyping by detecting relevant abnormalities at a price for 350.000 euro each, while it is uncertain what the knowledge of the chromosomal abnormalities adds, one may stick to the current RAD yield as societal acceptable. However, patient preferences are not incorporated in such a strategy. It is also possible to offer pregnant women a free choice. Our primary argument is that such a decision is of lifelong interest for the family, rather than on short term for the pregnant women alone, and also we believe non-health arguments prevail. Such a decision - within reasonable economic constraints - is most appropriately made by individuals who will bear the responsibility of raising the child.

Caregivers (obstetricians, clinical geneticists, clinical cytogeneticists, midwives, and general practitioners) prefer RAD over karyotyping. However, when realistic clinical cases are placed, experts in prenatal diagnosis agree that all chromosomal abnormalities with major consequences should be detected and chromosomal abnormalities without consequences should not be detected.

We prefer the introduction of individual choice in prenatal diagnosis. So that parents can decide, after nondirective prenatal counselling, which test meets their individual risk and needs most.

Future research should focus on promising future techniques, such as microarray genomic analysis and non-invasive prenatal diagnosis. In these clinical comparative studies, patient preferences and ethical discussion should be incorporated. Future research should assess the feasibility of shared informed decision in clinical practice and focus on the development of clear and structured information services for patients and physicians.

Chapter 11

Nederlandse samenvatting

DOELSTELLING VAN DIT PROEFSCHRIFT

Prenatale diagnostiek wordt routinematig aangeboden aan alle zwangere vrouwen, die een verhoogd risico hebben op het krijgen van een kind met een chromosomale afwijking. Het doel van de prenatale diagnostiek is informatie te verstrekken over de aanwezigheid en de aard van chromosomale afwijkingen, zodat ouders een weloverwogen keuze kunnen maken over het verloop van de zwangerschap. Ouders kunnen hierna besluiten om de zwangerschap te beëindigen, maar zij kunnen ook regelingen treffen voor tijdige medische behandeling of psychische ondersteuning. Bovendien kunnen zij zich op de situatie voorbereiden.

De selectie van vrouwen met een hoger dan gemiddeld risico voor foetale chromosomale afwijkingen kan gebaseerd zijn op verschillende informatie: de hogere leeftijd van de moeder, een belaste familiegeschiedenis, afwijkende prenatale screening testen en dan wel afwijkende bevindingen bij echoscopisch onderzoek. In Nederland zijn de belangrijkste indicaties voor prenatale diagnostiek leeftijd van de moeder van 36 jaar en ouder, en een verhoogd risico op Down syndroom na een eerste trimester prenatale screening test. Prenatale diagnostiek voor deze indicaties is voor het grootste deel gericht op de detectie van het syndroom van Down.

Sinds bijna 50 jaar is karyotypering de diagnostische test die gebruikt wordt om foetale chromosomale afwijkingen te detecteren. Karyotypering is een zeer nauwkeurige test. Er zijn gekweekte cellen nodig om cellen in de metafase te kunnen beoordelen. Deze kweektijd leidt tot een wachttijd van twee tot drie weken voordat de cellen beoordeeld kunnen worden en een uitslag bekend is. Karyotypering is arbeidsintensief en leidt tot hoge kosten. Karyotypering kan zowel numerieke als structurele microscopische chromosomale afwijkingen detecteren. Soms worden zo chromosomale afwijkingen gedetecteerd die geen, lichte, onzekere of onduidelijke klinische consequenties hebben. Deze bevindingen leiden tot lastige counselinggesprekken en emotionele dilemma's voor ouders die moeten beslissen om de zwangerschap voort te zetten of te beëindigen.

In 2003, kwam MLPA beschikbaar, een nieuwe test die de meest voorkomende chromosomale afwijkingen (trisomie 13, 18, 21 en niet-mozaïek geslacht chromosomale afwijkingen) kan detecteren op ongekweekte vruchtwatercellen. Preklinische studies lieten een hoge sensitiviteit en specificiteit zien. De techniek leek veelbelovend en zou eventueel een vervanger kunnen zijn van de karyotypering. Er waren toen echter nog geen grote klinische vergelijkende studies beschikbaar om de vervanging te ondersteunen. Bovendien speelden ook andere aspecten dan de diagnostische kenmerken een rol: de

impact op de kwaliteit van leven van patiënten, de kosten en de voorkeur van patiënten en artsen. Daarom werd de M.A.K.E. (MLPA En Karyotypering, een evaluatie) studie opgezet, een landelijke studie waarin MLPA en karyotypering vergeleken worden in de klinische prenatale diagnostiek.

