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Prenatal screening on B·R·A·H·M·S KRYPTOR instruments

Study overview on key publications using Thermo Scientific B·R·A·H·M·S KRYPTOR prenatal screening assays

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Content

Prenatal screening on Thermo Scientific B·R·A·H·M·S KRYPTOR instruments

The measurement of maternal serum biochemistry markers is an important component of first trimester prenatal screening and it is necessary that this step provides the most precise and reproducible data.

The Thermo Scientific™ B·R·A·H·M·S™ KRYPTOR™ instrument with B·R·A·H·M·S PAPP-A KRYPTOR, B·R·A·H·M·S Free βhCG KRYPTOR and B·R·A·H·M·S PlGF plus KRYPTOR assays fulfils the strict quality requirements of the Fetal Medicine Foundation (FMF) and provides continuously the highest inter assay and intra assay precision with the lowest biomarker CVs proven by independent UK NEQAS data since 2003.

The B·R·A·H·M·S KRYPTOR instruments are a fully automated random-access immunoassay system which ensures optimal analytical precision as well as maximum economic efficiency. The advantages of the system are the results of its unique TRACE™ (time-resolved amplified cryptate emission) technology – an elegant method based on the basic research for which French chemist Jean-Marie Lehn received the Nobel Prize for chemistry.

The B·R·A·H·M·S KRYPTOR instruments are user-friendly bench-top analyzers that can easily be integrated into any laboratory. Fast processing times mean that results for maternal serum biochemistry markers are available in just 30 minutes.

Due to the highest assay precision, ease of use and ideal suitability of the system for biochemistry laboratories, the B·R·A·H·M·S KRYPTOR analyzers are instruments of choice for prenatal screening in many hospitals and screening laboratories around the world.

The B·R·A·H·M·S KRYPTOR analyzer was introduced in 1999 as the first fully automated immunoassay instrument for prenatal screening. Today there are more than 150 publications covering different aspects of prenatal screening on B·R·A·H·M·S KRYPTOR instruments from the earliest studies by the FMF in the late 1990's when the concept of first trimester screening was introduced, to the latest developments in the area such as screening and the management of pre-eclampsia and other adverse outcome conditions. Current literature review presents selected key publications on different topics.

For information on the B·R·A·H·M·S KRYPTOR analyzers please visit www.thermoscientific.com/kryptor

1. First trimester trisomy screening on B·R·A·H·M·S KRYPTOR instruments from development of the concept till our days

Screening for trisomy 18 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation

Tul N, Spencer K, Noble P, Chan C, Nicolaides K
Prenat Diagn 1999 Nov;19(11):1035-42

Abstract

In a study of 50 cases of trisomy 18 compared with 947 controls we have found the median multiple of the median (MoM) of maternal serum free beta human chorionic gonadotrophin to be significantly decreased (0.281 MoM) in samples collected between the 10th and 14th week of gestation. Similarly, maternal serum pregnancy associated plasma protein A (PAPP-A) levels are also decreased (0.177 MoM), whilst the median nuchal translucency is significantly higher (3.272 MoM). Free beta-hCG MoM was less than the 5th centile of normal in 64 per cent of cases of trisomy 18 and for PAPP-A was less than the 5th centile in 78 per cent of cases. Also, in 78 per cent of cases the nuchal translucency was above the 95th centile. When combined together in a multivariate algorithm with maternal age, we predict that 89 per cent of cases of trisomy 18 could be detected at a 1 per cent false-positive rate.

We conclude that specific trisomy 18 risks should be part of developing risk algorithms combining maternal serum biochemistry and nuchal translucency for use in first trimester screening alongside those for trisomy 21.

A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A

Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH

Ultrasound Obstet Gynecol 1999 Apr;13(4):231-7

Objective

To examine the potential impact of combining maternal age with fetal nuchal translucency thickness and maternal serum free beta-human chorionic gonadotropin (beta-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in screening for trisomy 21 at 10-14 weeks of gestation.

Methods

Maternal serum free beta-hCG and PAPP-A were measured by KRYPTOR, a random access immunoassay analyzer using time-resolved amplified cryptate emission, in 210 singleton pregnancies with trisomy 21 and 946 chromosomally normal controls, matched for maternal age, gestation and sample storage time. In all cases the fetal crown-rump length and nuchal translucency thickness had been measured by ultrasonography at 10-14 weeks of gestation and maternal blood had been obtained at the time of the scan. The distributions (in multiples of the median; MoM) of free beta-hCG and PAPP-A (corrected for maternal weight) and fetal nuchal translucency (NT) were determined in the trisomy 21 group and the controls. Likelihood ratios for the various marker combinations were calculated and these were used together with the age-related risk for trisomy 21 in the first trimester to calculate the expected detection rate of affected pregnancies, at a fixed false-positive rate, in a population with the maternal age distribution of pregnancies in England and Wales.

Results

In a population with the maternal age distribution of pregnancies in England and Wales, it was estimated that, using the combination of maternal age, fetal nuchal translucency thickness and maternal serum free beta-hCG and PAPP-A, the detection of trisomy 21 pregnancies would be 89% at a fixed false-positive rate of 5%. Alternatively, at a fixed detection rate of 70%, the false-positive rate would be 1%. The inclusion of biochemical parameters added an additional 16% to the detection rate obtained using NT and maternal age alone.

KEY FACTS

Rapid diagnostic technology like KRYPTOR, which can provide automated reproducible biochemical measurements within 30 min of obtaining a blood sample, will allow the development of interdisciplinary one-stop clinics for early fetal assessment. Such clinics will be able to deliver improved screening sensitivity, rapidly and more efficiently, leading to reduced patient anxiety and stress.

Accuracy of Down syndrome risks produced in a first-trimester screening programme incorporating fetal nuchal translucency thickness and maternal serum biochemistry

Spencer K

Prenat Diagn 2002 Mar;22(3):244-6

Abstract

Over the past three years approximately 12 000 women have been screened in the first trimester through our OSCAR programme, which utilizes fetal NT and maternal serum free beta-hCG and PAPP-A. During this time 30 cases of Down syndrome were identified either prenatally or postnatally. Using an established procedure the accuracy of predicted risk for Down syndrome was assessed in a population of 30 cases of Down syndrome and 11 758 unaffected pregnancies. The correlation between predicted risk and prevalence of Down syndrome was very high (r=0.9995).

KEY FACTS

It is concluded that risks produced by the Fetal Medicine Foundation combined risk algorithm agree very closely with Down syndrome prevalence and can be used with confidence when counselling women of their risk.

One-stop clinic for assessment of risk for trisomy 21 at 11-14 weeks: a prospective study of 15 030 pregnancies

Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH

Ultrasound Obstet Gynecol 2002 Sep;20(3):219-25

Objective

To evaluate the performance of a one-stop clinic for assessment of risk (OSCAR) for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11-14 weeks of gestation.

Methods

Screening for trisomy 21 was carried out by OSCAR in 15 030 singleton pregnancies with live fetuses at 11-14 weeks. The estimated risk for trisomy 21 was calculated, and the women were counseled regarding this risk and the option of invasive testing or expectant management. Follow-up of the outcome of all pregnancies was carried out. The detection and false-positive rates for different risk cut-offs were calculated.

