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Atypicality index: avoiding false reassurance in prenatal screening

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KEYWORDS: adverse outcome; atypicality; combined first-trimester screening; incidental findings; prenatal screening; risk assessment

CONTRIBUTION

What are the novel findings of this work?
In women with a singleton pregnancy undergoing first-trimester screening for major trisomies by a combination of maternal age, fetal nuchal translucency thickness and maternal serum pregnancy-associated plasma protein-A and free β-human chorionic gonadotropin, a highly atypical biomarker profile, reflected by a high atypicality index, in the presence of low estimated risk for major trisomies was associated with a higher frequency of adverse pregnancy outcome.

What are the clinical implications of this work?
Low risk for trisomies in the presence of a highly atypical profile should not be wrongly considered to indicate a low-risk pregnancy but rather highlight the need for further investigation. The atypicality index is a measure of the degree of atypicality of a profile and can be employed as an adjunct to the already used and available variables in prenatal care. Its use should provide clinicians with a systematic way of dealing with multiple variables and mitigate the handling of incidental findings.

ABSTRACT

Objective *To demonstrate the application of the atypicality index as an adjunct to first-trimester risk assessment for major trisomies by the combined test.*

Methods *This was a study of 123 998 Danish women with a singleton pregnancy who underwent routine first-trimester screening, including risk assessment for major trisomies. An atypicality index, which is a measure of the degree to which a profile is atypical, was produced for measurements of fetal nuchal translucency thickness and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. The incidence of adverse pregnancy outcome, including miscarriage, intrauterine death and termination of pregnancy, was tabulated according to the screening result and atypicality index.*

Results *In pregnancies with low risk and those with high risk for major trisomies according to the combined screening test, the incidence of adverse pregnancy outcome increased with increasing atypicality index. In pregnancies with a low risk for trisomies and atypicality index of ≥ 99%, the incidence of adverse outcome was 5.1 (95% CI, 3.4–7.6) times higher compared with that in low-risk pregnancies with a typical measurement profile, reflected by an atypicality index of < 80%. Similarly, in high-risk pregnancies, the incidence of adverse outcome was 7.9 (95% CI, 4.4–14.5) times higher in those with an atypicality index of ≥ 99% compared to those with an atypicality index of < 80%. Using individual profile plots, we were able to demonstrate a transparent and intuitive method for visualization of multiple variables, which can help interpret the individual combination of measurements and level of atypicality.*

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Conclusions *In pregnancies undergoing first-trimester combined screening and classified as being at low risk for major trisomies, profiles that are typical of pregnancies with normal outcome provide additional reassurance, whereas those with an atypical profile may warrant further investigation.* © *2022 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Prenatal screening relies on a plethora of data from ultrasound examination of the fetus, analysis of blood samples from the mother and results from invasive testing, such as amniocentesis and chorionic villus sampling. Reference-range charts, multiples of the median (MoM) values, *Z*-scores and percentiles are used to assess a set of measurements relative to the reference distribution, which is specific to characteristics such as gestational age at the time of measurement. These data provide a way of assessing to what extent a measurement is small, typical or large relative to the reference population and can be used to define outliers. With an increase in the number of measurements, the probability of finding one or more abnormal value increases. This is similar to the multiple comparisons problem encountered in studies with multiple tests.

This study used a global measure of atypicality to classify individual measurement profiles on the basis of their atypicality relative to a multivariate reference distribution. This measure of atypicality allows assessment of the extent to which a set of measurements for an individual is unusual[1,2]. Knowing that the profile of measurements is typical of patients with normal outcome provides reassurance and indicates that there is nothing unusual in the measurements taken to date. In contrast, although the risks for screened conditions may be low, an atypical profile may necessitate further investigation.

The objective of this study was to demonstrate the application of the atypicality index as an adjunct to first-trimester risk assessment for major trisomies by the combined test of fetal nuchal translucency thickness (NT) and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A).

METHODS

Study population

This was a study of 123 998 women with a singleton pregnancy undergoing routine combined screening for trisomies 21, 18 and 13 at 11 + 0 to 13 + 6 weeks' gestation in the Central Denmark Region between 2008 and 2018. In Denmark, more than 90% of all pregnant women attend first-trimester combined screening and 95% attend an 18–21-week scan for diagnosis of structural malformations[3,4]. For the combined testing, risk estimates for individual trisomies are obtained using The Fetal Medicine Foundation (FMF) algorithm[5,6] within the Astraia software (Astraia, Astraia GmbH, Munich, Germany). In the case of a high-risk first-trimester combined screening result or in the case of fetal structural malformations, invasive testing and chromosomal microarray are offered; alternatively, non-invasive prenatal testing by analysis of cell-free DNA in maternal blood is carried out.

