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A Systematic Review of the Current Policies and Implementation of Screening for Foetal Abnormalities; the Use of Ductus Venosus Flow in Screening for Down's Syndrome

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Abstract: Down's syndrome is one of the commonest causes of mental retardation. It is associated with abnormal ductus venosus flow. We aimed to evaluate in a systematic review of the literatures available, the performance of screening for Down's syndrome using ductus venosus assessment. Studies were got from medline, web of science and pubmed using the keywords above. We selected works that studied the diagnostic performance of ductus venosus for screening for Down's syndrome and other foetal aneuploidies. About 20 studies were reviewed. About 9 studies worked on populations with high risk for Down's syndrome. About 16 studies found the addition of ductus venosus assessment valuable in the screening for Down's syndrome. Most studies however did not notice improvement in detection rate when ductus venosus was used alone. The best results were seen when ductus venosus was used alongside nuchal translucency. We concluded that the addition of ductus venosus to screening strategies for Down's syndrome should be considered.

Key words: Ductus venosus, Down's syndrome, chromosomal abnormalities, DV, screening

INTRODUCTION

Down's syndrome affects about 1 in 1000 live born babies in the United Kingdom. It occurs when an individual gets 3 rather than 2 copies of chromosome 21 as a result of translocation or trisomy. A great majority of mental retardation is caused by Down's syndrome. It is associated with cardiac defects including abnormal ductus venosus amongst other abnormalities. In the 1st trimester, 65% of trisomy 21 foetuses have cardiac atrioventricular or ventricular septa defects and 49% narrowing of the aortic isthmus (Hyett *et al*., 1995a, b).

Down's syndrome has no cure so prenatal screening and diagnosis are the options available for parents, affording them the opportunity to make a choice. Excellent screening measures are therefore required in order to reduce exposure to invasive prenatal diagnosis with all its attendant consequences.

The ductus venosus is a trumpet shaped structure that links the intra-abdominal umbilical vein to the inferior vena cava. It allows blood containing oxygen leaving the placenta to bypass the liver. It also allows blood leaving the inferior vena cava to go to the left atrium without mixing with deoxygenated blood coming from the right chamber (Ritter *et al*., 2004).

Assessment of ductus venosus doppler findings can be performed either by the use of venous doppler index or by waveform pattern commonly. The ductus venosus waveform in normal foetuses in ventricular systole and diastole will show a peak projection of velocity and in atrial contraction will give a nadir (Antolin *et al*., 2001). A triphasic pattern during atrial contraction is regarded as normal and a reversed or absent flow during the phase of contraction of the atrium is abnormal and has been linked with aneuploidies (Matias *et al*., 1998) hence, the use of ductus venosus assessment for the screening of aneuploidies.

Assessing the ductus venosus flow: The ductus venosus is recognised by colour doppler mapping in the first trimester as the part with the higher velocity following the umbilical vein. It should be assessed using the following criteria:

- Foetal quiescence is necessary for examination
- Foetal Thorax and abdomen should occupy the screen, after adjusting the magnification
- A right ventral mid sagittal view of the trunk of the foetus should be gotten and the umbilical vein, foetal heart and ductus venosus should be demonstrated using colour flow mapping
- Pulsed doppler sample ought to be between 0.5 and 1 mm to prevent being contaminated by adjacent veins. It can be positioned in the area immediately above the umbilical sinus

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- The <30% insonation angle should be used
- Filter frequency should be between 50-70 Hz
- Sweep speed of 2-3 cm/sec is used in order to allow for better assessment of the A wave

The UK NHS policy recommendations for Down's syndrome currently upholds the use of the combined test for patients who present early and quadruple test for patients who present late for antenatal care. They have recently incorporated assessment of nuchal translucency for Down's syndrome screening. So far they aim to achieve 90% rate of detection with a 2% screen positive rate for the combined test and 75% detection rate with screen positive rate of 3% for quadruple test. In estimating the risk of Down's syndrome, the combined test uses maternal age, first trimester blood markers with nuchal translucency. It is believed that this detection rates can be improved on. Hence, the constant search for other methods that might improve the detection rate and reduce invasive testing. Assessment of the ductus venosus is one of such methods. Several studies have looked into its practicability and its accuracy in detecting chromosomal abnormalities and it is still debatable if assessment of ductus venosus should be added into the routine screening programme for antenatal patients. It is expected that this work will attract further studies in this regard especially in practical clinical scenarios and will help policy makers come to a decision on this matter.

