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Comparison of two immunoassay systems for hCGβ and PAPP-A in prenatal screening for trisomy 21, 18, and 13 in the first trimester

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ABSTRACT

Objectives: The biochemical serum markers free β-human chorionic gonadotropin (hCGβ) and pregnancy associated plasma protein A (PAPP-A), used in screening for trisomy 21 (T21), trisomy 18 (T18), and trisomy 13 (T13) during the first trimester, can be measured on different laboratory instruments e.g. Kryptor (Brahms) and Cobas (Roche). We compared the performance of these two analytical instruments when used for first trimester combined testing.

Design and methods: Serum samples from 944 singleton pregnant women attending for first trimester combined testing were routinely assayed for hCGβ and PAPP-A on Kryptor, and re-analyzed on Cobas. In addition, serum samples from 70 pregnant women carrying a fetus affected by T21, T18 or T13, were re-assayed on Cobas.

Results: For the screening population, the hCGβ and PAPP-A results in multiples of the median (MoM) from Kryptor and Cobas were significantly lower on Cobas when compared to Kryptor. The number of pregnant women with a risk above 1:300 for T21 was 48 for both Cobas and Kryptor, although a few patients only had a high risk with one of the methods. Overall, the screen positive rate was 5.1% for both instruments. In the trisomy groups the calculated risks for T21, T18, and T13 agreed well between Cobas and Kryptor.

Conclusions: The screen positive rate for T21 (5.1%) did not differ between the two analytical platforms in our screening population, although PAPP-A measurements form Cobas were significantly lower than those from Kryptor. The calculated risks for the pregnancies affected by trisomies using hCGβ MoM and PAPP-A MoM from Kryptor agreed well with those from Cobas.

1. Introduction

The combined first trimester screening program for trisomy 21 (T21), trisomy 18 (T18), and trisomy 13 (T13) in Denmark is based upon maternal age, measurement of the thickness of fetal nuchal translucency (NT), and the maternal serum concentrations of free β-human chorionic gonadotrophin (hCGβ), and pregnancy associated plasma protein-A (PAPP-A) [1,2]. The ultrasound measurement of the NT thickness is performed between 11 + 2 and 14 + 1 weeks of gestation and the biochemical serum markers are measured from 8 + 1 to 14 + 0 weeks of gestation. hCGβ and PAPP-A concentrations vary during gestation and are therefore converted to gestational age-adjusted multiples of the median (MoM) values. In all three trisomies, the PAPP-A concentration is decreased, while hCGβ concentration is usually decreased in T18 and T13, and increased in T21 pregnancies [1]. If the risk is at or above 1:300 for

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T21, or at or above 1:150 for T18 or T13 an invasive diagnostic test (amniocentesis or chorionic villus sampling) is offered [2]. Both invasive procedures have a risk of fetal loss of 0.5–1.0% [3]. First trimester combined screening for T21 was found to have an average detection rate of 89.5% with a false positive rate of 3.6% over 2 years [4]. In T18 or T13 the detection rate was found to be 78.8% for an estimated risk of > 1:150 [2]. Lowering the cut-off risk threshold would allow more trisomy-affected pregnancies to be detected, but would also increase the number of false positive pregnancies with increased risk of T21, T18, and T13 leading to increased numbers of invasive diagnostic tests being offered.

The biochemical analysis of hCGβ and PAPP-A can be performed on different analytical systems and it is important not only to know the analytical variation but also the screen positive rate and the detection rate for these different systems. The aim of this study was to compare the clinical performance of the two analytical instruments, Kryptor and Cobas, in the risk assessment of T21, T18, and T13 during the first trimester.

2. Materials and methods

2.1. Study group - screening population

Data were collected from 1024 pregnant women attending for first trimester combined testing at Copenhagen University Hospital Hvidovre during a two-month period between April 2014 and June 2014. A number of pregnancies (n = 80) were excluded from the study, due to twin pregnancy (n = 23), missed abortion (n = 8), sample taken outside the screening window (n = 9), lack of a nuchal translucency scan (n = 36) and other reasons (n = 4). A total of 944 pregnancies were therefore included in the study. The median maternal age was 31 years (range 17–47 years) and the median gestational age was 11 + 2 (range 8 + 1 – 14 + 0 weeks of gestation) at time of sampling.

