Research article

Fetal structural and genetic anomaly screening. Are we doing it correct at the age of NIPT?

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Key content

- History and Evolution of screening for trisomy 21 and other aneuploidies.
- Genetic, Structural, and biochemical screening.
- First trimester combined test. detection rate of 90% at an FPR of 5%.
- Second trimester serum biochemistry- detection rate of 60-75% at an FPR of 5%.
- NIPT as a test – detection rate 99% with a reduction in FPR to 0.1%.
- NIPT-how best to incorporate in to screening protocols.
- Growth and placental disorders- PlGF and sFlt-1

Food for thought

- First trimester USS at 10 weeks.
- NT- should we be investing on it any further, or is it getting obsolete?
- NIPT- can it be universal?
- NIPT- How best to introduce?
- Place of genetic screening with NT and USS in the age of NIPT?
- NIPT- is it state funded Eugenics?
- Genetic vs Structural screening-together or should it be separate?
- Early dating scan followed by NIPT
- Can we scan early-for structural anomalies?
- Parental choice in a setting with limited options.
- Best way of screening?

Key words: prenatal diagnosis, antenatal care, fetal screening, nuchal translucency, NIPT, early fetal scan, anomaly scan.

Sri Lanka Journal of Obstetrics and Gynaecology 2021; **43:** 166-173

DOI: http://doi.org/10.4038/sljog.v43i3.7958

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Received 3rd September 2021

Accepted 3rd November 2021

1. Screening the Fetus

Currently, fetal screening can be looked in three broad areas. Screening for genetic anomalies, structural anomalies, and growth/placental disorders. Screening should be accessible universally. Any protocol that requires expensive laboratory testing, high end equipment and/or tertiary level training for operators cannot be considered as useful screening. Screening is primarily done to allow the parents to better execute reproductive choices including safe termination of an affected fetus. Consequently, current uncertainty of the Sri Lankan legal framework may severely limit usefulness of such programs. It is noteworthy, present trisomy screening criteria in the UK involves combined screening. Whoever is detected to have a high risk by combined testing, is offered Amniocentesis. Women who do not accept invasive screening at this stage are offered NIPT. Different strategies are adopted around the globe based on slightly varied principles.

❖ Genetics

In 1866 doctor "John Langdon Down" described syndromic babies with excess skin, flat face and small nose in his ever-famous article, "Observations on an ethnic classification of idiots"[1]. Nearly 150 years has passed by, and the humanity has come a long way, in fetal screening and identifying fetal anomalies. Today it is an integral part of modern obstetrics. With the development of modern ultrasound machines, there's sound argument for screening in the first trimester, hence, most protocols around the world are based on combined screening. Maternal Serum markers, (β hCG and PaPP-A) in combination with ultrasound evaluation of Nuchal translucency, added up with "a Priori" risk based on maternal age has been the accepted practice[2,3]. The best results are observed around 11-13+6 Weeks of gestation with this strategy, nearing 90% detection rate at a 5% false positive rate for fetal aneuploidy (Trisomy[21,18&13], monosomy X and Tri-ploidy)[4,5,6,7]. Screening is performed in a single visit avoiding prolonged waiting times, minimising the maternal anxiety that comes with it. For late bookers second trimester biochemical screening carried out between 15-20 weeks has been the norm with nearing 70% to 76% detection rates for triple and quadruple tests, respectively[8,9,10]. (Comprising MSAFP, β hCG, uE3 and Inhibin.)

Table 1. Comparison of different methods of screening for fetal trisomy 21

Method of screening	Detection rate (%)	False positive rate (%)
1. Maternal age 30	30	5
2. First trimester		
2.1 Combined test	90	5
2.2 Cell-free DNA test	99	0.1
3. Second trimester – Serum biochemistry.		
3.1 Double test (AFP, free β-hCG)	60-65	5
3.2 Triple test (AFP, free β-hCG, uE3)	65-70	5
3.3 Quadruple test (AFP, free β-hCG, uE3, inhibin A)	70-75	5

❖ Anomaly screening

With high resolution ultrasound machines, generating real-time high-quality visualisation ability, with relative ease of use, structural screening is almost exclusively ultrasound based today. Use of MRI in fetal screening is limited to highly specific areas only. With the development of 3D and 4D ultrasound machines acceptability has become even greener. Moreover, USS has a fairly short learning curve.

❖ Growth and placental disorders

Screening for growth disorders are based on screening the fetus over time, starting from 24 weeks onwards. This strategy has fair space for improvement. More useful screening is likely to shift to first trimester, and identify women with high risk. Studying the physiological changes of the uterine artery with doppler flow, serum biochemistry with PlGF and sFlt-1 (placental growth factor and soluble fms-like tyrosine kinase I) and other molecular candidates have opened up whole new depths for early screening for pre-eclampsia/ IUGR and other placental disorders[11,12,13].

