Original Articles

Role of ultrasound evaluation of nuchal translucency in prenatal diagnosis

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Summary

Background: Nuchal translucency (NT) is the ultrasonographic pattern of the accumulation of subcutaneous fluid (≥ 3 mm) behind the fetal neck. The measurement of NT thickness by ultrasound examination at 11-14 weeks of gestation has been associated with maternal age and to be an effective screening tool for trisomy 21; with an invasive method rate of 5%, about 75% of trisomical pregnancies can be identified. With the association of some biochemical markers like maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) to ultrasonography at 11-14 weeks, it is possible to identify about 90% of chromosomal abnormalities. An increase of NT also allows us to identify most other chromosomal abnormalities, a large number of major cardiac defects, skeletal dysplasias, and genetic syndromes. In monochorial twins the discordance in the measurement of NT represents an early sign of twin-to-twin transfusion syndrome (TTTS).

Methods: The objective of our study was to assess the detection of fetal structural defects with an ultrasound scan at 11-14 weeks of gestation. We submitted 3,157 pregnant women to a routine ultrasound examination at 11-14 weeks. The patients were then submitted to ultrasound scan in the second or third trimester of pregnancy. An isolated increased NT thickness was not considered an abnormality, but these patients, nonetheless, were submitted to an early echocardiographic evaluation. Fetal structural abnormalities were classified as major or minor and of early or late onset.

Results: A detection rate of 4.3% (135 cases) of abnormalities was found and 22.6% of these (30 cases) were diagnosed by ultrasound scan at 11-14 weeks, including seven cardiac defects associated with increased NT. The antenatal ultrasound detection rate was 73.5%, and 33.2% were diagnosed in the first trimester assessment. A rate of 76.8% of the major defects were diagnosed by the prenatal scan and 35.8% by the scan at 11-14 weeks. Fetal structural defects with the ultrasonography at 11-14 weeks were diagnosed in about 24.3% of the cases, therefore, a second trimester abnormality is important in routine antenatal care to increase the prenatal assessment of fetal anomalies.

Conclusions: As for the introduction of every new technique in routine clinical practice, the operators who perform the ultrasound scan at 11-14 weeks should be submitted to adequate training and to strict quality control.

Key words: Nuchal translucency; Chromosomal abnormalities; Fetal malformations; Ultrasonography; Screening; β-human chorionic gonadotropin; Pregnancy-associated plasma protein-A.

Introduction

First trimester nuchal translucency (NT) and second trimester triple test (TT) are common screening programmes for trisomy 21. Sonographic findings of an increased NT in early pregnancy is associated with fetal aneuploidy and various structural and genetic abnormalities. Increased NT may identify fetuses that require vigilant assessment, especially when found in association with other abnormalities [1, 2].

The measurement of NT thickness by ultrasound examination at 11-14 weeks of gestation has been associated with maternal age and to be an effective screening tool for trisomy 21; with an invasive method rate of 5%, about 75% of trisomical pregnancies can be identified [3]. Increased fetal NT thickness at 11-14 weeks of gestation can identify 80% of trisomy 21 pregnancies for a false-positive rate of less than 5% [4]. However, a potential disadvantage of screening in the first trimester of pregnancy is that earlier screening preferentially identifies those chromosomally abnormal pregnancies that are destined to miscarry [5].

Thus, measurement of nuchal translucency (NT) is a widely used method of screening for chromosomal abnormalities. In fact, increased NT is seen in a diversity of fetal malformations. The mechanism explaining the abnormal fluid accumulation and the transient nature of NT remains unexplained. The cause of increased NT may be mesenchymal oedema in the presence of distended jugular lymphatic sacs. The delayed organization and connection of these lymphatic sacs to the venous circulation might explain the transient nature of NT. Disturbance in timing of endothelial differentiation might be a common denominator in the origin of NT, linking cardiovascular and haemodynamic abnormalities [6].

Sonographic and biochemical methods for Down's syndrome (DS) screening have developed simultaneously, but independently. As a consequence, the rate of

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invasive procedures for fetal karyotyping has dramatically increased and become an important public health issue which needs to be controlled. One approach is to combine sonographic and biochemical results into a single risk assessment [7].