The Central Denmark Region is the second largest region in Denmark with approximately 15 000 annual births. From the regional fetal medicine Astraia database, we retrieved data on all singleton pregnancies with a first-trimester scan, including maternal characteristics and first-trimester combined screening results. Data on pregnancy outcome (live birth, termination of pregnancy, miscarriage or stillbirth) were retrieved from the Danish Fetal Medicine Database[7], which holds information from various registries, including the Danish National Birth Registry[8]. Data were linked across registries using a unique personal identifier (the CPR-number) given to all citizens at birth and to residents at the time of immigration[9].

Pregnancies with incomplete first-trimester combined screening data or missing pregnancy outcome were excluded. In the case of repeated measurements, the first set of complete first-trimester combined screening data was used for analysis.

Atypicality index

The atypicality index[1,2] is a measure of how unusual an observation is relative to a reference distribution and is quantified on a scale of 0% to 100%; the larger the index, the less likely it is that the measured value arises from the reference distribution. Figure 1 shows the bivariate distribution of PAPP-A and β-hCG MoM values in the study population. The contours that contain 50%,

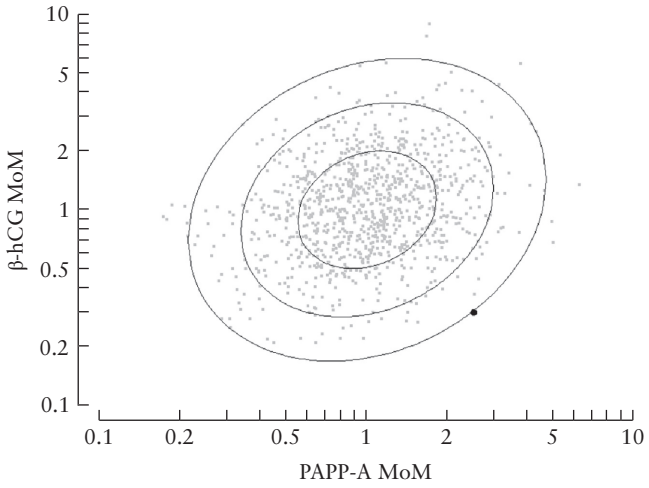

Figure 1 Sample of 1000 observations (gray circles) of pregnancy-associated plasma protein-A (PAPP-A) multiples of the median (MoM) and β-human chorionic gonadotropin (β-hCG) MoM. The contours contain 50% (inner), 90% (middle) and 99% (outer) of values from the reference distribution. The black circle represents PAPP-A MoM of 2.5 and free β-hCG MoM of 0.3; the datapoint is on the 99% contour and therefore has atypicality index of 99%.

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90% and 99% of the observations were obtained from a bivariate Gaussian distribution of log-transformed MoM values of PAPP-A and free β-hCG. The atypicality index for observations outside the 99% contour is > 99%.

The measure of atypicality can be extended to any number of dimensions and other reference distributions. In the case of the first-trimester combined test for trisomies, a trivariate distribution including PAPP-A, β-hCG and NT was used and defined by the models for unaffected pregnancies in the FMF algorithm. A bivariate Gaussian distribution with parameters from the FMF algorithm was used for PAPP-A and β-hCG[6]. NT was assumed to be independent of PAPP-A and β-hCG and distributed according to the mixture model[5]. In contrast to PAPP-A and β-hCG, for which both unusually small and large values are of concern, it is only large values of NT that are of concern. To focus the assessment of atypicality on larger values of NT, values were truncated at the most probable value of NT for the given crown–rump length; values of NT below this were replaced by this modal value.

In addition to the atypicality index, the study population was divided into low- and high-risk groups according to the risk of trisomies 21, 18 and 13. Risk estimates were collected prospectively from the combined testing. The high-risk group comprised pregnancies with at least one risk estimate < 1 in 100. In the low-risk group, all risk estimates were ≥ 1 in 100. Within each risk category, atypicality indices were stratified into four groups: < 80%, [80–90%), [90–99%) and ≥ 99%. We then examined the association between atypicality and adverse pregnancy outcome, defined as miscarriage (fetal loss before 24 weeks), intrauterine death (fetal loss at or after 24 weeks) or termination of pregnancy. The adverse outcomes by risk group (high or low) and atypicality-index stratum were tabulated, and relative and absolute risks with 95% CIs were calculated for both low-risk and high-risk pregnancies. All analyses were undertaken using the statistical software R[10,11].