2. First trimester ultrasound scan

Scanning in the first trimester was introduced primarily for accurate dating and assessment of fetal viability. It also had the advantage of detecting multiple pregnancies accurately as chorionicity and amnionicity could be determined. Accurate establishment of chronicity at an early stage became the cornerstone of managing multiple gestations.

A clear gestational sac can be visualized as early as 4 to 5 weeks with a transvaginal scan. However, dating is best done at 11-13+6 weeks as it allows the integration of genetic screening in a single encounter. By this time, the embryonic development is relatively complete, and the cranial Vault is ossified. Beyond the upper cut-off, image acquisition is thought to be difficult due to fetal lie reducing the effectiveness of NT as a marker for aneuploidy[14]. Nuchal translucency, (the sonolucent area beneath the skin at the nape of the neck) is best assessed with the dating scan at 11 to 13+6 weeks corresponding to a CRL measurement of 45-84 mm[4,5]. Mid sagittal section of the fetus is taken with the head in a neutral position. Increase in nuchal thickness increase the risk of the fetus, being affected with a chromosomal/ structural anomaly. Visualizing some of the gross fetal anomalies became a possibility with the modern high-resolution scanners, specially the cerebral and brain anomalies. Anencephaly, holoprosencephaly, encephalocele, dandy walker Syndrome, gastroschisis, exomphalos, megacystis and diaphragmatic hernia are recognised examples[15,16]. It has been shown that using TAS and TVS in combination may detect up to 59% of major structural defects at 11-13+6 weeks[15].

3. NIPT as a Test

Dead and dying cells release DNA fragments to the plasma. In 1990s these fragments were first identified. Lo et al. in 1997 demonstrated the presence of cell free fetal DNA in plasma of pregnant women[17]. Later the yielding mechanism was developed and today it has come into clinical practice. Exact chromosomal origin of each DNA fragment can be identified and quantified Since the completion of the Human Genome Project. Cf-DNA in maternal plasma contains both fragments arising from maternal cells as well as fetal cells. The fetal fraction (Proportion of cell free fetal DNA) is generally around 10%[18]. NIPT depends on the ability to detect the increase in total amount of DNA. With current technology standards minimum of 4% fetal fraction is needed for a successful test result. Cost of the test has undergone dramatic reduction since its first introduction making it a feasible option to be introduced in to screening protocols worldwide.

Analysing Fetal DNA released by the trophoblastic cells into the maternal plasma (cell-free fetal DNA) yielded a high sensitivity coupled with remarkably low false positive rate of less than 0.1%[18,19,20]. This has not only improved the detection of main three trisomies to more than 99%, but also detection of sex chromosomal abnormalities as well as some microdeletions, such as DiGeorge syndrome[20,21]. In the view of slightly less than 100% detection rate, NIPT still remains as a screening test. However, this significantly reduces the false positive rate and the maternal anxiety as well as the inadvertently aborted babies with diagnostic testing. If the NIPT showed a high-risk then the possibility of chorionic villus sampling at 12 weeks is still feasible versus opting for amniocentesis at a later gestation. On the contrary, if the NIPT yielded low risk for trisomies, couple can be reassured that it is extremely unlikely that the pregnancy is affected. However, NIPT is not without its unique technical challenges. Maternal

chromosomal rearrangements, Confined placental mosaicism, Maternal malignancies could release abnormal maternal cf-DNA, causing discordant NIPT results, demanding conformation with maternal karyotype. NIPT is comparatively expensive and is the main limitation in broader application for genetic anomaly screening worldwide.

4. Is there a place for genetic screening with NT and USS in the age of NIPT?

The development of a screening tool with a high credibility needs to have number of essential components. Firstly, there should be clearly defined non operator dependent marker variables. These should be easily reproducible and the cost of training as well as the learning curve must be a minimum. These shouldn't be population based for widespread adaptability. More importantly the variables should have high likelihood ratios, high sensitivity with minimum false positive rate.

In combined screening, NT is an operator dependent variable. Assessment is challenging in the obese population. It is not a diagnostic marker but merely gross indicator for a potential fetal anomaly. It's true that it has served the obstetricians commendably over the last couple of decades. However, when a better testing modality becomes available, the million-dollar question that arises is "Should we be investing time, skill and the finances into a test that gives lesser diagnostic accuracy?". Ultimately the overall cost effectiveness for the healthcare system has to be conciderd, not only the cost of a test in its face value.

