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Uncertainty of the measurements in antenatal screening for Down's syndrome

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Review article

Abstract
Prenatal screening for Down's syndrome and some other chromozomal abnormalities is currently based on maternal blood tests and ultrasound examinations. Results of quantitative measurements are used for the risk evaluation. Effectivity of screening models is influenced by the selection of markers and by the quality of measurements. The article gives a basic overview of the uncertainty of measurements and shows how it could influence the whole screening process. The preanalytical phase of the biochemical tests is not discussed in this article, although it could also affect the screening process.

Key words:
measurement, uncertainty, biological variation, screening, Down's syndrome

VÝZNAM NEJISTOTY MĚŘENÍ PŘI ANTENATÁLNÍM SCREENINGU DOWNOVA SYNDORMU

Přehledový článek

Abstrakt
Prenatální screening Downova syndromu a některých dalších chromozomálních aberací je v současnosti prováděn pomocí biochemických testů, případně kombinovaných s ultrazvukovým vyšetřením. V obou případech se provádějí kvantitativní měření veličin, která se dále využívají pro stanovení rizika přítomnosti hledaného onemocnění plodu. Účelem článku je upozornění na skutečnost, že efektivita screeningových programů nemusí být ovlivněna pouze volbou konkrétních parametrů, ale také kvalitou měření těchto parametrů. Článek poskytuje základní informaci z oblasti nejistot měření a uvádí je do souvislosti s běžnou praxí při provádění screeningu Downova syndromu. V článku není diskutována preanalytická fáze, která biochemickým měřením předchází a výsledky těchto měření může také ovlivnit.

Klíčová slova:
měření, nejistota, biologická variabilita, screening, Downův syndrom

Prenatal Screening as a Result of Interdisciplinary Cooperation

There are ways of gaining information based on combining knowledge and measurements from various sources in the biological sciences. One of the examples of interdisciplinary cooperation in medicine is screening for Down's syndrome and other chromosomal abnormalities. Gynaecologists, biochemists, ultrasound specialists and geneticists participate in this kind of examination. All the above mentioned professionals contribute to screening with all required information. Those could be anamnestic data or results of specific maternal or fetal measurements. The evaluation process is quite difficult, the result of which is risk (probability) calculation and it is necessary to consider the fact that all input data influences the final results in some way. Basically there are two sources of results used in this type of screening. The results of measurements of a specific biochemical markers in the blood circulation of pregnant women and ultrasound measurements of fetal biometric parameters. The basic requirements for purposeful screening are quality monitoring of all measured parameters concerned in final results and being aware of all possible limits and error sources which can influence the measurements.

Metrologic Approach as a Basis of Quality of Measurement Monitoring

Traditional metrology was once used mainly in physics when defined standards were used to compare quality of measurements of various physical parameters. Metrology has also entered the world of chemical and biochemical laboratories in the past 15 years. The reason for this is to unify laboratory results and description of a laboratory procedure so that their examinations can be considered as consecutive as far as metrology is concerned, as well as that each laboratory is able to define the quality of each examination. The parameter used for this kind of evaluation in metrology is called **uncertainty in measurement.** The parameter covers various types of errors in all sorts of measurements. The term of uncertainty in measurement has generally been accepted and used in all kinds of quantitative test results (1). Some of the basic principles are:

- Evaluation of uncertainty is complex and includes all significant sources of errors in measurements
- All uncertainties of both coincidental and systematic phenomenon are treated in the same way, i.e. they are defined and combined in the same way as allowances associated with probability divisions

From a practical point of view, there are several basic requirements we should meet if we want to define uncertainty in any kinds of measurements (2):

- We should have a clear definition of measured parameters
- We should have a complete specification of the measuring method and measured items
- We should have a complete analysis of all the sources influencing results of measurements

Uncertainty in measurement is defined as a parameter associated with the results measured, characterizing diversion of values which are attributed to measured parameters on the basis of available information. We can also say that uncertainty in measurement defines limits in which the results are considered to be correct with a certain probability.