Results

Fetal NT and maternal serum free beta-hCG and PAPP-A were successfully measured in all cases. Pregnancy outcome, including karyotype results or the birth of a phenotypically normal baby, was obtained from 14 383 cases. The median maternal age of these cases was 34 (range 15-49) years and in 6768 (47.1%) the age was 35 years or greater. The median gestation at screening was 12 (range 11-14) weeks and the median fetal crown-rump length was 64 (range 45-84) mm. The estimated risk for trisomy 21 based on maternal age, fetal NT and maternal serum free beta-hCG and PAPP-A was 1 in 300 or greater in 6.8% (967 of 14 240) normal pregnancies, in 91.5% (75 of 82) of those with trisomy 21 and in 88.5% (54 of 61) of those with other chromosomal defects. For a fixed false-positive rate of 5% the respective detection rates of screening for trisomy 21 by maternal age alone, maternal age and serum free beta-hCG and PAPP-A, maternal age and fetal NT, and by maternal age, fetal NT and maternal serum biochemistry were 30.5%, 59.8%, 79.3% and 90.2%, respectively.

KEY FACTS

Screening for trisomy 21 by a combination of maternal age, fetal NT and maternal serum biochemistry at 11-14 weeks can be provided in an OSCAR setting and is associated with a detection rate of about 90% for a false-positive rate of 5%.

Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks

Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH
Prenat Diagn 2003 Apr;23(4):306-10

Background
Screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free beta-hCG and pregnancy-associated plasma protein-A (PAPP-A) at 11 to 14 weeks of gestation is associated with a detection rate of 90% for a false-positive rate of 5%. Recent evidence suggests that in about 70% of fetuses with trisomy 21, the nasal bone is not visible at the 11th- to 14th-week scan (Cicero et al., 2001). The aim of this study was to examine whether fetal NT thickness and the level of maternal serum biochemical markers is independent of the presence or absence of the nasal bone, and to estimate the performance of a screening test that integrates the two sonographic and the two biochemical markers.

Methods
This was a retrospective case-control study comprising 100 trisomy 21 and 400 chromosomally normal singleton pregnancies at 11 to 14 weeks of gestation. Ultrasound examination was carried out for measurement of fetal NT and assessment of the presence or absence of the fetal nasal bone. Maternal serum free beta-hCG and PAPP-A were measured using the KRYPTOR rapid random-access immunoassay analyser (B·R·A·H·M·S Diagnostica GmbH, Berlin). The distribution of fetal NT, maternal serum free beta-hCG and PAPP-A in trisomy 21 fetuses with absent and present nasal bone was examined.

Results
The nasal bone was absent in 69 and present in 31 of the trisomy 21 fetuses. There were no significant differences in median maternal age, median gestational age, NT delta, free beta-hCG MoM and PAPP-A MoM in trisomy 21 fetuses with and without a visible nasal bone. For a false-positive rate of 5%, it was estimated that screening with the four markers in combination with maternal age would be associated with a detection rate of 97%. For a false-positive rate of 0.5%, the detection rate was 90.5%.

KEY FACTS

An integrated sonographic and biochemical test at 11 to 14 weeks can potentially identify about 90% of trisomy 21 fetuses for a false-positive rate of 0.5%.

Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy

Spencer K, Bindra R, Nicolaides KH
Prenat Diagn 2003 Oct;23(10):851-5

Objective
To assess the suitability of either the log-linear or reciprocal-linear regression procedure for maternal weight correction of biochemical marker MoMs in the first trimester.

Methods
Data from two prospective first-trimester OSCAR screening programmes including 32,010 women with first-trimester maternal serum-free beta-hCG and PAPP-A measured by the KRYPTOR analyser was analysed by regression analysis to provide parameters for the log-linear and reciprocal-linear MoM correction procedures. Assessment was made by goodness of fit to the data. The impact on detection rate and false-positive rate of the different correction procedures was assessed using statistical modelling with biochemical markers alone.

Results
Both log-linear and reciprocal-linear correction were shown to fit the data well. For free beta-hCG, the log-linear procedure was marginally superior to the reciprocal-linear procedure (r2=0.986 v 0.980), whilst for PAPP-A the reciprocal-linear procedure was marginally better (r2=0.991 v 0.985). Log-linear correction reduced the variance for both markers more than did the reciprocal-linear procedure. For free beta-hCG, the sd was reduced from 0.2675 to 0.2605 and for PAPP-A, it was reduced from 0.2545 to 0.2336. Correcting for maternal weight was shown to reduce the population false-positive rate from 7.0 to 6.5%, whilst maintaining the same detection rate at a risk cut-off of 1 in a 100. At individual levels, a two-fold variation in risk was demonstrated depending upon the individual's weight.

KEY FACTS

To provide accurate individual patient-specific risks for trisomy 21, maternal weight must be taken into account and should be a mandatory data item for screening programmes. Maternal weight correction in the first trimester using free beta-hCG and PAPP-A can be best achieved using the log-linear procedure.

Dose dependency between cigarette consumption and reduced maternal serum PAPP-A levels at 11-13+6 weeks of gestation

Kagan KO, Frisova V, Nicolaides KH, and Spencer K
Prenat. Diagn 2007 Sep;27(9):849-53

Objective
To examine whether in smokers there is a significant dose dependency between the number of cigarettes per day and levels of free ss-hCG and pregnancy-associated plasma protein A (PAPP-A) at 11-13(+6) weeks of gestation.

Methods
This was a retrospective analysis of the maternal serum free ss-hCG and PAPP-A levels in relation to the maternal smoking status in 109 263 chromosomally normal singleton pregnancies that had undergone first-trimester screening for Down syndrome by a combination of fetal nuchal translucency thickness and maternal serum biochemistry.

Results
There were 95 287 nonsmokers and 13 976 cigarette smokers. The overall median PAPP-A MoM among cigarette smokers was 0.827, which was 19.6% lower than the value of 1.029 in nonsmokers ($p < 0.0001$ for log(10) MoM). The respective values for beta-hCG MoM were 1.003 for smokers and 1.035 for nonsmokers ($p < 0.0001$ for log(10) MoM) which corresponds to a reduction of 3.1%. There was a significant inverse relationship between the number of cigarettes per day and the level of PAPP-A MoM ($r = 0.989$, $p < 0.0001$) but not the level of free beta-hCG MoM ($r = 0.733$; $p = 0.098$). Using a statistical modeling approach we found that the screen-positive rate when correcting the PAPP-A MoM by an all or nil smoking factor was reduced by only 0.1% (3.75 vs 3.85%) when compared to correcting with a factor related to the smoking dose per day.

KEY FACTS

In first-trimester screening for Down syndrome by maternal serum PAPP-A and free beta-hCG the impact of correcting for the dose dependent rather than the all or nil effect of smoking is marginal. However, a dose dependent correction improves the accuracy of the individual patient-specific risk.