There is a significant correlation between NT and ductus venosus pulsatility index (PI). In chromosomally normal fetuses with an enlarged NT an abnormal ductus venosus flow is associated with a nearly nine-fold increase in adverse outcome (odds ratio, OR, 11.7). In fetuses with NT of 4 mm or more and normal karyotype, there is a high association with other defects and the prognosis is often poor, whereas the NT resolves for those with 3 mm and the pregnancy outcome is usually normal.

Transvaginal sonography (TVS) enables imaging of the fetal heart in various planes and directions in early pregnancy. Examination of the cardiovascular system does not rely on still images of the classic views but instead must be performed in a dynamic mode visualizing the heart and great vessels from different directions and in various scanning planes. Early detection of fetal cardiac anomalies is now possible. Most anomalies occur in low-risk pregnancies. It is advisable to perform a detailed early multidirectional dynamic continuous sweep ultrasound examination of the fetal cardiovascular system in all pregnancies [8].

The debate over the application of NT measurement in DS screening is still unresolved in some clinicians' minds. Although different authors report a range of sensitivities for DS, none question the validity of the association between increased NT and fetal aneuploidy. The published literature reveals a lack of congruence over a standard, reproducible method for measuring NT. Only with the adoption of uniform methodology, and the establishment of international standards for NT measurement, is the true potential of this test likely to be realized. The increasing use of first trimester ultrasound has focused attention on the value of this investigation in confirming fetal viability, estimation of gestational age, and screening for congenital abnormalities [9].

With the association of some biochemical markers like maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) to ultrasonography at 11-14 weeks, it is possible to identify about 90% of chromosomal abnormalities.

An increase of NT also allows us to identify most other chromosomal abnormalities, a large number of major cardiac defects, skeletal dysplasias, and genetic syndromes [10].

The wide use of assisted conception methods has risen dramatically. The greater proportion of singletons, twins and high order of multiplicity conceived by those methods have already focused the medical community on various obstetric complications. Recently, there have been suggestions that the levels of mid-gestation serum markers, particularly β-hCG, might be affected by assisted conception, leading to higher false-positive results. Furthermore, women who conceived after assisted reproduction methods are on average older, and in many cases their current pregnancy was achieved after long-standing infertility and might even be their last one. This is why they are extremely wary of any invasive fetal karyotyping. Therefore, every effort should be made to provide them with the most accurate screening of DS risk. In this respect, NT measurement, which has been reported to be another effective screening method, might be a more reliable marker in these pregnancies [11, 12].

It has been reported that second-trimester serum markers may be affected by assisted reproduction, leading to a higher false-positive rate [13].

In monochorial twins the discordance in the measurement of NT represents an early sign of twin-to-twin transfusion syndrome (TTTS).

Despite the definition of new screening policies for fetal trisomies, based on NT thickness or maternal serum, the prevalence of trisomy 21 remains high [14].

There is a need for a simple method of expressing NT measurement in early pregnancy that will allow for gestational age and be useful in screening for DS [15].

Materials and Methods

The objective of our study was to assess the detection of fetal structural defects with an ultrasound scan at 11-14 weeks of gestation performed on fetuses of women with singleton pregnancies undergoing first trimester fetal karyotyping by amniocentesis or chorionic villus sampling because of advanced maternal age, parental anxiety, or family history of a chromosomal abnormality in the absence of balanced parental translocation. In the 4-year period from January 1998 to December 2001 we submitted 3,157 pregnant women to a routine ultrasound examination at 11-14 weeks. The patients were then submitted to an ultrasound scan in the second or third trimester of pregnancy.

The same type of ultrasound machine and standardized approach were used in all patients. The measurement of fetal NT was performed by mid-sagittal section. Fetal NT thickness was measured twice by each examiner on a regular-sized image and subsequently on the same still image magnified.

An isolated increased NT thickness [> 95th percentile thickness of the normal range for crown-rump length (CRL) between 38 and 84 mm] was not considered an abnormality. These patients, however, were submitted to an early echocardiographic evaluation. Fetal structural abnormalities were classified as major or minor and of early or late onset.

The study protocol included first-trimester combined NT and maternal serum markers (MSM) (free beta-HCG and PAPP-A) testing. Karyotyping was advised when:

(a) NT was > or = 3 mm; or

(b) the MSM-related risk was ≥1 in 250 at term.