Literature review: In a study done by Aune and Moller (2012) "In I want a choice but I don't want to decide" a qualitative study of pregnant women's experiences in assessment of risk for Chromosomal abnormalities, we are able to appreciate how sensitive the issue of foetal anomaly screening is to the pregnant woman. Hence, the need for excellent screening methods with good sensitivity and specificity that will reduce the incidence of false negative or false positive cases during screening. There is an increasing urge to select with higher efficiency which parents should be offered prenatal diagnosis. Prenatal diagnosis (amniocentesis and chorionic villi sampling) carry inherent procedure related risk so that health care providers are in a continuous dilemma as to how to identify patients with increased risk and in so doing, not subject those with low risk to unnecessary diagnostic procedures (Kittichottipanich *et al*., 2016).

Down's syndrome affects about 1 in 800 live born babies. It occurs when a person gets 3 rather than 2 copies of chromosome 21 as a result of trisomy or translocation. It is the most common cause of mental retardation. It is associated with several congenital abnormalities especially affecting the heart. It could also predispose to Cancers, diabetes and thyroid disease. Having a baby with Down's syndrome can be physically and emotionally draining for the parents. Down's syndrome has no cure so prenatal screening and diagnosis helps parents-to-be prepare for the birth and subsequent care of the child or for the possible offer of pregnancy termination.

Brief history of screening for Down's syndrome: Prenatal screening for Down's syndrome has evolved over the last years. A maternal age of >35 years at expected date of delivery was used as the basis for Down's syndrome screening in the 1970's. Women older than 35 years were offered prenatal diagnosis. Using maternal age alone identifies only approximately 30% of affected foetuses (Wald *et al*., 2003). However, in a study done reviewing the pattern in Down's syndrome live births and prenatal diagnosis from 1989-2008, they found that maternal age is a very powerful predictor of Down's syndrome hence its importance in Down's syndrome screening cannot be overemphasized especially in risk assessment although after the age of 45, prevalence of Down's syndrome begins to decline with increasing maternal age this as against previous belief that there is a steady increase in prevalence of Down's syndrome with increasing maternal age (Morris *et al*., 2002).

In Glasgow, the issue of younger women getting more and more associated with down syndrome is seen as represented by the increase in termination of pregnancy among these women who are supposedly low risk. This shows that antenatal screening is now beneficial even to the lower risk younger age group of women. The epidemiological effect of antenatal screening and hence ensuing antenatal diagnosis is clear with birth and pregnancy prevalence rates going in different directions steadily over time since the late 1980s (Ilayasu *et al*., 2002). Hence, there is a need for even more accurate screening methods.

In 1988, screening using maternal age was made better by introducing the second trimester triple test. The test was also known as, the Kettering test, triple screen or the Bart's test. The triple test measures maternal blood levels of alpha fetoprotein, oestriol and beta-human chorionic gonadotrophin with 70% sensitivity and 5% false negative rate. Some centres at this time adopted the double test where only alpha fetoprotein and human chorionic gonadotrophin are measured in addition to maternal age, weight and ethnicity. Further, improvement on the triple screen was achieved by the addition to inhibin A to form the quadruple test. About 3 first trimester markers; free beta Human Chorionic Gonadotrophin (beta-HCG), Pregnancy Associated

Plasma Protein A (PAPP-A) and the ultrasound marker nuchal translucency were also discovered to be of value for foetal anomaly screening during this period.

Recent strategies for screening: A systematic review done in 1997 recommended that the quadruple test or a second trimester triple screen should be the screen test of choice (Wald *et al*., 2003). SURUSS in perspective suggested that integrated test is the best and safest method of screening for women who attend antenatal clinic in the first trimester. Although, it has been suggested that this result was because the nuchal translucency scan was poorly done. It is still believed that the combined test is the best (National screening committee). The serum integrated test is thought to be the next best test. The quadruple test was suggested to be the best test for those who attend antenatal clinic for the first time in second trimester. They concluded that there was no true reason to retain the double test (alpha fetoprotein and human chorionic gonadotrophin) or triple test (alpha fetoprotein, estriol and human chorionic gonadotrophin) or nuchal translucency alone (with or without maternal age) in screening for Down's syndrome. In 1999, a combination of markers from first and second trimesters was introduced and this was called the integrated test. This was discovered to get better results than using either trimester alone. In addition, beta-core fragment and invasive trophoblast antigen are urinary Down's syndrome markers which have been recently recommended as possible screen tests for Down's syndrome.