A mixture model of nuchal translucency thickness in screening for chromosomal defects

Wright D, Kagan KO, Molina FS, Gazzoni A, and Nicolaides KH

Ultrasound Obstet Gynecol 2008 Apr;31(4):376-83

Objective

Fetal nuchal translucency (NT) thickness increases with crown–rump length (CRL). In screening for chromosomal defects patient-specific risks are derived by multiplying the apriori maternal age-related risk by a likelihood ratio, determined from the deviation of the measured NT from the expected median. To quantify this deviation the measured NT is either subtracted (delta NT) or divided by the expected median (multiple of the median method, MoM). This study examines the validity of these methods.

Methods

NT was prospectively measured at 11+0 to 13+6 weeks in screening for chromosomal defects. The distribution of NT in euploid and chromosomally abnormal fetuses was examined.

Results

There were 37078 normal pregnancies and 264 with trisomy 21, 81 with trisomy 18, 38 with trisomy 13 and 27 with Turner syndrome. We found that firstly, contrary to the assumption underlying the delta NT method, the distribution of delta NT changes with CRL and secondly, contrary to the assumption underlying the MoM method the distribution of NT was not Gaussian. Fetal NT followed two distributions, one that was dependent on CRL and one that was independent of CRL. The distribution in which NT increases with CRL was observed in about 95% of euploid fetuses, 5% with trisomy 21, 30% with trisomy 18, 15% with trisomy 13 and 10% with Turner syndrome. The median CRL-independent NT was 2.0 mm for the euploid group and 3.4, 5.5, 4.0 and 7.8 mm for trisomies 21, 18, 13 and Turner syndrome, respectively.

KEY FACTS

The NT thickness in chromosomally normal and abnormal fetuses follows a mixture of a gestation-dependent and gestation-independent distribution.

Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study

Ekelund CK, Jørgensen FS, Petersen OB, Sundberg K, Tabor A, Danish Fetal Medicine Research Group

BMJ 2008 Nov 27;337:a2547

Objective
To evaluate the impact of a screening strategy in the first trimester, introduced in Denmark during 2004-6, on the number of infants born with Down's syndrome and the number of chorionic villus samplings and amniocenteses, and to determine detection and false positive rates in the screened population in 2005 and 2006.

Design
Population based cohort study.

Setting
19 Danish departments of gynaecology and obstetrics and a central cytogenetic registry 2000-7.

Participants
65 000 pregnancies per year.

Main outcome measures
The primary outcomes measured were number of fetuses and newborn infants with Down's syndrome diagnosed prenatally and postnatally and number of chorionic villus samplings and amniocenteses carried out. Secondary outcomes measured were number of women screened in 2005 and 2006, screen positive rate, and information on screening in 2005 and 2006 for infants with a postnatal diagnosis of Down's syndrome.

Results
The number of infants born with Down's syndrome decreased from 55-65 per year during 2000-4 to 31 in 2005 and 32 in 2006. The total number of chorionic villus samplings and amniocenteses carried out decreased from 7524 in 2000 to 3510 in 2006. The detection rate in the screened population in 2005 was 86% (95% confidence interval 79% to 92%) and in 2006 was 93% (87% to 97%). The corresponding false positive rates were 3.9% (3.7% to 4.1%) and 3.3% (3.1% to 3.4%).

KEY FACTS

The introduction of a combined risk assessment during the first trimester at a national level in Denmark halved the number of infants born with Down's syndrome. The strategy also resulted in a sharp decline in the number of chorionic villus samplings and amniocenteses carried out, even before full implementation of the policy.

Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free β-hCG and pregnancy-associated plasma protein-A

Kagan KO, Wright D, Valencia C, Maiz N, and Nicolaides KH
J Hum Reprod 2008;23(9):1968-75

Background
A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13. Our objective was to examine the performance of first-trimester screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency (NT) thickness, fetal heart rate (FHR) and maternal serum-free β-hCG and pregnancy-associated plasma protein-A (PAPP-A).

Methods
Prospective screening for trisomy 21 by maternal age, fetal NT, free beta-hCG and PAPP-A at 11(+0)-13(+6) weeks in singleton pregnancies, including 56 376 normal cases, 395 with trisomy 21,122 with trisomy 18 and 61 with trisomy 13. Risk algorithms were developed for the calculation of patient-specific risks for each of the three trisomies based on maternal age, NT, FHR, free beta-hCG and PAPP-A. Detection (DR) and false positive rates (FPR) were calculated and adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000-2002.

Results
The DR and FPR were 90% and 3%, respectively, for trisomy 21, 91% and 0.2% for trisomy 18 and 87% and 0.2% for trisomy 13. When screen positivity was defined by an FPR of 3% on the risk for trisomy 21 in conjunction with an FPR of 0.2% on the maximum of the risks for trisomies 13 and 18, the overall FPR was 3.1% and the DRs of trisomies 21, 18 and 13 were 91%, 97% and 94%, respectively.

KEY FACTS

As a side effect of first-trimester screening for trisomy 21, approximately 95% of trisomy 13 and 18 fetuses can be detected with an 0.1% increase in the FPR.

First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: Impact of maternal and pregnancy characteristics

Kagan KO, Wright D, Spencer K, Molina FS, and Nicolaides KH

Ultrasound Obstet Gynecol 2008;31(5):493-502

Objective

To use multiple regression analysis to define the contribution of maternal variables that influence the measured concentration of free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), and the interaction between these covariates, in first-trimester biochemical screening for trisomy 21

Methods

This was a multicenter study of prospective screening for trisomy 21 by a combination of fetal nuchal translucency thickness, and maternal serum free β-hCG and PAPP-A at 11+0to 13+6 weeks of gestation. In the pregnancies subsequently found to have trisomy 21 and in those with no obvious chromosomal abnormality, we used multiple regression analysis to account for pregnancy characteristics that influence the measured concentrations of free β-hCG and PAPP-A. We fitted Gaussian distributions to the distribution of log multiples of the median (MoM) values in trisomy 21 and in unaffected pregnancies.

Results

There were 491 cases of trisomy 21 and 96803 chromosomally normal pregnancies. Compared with values in Caucasian women, those who were parous, non-smokers and those who conceived spontaneously, PAPP-A was 57% higher in women of Afro-Caribbean origin, 3% higher in South Asians, 9% higher in East Asians, 2% higher in nulliparous women, 17% lower in smokers and 10% lower in those conceiving by in-vitro fertilization (IVF). Free β-hCG was12%higher in women of Afro-Caribbean origin, 9%lower in South Asians, 8% higher in East Asians, 2% higher in nulliparous women, 4% lower in smokers and 9% higher in those conceiving by IVF. In screening for trisomy 21 by maternal age and serum free β-hCG and PAPP-A the estimated detection rate was 65% for a false-positive rate of 5%.

KEY FACTS

In first-trimester biochemical screening for trisomy 21 it is essential to adjust the measured values of free β-hCG and PAPP-A for maternal and pregnancy characteristics.