Karyotyping was delayed until after a maternal blood sample had been taken. NT and MSM were expressed as multiples of the medians (MoM), and risks were calculated and tailored to the study population. A combined risk for NT and MSM was estimated retrospectively.

The second trimester triple serum screening included alpha-fetoprotein (AFP), intact hCG and unconjugated estriol (uE3). After excluding aneuploidies, miscarriages, anatomical anomalies, and cases with incomplete follow-up, the serum samples of normal cases were assessed and correlated.

To determine if the risk for fetal trisomies during the first trimester of pregnancy can be derived by combining data from

maternal serum PAPP-A and fetal NT thickness, likelihood ratios for trisomies 21, 18, and 13 in relation to PAPP-A, in MoM for CRL, were derived from the overlapping Gaussian frequency distribution curves for normal and abnormal pregnancies.

Moreover, we compared the potential value of maternal serum total hCG and free beta-hCG in predicting the risk for fetal trisomies during the first trimester of pregnancy and to examine whether data on maternal hCG and fetal NT thickness can be combined to derive risks.

Clinical examination of the neonates was performed by a paediatrician. Long-term follow-up was completed through a questionnaire filled in by parents and paediatricians.

Results

A detection rate of 4.3% (135 cases) of abnormalities was found and 22.6% of these (30 cases) were diagnosed with the ultrasound scan at 11-14 weeks, including seven cardiac defects associated with increased NT. The antenatal ultrasound detection rate was 73.5%, and 33.2% were diagnosed in the first trimester assessment. A rate of 76.8% of the major defects were diagnosed by the prenatal scan and 35.8% by the scan at 11-14 weeks. Fetal structural defects with ultrasonography at 11-14 weeks were diagnosed in about 24.3% of the cases, therefore, a second trimester abnormality is important in routine antenatal care to increase the prenatal assessment of fetal anomalies.

The incidence of trisomies 21, 18, or 13 was significantly associated with both maternal age ($r = 0.92$) and fetal NT thickness ($r = 0.70$).

The sensitivity, specificity, and relative risk (RR) for all aneuploidies were 25%, 91%, and 5.77, respectively, and no difference was found between trisomy 21 and other types of aneuploidy. The sensitivity, specificity, and RR were significantly higher at 11 weeks than at 14 weeks.

The likelihood of aneuploidy increased with increasing thickness of the NT. Where the karyotype was found to be normal, there was complete resolution of this ultrasound appearance by the second trimester.

Spontaneous abortion, intrauterine and neonatal death occurred in 2.9%, 1.6%, and 1.0%, respectively. The incidence of immature delivery was 1.6% and of premature delivery 6.5%. The only adverse pregnancy outcome recorded that was associated with increased NT was spontaneous abortion. The likelihood ratio for the occurrence of a spontaneous abortion was 3.6 for measurements between 3.0 and 3.9 mm, and 7.3 for measurements ≥ 4 mm.

NT measurement increased by about 12% per week. Expressing the result as a (MoM) NT for a given CRL allowed for this increase with gestational age and yielded a distribution of values that was approximately Gaussian. About 91% of values lay between 0.5 and 2.0 MoM. The variance and therefore the false-positive rate of NT were significantly reduced by recording several measurements and using the average: for example, the false-positive rate reduced from 7.8% to 4.5% if the average of six measurements were used instead of one (a potential 40% reduction in the false-positive rate if the test was used in screening).

Interobserver repeatability of regular-sized image measurements showed a significant difference ($p < 0.01$) but the mean difference (+/– standard deviation, SD) of 0.05 (0.13) mm was negligible. Similarly, inter-observer repeatability of the magnified-image measurements yielded a significant difference ($p < 0.02$), but again with a small mean difference of 0.08 (0.21) mm.

NT increases with gestational but not maternal age. Reproducibility is poor: by repeating measurements with a different operator, the same operator using a different still image, or the same operator using the same still image, 13.8%, 12.5%, or 11.9% of NT measurements, respectively, change their classification as normal or abnormal.