All the above mentioned facts are applicable in measurements in physical, testing and analytic laboratories, in which the measurements in inanimate systems are carried out.

Quantitative Parameter Measurements in Biological Systems

When it comes to biological systems, the situation is more complicated than with inanimate matter, both have intrinsic inaccuracy in measurements but there are additional issues with living entities.

On one hand there is an analogy between the measuring act and the facts described above, on the other hand we have to consider the living system characteristics known as biological variation (3). Biological variation is an organic system characteristic which reflects the unique status of a monitored specimen depending on time, conditions and also compared with other individuals of comparable group. From this point of view, the biological variation can be divided into:

- Intra-individual
- Inter-individual

Intra-individual biological variation is defined as variation conditional on physiologic and pathologic changes concerning an individual and it is variation with high ratio of genetic background. On the contrary, inter-individual biological variation is conditional on physiologic and pathologic differences among individuals and influences how wide reference range is. This fact is very important when it comes to common interpretation of biochemical examinations, when we consider whether the result belongs to a reference range which is considered to be physiological or the result is beyond the reference interval and can therefore reflect the presence of pathologic processes. As far as screening for Down's syndrome is concerned, the inter-individual biological variation is applicable as a factor, creating distribution curves of values, of individual biochemical or ultrasound parameters, with a normal fetus as well those with Down's syndrome ***(Fig. 1)***. If an ideal marker for Down's syndrome screening had existed, a distribution curve sheet of pregnancies with a normal fetus and fetus affected by Down's syndrome would not have overlain each other and this screening marker would have become a dia-

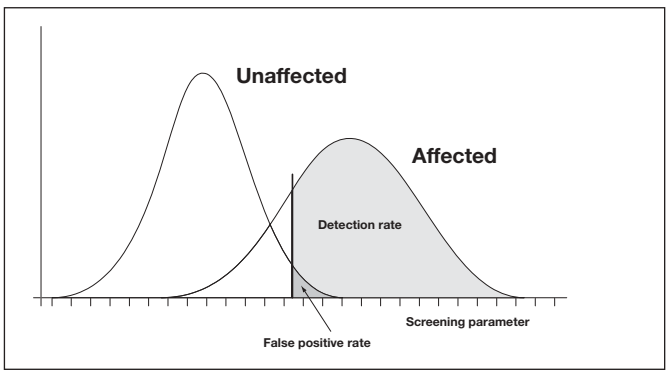

Fig.1 *Example of distributional curves of the screening parameters.*

gnostic marker ***(Fig. 2)***. In practical terms, there are always situations with higher or lower intersections which can influence screening results in the way that a normal fetus can have an atypical value of some markers which can show higher Down's syndrome risk or the other way round, when there is a fetus affected by Down's syndrome which has results without higher Down's syndrome risk. It is clear that measurements of any quantitative parameters of living systems are influenced by uncertainty in measurement and inter-individual biological variation. The relation between those two factors can be mathematically stated as the following (3): $SD_T = \sqrt{SD_A + SD_T}$, kde SD_T, where SD_T is the total variation of measurement, SD_A is analytic variation of measurement (uncertainty) and SD_I is inter-individual biological variation.

Down's Syndrome Screening Types and Uncertainty in Measurement Influence

Several protocols of Down's syndrome screening exist at present which are defined according to parameters used. There is a biochemical screening type, when only biochemical parameters are stated, or combined, when biochemical and ultrasound parameters are used. The number of biochemical and ultrasound parameters can differ, depending on regional facilities and access to a quality work place carrying out ultrasound examinations. It has already been said that the result of this type of screening depends on the quality of measurement of individual screening markers. The age of mothers examined is the only parameter in Down's syndrome screening which is not influenced by measurement. Benn and Collins (4) have monitored how the uncertainty in biochemical measurements can influence the screening results. Their work has clearly shown that at the defined confidence interval (CI) screening results could vary greatly. It shows that mainly border results can change from positive to negative and vice versa, depending on the quality of measurement. The situation is complicated by age of the mother examined. The same biochemical result with certain uncertainty in measurement influences the final screening result in a different way for mothers of different age.