Blood samples were collected into tubes with clot activator and gel separator (4 mL;Greiner Bio-One, Kremsmunster, Austria) and centrifuged at 1850 g for 10 min within a few hours of collection. Serum was stored at −20 °C until analysis. hCGβ and PAPP-A were routinely analyzed on the Kryptor system (Thermo Fisher Scientific, Clinical Diagnostics, Brahms GmbH, Henningsdorf, Germany). Samples were subsequently stored at – 20 °C for 5–6 months and re-analyzed on Cobas 6000 (Roche Diagnostics, Basel, Switzerland). The measurement principle of Kryptor is time-resolved amplified cryptate emission technology while the Cobas employs electro-chemiluminescence immunoassay.

2.2. Study group - trisomy population

Serum samples from 70 women carrying a fetus affected by T21 (n = 50), T18 (n = 14) or T13 (n = 6) were compared. These women attended for first trimester combined testing at Copenhagen University Hospital Hvidovre between December 2004 and November 2010.

The median maternal age at time of sampling for T21 pregnancies was 33 years (range 23–44), for T18 pregnancies 37 years (range 27–43), and for T13 pregnancies 31 years (range 26–40). The median gestational age was 11 + 1 (range 9 + 3 – 13 + 4) for T21, 10 + 6 (range 9 + 0 – 12 + 5) for T18, and 10 + 6 (range 10 + 2 −11 + 6) for T13 pregnancies.

hCGβ and PAPP-A concentrations were routinely analyzed on Kryptor at the time of testing. After storage at −80 °C, the samples were re-analyzed on Cobas in the autumn of 2015. The karyotype was determined with either amniocentesis or chorionic villus sampling or by karyotyping after birth.

2.3. Risk calculation

All measurements of hCGβ and PAPP-A were transformed to multiples of the median (MoM) values for each analytical platform by a commercial software program and fetal database (Astraia, Gmbh, Munich, Germany) using the latest default medians for the two instruments. The parameters maternal weight, smoking status, conception method and ethnicity were included in the risk calculation for T21, T18, and T13.

2.4. Statistical analysis and ethical approval

Data were analyzed using GraphPad Prism, (GraphPad Software, San Diego, CA, USA). We compared the results from Kryptor and Cobas by plotting the data into a scatter plot and assessing the slopes using Deming and linear regression analysis for MoM and risk values, respectively. Correlation was measured using nonparametric Spearman r.

The study was conducted following informed consent and was approved by the Danish Data Protection Agency (AHH-2014-014, I-Suite no 03069).

3. Results

3.1. Screening population

hCGβ MoM and PAPP-A MoM results from Kryptor correlated well with those from Cobas, and the slopes for the regression lines were 1.04 (95% CI 1.02–1.05) and 0.96 (95% CI 0.94–0.97), respectively (Fig. 1). The median hCGβ MoM was 0.99 for Kryptor and

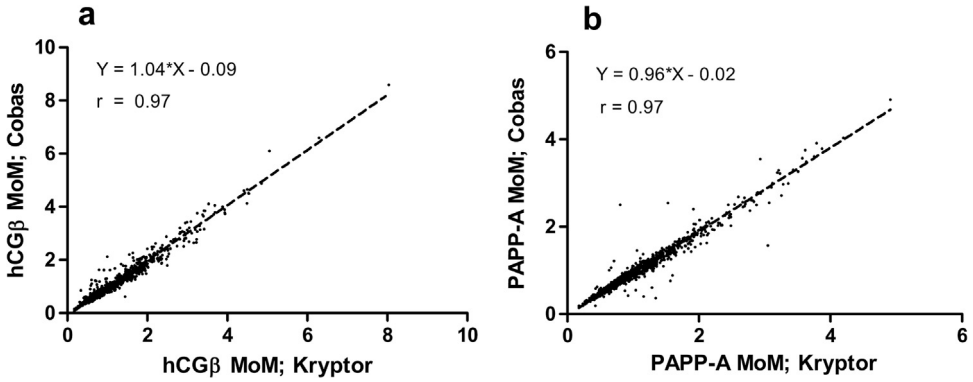

Fig. 1. Comparison plots of hCGβ (a) and PAPP-A (b) MoM values analyzed on Kryptor and Cobas in the screening population (n = 944). The regression lines and corresponding equations are shown.