Medians and correction factors for biochemical and ultrasound markers in Chinese women undergoing first-trimester screening for trisomy 21

Sahota DS, Leung TY, Fung TY, Chan LW, Law LW, Lau TK
Ultrasound Obstet Gynecol 2009 Apr;33(4):387-93

Objective
To establish normative values and distribution parameters of first-trimester maternal serum free beta-human chorionic gonadotropin (beta-hCG), pregnancy-associated plasma protein-A (PAPP-A) and fetal nuchal translucency (NT) thickness in Chinese women and to examine the effects of covariates on their levels.

Methods
Maternal serum free beta-hCG, PAPP-A and fetal NT were measured in 9762 women presenting for first-trimester combined screening for Down syndrome at 11 to 14 weeks of gestation. Individuals' markers were converted to multiples of the median (MoM) using expected medians estimated by performing a weighted regression analysis. Multivariate regression analysis was performed to assess the influence of maternal weight, parity, ethnicity, chorionicity in twin pregnancies, smoking, insulin-dependent diabetes and mode of conception on individual marker MoM levels.

Results
Both free beta-hCG and PAPP-A median values demonstrated an exponential relationship with gestational age in days. Multivariate regression analysis indicated that free beta-hCG MoM was statistically significantly dependent on maternal weight ($P < 0.0001$) and chorionicity in twin pregnancy (both monochorionic and dichorionic $P < 0.0001$), that PAPP-A MoM was dependent on maternal weight ($P < 0.0001$), parity ($P < 0.0001$), chorionicity in twin pregnancy (both monochorionic and dichorionic $P < 0.0001$) and mode of conception ($P = 0.002$), and that fetal NT-MoM was dependent on maternal weight ($P = 0.0006$) and mode of conception ($P = 0.012$).

KEY FACTS

Normative values have been generated to allow conversion of NT, free beta-hCG and PAPP-A to their MoM equivalents and correction factors have been determined to adjust for maternal and pregnancy characteristics for use in ethnic Chinese women undergoing first-trimester screening for aneuploidy.

First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI

Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A
Ultrasound Obstet Gynecol 2009 Jan;33(1):8-17

Objective
To determine the levels of first-trimester screening markers and to assess the false-positive rate for first-trimester combined screening for Down syndrome in a large national population of women pregnant after assisted reproductive technology (ART), in order to decide whether or not to correct risk calculation for mode of conception.

Methods
A national prospective cohort study of 1000 pregnancies achieved after ART was compared with a control group of 2543 pregnancies conceived spontaneously. All women completed a first-trimester combined screening program. Risk calculation was performed retrospectively based on the screening parameters to avoid bias due to the use of different algorithms of risk calculation.

Results
In chromosomally normal pregnancies conceived after in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), the pregnancy-associated plasma protein-A multiples of the median value was significantly decreased when compared with that of pregnancies conceived spontaneously (0.78 and 0.79 vs. 0.98), while there was no difference in the group treated by frozen embryo replacement. There was no difference in the level of free beta-human chorionic gonadotropin between groups. The median nuchal translucency thickness was smaller in the overall ART group compared with controls. The false-positive rate of first-trimester combined screening in the overall ART group, adjusted for maternal age, was significantly higher when compared with controls (9.0% vs. 6.0%).

KEY FACTS

It seems advisable to use a population of IVF/ICSI pregnancies to establish median curves for the first-trimester serum screening parameters and perhaps also for nuchal translucency thickness. However, care must be taken, as different ART treatment methods and aspects of medical history seem to alter the screening parameters in different ways.

First-trimester combined screening for trisomy 21 at 7-14 weeks' gestation

Wright D, Spencer K, Kagan K, Tørring N, Petersen OB, Christou A, Kallikas J, and Nicolaides KH

Ultrasound Obstet Gynecol 2010;36(4):404-11

Objective

To establish an algorithm for first-trimester combined screening for trisomy 21 with biochemical testing from 7 to 14 weeks' gestation and ultrasound testing at 11–13 weeks.

Methods

This was a multicenter study of 886 pregnancies with trisomy 21 and 222 475 unaffected pregnancies with measurements of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 7–14 weeks' gestation. Multiple regression modeling of log-transformed marker values was used to produce log multiples of the median (MoM) values for PAPP-A and free β-hCG. The models included terms for the center attended and the machine used for biochemical analysis, gestational age, maternal racial origin, maternal weight, smoking status and method of conception. Bivariate Gaussian distributions were fitted to log MoM PAPP-A and log MoM free β-hCG in trisomy 21 and in unaffected pregnancies. In each case the patient-specific risk for trisomy 21 was estimated by multiplying the individual maternal age-related risk with the likelihood ratio (LR) for fetal nuchal translucency (NT) according to the mixture model and the combined LR for maternal serum free β-hCG and PAPP-A. Estimates of detection rates for trisomy 21 and false-positive rates were calculated for combined screening with measurements of NT at 12 weeks together with measurements of free β-hCG and PAPP-A from 8 to 13 weeks.

Results

In trisomy 21 pregnancies the mean log MoM free β-hCG increased linearly with gestation between 7 and 14 weeks, whereas the relation between log MoM PAPP-A and gestation was fitted by a quadratic equation such that the maximum separation between trisomy 21 and unaffected pregnancies occurs at 9–10 weeks. At a false-positive rate of 3% the detection rate of combined screening at 12 weeks was 86% and this increased to 90% by biochemical testing at 9 weeks and ultrasound scanning at 12 weeks. The detection rate increased to 92% by measuring PAPP-A at 9 weeks and free β-hCG at the time of the scan at 12 weeks.

KEY FACTS

The performance of first-trimester biochemical screening for trisomy 21 is best at 9–10 weeks rather than at 7–8 or 11–14 weeks.

First-trimester screening for trisomy 21 with adjustment for biochemical results of previous pregnancies

Wright D, Syngelaki A, Birdir C, Bedei I, and Nicolaides KH
Fetal Diagn Ther 2011;30(3):194-202

Objective
To investigate the effect of associations in serum free β-hCG and PAPP-A between successive pregnancies on the performance of screening for trisomy 21 at 11-13 weeks' gestation.

Methods
In 8,499 women with two consecutive pregnancies, including 49 women with fetal trisomy 21 in the second pregnancy, the correlation in serum free β-hCG multiples of the median (MoM) and PAPP-A MoM between pregnancies was determined, and the effects of correcting for the correlation on the performance of screening was estimated.

Results
There were significant associations between pregnancies in free β-hCG MoM (r = 0.4435) and PAPP-A MoM (r = 0.4796). In screening by maternal age and biochemistry at a risk cutoff of 1 in 100, in the second pregnancies the false-positive rate was 35.5% for those with screen-positive results in the first pregnancy, and this was reduced to 17.1% after adjustment for the results of the first pregnancy. Similarly, in women with screen-negative results in the first pregnancy, adjustment for the results improved the detection rate in the second pregnancy from 66.7 to 81.2%.

KEY FACTS

In screening for trisomy 21, adjustment for the biochemical findings in a previous pregnancy has major effects on individual patient-specific risks, increases the detection rate and reduces the false-positive rate.