RESULTS

The study population comprised a total of 123 998 women with available data on the measurements of NT, PAPP-A and β-hCG. Population characteristics are summarized in Table 1. In the high-risk *vs* low-risk pregnancies, there were a higher median maternal age, higher median NT thickness and lower median PAPP-A MoM. The median free β-hCG MoM was not significantly different between the two groups, but in the high-risk group, the values were more spread out. This reflects the way in which high β-hCG MoM levels are associated with a high risk of trisomy 21, while low β-hCG MoM levels are associated with a high risk of trisomies 18 and 13.

Adverse outcomes according to atypicality index

The risk for trisomies was low in 120 855 (97.5%) and high in 3143 (2.5%) pregnancies according to the combined screening test. An adverse outcome was recorded in 2073 pregnancies, including 1477 (1.2%) low-risk and 596 (19.0%) high-risk pregnancies. The adverse outcomes included 662 cases of miscarriage, 354 cases of intrauterine fetal death and 1057 cases of pregnancy termination. Among cases of pregnancy termination, 551 (52.1%) were low-risk and 506 (47.9%) were high-risk pregnancies.

In both low-risk and high-risk pregnancies, the frequency and risk of adverse pregnancy outcome increased with atypicality index (Table 2, Figure 2). In the low-risk group, the relative risk for adverse pregnancy outcome in the subgroup with an atypicality index of ≥ 99%, compared to those with an atypicality index of < 80%, was 5.1 (95% CI, 3.4–7.6). Similarly, in the high-risk group, the relative risk for adverse pregnancy outcome in the subgroup with an atypicality index of ≥ 99%, compared to those with an atypicality index of < 80%, was 7.9 (95% CI, 4.4–14.5).

Figure 3 presents a way of visualizing percentile profiles for a sample of four pregnancies. Figures 3a and b show two highly atypical profiles, with an atypicality index of > 99%. Both pregnancies were at low risk of trisomies, but the pregnancy from Figure 3a resulted in intrauterine fetal death and the pregnancy from Figure 3b resulted in miscarriage. Figures 3c and d show relatively typical profiles with atypicality index of 50%, and in both cases, the outcome was healthy live birth.

Table 1 Characteristics of study population, overall and according to risk for major trisomies on first-trimester combined screening

Characteristic	*High risk* (n = *3143*)	*Low risk* (n = *120 855*)	*All* (n = *123 998*)
Maternal age (years)	34 (30–37)	29 (26–33)	30 (26–33)
Crown–rump length (mm)	63 (57.4–68.2)	63 (57.7–68.5)	63 (57.7–68.5)
Nuchal translucency (mm)	2.1 (1.6–3.1)	1.7 (1.4–1.9)	1.7 (1.4–2.0)
PAPP-A MoM	0.36 (0.25–0.55)	1.00 (0.70–1.42)	0.99 (0.69–1.41)
Free β-hCG MoM	1.01 (0.60–1.62)	1.01 (0.70–1.45)	1.01 (0.70–1.45)
Adverse outcome	596 (18.96)	1477 (1.22)	2073 (1.67)
Pregnancy termination	506 (16.10)	551 (0.46)	1057 (0.85)
Miscarriage	70 (2.23)	592 (0.49)	662 (0.53)
Intrauterine fetal death	20 (0.64)	334 (0.28)	354 (0.29)

Data are given as median (interquartile range) or *n* (%). β-hCG, β-human chorionic gonadotropin; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A.

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DISCUSSION

Principal findings

This study demonstrates the application of the atypicality index as a tool for assessing the extent to which the profile of measurements of cases with a low risk of major trisomies according to the combined test is atypical. This index can be used as an adjunct to current risk assessment and profile plots but not as an alternative to screening already implemented.

Modern prenatal care involves risk assessment for several conditions, such as aneuploidy, pre-eclampsia and fetal growth restriction. Furthermore, fetal anatomy and biometry are thoroughly evaluated. Pregnancies classified as high risk are followed up on several extra scans and examinations, often including invasive diagnostic testing. The low-risk group undergoes routine care. However, there are pregnancies that fall into the low-risk group that show deviation from the reference distribution and may warrant further clinical investigation. One approach is to compare individual measurements with percentiles of the reference distribution, as we have illustrated in the profile plots in Figure 3. The problem with this approach is the increased number of apparent outliers arising because of multiple comparisons. For example, with three measurements in each pregnancy and assuming independence, 49% would have one or more measurements below the 10th or above the 90th percentile just by chance. With 10 measurements, 89% will have one or more measurements below the 10th or above the 90th percentile. The use of the atypicality index enables outliers to be identified in multivariate data, avoiding the problems of multiple comparisons and aiding the interpretation of measurement profiles.