0.93 for Cobas. For PAPP-A the median MoM was 0.98 for Kryptor and 0.91 for Cobas. Both hCGβ and PAPP-A MoM values from Cobas were significantly lower when compared to those from Kryptor (p = 0.02 and 0.004, respectively). These differences can be seen in the difference plots (Fig. 2). Further analysis was performed to investigate whether this observed difference between Kryptor and Cobas measurements was present at all weeks of gestation by dividing the screening population into subgroups depending on their week of gestation. Both analytical platforms had low mean $\log_{10}$ hCGβ MoMs and $\log_{10}$ PAPP-A MoMs in weeks 8 and 9 of gestation, probably because of fewer values (Fig. 3), whereas in weeks 10–12 with more values only $\log_{10}$ PAPP-A MoMs from Cobas were below 0.

The calculated risks of T21, T18, and T13 in the screening population using results from Kryptor and Cobas agreed well (Fig. 4). Of 55 pregnancies with a high risk for T21, 41 had an increased risk of T21 regardless of whether the samples were analyzed on either Kryptor or Cobas, seven only had a high-risk pregnancy when measurements from Kryptor were used, and seven only had a high risk pregnancy when results from Cobas were used. All 55 pregnancies with a high risk for T21 had a normal karyotype determined by CVS or AC or no sign of T21 after birth. The screen positive rate for T21 was 5.1% for both Kryptor and Cobas (95% CI 3.8–6.7%).

3.2. Trisomy population

hCGβ MoM values from Kryptor agreed with those from Cobas, and the slopes of the regression lines were 0.73 (95% CI 0.47–0.99) for T13 (n = 6), 1.04 (95% CI 0.95–1.12) for T18 (n = 14) and 1.21 (95% CI 1.06–1.35) for T21 (n = 50) (Fig. 5a–c). PAPP-A MoM values from Kryptor and Cobas agreed much better, and the slopes of the regression lines were 0.97 (95% CI 0.88–1.07) for T13, 0.99 (95% CI 0.92–1.05) for T18, and 1.04 (95% CI 0.95–1.13) for T21 (Fig. 5d–f).

As with the screening population, we also compared the calculated risk for T21, T18, and T13 in the three trisomy groups using results from Kryptor and Cobas. These also agreed well (Fig. 6a–c).

In evaluating the clinical performance in detection of trisomy positive pregnancies, two out of six T13 pregnancies were not detected by either Kryptor or Cobas when serum markers were included in the first trimester combined test with NT thickness and maternal age. However, both analytical instruments picked up these two T13 pregnancies as the results corresponded to a high risk for T18. Three out of 14 T18 pregnancies and five out of 50 T21 pregnancies failed to be identified using measurements from either Kryptor or Cobas (the risk values from Kryptor were 1:306, 1:322, 1:593, 1:768, 1:4363).

4. Discussion

We compared hCGβ and PAPP-A MoM values derived from two analytical instruments, Kryptor and Cobas, from pregnant women in a screening population attending first trimester screening for T21, T18, and T13 and in a population of known trisomy pregnancies.

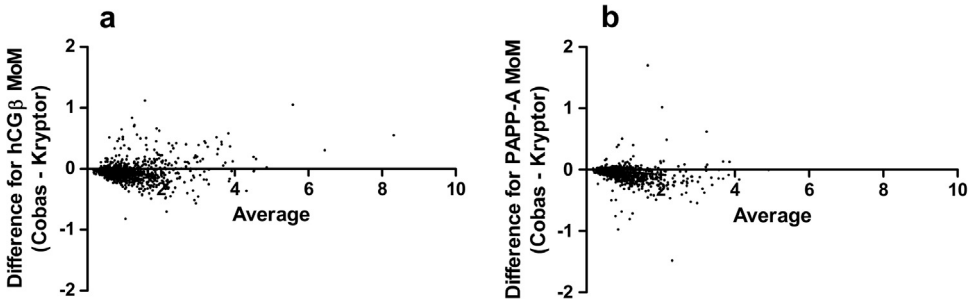

Fig. 2. Difference plot of hCGβ (a) and PAPP-A (b) MoM values for Cobas compared with Kryptor.