A reassessment of biochemical marker distributions in trisomy 21-affected and unaffected twin pregnancies in the first trimester

Madsen HN, Ball S, Wright D, Tørring N, Petersen OB, Nicolaides KH, Spencer K

Ultrasound Obstet Gynecol 2011 Jan;37(1):38-47

Objective

To estimate the difference between levels of the two biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and maternal serum free β-human chorionic gonadotropin (free β-hCG) in twin pregnancies relative to singleton pregnancies and establish an improved screening procedure for chromosomal abnormalities such as trisomy 21 in twin pregnancies.

Methods

4843 unaffected and 47 trisomy 21-affected twin pregnancies were included in the study. Chorionicity-specific medians were generated for PAPP-A and free β-hCG from gestational ages 8 to 14 weeks. Multiple of the median values for each of the biochemical markers were calculated. Detection rates and false-positive rates were estimated for screening tests incorporating nuchal translucency and maternal age, with and without biochemistry.

Results

Medians for the two biochemical markers for monochorionic and dichorionic twins in unaffected pregnancies show a gestational age-specific increase relative to singleton medians. Allowing for gestation and chorionicity, twin pregnancies affected with trisomy 21 had higher levels of free β-hCG and lower levels of PAPP-A. Adding biochemistry into the risk assessment using a fixed risk cut-off of 1 in 100 increased the detection rate for fetal trisomy 21 in dizygotic twin pregnancies from 78 to 90%, and decreased the false-positive rate from 8.0 to 5.9%.

KEY FACTS

Generation of chorionicity-specific medians for the biochemical markers and their use in risk assessment can improve the performance of first-trimester screening for chromosomal abnormalities in twins to a level comparable with that in singleton pregnancies.

Prospective study evaluating performance of first-trimester combined screening for trisomy 21 using repeat sampling of maternal serum markers PAPP-A and free β-hCG

Ekelund C, Wright D, Ball S, Kirkegaard I, Nørgaard P, Sørensen S, Friis-Hansen L, Jørgensen FS, Tørring N, Bech BH, Petersen OB, Tabor A
Ultrasound Obstet Gynecol 2012 Sep;40(3):276-81

Objective
To prospectively evaluate the performance of first-trimester combined screening for trisomy 21 using the biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (free β-hCG) obtained before and at the time of the nuchal translucency (NT) scan.

Methods
Three fetal medicine departments in Denmark participated in the study. Screening for trisomy 21 was set up as a two-step approach with blood sampling performed before the NT scan (early sample) and again at the time of the NT scan (late sample). PAPP-A and free β-hCG were measured on both the early and late samples. Age-standardized detection and false-positive rates for different screening protocols were calculated.

Results
We collected two blood samples in 27 pregnancies affected by trisomy 21 and in 3891 control pregnancies. The early samples were taken between gestational ages 8 + 0 and 13 + 6 weeks, and the late samples between 11 + 3 and 14 + 6 weeks. The median interval between the samples was 17 (range, 1-40) days. We found a significantly better estimated screening performance when using early sampling vs late sampling ($P < 0.05$). With a risk cut-off of 1 in 100, at the time of the risk assessment the estimated detection and false-positive rates when using the early sample were 91% (95% CI, 81-98%) and 1.6% (95% CI, 1.3-2.0%), respectively. For fixed false-positive rates the highest detection rates were achieved using both blood samples. When comparing early sampling vs double sampling there was no significant difference in screening performance.

KEY FACTS

In combined first-trimester screening for trisomy 21, use of early sampling with measurement of PAPP-A and free β-hCG before the time of the NT scan can optimize screening performance. Using maternal serum markers obtained both before and at the time of the NT scan has the potential to further improve performance, but larger studies are needed to confirm this potential.

Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks' gestation and the combined test at 11-13 weeks

Quezada MS, Gil MM, Francisco C, Oròsz G, Nicolaides KH

Ultrasound Obstet Gynecol 2015 Jan;45(1):36-41

Objective

To examine in a general population the performance of cell-free DNA (cfDNA) testing for trisomies 21, 18 and 13 at 10-11 weeks' gestation and compare it to that of the combined test at 11-13 weeks.

Method

In 2905 singleton pregnancies, prospective screening for trisomies was performed by chromosome-selective sequencing of cfDNA in maternal blood at 10-11 weeks' gestation and by the combined test at 11-13 weeks' gestation.

Results

Median maternal age of the study population was 36.9 (range, 20.4-51.9) years. Results from cfDNA analysis were provided for 2851 (98.1%) cases and these were available within 14 days from sampling in 2848 (98.0%) cases. The trisomic status of the pregnancies was determined by prenatal or postnatal karyotyping or clinical examination of the neonates. Of the 2785 pregnancies with a cfDNA result and known trisomic status, cfDNA testing correctly identified all 32 cases with trisomy 21, nine of 10 with trisomy 18 and two of five with trisomy 13, with false-positive rates of 0.04%, 0.19% and 0.07%, respectively. In cases with discordant results between cfDNA testing and fetal karyotype, the median fetal fraction was lower than in those with concordant results (6% vs 11%). Using the combined test, the estimated risk for trisomy 21 was ≥ 1/100 in all trisomic cases and in 4.4% of the non-trisomic pregnancies.

KEY FACTS

The performance of first-trimester cfDNA testing for trisomies 21 and 18 in the general population is similar to that in high-risk pregnancies. Most false-positive and false-negative results from cfDNA testing could be avoided if the a priori risk from the combined test is taken into account in the interpretation of individual risk.

Pregnancy outcomes regarding maternal serum AFP value in second trimester screening

Bartkute K, Balsyte D, Wisser J, Kurmanavicius J
J Perinat Med 2017 Oct 26;45(7):817-820

The aim of this study was to evaluate the predictive value of α-fetoprotein in maternal serum (MS-AFP) as a marker for diverse pregnancy outcomes.

Methods

The study was based on pregnancy and delivery data from 5520 women between 1999 and 2014 at University Hospital of Zurich (UHZ).

Inclusion criteria

Both MS-AFP and pregnancy outcome were known for the same pregnancy. Pregnancy outcomes and characteristics such as fetal malformation, intrauterine fetal death (IUFD) and intrauterine growth retardation as well as maternal age, weight before pregnancy, gestational age (GA) at delivery, newborn weight, length and head circumference were analyzed with respect to the MS-AFP value. MS-AFP value was categorized into three groups: elevated MS-AFP >2.5 multiples of the median (MoM), normal 0.5-2.49 MoM and decreased <0.5 MoM.

Results

Newborn weight (g) and length (cm) were significantly lower in the elevated MS-AFP ($P<0.001$) group, and infants had 1 week lower GA at delivery ($P<0.05$). In the group of elevated MS-AFP (n=46), 26.1% of pregnancies were significantly related to adverse pregnancy outcomes, such as fetal malformations, fetuses small for gestational age (SGA) and IUFD. Adverse pregnancy outcomes of 5.6% were registered in the group of normal MS-AFP and 7.3% in the group of low MS-AFP ($P<0.05$).

KEY FACTS

MS-AFP level in the second trimester is still an important indicator of fetal surface malformations; however, ultrasound still outweighs as a screening method. Nevertheless, pregnant women with elevated MS-AFP values and with no sonographically detected fetal malformations should additionally receive the third trimester ultrasound examination to exclude other possible complications of pregnancy.