Patiënten

Zwangere vrouwen werden in de M.A.K.E. studie geïncludeerd als zij een vruchtwaterpunctie ondergingen op basis van hun leeftijd (36 jaar of ouder), of op basis van een verhoogd risico op het syndroom van Down na prenatale screening of op basis van ouderlijke angst. Vrouwen met andere indicaties (echoscopische afwijkingen, waaronder een nekplooidikte vanaf 3.5 mm, een ouder met een chromosomale afwijking, of een eerder kind met een chromosoomafwijking) voor een vruchtwaterpunctie werden niet geïncludeerd, omdat zij een verhoogd risico op andere chromosomale afwijkingen hebben dan de meest voorkomende aneuploidieën. Deze andere chromosomale afwijkingen worden die niet gedetecteerd door MLPA, maar wel met karyotypering.

Interventies

Voor de MLPA procedure werd 1 tot 8 ml, afhankelijk van de totale hoeveelheid ontvangen vruchtwater ontvangen, DNA geïsoleerd uit ongekweekte vruchtwatercellen. De commercieel verkrijgbare kit, de Salsa MLPA P095 (MRC Holland, Nederland) werd gebruikt. Voor elke target genoom, is een set van 2 probes ontworpen, die onmiddellijk naast elkaar hybridiseren op dezelfde target streng. Beide probes bestaan uit een korte target sequentie en een universele polymerase chain reaction (PCR) primer-bindende site. Een van de probes bevat een stuffer sequentie met een unieke lengte en volgorde. Na hybridisatie, wordt elk paar aangrenzende probes verbonden door een ligatiereactie. Vervolgens wordt de PCR uitgevoerd met behulp van een fluorescent gelabeld primerpaar, dat ervoor zorgt dat de relatieve opbrengst van elk van de PCR-producten evenredig is met de hoeveelheid van de target sequenties. De verschillende producten worden op basis van hun lengte gescheiden op een geautomatiseerde capillaire sequencer. De grootte en de maximale oppervlakte voor elke probe worden gekwantificeerd en geanalyseerd met behulp van software (GeneMarker, SoftGenetics, LLC, State College, PA, USA of Genescan en Genemapper versie 3.7/4.0, Applied Biosystems, CA, USA). Relatieve probe signalen worden berekend en vergeleken ten opzichte van monsters van normaal mannelijk en vrouwelijk geslacht. Als er een normale hoeveelheid chromosomen aanwezig is, zal het relatieve probe signaal naar verwachting 1 zijn. Een normale waarde wordt gedefinieerd als een relatief probe-signaal tussen de 0,7 en 1,3. Een relatieve waarde van $<0,7$ duidt op een monosomie, terwijl een relatieve waarde van $>1,3$ wijst

op een trisomie. MLPA detecteert geen laaggradig mosaïcisme. Voor karyotypering, de standaardtest, worden foetale cellen gekweekt en in de metafase onderzocht. Daarna worden ze gekleurd voor chromosomale bandering. Routinematig worden 10 kolonies onderzocht. Alle deelnemende centra volgden de nationale kwaliteitseisen. Kleine verschillen in het aantal gekweekte cellen, de manier van kleuren en de rapportage van de resultaten waren toegestaan.

RESULTATEN VAN DE M.A.K.E. STUDIE

Diagnostische nauwkeurigheid (hoofdstuk 2)

In een nationaal vergelijkende cohort studie werden 4585 vruchtwater monsters onderzocht. Alleen vrouwen die een vruchtwaterpunctie ondergingen op basis van hun leeftijd (75,6%), een verhoogd risico na prenatale screening (23,4%) of angst (1%) werden geïncludeerd. Vruchtwater monsters werden onafhankelijk getest met zowel MLPA als karyotypering. In 4484/4585 monsters (97,8%) kwamen karyotypering en MLPA overeen. Bij 4386 van de 4585 samples (95,7%) was er sprake van een normaal resultaat en bij 98 van de 4585 (2,1%) was er een aneuploidie. Bij 26 van de 4585 (0.6%) werden discordante resultaten gevonden; karyotypering liet een abnormaal karyotype zien dat niet gedetecteerd kon worden door MLPA. De diagnostische accuratesse van MLPA was 1.0 (95% betrouwbaarheidsinterval 0.99 tot 1.0), de sensitiviteit was 100% (95% betrouwbaarheidsinterval 0.96 tot 1.0) en de specificiteit 100% (95% betrouwbaarheidsinterval van 0.999 tot 1.0). De diagnostische accuratesse van MLPA was statistisch vergelijkbaar (noninferieur) met die van karyotypering ($P < 0.001$). In 75 gevallen gaf MLPA geen uitslag (1,6%); karyotypering gaf een keer geen uitslag (0.02%). Vergeleken met karyotypering, verkortte MLPA de wachttijd met 14.5 dagen ($p < 0.001$, 95% betrouwbaarheidsinterval 14.3-14.6).