Gestation Age Estimation by Ultrasound

This exemplary situation is applicable only when an exactly defined and accurate way of gestation age estimation exists at the time when biochemical and ultrasound parameters are measured for the sake of screening. The quality of the whole screening examination depends on accuracy of the estimation. Let us assume that there is a situation with an incorrectly estimated gestation age, and very low uncertainty, correctly done biochemical examinations, and/or ultrasound examinations used in an algorithm of Down's syndrome risk calculation. In such a case, the final results will be highly influenced by incorrectly estimated gestation age, in spite of the fact that all the measurements of used screening magnitudes, have been carried out correctly. The correct gestation age estimation is a basic factor, without which it is absolutely useless and impossible to carry out this type of screening. One of the common ways of gestation age estimation is CRL measurement

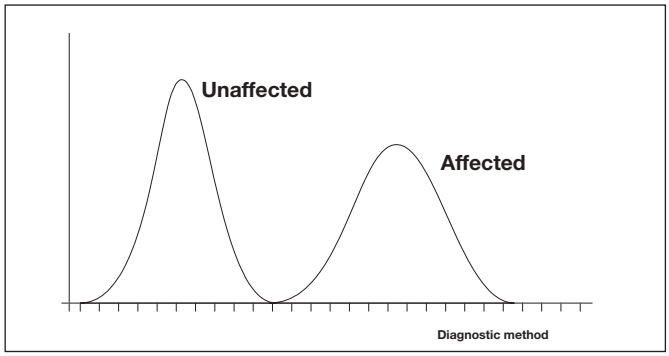

Fig. 2 *Hypothetical example of distributional curves of the diagnostical method.*

according to Robinson, when fetal biometry tables are used to deduce gestation age. It is clear from the above mentioned information that it is necessary to focus on the first step which is absolutely essential for carrying out quality Down's syndrome screening from the point of good quality measurement monitoring. Carneiro G. et al (5) described a method of gaining more accurate ultrasound biometrical measurement results of the fetus, in order to eliminate one type of error and so that there is the lowest possible uncertainty in the final estimation of the gestational age of the fetus. This method has not been used in practice yet but experience has shown that ultrasound specialists have been considering this situation, and searching for options of how to eliminate all possible errors. From the metrological point of view it is very difficult to define any measurements of fetal biometrical parameters in addition; it is difficult or almost impossible to estimate their uncertainty in measurement.

Choice of Parameters in Order to Monitor Ultrasound Examination Quality and Evaluation among Operators

An article has been published (6), dealing not exactly with uncertainty in measurement but describing a big database of how ultrasound measurements are comparable among operators. The authors have chosen three basic parameters in order to evaluate the quality of ultrasound NT marker measurements.

- they have considered a week's increase in NT values
- they have compared how measurements are done by individual ultrasound specialists getting closer to an average median of all the data
- they have monitored a parameter characterizing a statistic division of measured values using the Gauss curve– $\log^{10}$ SD

NT measurement results done by individual ultrasound operators have shown high variation in a file of more than 23 thousand measurements ***(Fig. 3)***. An optimal week's increase in NT values should be around 20% of parameter value. It is clearly shown that dispersion of measured values is very significant. On one hand there are measurements done by some ultrasound operators which have shown an increase in units of percentage, whilst on the other hand there are measurements with results exceeding an optimal value by one or two times. A similar situation occurs when a median of NT measurements is compared with an average median of the