2. Pre-eclampsia and other adverse outcome conditions

Use of the combined first-trimester screen result and low PAPP-A to predict risk of adverse fetal outcomes

Barrett SL, Bower C, Hadlow NC
Prenat Diagn 2008 Jan;28(1):28-35

Objective

To investigate associations between combined first-trimester screen result, pregnancy associated plasma protein-A (PAPP-A) level and adverse fetal outcomes in women.

Methods

Pregnancy outcomes for 10,273 women participating in a community based first-trimester screening (FTS) programme in Western Australia were ascertained by record linkage to birth and birth defect databases. A first-trimester risk cut-off of > or = 1 in 300 defined screen positive women.

Results

Screen positive pregnancies were more likely to have Down syndrome and birth defects (chromosomal or nonchromosomal) than screen negative pregnancies. When birth defects were excluded, screen positive pregnancies were at increased risk of pregnancy loss, low birth weight and preterm birth. Pregnancies with low PAPP-A (< or =0.3 multiples of the median (MoM)) had higher risk of chromosomal abnormality, birth defect, preterm birth, low birth weight, or pregnancy loss, compared to those with PAPP-A > 0.3 MoM. In pregnancies without birth defects, low PAPP-A was a stronger predictor of preterm birth, low birth weight or pregnancy loss than a screen positive result.

KEY FACTS

Women with positive screen or low PAPP-A were at increased risk for some adverse fetal outcomes. The sensitivity of these parameters was insufficient to support primary screening, but increased surveillance during pregnancy may be appropriate.

Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler and PAPP-A at 11-14 weeks' gestation

Pilalis A, Souka AP, Antsaklis P, Daskalakis G, Papantoniou N, Mesogitis S, Antsaklis A

Ultrasound Obstet Gynecol 2007 Feb;29(2):135-40

Objective

To assess the role of maternal demographic characteristics, uterine artery Doppler velocimetry, maternal serum pregnancy-associated plasma protein-A (PAPP-A) and their combination in screening for pre-eclampsia and small-for-gestational age (SGA) fetuses at 11-14 weeks.

Methods

This was a prospective study of 878 consecutive women presenting for a routine prenatal ultrasound examination at 11-14 weeks. Pulsed wave Doppler was then used to obtain uterine artery flow velocity waveforms and the mean pulsatility index (PI) of the uterine arteries was calculated. Maternal serum samples for PAPP-A were assayed. Along with maternal history, these measurements were compared in their ability to predict adverse outcome, defined as pre-eclampsia and/or SGA and/or placental abruption.

Results

Mean uterine artery PI > or = 95(th) centile and PAPP-A < or = 10(th) centile each predicted 23% of the women that developed pre-eclampsia and 43% of cases of placental abruption. For SGA < or = 5(th) centile, mean uterine artery PI > or = 95(th) centile predicted 23% of cases and PAPP-A < or = 10(th) centile predicted 34%. Independent predictors for subsequent development of pre-eclampsia were increased mean uterine artery PI > or = 95(th) centile (OR, 2.76; 95% CI, 1.11-6.81) and maternal history of pre-eclampsia/hypertension (OR, 50.54; 95% CI, 10.52-242.73). The predicting factors for SGA < or = 5(th) centile were increased mean uterine artery PI > or = 95(th) centile (OR, 2.0; 95% CI, 1.07-3.74) and low PAPP-A (OR, 0.43; 95% CI, 0.20-0.93). Increased uterine artery PI was the only independent factor in the prediction of placental abruption (OR, 8.49; 95% CI, 2.78-25.94). The combination of uterine artery PI and maternal history of pre-eclampsia/hypertension was better than was using uterine artery Doppler alone in predicting pre-eclampsia. Similarly, for the prediction of SGA < or = 5(th) centile, combining uterine artery Doppler and maternal serum PAPP-A was better than was uterine artery Doppler alone. In both cases, the difference approached statistical significance.

KEY FACTS

The combination of maternal history with abnormal uterine artery Doppler and low PAPP-A level at 11-14 weeks achieves better results than does either test alone in the prediction of pre-eclampsia and SGA.

First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of preterm or early preterm delivery

Spencer K, Cowans NJ, Molina F, Kagan KO, Nicolaides KH
Ultrasound Obstet Gynecol 2008 Feb;31(2):147-52

Objective
To examine the clinical utility of the first-trimester markers of aneuploidy in their ability to predict preterm delivery.

Methods
We examined 54 722 singleton pregnancies with no chromosomal abnormality and with complete outcome data that had undergone screening for trisomy 21 by a combination of fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (beta-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 and 13 + 6 weeks' gestation. The biochemical markers were converted to multiples of the median (MoM) of the expected normal median for a pregnancy of the same gestation and the measurements of fetal NT were expressed as the difference (delta) from the normal median NT for crown-rump length. The association between free beta-hCG, PAPP-A and delta NT and the incidence of preterm delivery before 37 weeks or early preterm delivery before 34 weeks was assessed by comparing the relative incidence at a number of MoM or delta NT cut-offs and at various centile cut-offs. At various marker levels the likelihood ratios (LR) for preterm delivery and early preterm delivery were also calculated after excluding other adverse pregnancy complications.

Results
The risk of preterm delivery increased with decreasing maternal serum PAPP-A. In the 3132 cases delivering before 37 weeks the PAPP-A MoM was 0.91 and in the 1060 cases delivering before 34 weeks the PAPP-A MoM was 0.90. At the 5th centile of the normal outcome group for PAPP-A (0.415 MoM) the odds ratios for delivery before 37 weeks and before 34 weeks were 1.92 and 2.35, respectively. The respective values for the 5th centile of free beta-hCG (0.41 MoM) were 1.18 and 1.08 and for the 95th centile of delta NT they were 0.91 and 0.77, respectively.

KEY FACTS

Low levels of maternal serum PAPP-A are associated, in the absence of an abnormal karyotype, with an increased risk of preterm or early preterm delivery. The LR profiles provided at various levels of PAPP-A may be of some help in counseling women with such results and may raise awareness among healthcare professionals for increased surveillance in such cases.

First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses

Spencer K, Cowans NJ, Avgidou K, Molina F, Nicolaides KH

Ultrasound Obstet Gynecol 2008 Jan;31(1):15-9

Objective

To examine the clinical utility of the first-trimester biochemical markers of aneuploidy in their ability to predict subsequent delivery of a small-for-gestational age (SGA) infant.

Methods

We examined singleton pregnancies with no chromosomal abnormality and with complete outcome data that had undergone screening for trisomy 21 by a combination of fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (beta-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 and 13 + 6 weeks' gestation. The biochemical markers were converted to multiples of the expected normal median (MoM) for a pregnancy of the same gestation. The association between free beta-hCG and PAPP-A and the incidence of SGA were assessed by comparing the relative incidence at MoM cut-offs and birth-weight centile cut-offs. At various marker levels the likelihood ratios (LR) for SGA were also calculated after excluding other adverse pregnancy complications.