Kwaliteit van leven (hoofdstuk 3 en 4)

In de eerste studie werden angst, persoonlijke controle, stress en gezondheidgerelateerde kwaliteit van leven gemeten met behulp van gevalideerde vragenlijsten. Resultaten werden vergeleken van vrouwen die alleen een karyotype kregen ($n = 132$) en vrouwen die zowel een MLPA en karyotypering kregen ($n = 181$). Er waren geen systematische verschillen in de tijd van de gecombineerde strategie versus karyotypering alleen in angst ($p = 0.91$), algemene fysieke gezondheid ($p = 0.76$, $p = 0.46$), algemene geestelijke gezondheidszorg ($p = 0.52$, $p = 0.72$), persoonlijke controle ($p = 0.91$), en stress ($p = 0.13$). De gecombineerde strategie verminderde angst en stress twee weken eerder in

vergelijking met karyotypering. Concluderend verminderde MLPA met karyotypering angst en stress op de korte termijn, maar beïnvloedde het de totale angst, stress, persoonlijke controle, en generieke gezondheid niet in vergelijking met alleen karyotypering In de tweede studie werd de impact van standalone MLPA en karyotypering beoordeeld, nadat vrouwen een keuze was aangeboden tussen MLPA en karyotypering. Opnieuw werden angst, persoonlijke controle, stress en gezondheidgerelateerde kwaliteit van leven gemeten met behulp van gevalideerde vragenlijsten. Ook werden de redenen en overwegingen van vrouwen in kaart gebracht bij de keuze tussen standalone MLPA (n=61) en karyotypering (n=68). De meest genoemde reden om voor karyotypering te kiezen was het krijgen van zoveel mogelijk informatie, terwijl de korte wachttijd voor standalone MLPA werd genoemd. Prenatale screening (OR 7,09), onbekend zijn met karyotypering (OR 4,2) en een niet klein en niet groot ervaren risico op chromosomale afwijkingen (OR 3,6) was geassocieerd met het kiezen van standalone MLPA. Er waren geen systematische verschillen in de tijd tussen karyotypering en standalone MLPA in angst ($p = 0.11$), algemene lichamelijke en geestelijke gezondheid ($p = 0.94$, $p= 0.52$, $p = 0.66$, $p = 0.07$), persoonlijke gepercipieerde controle ($p = 0.69$), en stress ($p = 0.66$). Concluderend, heeft de keuze tussen karyotypering en standalone RAD geen invloed op angst, stress, persoonlijke controle, en generieke gezondheid.

Kosten (hoofdstuk 5)

Dit hoofdstuk beschrijft de vergelijking van de kosten van MLPA en karyotypering. Alleen vrouwen die een vruchtwaterpunctie ondergingen op basis van hun leeftijd (≥ 36 jaar), een verhoogd risico na prenatale screening of angst werden geïncludeerd. Wij gebruikten de klinische gegevens en gerealiseerde zorg op basis van de klinische MAKE studie. De kosten-minimisatieanalyse toonde aan dat MLPA op de korte termijn (de tijd vanaf de vruchtwaterpunctie tot aan de beslissing de zwangerschap te beëindigen) € 315,68 (95% CI: € 315,63 tot € 315,74; -44,4%) per sample minder kost vergeleken met karyotypering. Op de lange termijn - dit is de tijd vanaf de beslissing om de zwangerschap voort te zetten of te beëindiging - is MLPA € 76,42 (95% CI: 71,32 tot 81,52; +8,6%) duurder per monster. Als de korte en lange termijn worden samengenomen, is MLPA € 240,13 (bootstrap 95% CI: 235,02 tot 245,23; -14,9%) per monster goedkoper. De kosten werden beïnvloed door het aantal zwangerschapsafbrekingen, de hoeveelheid monsters en de hoeveelheid laboratoria. Vanuit een economisch perspectief, heeft de MLPA strategie de voorkeur bij vrouwen die een vruchtwaterpunctie te ondergaan wegens van hun leeftijd, na prenatale screening of wegens angst.