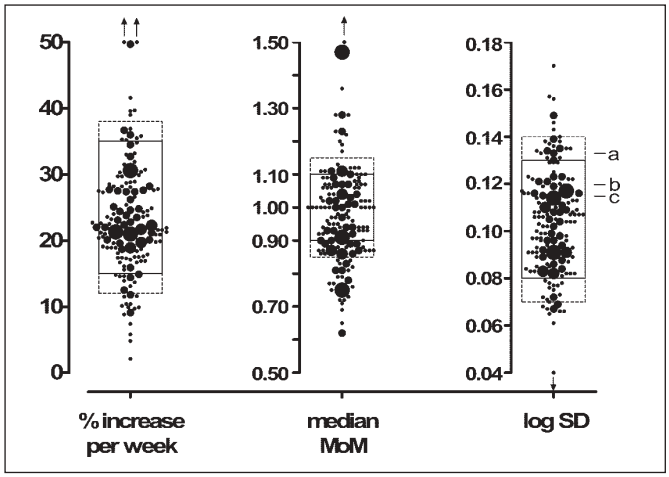

Fig. 3 *Results published in the article (6). (with permition of: Glenn Palomaki, Louis Neveux, Alan Donnenfeld, et al. Quality assessment of routine nuchal translucency measurements: a North American laboratory perspective. Genet Med. 2008 Feb;10(2):131-8)*

whole file among individual ultrasound specialists.
We have experienced an identical situation while evaluating the NT measurements by various specialists in our screening centre. An evaluation of the above mentioned qualitative parameters should be included in computer programs which are used for Down's syndrome risk calculation.

Ultrasound Measurement in Practice

Let us try to apply a common metrological terminology in ultrasound practice. Generally speaking, uncertainty in measurement occurs when the measurements are accompanied by a coincidence of various error types (7). The ultrasound measurement results can be influenced by the following:

- coincidental errors
- constant errors
- proportional errors
- other systematic errors

The coincidental errors occur due to various effects which cannot be systematically described. It can be due to e.g. environmental diversity in which ultrasound is carried out, when unexpected physical interferences can occur which might not have been noticed by an operator.
The constant errors occur due to e.g. various ultrasound equipments, which means different ultrasound scan specifications. The proportional errors occur when the same parameter is being measured but they depend on the size of the measured parameter. A good example can be the CRL measurement in the 10^{th} and 13^{th} weeks of pregnancy. Some errors appear in both measurements but those errors are not necessarily the same. The appearance of other systematic errors in ultrasound measurements can depend on the operators. It is clear that operators are very important when it comes to evaluation of the measured ultrasound parameters but it is impossible to quantify the degree of subjective estimation. Certain methods of ultrasound marker measurements have been described (8) but those methods cannot, by any means, give information about the exact type of uncertainty in measurement. As far as uncertainty in measurement is concerned, the basic problem of ultrasound examinations is that in practice it is basically impossible to carry out measurements which are repeated several times. The following example can help in order to be able to compare such situations: If there is a sample specimen in a biochemical laboratory, it can be examined 10 times in a row, then the measurements can be repeated the next day, calibration uncertainty stated by the producer can be added , etc. Using all those gained values, it is possible to calculate the type of uncertainty in measurement. The character of ultrasound examinations and the whole measurement method even in respect to impossible fetus fixation to one ideal position, do not allow a correct estimation of uncertainty in measurement at present, and NT measurement dependence on CRL (gestational age length) shows a high variation amongst various ultrasound specialists (9). Usage of video could be an alternative for estimation of operator subjective influence. In such cases, an operator could measure an examined parameter several times and store the values, similarly as with biochemical measurements. The second option how to get more precise results of ultrasound measurements is mathematical processing of an image with the help of a function which would evaluate automatically shade of grey of the image, and on the basis of it would deduce the relevant parameter, e.g. NT (10).

Conclusion

Uncertainty in measurement is an accompanying parameter of all activities which are used in order to gain quantitative information about a certain magnitude measured. In the case of Down's syndrome screening, an amount of specific biochemical substances is measured in the mother's blood, and some chosen biometrical parameters of the fetus are measured. As far as biochemical estimations are concerned, uncertainty in measurement can be estimated through the described methods which include validation and verification of individual methods. Long-term monitoring of quality of biochemical tests can be carried out through both internal and external quality controls. Requirements for an amount of tests and their quality are recommended by scientific bodies (11). As far as ultrasound examinations with quantitative information output are concerned, it is necessary to consider subjective elements which are added to a measurement by a particular operator. When NT measurement data are implemented, it would be advisable to consider each operator as an independent source of measurements and process their data separately. A small amount of measurement influences the quality of the whole screening process negatively, which is valid for both types of examinations.

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