Use of atypicality index in previous studies

The concept captured by the atypicality index can be traced back to over 80 years ago[12]. Although applications in clinical laboratories and screening have been suggested[1,2], the atypicality index is not used routinely alongside prenatal screening programs. Over recent years, however, there has been a considerable debate[13] about

Table 2 Distribution of pregnancies and frequency of adverse pregnancy outcome according to risk for major trisomies on first-trimester combined screening and atypicality index

		Adverse outcome	
Atypicality index	*Total* (n *(%))*	n	*Absolute risk (95% CI) (%)*
Low risk ($n = 120\,855$)			
< 80%	99 310 (82.2)	1079	1.1 (1.0–1.2)
[80–90%)	12 127 (10.0)	178	1.5 (1.3–1.7)
[90–99%)	9002 (7.4)	197	2.2 (1.9–2.5)
≥ 99%	416 (0.3)	23	5.5 (3.3–7.7)
High risk ($n = 3143$)			
< 80%	170 (5.4)	10	5.9 (2.3–9.4)
[80–90%)	297 (9.4)	18	6.1 (3.3–8.8)
[90–99%)	1887 (60.0)	200	10.6 (9.2–12.0)
≥ 99%	789 (25.1)	368	46.6 (43.2–50.1)

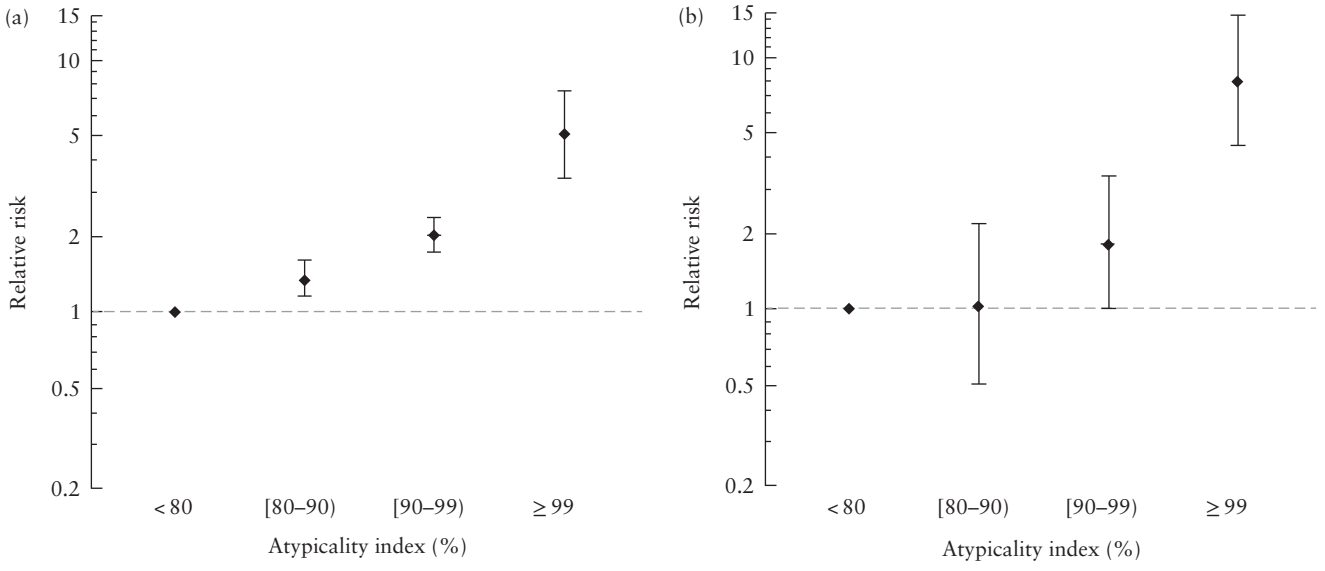

Figure 2 Relative risks with 95% CI of adverse pregnancy outcome in pregnancies with low (a) and high (b) risk of major trisomies on first-trimester combined screening according to atypicality index. Reference category for atypicality index is < 80%.