Voorkeuren van patiënten (hoofdstuk 6 en 7)

De voorkeuren van patiënten werden onderzocht in twee hoofdstukken. In hoofdstuk 5 hebben we de voorkeuren van patiënten gemeten voor drie strategieën (een test op trisomie 21, een test op trisomies 13, 18, 21, X en Y, en een test vergelijkbaar met karyotypering). De detectie capaciteit, de gevolgen van het resultaat indien een afwijking werd geconstateerd en de wachttijd op het testresultaat werden beschreven. We nodigden 150 zwangere vrouwen uit die een vruchtwaterpunctie ondergingen op basis van hun leeftijd (84,1%), een verhoogd risico na prenatale screening (11,5%) of vanwege angst (2,7%). Vijfenzeventig procent (113/150) vulde de vragenlijst in. Vrouwen gaven de voorkeur aan de test die lijkt op karyotypering (50,4%) of aan RAD techniek (43,4%). Een minderheid van de vrouwen koos voor de test op syndroom van Down. In hoofdstuk 7 evalueerden we patiënten preferenties en werd bepaald welke testeigenschappen van invloed zijn op de voorkeur met behulp van een discrete choice experiment. Zwangere vrouwen die voor prenatale counseling naar het ziekenhuis gingen werden uitgenodigd om deel te nemen. In totaal namen 103/118 (87%) vrouwen deel. Vrouwen waardeerden de detectie van chromosomale afwijkingen met ernstige gevolgen voor hun kind het meest ($P < 0.01$). Het percentage niet gelukte testen, de wachttijd en de ervaren angst beïnvloeden de voorkeuren van vrouwen significant ($p < 0.05$). Voor de huidige beschikbare testen, verkozen vrouwen karyotypering boven RAD ($p < 0.01$) als de gedetecteerde chromosomale afwijkingen maar ernstige gevolgen hadden voor hun kind. Als karyotypering chromosomale afwijkingen detecteert met onzekere of geen klinische gevolgen voor hun kind, gaven vrouwen de voorkeur aan RAD ($p < 0.01$). Angst en wachttijd hadden een gering effect op de voorkeuren van vrouwen, maar de mogelijke klinische gevolgen van de gedetecteerde chromosomale afwijking en het percentage niet gelukte testen beïnvloeden de keuze het meest. Omdat RAD en karyotypering allebei de meest voorkomende chromosomale afwijkingen detecteren met ernstige gevolgen, zijn beide testen geschikt voor prenatale diagnostiek.

De voorkeuren van experts (hoofdstuk 6 en 8)

In twee hoofdstukken bespreken we de mening van zorgverleners over karyotypering en MLPA. In hoofdstuk 6 hebben we de zorgverleners voorkeuren onderzocht voor drie testen (een test op trisomie 21, een test op trisomies 13, 18, 21, X en Y, en een test overeenkomend met karyotypering). De detectie capaciteit, de gevolgen van het resultaat als een afwijking werd geconstateerd en de wachttijd voor het testresultaat werden beschreven. We nodigden 140 zorgverleners uit die betrokken zijn bij prenatale diagnostiek. Dat waren verloskundigen, vroedvrouwen, klinisch

genetici, klinisch cytogeneticists en huisartsen. Van de zorgverleners (70% vrouwen; gemiddelde leeftijd 44 jaar, 26 tot 63 jaar), antwoordde 55% (77/140) op de vragenlijst. Het merendeel gaf de voorkeur aan voor een RAD test (77.9%), terwijl de resterende voorkeuren voor de andere testen gelijk waren verdeeld (10.4%). In hoofdstuk 8 werd gestreefd naar consensus tussen prenatale experts over welke chromosomale afwijkingen wel en niet gedetecteerd moeten worden in de prenatale diagnostiek. Een deskundig panel van 24 prenatale experts (8 klinische cytogenetici, 8 klinisch genetici en 8 verloskundigen) stemden over 15 chromosomale afwijkingen in twee individuele anonieme rondes en een gezamenlijke bijeenkomst. De experts moesten voor of tegen detectie stemmen. Consensus werd gedefinieerd als minstens 80% van de deskundigen het eens was. Consensus werd bereikt in 12 van 15 gevallen. In 10 gevallen werd er voor detectie gestemd en in twee gevallen tegen detectie. Aan het einde van de 3de ronde bleef er voor drie afwijkingen dissensus bestaan. Experts zijn het eens dat chromosomale afwijkingen met ernstige gevolgen gedetecteerd moeten worden en chromosomale afwijkingen zonder klinische consequenties niet gedetecteerd moeten worden. Voor chromosomale afwijkingen met milde of onzekere uitkomsten werd geen consensus bereikt. Geen van de momenteel beschikbare testen (MLPA en karyotypering) komen overeen met de eisen van alle experts, tenzij men bij karyotypering non-disclosure toepast voor bepaalde onzekere bevindingen.