Discussion

NT measurement of 3 mm or more (≥ 95th percentile for gestation age), hydrops fetalis, or hygroma colli between the 11th and 14th weeks of gestation is associated with a higher risk of fetal DS and other aneuploidies. So far, chromosome preparation of chorionic villi samplings (CVS) after short-term (or direct) culture is the only valid, reliable, and rapid method of choice for the early detection of chromosomal aberrations. However, because of the placental mosaicisms detected after short-term culture, CVS has to be confirmed by a second method. Moreover, short-term villi preparation does not always provide a sufficient quantity and quality of metaphases to enable cytogenetic analysis. Unfortunately, a predicative cytogenetic result will be available only after long-term cultivation (usually after 1-2 weeks).

An alternative rapid method, inexpensive and suitable for diagnosing autosomal trisomies, is quantitative fluorescence polymerase reaction (QF-PCR) using different polymorphic small tandem repeats (STRs) on CVS-DNA. All early pregnancies with a clinically relevant autosomal trisomy could be detected prenatally in routine practice by QF-PCR. The combined use of both rapid methods (QF-PCR and short-term CVS chromosome analysis) optimise the results by minimising the possibility of false-positive or false-negative findings. After verification of a pathological result obtained by the two independent methods, long-term villi cultivation is no longer necessary. However, in all cases with discrepancies, especially in samples with mosaic findings at short-term CVS cultivation, further studies are still necessary [16].

The risk of fetal trisomy can be derived by combining maternal age and fetal NT thickness at 11 to 14 weeks of gestation. It is predicted that for a false positive rate of 5%, the sensitivity of the new method of screening would be at least 85%, which compares favourably with the respective 20 to 30% and 50 to 60% of screening based on maternal age alone or the combination of maternal age with maternal serum biochemistry [17].

At 11-14 weeks' gestation, fetal NT of 3 mm is associated with a fourfold increase, and NT of greater than 3 mm with a 29-fold increase, in the maternal age-related risk for trisomies 21, 18, and 13. Fetal NT of 4 mm or

more is associated with poor pregnancy outcome even when the fetal karyotype is normal [18].

Increased lethality in fetuses with enlarged NT and normal chromosomes may provide evidence that the same insult causing excessive fluid collection in the nuchal region may also be responsible for fetal demise [19].

Although it is not possible to correlate the sonographic data with post-evacuation microdissection findings, it is possible that a uniformly shaped, increased NT may be more representative of a developmental delay in a normal fetus. Conversely, a "notched" nuchal surface may represent abnormal lymphatic or cardiovascular development more commonly seen in DS fetuses [20].

Sonographic assessment of the umbilical cord too in early gestation appears to identify a subset of fetuses at increased risk of chromosomal abnormalities [21].

The umbilical cord can be measured together with the NT thickness when it passes around the fetal neck and this adds a mean of 0.8 mm to the actual NT measurement. After the thickness of the cord was subtracted, the measurements of NT thickness did not differ from those in the overall population studied. These findings indicate that the presence of a nuchal cord may bias the results of fetal NT measurement and that the use of color Doppler (CD) might decrease the false-positive rate in screening for fetal aneuploidy by NT measurement at 11-14 weeks' gestation [22].

The incidence of ventricular and atrioventricular septal defects is much higher in fetuses with trisomy 21 and increased NT thickness at 11 to 14 weeks' gestation than in live-born infants with this chromosomal abnormality. The incidence of cardiac septal defects increases with NT thickness [23].

Ductus venosus Doppler velocimetry can be used in addition to NT measurement as a predictor of chromosomal anomalies. However, as the ductus venosus blood flow pattern is correlated with NT measurement it cannot be used as an independent variable to reduce the indication for fetal karyotyping. Ductus venosus Doppler velocimetry may have a role in the counseling of parents in the case of an enlarged NT and normal karyotype by identifying those fetuses in need of an intensive follow-up due to an increased risk of adverse outcome [24].

These preliminary results suggest that evaluation of the ductus venosus PI at 10-16 weeks' gestation is a useful second-line screening test for chromosomal defects. A combination of NT measurement and ductus venosus assessment might increase specificity while maintaining an optimal detection rate for chromosomal abnormalities [25].