5. Screen for genetic or structural anomalies simultaneously or should it be clearly separate?

Described incidence of major structural anomalies is 2-3 percent. Unsurprisingly, this incidence far exceeds all babies born with chromosomal abnormalities or single gene defects[22]. In the current strategy, anomaly scan is deployed at 18-22+6 weeks in many protocols for structural screening. Since fetal structures are best assessed visually, no other method can compete with modern ultrasound in quality, accessibility, safety, and cost.

There is little doubt, from an assessment based on maternal age alone to the advent of serum screening and finally combined screening including NT, have achieved a remarkable feat over the past 2 decades. This improvement of detection of aneuploidy approximately from 30% to greater than 90%, in itself is a testimony to the rapid pace of development of modern medicine. However, with the above testing, false positive rate remained fairly constant around 5 % until the new technology, Non-invasive prenatal testing (NIPT) gave it advances never achieved before. Combined test inclusive of NT measurement as a marker for chromosomal anomaly would give more than 5% of women false positive test result pushing them for an invasive diagnostic test (CVS, Amniocentesis or cordocentesis best done at 11-13+6, 15-16, ≥ 20 weeks respectively)[23]. Since invasive testing carries a small yet measurable risk of inducing miscarriage, a 5% false positive means there will be a number of normal foetuses that will be affected by performing the test. The best way of fetal screening is yet to be established. Few of the draw backs of implementing NIPT as the screening test would be its high cost, challenges faced with twin pregnancies and higher gestation pregnancies, poor fetal fraction in monosomies, still being a screening test irrespective of the high detection rates as well as the maternal anxiety related to relatively long hold until the results become available. Bearing this important fact in mind there may be a clear argument that we should separate structural and genetic screening in the age of NIPT, rather than transpiring risk assessed using an ultrasonic marker (NT) for genetic screening.

6. A proposed strategy to be time tested

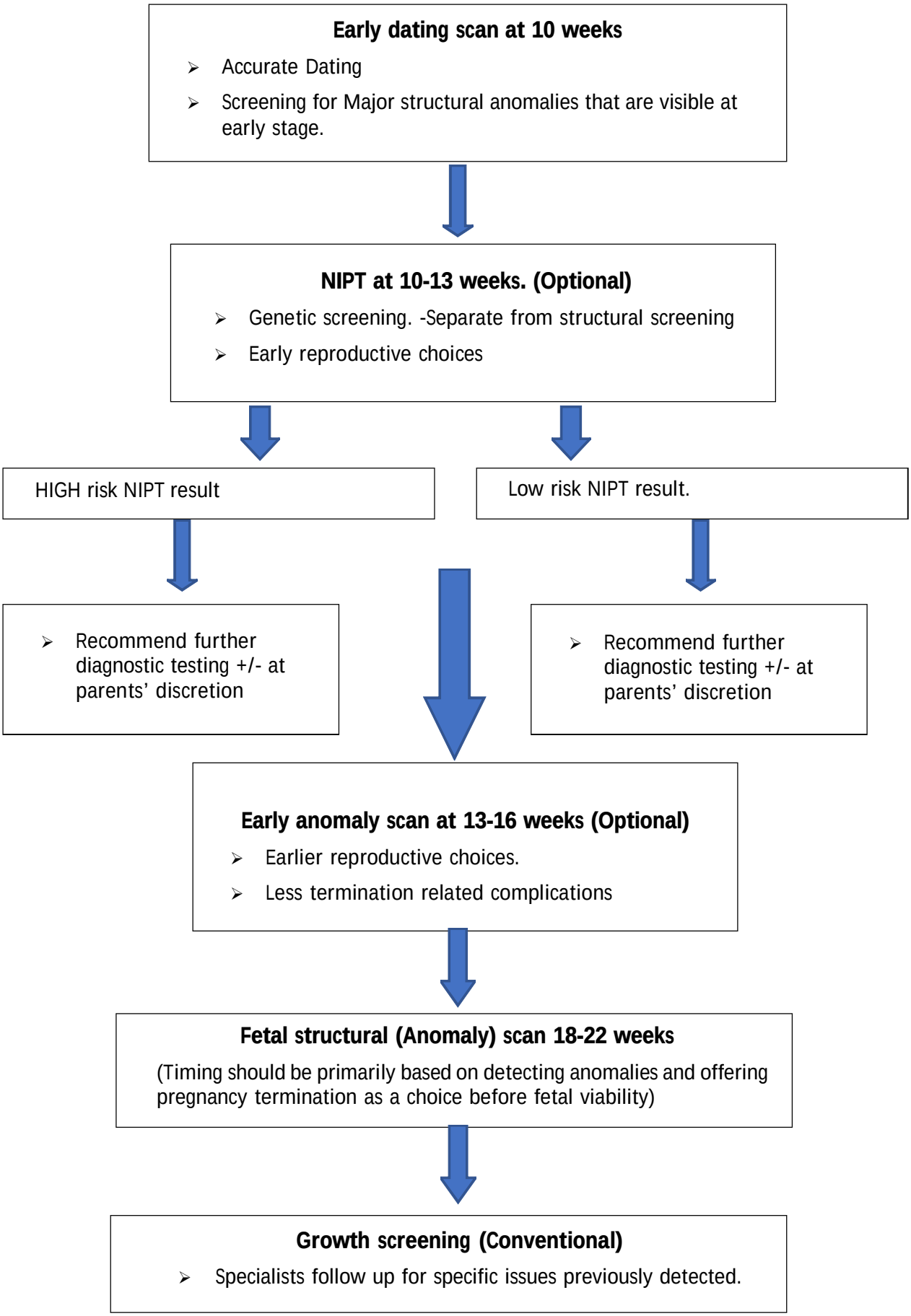
Place of an early dating scan around 10 weeks is gaining credibility with accumulating evidence that, it has all the benefits of a scan between 11-13+6 for dating purposes[24,25,26]. Most of the cerebral structural anomalies would be visible even in an earlier stage around 10 weeks with modern high-resolution scans. Current anomaly scan may be brought down by few weeks from 18-20 +6 weeks (to around 14- 16 weeks). This will facilitate earlier screening for structural anomalies giving earlier choices to parents. The downside of a strategy like this would be, necessitating the need for an anomaly scan by a highly trained specialist in fetal medicine, with high-end USS equipment to avoid missing some of the anomalies that would have otherwise easily been detected at 18-20+6