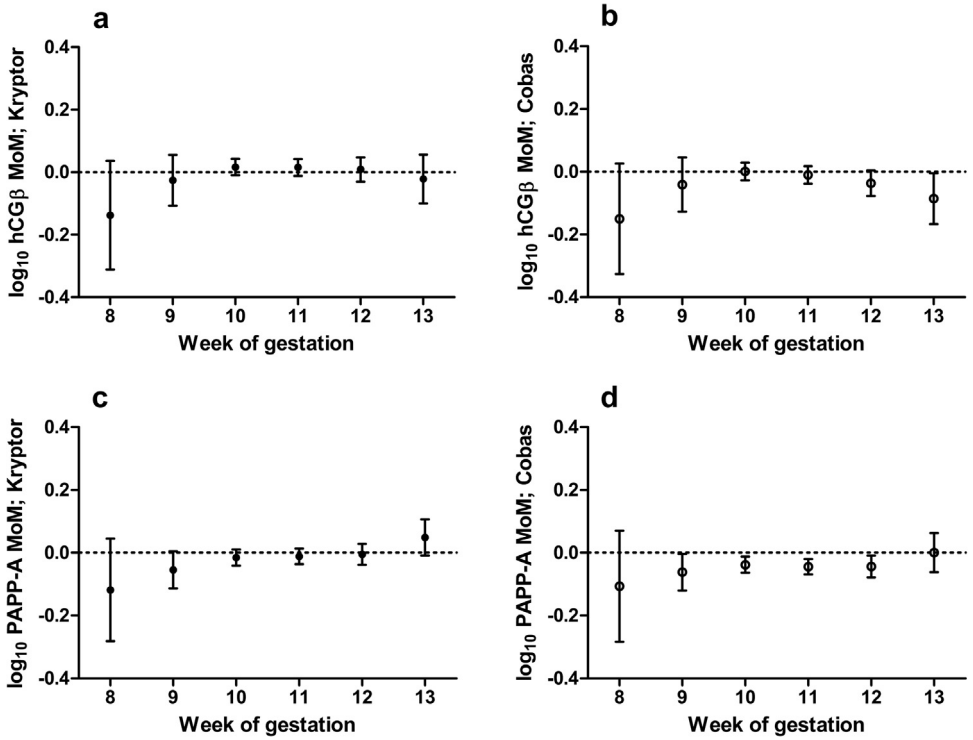

Fig. 3. Log_{10} hCGβ MoM for Kryptor (a) and Cobas (b) and log_{10} PAPP-A MoM for Kryptor (c) and Cobas (d) in gestational week 8 (n = 9), week 9 (n = 40), week 10 (n = 325), week 11 (n = 350), week 12 (n = 170) and week 13 (n = 50). Means with 95% CI are shown.

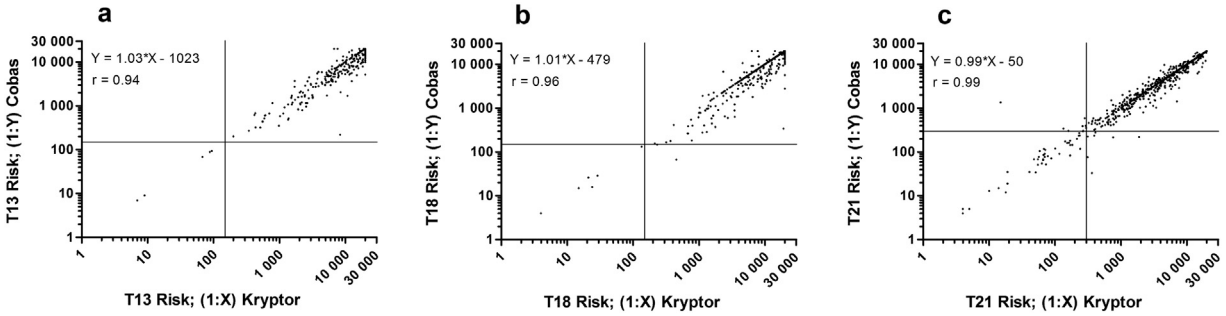

Fig. 4. Comparison of the calculated risk of T13 (a), T18 (b), and T21 (c) between Kryptor and Cobas in the screening population (n = 944) using cut-offs of 1:150 for T13 and T18, and 1:300 for T21.