Cardiac defects are the most prevalent congenital anomalies. Screening policies have adopted an 18- to 22-week ultrasound scan to detect such anomalies. However, diagnosis may be feasible early in pregnancy using transvaginal Doppler ultrasound. Increased NT has been associated with major chromosomal anomalies and is being increasingly related to cardiac defects. Considering that venous blood flow patterns may provide additional clues to cardiac function, it may be useful as a complementary tool for the earlier diagnosis of structural cardiac anomalies [26].

Abnormal ductus venosus blood flow in chromosomally normal fetuses with increased nuchal translucency identifies those with an underlying major cardiac defect [27].

The significance of the association between isolated echogenic intracardiac foci and DS is a matter of ongoing debate. The data of this study suggest that in an unselected obstetric population with prior, effective, routine DS screening, the association between isolated echogenic intracardiac foci and DS is no longer significant [28].

Real-time 2D ultrasound is still the best way to examine fetal anatomy in the first trimester. However, 3D ultrasound can be a useful addition to clinical practice, providing views not easily obtained by conventional 2D ultrasound. It can potentially minimize actual scanning time and provide an excellent way to store scanned data [29].

We propose to define a high-risk group associated to the NT marker by using a cut-off risk of 1/250 for the simultaneous risk. This criteria may, as well, be expressed by a pathological threshold of NT varying with maternal age and gestational age. Without questioning that women aged 38 years or older are a high-risk group, this approach should allow an improvement of the prenatal screening for trisomy 21.

Other findings suggest that there is an added role of fetal heart rate (FHR) in ultrasound screening of chromosomal abnormalities, specifically for those other than trisomy 21 and 18. The value of a single measurement of FHR for screening purposes needs to be confirmed by further investigation in a low-risk population [30].

Estimating the distribution of NT in MoM values will assist in specifying the statistical parameters to be used in prenatal screening for DS and the use of repeated NT measurements is expected to have a useful effect on reducing the screening false-positive rate at a given MoM cut-off level.

For singletons and twins, a sequential NT and second trimester serum marker screening can be offered, thus producing a single-risk estimation which seems to be more accurate. For the high order of multiplicity, NT offers additional important data, which can be taken into consideration both as a screening tool for DS and if fetal reduction is planned.

Assisted reproduction may adversely affect second trimester screening results, which did not affect the NT screening test [31].

The benefits of first-trimester genetic sonography depend on its diagnostic accuracy [32]. NT image magnification does not contribute to the reproducibility of the measurement. Despite significantly smaller mean values obtained from the magnified images, compared to the regular-sized measurements, those differences do not justify modification of the criteria for caliper placement on magnified images. Blind repeated measurements on a regular-sized and/or magnified image are recommended as a tool for self-assessment, quality control, and training [33].

In 95% of the time the intraobserver, interobserver, and caliper placement repeatability of measuring fetal NT are less than 0.54 mm, 0.62 mm, and 0.58 mm, respectively. In addition, the repeatability is unrelated to the size of the NT. These findings demonstrate that, when the NT thickness is measured by well-trained operators, the measurement is highly reproducible [34].

Global qualitative review of images from one sonographer may be preferable to assessment of individual aspects of images. Results from global qualitative review correspond well with findings from quantitative analysis, indicating that the latter can be applied for ongoing audit. Observation of divergent results should prompt extensive personal feedback, rather than a written report, to prevent sonographers from settling in their own, inappropriate technique [35].

As for the introduction of every new technique in routine clinical practice, the operators who perform the ultrasound scan at 11-14 weeks should be submitted to adequate training and to strict quality control.

The results obtained confirm the potential application of the measurement of NT thickness for fetal aneuploidy screening before the end of the first trimester and suggest that a multiplicity of individual, structural, and organizational factors may interact and play a crucial role in determining the actual efficiency of ultrasound screening programs [36].

Implementation of an ongoing audit, using the image-scoring method, proves to be an efficient method for surveillance and improving the quality of NT images. We recommend centres or individuals practicing first trimester ultrasound screening to consider its routine utilization, in an unbiased and strict manner [37].

The findings of this study demonstrate the feasibility of introducing scanning at 11-14 weeks' gestation and the measurement of fetal NT thickness in routine maternity units.

The risks for fetal trisomies at 11-14 weeks' gestation can be derived by combining data on maternal age, maternal serum PAPP-A, and fetal NT thickness [38].