weeks by a generalist. This may be a commodity that we may not be able to afford, in a universal screening program in today's context.

It has been shown that non-lethal cardiac abnormalities, especially the late onset conditions are best detected at 22-24 weeks**27**. So, one might argue morphology scanning beyond 20 weeks is not totally obsolete and it may be too early to bring down the anomaly scan to an earlier gestation. However, its usability in a specialized centre cannot be ruled out. A strategy of genetic screening with NIPT, and structural screening with an earlier anomaly scan, clearly separates genetic and structural screening with higher detection. With the reducing costs of NIPT it can be implemented around 10-13 weeks giving the couple their reproductive choices at an earlier stage with higher accuracy. It will open-up choices on an individual basis whether to go for diagnostic test earlier at the couple's discretion. In a setting where government funding is limited, should a clinic based prior risk stratification approach leading to NIPT hold more productivity is an important thought. However, there looms an important ethical dilemma. If this is eugenics practiced by state? and if it is justifiable to use public funding for that purpose. In a culture where iatrogenic abortion for a lethal fetal anomaly is not allowed, NIPT will only prepare the parents for what to expect at an earlier stage. These issues need further discussion prior to implementation of any state funded screening program.

NIPT fulfils most of the screening qualities. However, there will always be an argument against a test that needs advanced genetic platforms like Next Generation Sequencing technology, while demanding at least 10 days for results. In Sri Lankan context there are few laboratories which offer NIPT as a screening tool for aneuploidies. Two laboratories offer in-house NIPT testing, while others being collection centres of overseas laboratories. Currently, all these are commercial labs and are virtually based on revenue generation. The cost of NIPT in Sri Lanka ranges between 35,000-75,000 LKR which precludes it being incorporated in the public sector expenses. How best to adopt NIPT in a wider scale and cost analysis in screening preview, would be a topic for a separate article.

Anomaly detection will open better treatment and follow up options for a fetus with a salvageable condition considering the advancements made on fatal therapy as well as time-tested treatment modalities. Finding the best path of fetal screening is a complex undertaking. Screening for genetic, structural and placental disorders will be the three-pronged trident for the future, each component holding merit on its own. Screening for placental disorders is less clear at the moment and mandates further research. How best to incorporate NIPT into a screening protocol? If it should be introduced with public funding? Or should it be at the parent's discretion or how cost effective would it be? is an open debate to be proven with scientific evidence in the years to come in our quest to find the optimum fetal screening tool.


Early dating scan at 10 weeks
Accurate Dating
Screening for Major structural anomalies that are visible at early stage.
NIPT at 10-13 weeks. (Optional)
Genetic screening. -Separate from structural screening
Early reproductive choices
HIGH risk NIPT result
Low risk NIPT result.
Recommend further diagnostic testing +/- at parents' discretion
Recommend further diagnostic testing +/- at parents' discretion
Early anomaly scan at 13-16 weeks (Optional)
Earlier reproductive choices.
Less termination related complications
Fetal structural (Anomaly) scan 18-22 weeks
(Timing should be primarily based on detecting anomalies and offering pregnancy termination as a choice before fetal viability)
Growth screening (Conventional)
Specialists follow up for specific issues previously detected.

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