In the screening population, both hCGβ and PAPP-A MoM values were lower on Cobas when compared to Kryptor, and these differences varied depending on the week of gestation. These results for PAPP-A MoM values are in contrast to a small study by Hörmansdörfer et al., who obtained PAPP-A MoM values higher on Cobas compared to Kryptor ($p < 0.0001$) [5]. However, similar to our results, a previous study also found a difference in hCGβ MoM and PAPP-A MoM values between Kryptor and Cobas in T21 pregnancies in the later weeks of screening gestation (week 10 + 0 to 14 + 0) [6].

In general, one would assume that lower PAPP-A MoM in clinical practice would result in more pregnancies considered to be at increased risk of trisomy and therefore more invasive diagnostic tests. However, when screening for T21 a corresponding decreased hCGβ value would result in a reduction in the calculated risk of T21, which might offset the differences in hCGβ and PAPP-A values. Furthermore, our study demonstrates that the risk assessment, when including NT and maternal age, is not affected and the same screen positive rate of 5.1% is found when using results from both analytical instruments.

In the T21 trisomy group a significant difference was found for hCGβ MoM values between Kryptor and Cobas ($p < 0.001$) but not for T18 and T13. Overall, as observed in the screening population, the calculated risk of T21, T18, and T13 agreed well between the two instruments when the biochemical markers were included in the first trimester combined test with NT and maternal age. The two instruments detected the same number of trisomy pregnancies and each failed to identify the same numbers of false negative cases.

To our knowledge, this is the first study comparing samples from T18 and T13 pregnancies on Cobas and Kryptor. A fewer other studies have compared hCGβ and PAPP-A values measured on Cobas and Kryptor in screening populations, and in T21 affected