Results

There were 46,262 pregnancies resulting in live births with birth weight at or above the 10(th) centile, and 3,539 below the 10(th) centile for gestation (SGA). There was a significant inverse association between the risk for SGA and maternal serum PAPP-A MoM but not free beta-hCG MoM. At the 5(th) centile of the normal outcome group for PAPP-A (0.415 MoM) the odds ratios for SGA below the 10(th), 5(th) and 3(rd) centiles of normal were 2.70, 3.21 and 3.66 and the respective detection rates for SGA were 12.0%, 14.0% and 16.0%.

KEY FACTS

Low levels of maternal serum PAPP-A are associated, in the absence of an abnormal karyotype, with an increased risk for subsequent delivery of an SGA infant.

Early fetal growth, PAPP-A and free β-hCG in relation to risk of delivering a small-for-gestational age infant

Kirkegaard I, Henriksen TB, Uldbjerg N

Ultrasound Obstet Gynecol 2011 Mar;37(3):341-7

Objective

To examine early fetal growth, pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) in relation to the risk of delivering a small-for-gestational age (SGA) infant.

Methods

Included in the study were 9450 singleton pregnant women who attended the prenatal screening program at Aarhus University Hospital, Denmark, between January 2005 and December 2007. Maternal serum levels of PAPP-A and free β-hCG were measured between gestational weeks 8 and 13. Two ultrasound examinations were performed, the first at 11-13 weeks and the second at 18-22 weeks, from which gestational age was estimated based on crown-rump length and biparietal diameter, respectively. Early fetal growth was expressed as an index: the ratio between the estimated number of days from the first to the second scan and the actual calendar time elapsed in days. SGA was defined as birth weight < 5(th) centile for gestational age, and the risk of SGA was evaluated according to different cut-offs of the early fetal growth index and the serum markers.

Results

PAPP-A < 0.4 MoM combined with an early fetal growth index < 10(th) centile resulted in an increased risk of SGA (odds ratio (OR), 5.8; 95% CI, 2.7-12.7). Low PAPP-A, low free β-hCG and slow early fetal growth were statistically, independently associated with SGA, and the association between free β-hCG < 0.3 MoM and SGA was as strong as that between PAPP-A < 0.3 MoM and SGA (OR, 3.1 and 3.0, respectively).

KEY FACTS

The combination of slow early fetal growth and low PAPP-A resulted in a nearly six-fold increased risk of delivery of an SGA infant. These findings might improve our chances of early identification of fetuses at increased risk of growth restriction.

Maternal Serum PlGF Isoforms 1 and 2 at 11-13 Weeks' Gestation in Normal and Pathological Pregnancies

Nucci M, Poon LC, Demirdjian G, Darbouret B, Nicolaides KH

Fetal Diagn Ther 2014;36(2):106-16

Rationale

To compare the maternal serum concentration of PlGF-1 and PlGF-2 at 11-13 weeks' gestation in normal pregnancies and in those complicated by pre-eclampsia, delivery of small for gestational age neonates and fetal trisomies 21, 18 and 13.

Methods

Serum PlGF-1 and PlGF-2 were measured in 270 pathological pregnancies (PE, n = 80; SGA, n = 80; trisomy 21, n = 44; trisomy 18, n = 38; trisomy 13, n = 28) and 590 normal controls. The values were expressed as multiple of the median after adjustment for maternal characteristics and corrected for adverse pregnancy outcomes and the median MoM values in each pathological pregnancy were compared to the normal group.

Results

There were significant contributions to PlGF-1 and PlGF-2 from gestational age, smoking and racial origin. In addition, there were significant contributions to PlGF-1 from parity and method of conception.

The median MoM of PlGF-1 and PlGF-2 was significantly decreased in

- PE (0.783 and 0.916 MoM)
- SGA (0.891 and 0.851 MoM)
- trisomy 21 (0.609 and 0.749 MoM)
- trisomy 18 (0.529 and 0.730 MoM)
- trisomy 13 (0.373 and 0.699 MoM)

KEY FACTS

In pathological pregnancies, except SGA, the decrease in serum PlGF-1 at 11-13 weeks' gestation is more marked than the decrease in PlGF-2.

Analytical evaluation of the novel soluble fms-like tyrosine kinase 1 and placental growth factor assays for the diagnosis of pre-eclampsia

van Helden J, Weiskirchen R

Clin Biochem 2015 Nov;48(16-17):1113-9

Objective

Performance evaluation of the novel B·R·A·H·M·S KRYPTOR soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) assays.

Design and Methods

Intra- and inter-assay imprecision, functional sensitivity, linearity in dilution, method comparison, and diagnostic capacity were evaluated.

Results

Intra-assay coefficient of variations (CVs) were between 1.1% and 5.3% and inter-assay CVs between 3.9% and 11.1%. Functional sensitivity was 6.7ng/L for PlGF and 34ng/L for sFlt-1, respectively. The linearity in dilution was excellent (r>0.995) in the assay-specific relevant range of concentration.

The KRYPTOR assay correlated well with the Elecsys sFlt-1 (r=0.996), Elecsys PlGF (r=0.990) and the Elecsys sFlt-1/PlGF ratio (r=0.947) with partially high mean bias values. The optimal cut points for diagnosis of pre-eclampsia were calculated for KRYPTOR assays at: 60.5ng/L (PlGF), 4725ng/L (sFlt-1), and 99.2 (sFlt-1/PlGF ratio) which were different with the corresponding Elecsys cut points.

Nevertheless, the sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), and areas under the curves (AUCs) were completely comparable in both assay platforms, even when applying the standard cut-off of 85 for sFlt-1/PlGF ratio or gestational age specific "rule in-rule-out" cut-offs for early and late onset pre-eclampsia.

KEY FACTS

The new B·R·A·H·M·S KRYPTOR sFlt-1 and PlGF immunoassay show excellent precision and reliability. The assay results and the diagnostic capacity were highly comparable to established fully automated immunoassays (Elecsys). Hence, sFlt-1/PlGF ratio generated on KRYPTOR immunoassay platform should be suitable for diagnosing pre-eclampsia in clinical routine laboratory.

Diagnosis of pre-eclampsia with soluble Fms-like tyrosine kinase 1/placental growth factor ratio: an inter-assay comparison

Andersen LB, Frederiksen-Møller B, Work Havelund K, Dechend R, Jørgensen JS, Jensen BL, Nielsen J, Lykkedegn S, Barington T, Christesen HT

J Am Soc Hypertens 2015 Feb;9(2):86-96

Abstract

The angiogenic factor ratio soluble Fms-kinase 1 (sFlt-1)/placental growth factor (PlGF) is a novel diagnostic tool for pre-eclampsia. We compared the efficacy of the KRYPTOR (B·R·A·H·M·S) automated assays for sFlt-1 and PlGF with the Elecsys (Roche) assays in a routine clinical setting. Pre-eclamptic women (n = 39) were included shortly after the time of diagnosis. Normotensive control pregnancies were matched by gestational age (n = 76).