Whether the good discriminatory power of PAPP-A can be realized in second trimester screening programmes will depend on developing two-stage screening algorithms. This approach is unlikely to be better than the excellent detection rates achievable with free beta-hCG, PAPP-A, and NT in the first trimester [39].

Measurement of maternal serum AFP concentration in the first trimester of pregnancy is not likely to be useful in the prediction of fetal trisomies [40].

In the same way, first trimester biochemical screening for trisomy 21, which is currently optimised using maternal serum free beta-hCG, PAPP-A, and fetal NT, will not benefit from the inclusion of inhibin A [41].

Second trimester prenatal screening for trisomy 21 and trisomy 18 using a simple two-marker approach incorporating free beta hCG can achieve high detection rates over a long period of time. Health authorities who still have not introduced trisomy 21 screening should be encouraged by what can be achieved and should consider making such screening available to all women. Established second trimester detection rates of 75% for a 5% false positive rate will be the benchmark by which first trimester screening using NT, PAPP-A, and free beta hCG will be judged [42].

The implications for such between pregnancy marker association is that women who have an increased risk of DS in their first pregnancy are 1.5-2 times more likely to repeat this event in their next pregnancy. This observation may be useful in counselling women in the first trimester screening of a subsequent pregnancy [43].

These findings show that in chromosomally normal fetuses increased NT thickness at 11-14 weeks of gestation is a marker for fetal abnormalities including structural defects and genetic syndromes [44]. But they also show that if fetal karyotype is normal, the incidence of congenital malformations seems to be the same by comparison with the general population [45].

In conclusion, this study demonstrates that first trimester NT measurement is an effective method of screening for fetal chromosomal abnormality [46].

Knowledge of NT could lead to a decrease in the demand for invasive diagnosis and to a more frequent diagnosis by first trimester transabdominal CVS [47].

The measurement of NT and the evaluation of other sonographic signs in the first trimester scan allow a detection rate of 70-80% of aneuploid pregnancies, significantly more than with consideration of the maternal age alone (30%). The sonographic signs include early growth retardation, deviations of the FHR, exomphalos, megacystis, holoprosencephaly, and enlargement of the cisterna magna. Maternal serum biochemistry alone (PAPP-A and beta-hCG or AFP, uE3 and beta-hCG) detects about 65% of aneuploid pregnancies. The best individual risk estimation is based on maternal age, measurement of the NT, and the maternal biochemistry [48].

In an unselected population, NT $\geq$ 3 mm is associated with a high incidence of chromosomal and non chromosomal abnormalities. Even when the fetal karyotype and serial ultrasound examinations are considered to be normal, the risk of fetal malformation and developmental delay should not be underestimated [49].

Targeted ultrasonographic screening for nuchal fluid accumulation during the first trimester (11-14 weeks) seems to be a recommendable method for the detection of DS and other chromosomal anomalies in pregnant women. It compares favourably with current methods of maternal serum screening performed during the second trimester [50].

The current trend in prenatal diagnosis is that trisomy screening is being moved to the first trimester and ultrasonographic NT measurement is included in risk calculation. It is likely that biochemical screening in the second trimester will gradually be given up [51].

Conclusions

NT is the sonographic appearance of a subcutaneous collection of fluid behind the fetal neck. The measure-

ment of fetal NT thickness at 11-14 weeks' scan has been combined with maternal age to provide an effective method of screening for trisomy 21; for an invasive testing rate of 5%, about 75% of trisomic pregnancies can be identified. When maternal serum free β-hCG and PAPP-A at 11-14 weeks are also taken into account, the detection rate of chromosomal defects is about 90%. Increased NT can also identify a high proportion of other chromosomal abnormalities and is associated with major defects of the heart and great arteries, and a wide range of skeletal dysplasias and genetic syndromes. In monochorionic twins, discordancy for increased NT is an early marker of TTTS. Thus, fetal NT ≥ 3 mm is a useful first trimester marker for fetal chromosomal abnormalities [52]. An improved estimate of risk for fetal trisomies at 11 to 14 weeks' gestation can be derived by combining data on maternal age, maternal serum total or free beta-hCG, and fetal NT thickness [53]. As with the introduction of any new technology into routine clinical practice, it is essential that those undertaking the 11 to 14 week scan are adequately trained and their results are subjected to rigorous audit [54].

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