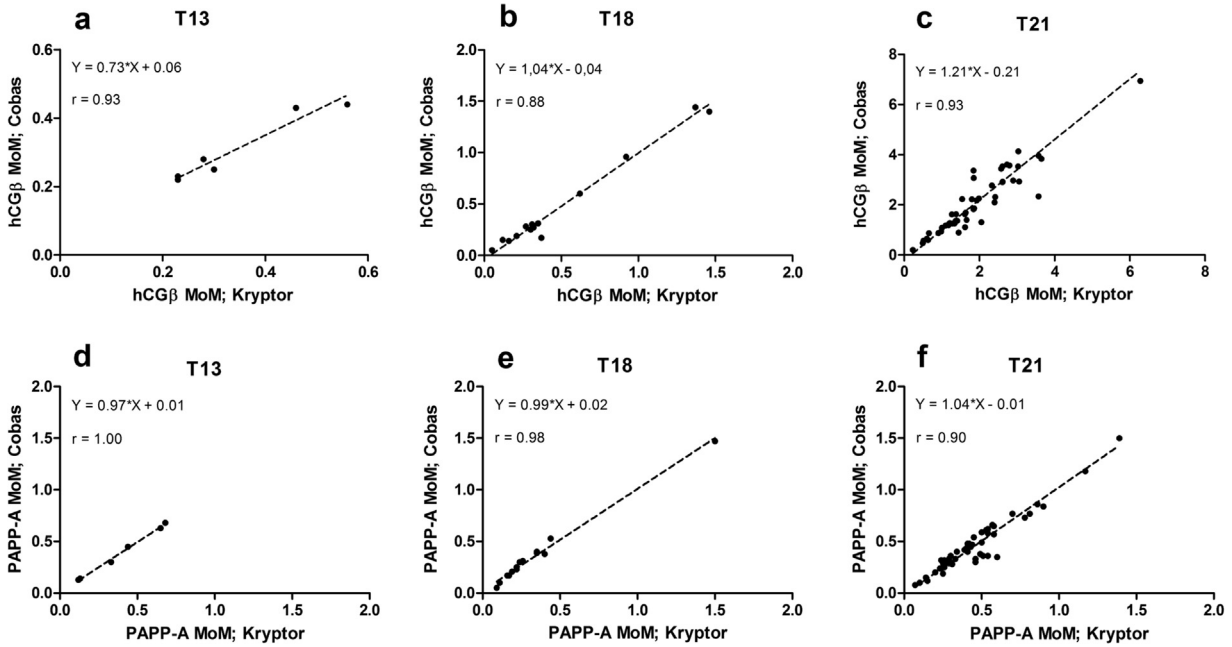

Fig. 5. Comparison of hCGβ MoM values from Kryptor and Cobas in pregnancies with (a) T13 (n = 6), (b) T18 (n = 14), and (c) T21 (n = 50), and of PAPP-A MoM values from Kryptor and Cobas in pregnancies with (d) T13 (n = 6), (e) T18 (n = 14), and (f) T21 (n = 50).

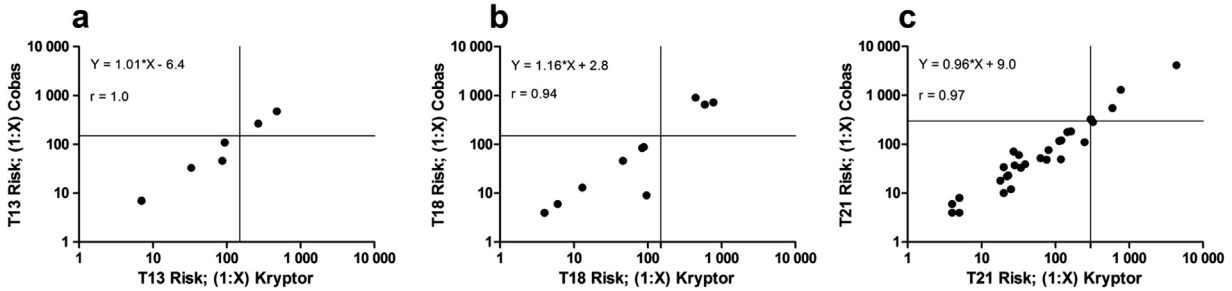

Fig. 6. Comparison of the calculated risk for T13 (a), T18 (b), and T21 (c) between Kryptor and Cobas in trisomy affected pregnancies using cut-offs of 1:150 for T13 (n = 6), T18 (n = 14), and 1:300 for T21 (n = 50).

pregnancies. Rossier et al. found hCGβ and PAPP-A concentrations to be well-correlated with no significant difference between the two analytical instruments [7]. The risk of T21 was calculated with two different software programs (not Astraia) for a screening population and T21 pregnancies. Both analytical instruments detected T21 with similar sensitivity and specificity. Variability in the results was mostly due to the software, not to the analytical instrument [7].

Other analytical platforms than Kryptor and Cobas exist for measuring hCGβ and PAPP-A in the first trimester combined test. Spencer compared the clinical and analytical performance of the DPC Immulite 2000 with Kryptor by analyzing 813 samples from normal pregnancies and 60 samples from T21 pregnancies [8]. In that study, Kryptor appeared to give a better clinical performance than DPC Immulite 2000.

One of the strengths of our study was the homogenous pre-analytical handling of blood samples and data, making the results, more comparable. The blood samples were all collected and analyzed at the same hospital and laboratory. In addition, the MoM values and the risk calculations were all generated and performed by the same software program, Astraia, minimizing bias due to software variation.

A potential limitation of the study is the differences in storage of the samples. The samples were analyzed prospectively on Kryptor and retrospectively on Cobas after 5–6 months of storage at −20 °C. However, previous studies about thermal stability have shown that freezing has no significant impact on the stability of hCGβ and PAPP-A [9,10]. By utilizing the latest default medians in the risk calculation comparisons, this should help eliminate any potential bias from having to use samples frozen for months, or years, in the retrospective analysis on Cobas.

5. Conclusion

This study demonstrates that although, in our screening population, hCGβ and PAPP-A measurements performed on Cobas were

significantly lower than on Kryptor, the same screen positive rate of 5.1% was found. The two analytical instruments performed equally well in detecting pregnancies with T21, T18, or T13 karyotypes when hCGβ and PAPP-A were included in the risk calculation.

Conflict of interest

None.

Acknowledgements

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