CONCLUSIES

In hoofdstuk 9 worden de bevindingen van dit proefschrift besproken, de klinische implicaties gegeven en worden aanbevelingen gedaan voor toekomstig onderzoek. De resultaten van dit proefschrift laten zien dat MLPA eeen zeer nauwkeurige test is voor de detectie van trisomie 13, 18, 21, X en Y. MLPA is vergelijkbaar (noninferieur) met karyotypering, geeft een vergelijkbare kwaliteit van leven en een significante kostenreductie. Men kan beredeneren op grond van besliskundige overwegingen en de uitkomsten uit de M.A.K.E. studie dat er 2 primaire varianten zijn waarin MLPA en of TKT kunnen worden toegepast: hetzij karyotypering, hetzij RAD gevolgd door karyotypering bij een positieve RAD uitslag.

MLPA gevolgd door karyotypering in geval van een chromosomale afwijking, leidt ten opzichte van karyotypering tot een aanzienlijke kostenverlaging met weinig verlies van informatie. Deze optie is te verdedigen indien maatschappelijk één variant de voorkeur

heeft: de meerkosten voor de detectie van de zeldzame ernstige afwijking die alleen met karyotypering wordt vastgesteld ligt boven de 350.000 euro per afwijking.
Men kan ook van mening zijn dat de keuze aan de ouders moet zijn, ongeacht de kosten. In onze preferentiestudie waren de meningen van zwangere vrouwen bijna gelijkwaardig verdeeld tussen RAD en karyotypering. Zorgverleners (verloskundigen, klinisch genetici, klinisch cytogenetici, verloskundigen en huisartsen) geven de voorkeur aan RAD boven karyotypering. Wanneer prenatale experts moeten stemmen over realistische klinische gevallen, zijn zij het erover eens dat alle chromosomale afwijkingen met ernstige gevolgen gedetecteerd dienen te worden en chromosomale afwijkingen zonder gevolgen niet gedetecteerd moeten worden.Wij prefereren de invoering van een vrije individuele keuze in de prenatale diagnostiek. Zo kunnen ouders, verantwoordelijk voor de zorg van hun kind besluiten na niet-directieve counseling welke test voldoet aan hun individuele behoefte en risico.Toekomstig onderzoek moet zich richten op veelbelovende toekomstige technieken, zoals microarray analyse en niet-invasieve prenatale diagnostiek.
In klinische vergelijkende studies moeten de voorkeuren van patiënten en de ethische discussie worden opgenomen. Toekomstig onderzoek moet de haalbaarheid van gedeelde besluitvorming in de klinische praktijk evalueren en zich richten op de ontwikkeling van duidelijke en gestructureerde informatie voor patiënten en artsen.

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CURRICULUM VITAE

Elisabeth Boormans was born on a Sunday, January 8th 1978 in Tilburg, the Netherlands. Elisabeth was raised in a "General Practioner's" family where patient care took place at the practice attached to her home. She has wanted to be a physician for as long as she can remember. In 1996 she graduated from secondary school (VWO) at the Theresialyceum in Tilburg. In the same year she started medical school at the Academic Medical Centre in Amsterdam.

The interest in Obstetrics and Gynaecology started during her internships. After graduating from medical school, she worked as a resident at the 'Meander Medisch Centrum', Amersfoort and at the 'Onze Lieve Vrouwe Gasthuis', Amsterdam. In 2003 prof. dr. J.M.M. van Lith gave her the opportunity to start her research on MLPA in prenatal diagnosis as a research fellow at the Department of Obstetrics and Gynaecology of the Onze Lieve Vrouwe Gasthuis. A nationwide study was set-up with the eight genetic centres and their prenatal satellites. At the end of her research internship, Elisabeth was invited to be trained in microarray analysis in prenatal diagnosis at Columbia University Medical Center in New York (head of department: prof. dr. RJ Wapner).

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Elisabeth Boormans lives with Willem van Wijngaarden in Amsterdam. They are looking forward to the arrival of their first child.