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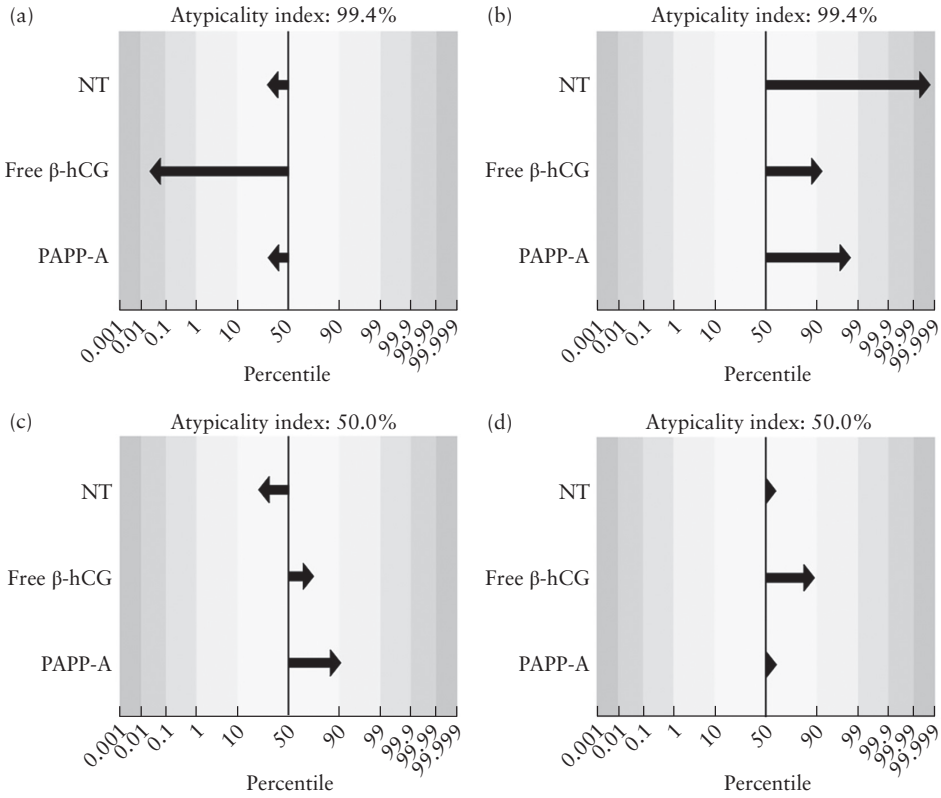

Figure 3 Examples of percentile profile and atypicality index for measurements of fetal nuchal translucency (NT), serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) in four pregnancies with low risk of major trisomies on first-trimester combined screening. Pregnancies in (a) and (b) had atypicality index of > 99% and resulted in fetal death and miscarriage, respectively. Pregnancies in (c) and (d) had atypicality index of 50% and, in both cases, outcome was healthy live birth.

incidental findings and the resultant further testing called a 'cascade of care'. Patients, clinicians, healthcare providers and policymakers have requested methods to manage incidental findings and mitigate the consequences. In the USA, the 2013 Presidential Commission for the Study of Bioethical Issues report on incidental findings recommended development of decision aids, point-of-care tools as well as clinical guidelines with population-based evidence[13]. The atypicality index has a role as a decision-aid tool that can be used alongside screening programs to assist in the interpretation of incidental findings arising from the increasingly high-dimensional multivariate data.

Strengths and limitations

The combined first-trimester assessment is offered to all pregnant women in Denmark by the public healthcare system and is accepted by more than 90% of women. The Danish Fetal Medicine Database serves as a national database for clinical quality control, and all departments are obliged to provide and validate data. Therefore, our results are based on data considered to be of high quality and with a low risk of selection bias. The atypicality index was produced using data on variables already included in the current combined first-trimester assessment.

In our study, the atypicality index was derived based on a limited number of variables to describe the method and as a proof of concept. Further clinical application of the atypicality index will require evaluation of relevant variables depending on the time of the assessment (e.g. second-trimester anomaly scan), type of pregnancy (e.g. multifetal pregnancy and specific chorionicity) and the defined pregnancy outcome measures.

Clinical implications of study findings

We believe that the atypicality index may contribute to mitigation of the multiple-comparisons dilemma caused by the many variables used in everyday fetal medicine by providing a 'safety net' around the increasing amounts of data obtained in clinical practice. The atypicality index is not a new screening test but a measure that facilitates management of the unintended consequences arising from multivariate data collected in contemporary prenatal screening and follow-up examinations. As a decision-aid tool, the atypicality index may provide reassurance to many patients and identify the few of those with a low-risk screening result but with a highly atypical combination of data, in which further testing may be necessary.

Conclusions

In conclusion, measures of atypicality should be considered as a decision aid and an adjunct to risk assessment and screening tests, as they can provide information regarding the extent to which a profile of measurements

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is unusual. By identifying highly atypical but low-risk profiles, atypicality measures provide a safety net that captures unusual pregnancies that are categorized as low risk for specific conditions.

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