The KRYPTOR assays performed comparably or superior to Elecsys (sFlt-1/PlGF area under the curve 0.746 versus 0.735; P = .09; for non-obese 0.820 versus 0.805, P = .047). For early-onset pre-eclampsia, KRYPTOR area under the curve increased to 0.929 with a 100% specificity for pre-eclampsia at cut-off 85 and an 88.9% sensitivity for pre-eclampsia at cut-off 33. For women with pre-eclampsia and preterm delivery or Hemolysis, Elevated Liver enzymes, Low Platelet count (HELLP) syndrome, the KRYPTOR sFlt-1/PlGF ratio was manifold increased (P < .01). The sFlt-1/PlGF ratio proved especially useful in early-onset pre-eclampsia, pre-eclampsia with preterm delivery or HELLP, and among non-obese women.

KRYPTOR-automated angiogenic factor assays and risk of pre-eclampsia-related adverse outcomes

Salahuddin S, Wenger JB, Zhang D, Thadhani R, Karumanchi SA, Rana S
Hypertens Pregnancy 2016 Aug;35(3):330-45

Objective
To evaluate KRYPTOR assays for circulating soluble fms-like tyrosine kinase-1 (sFlt1) and placental growth factor (PlGF) in risk assessment of adverse outcomes in women with suspected pre-eclampsia.

Methods
We studied 412 women carrying a singleton pregnancy from a previous study cohort who were evaluated for suspected pre-eclampsia. Another 434 nonpre-eclamptic patients with plasma samples drawn throughout pregnancy were used to derive normative data. Plasma sFlt1 and PlGF levels were measured on the automated KRYPTOR platform and evaluated for prediction of adverse maternal and perinatal outcomes within 2 weeks. Normative values were used to create a ratio of markers and these values were reported as multiples of median (MoM) for women with and without adverse outcomes. The KRYPTOR assay results were also compared with previously reported measurements obtained using the automated Elecsys platform.

Results
Among participants presenting at <34 weeks (N = 110), patients with subsequent adverse outcome had higher sFlt1, lower PlGF, and higher sFlt1/PlGF ratio compared with women without adverse outcomes: the median (25th, 75th centile) sFlt1 (pg/ml), 9030 (3197, 12,140) versus 1976 (1248, 2937); PlGF (pg/ml), 36 (16, 111) versus 318 (108, 629); and ratio, 285.6 (32.2, 758.5) versus 6.1 (2.3, 20.3) (all $p < 0.0001$). Higher sFlt1/PlGF ratio correlated negatively with timing of delivery ($r = -0.60$, $p < 0.001$) and the risk of adverse outcomes was markedly elevated among women in highest tertile compared with lower tertile (odds ratio, 14.77; 95% confidence interval (CI), 4.28-51.00). The addition of sFlt1/PlGF ratio (≥85) to hypertension and proteinuria significantly improved the prediction for subsequent adverse outcomes (AUC 0.89 (95% CI): 0.82, 0.95) for hypertension, proteinuria, and sFlt1/PlGF (AUC = 0.75 (0.65, 0.85)) for hypertension alone ($p = 0.002$). Compared with normative controls, women who were evaluated for pre-eclampsia without adverse outcomes had higher MoM for sFlt1/PlGF ratio; these values were further elevated in women with adverse outcomes. sFlt1/PlGF ratios measured on the KRYPTOR platform were highly correlated with measurements obtained using Elecsys platform ($r = 0.97$, $p < 0.001$).

KEY FACTS

In women with suspected pre-eclampsia presenting prior to 34 weeks of gestation, KRYPTOR assays for circulating sFlt1 and PlGF when used in conjunction with standard clinical evaluation performs well in the prediction of adverse maternal and perinatal outcomes occurring within 2 weeks of presentation.

Diagnosis of pre-eclampsia and fetal growth restriction with the sFlt-1/PlGF ratio: Diagnostic accuracy of the automated immunoassay KRYPTOR®

Dröge LA, Höller A, Ehrlich L, Verlohren S, Henrich W, Perschel FH
Pregnancy Hypertens 2017 Apr;8:31-36

Objective
We aimed to characterize the diagnostic accuracy of the KRYPTOR® assay for sFlt-1 and PlGF in maternal serum samples of uneventful singleton pregnancies and subjects with pre-eclampsia (PE) and PE-related outcomes such as fetal growth restriction (FGR). Longitudinal reference ranges of the sFlt-1 and PlGF level in the course of normal pregnancies were generated.

Methods
A cohort of subjects with PE and PE-related outcomes including FGR in the third trimester was compared to a cohort of women with uneventful outcome. Serum levels of sFlt-1, PlGF level as well as the sFlt-1/PlGF ratio was analysed with the KRYPTOR® assay and compared between the case- and control groups. Cut-off values were generated and diagnostic accuracy examined.

KEY FACTS

Longitudinal reference ranges of the sFlt-1 and PlGF level in healthy pregnancies were in line with those levels measured with other immunoassays. Comparison of the sFlt-1/PlGF ratio between PE-related outcomes including FGR or PE and healthy controls showed a high diagnostic accuracy with an area under the curve (AUC) of 0.917 for PE-related outcomes and 0.919 for PE.

Protocol for the prospective validation study: 'Screening programme for pre-eclampsia' (SPREE)

Tan MY, Koutoulas L, Wright D, Nicolaides KH, Poon LCY

Ultrasound Obstet Gynecol 2017 Aug;50(2):175-9

Abstract

Pre-eclampsia (PE), which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality. Early detection of PE can improve pregnancy outcome by providing timely intervention and closer monitoring. The current guideline from the UK National Institute for Health and Care Excellence (NICE) recommends that, at the booking visit, women identified with one major risk factor or more than one moderate risk factor for PE should be advised to take low-dose aspirin daily from 12 weeks until delivery. However, performance of the current method of screening is poor and identifies only about 35% of PE.

Extensive studies in the last decade have established that the best performance for early prediction of PE can be achieved by using a novel Bayes' theorem-based method that combines maternal characteristics and medical history together with measurements of mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI), serum placental growth factor (PlGF) and pregnancy-associated plasma protein-A (PAPP-A) at 11-13 weeks' gestation. This forms the 'combined test', which could be simplified to the 'mini combined test' when only maternal factors, MAP and PAPP-A are taken into consideration.

We present the protocol (version 3.1, 14 November 2016) for the 'Screening programme for pre-eclampsia' (SPREE) study, a prospective multicenter cohort study that will be carried out in seven National Health Service maternity hospitals in England. Eligible pregnant women attending their routine scan at 11-13 weeks' gestation will be invited to participate in this study. Maternal characteristics and history and measurements of MAP, UtA-PI, serum PAPP-A and PlGF will be recorded according to standardized protocols. The patient-specific risk for PE will be calculated and data on pregnancy outcomes collected. We hypothesize that the first-trimester mini combined test and combined test for PE screening, using the Bayes' theorem-based method, are likely to be superior to the current method recommended by NICE that is based on maternal demographics and history alone.

Enrollment for the study commenced in April 2016. The study is registered on the International Standard Randomised Controlled Trial Number (ISRCTN) registry.

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- In routine use by FMF since 1999

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