Efforts to make available screening methods to identify mothers at high risk of carrying a fetus with chromosomal abnormalities have focused on nuchal translucency and serum biochemistry as well as the presence of other sonographic markers. There have been a lot of controversies regarding the best combinations of screening tests and whether they should be offered in the first or second trimester or in both. In Australia, second trimester serum screening and first trimester serum in combination with nuchal translucency measurements are the established standards of care and have been utilized with increasing rates of uptake since 1980's (Chang, 2006).

The American college of Gynecologists recommends offering invasive testing to women older than 35 years and to women with a maternal serum screen that comes out positive (Slack *et al*., 2006). The United Kingdom national screening committee advocates that screening be done in the time window of 10 weeks+0 days-20 weeks+0 days gestation. Preferably screening should be complete by 14 weeks+2 days gestation. Recommended programme outcomes are that there should be "detection rate for Down's syndrome of greater than 90% of affected pregnancies with a screen positive rate of <2%". However, this projected outcome cannot be reached using the recommended screen tests. The present strategy is based on achievement of a detection rate >75% with a screen positive rate 2%. The recommended screening strategies from 2007 are first trimester combined tests using nuchal translucency assessment and serum biochemistry testing to measure free beta human chorionic gonadotrophin and pregnancy associated plasma Protein A. This test is the recommended method to allow for early antenatal diagnosis and risk assessment (before 14 weeks) hence giving parents opportunity to decide on what they want to do quite early on in pregnancy.

It allows for a 1 stage screen without need for another attendance. The recent revision of the nice clinical guideline on antenatal care also advises on the use of first trimester combined test. With increase in crown-rump length, nuchal translucency increases. If a fetus has a given crown rump length, a new risk can be calculated by multiplying every nuchal translucency measurement which represents a factor by the background risk. It is usually done at 11 weeks+0 days and before 13 weeks+6 days gestation.

A gestational age estimate gotten by ultrasound if available at the time of Down's syndrome screening is beneficial. Adjusting for maternal weight also adds additional value. Although it is not so beneficial to routinely adjust for maternal weight for serum markers (Wald *et al*., 1992). A study done by Brizot *et al*. (1995) showed that trisomy 21 resulted in significantly higher values of total human chorionic gonadotrophin and free beta HCG and trisomy 13 and 18 resulted in lower values as compared to normal controls. They found no significant association between HCG and nuchal translucency in either chromosomally abnormal or normal foetuses. Another study by Zoppi *et al*. (2002), suggests that nuchal translucency identifies 80% of foetuses with trisomy 21 for a false positive rate of 5%.

Results from a demonstration project showed that prenatal Down's syndrome screening using maternal serum biochemistry is effective in practice and that it could be readily introduced into routine antenatal care. They also demonstrated that it was cost effective (Wald *et al*., 1992). A short coming of the use of nuchal translucency is that it may also increase in normal karyotype. Increased foetal nuchal translucency thickness can also be seen in foetuses that are chromosomally normal and could be indicative of foetal malformations, dysgenesis, etc. Note also that nuchal translucency

increases with increase in crown rump length of normal foetuses. This is of value when you have to counsel parents of pregnancies with increased foetal nuchal translucency and in making arrangements for possible follow up investigations.

Nuchal translucency usually resolves after 14 weeks although it may lead to cystic hygromas or nuchal oedema in some cases (Niclaides, 2004). In a population with maternal age distribution of pregnancy in England and Wales, a detection rate of 89% with a fixed false positive rate of 5% can be gotten by using foetal nuchal translucency, maternal age and serum free beta HCG and also PAPP-A. If the detection rate is changed to 70% then you get a fixed false positive rate of 1%. The addition of biochemical markers improved the detection rate gotten by using maternal age and nuchal translucency alone by 16% (Spencer *et al*., 2005).

Integrated testing which involves measuring nuchal translucency thickness and maternal serum biochemistry for PAPP-A in the first trimester and serum testing for HCG (all types), Eu3, alpha fetoprotein in the second trimester. First trimester serum test for PAPP-A is best done between 10 weeks+0 days and 12 weeks+0 days. Though, the full screening window for PAPP-A testing in the first trimester is between 10 weeks+0 days and 13weeks and 6 days gestation. The screening time limit for serum biochemistry testing during the second trimester is between 15 and 20 weeks+0 days.

Serum integrated testing. This requires two attendances. It involves HCG, testing for PAPP-A in the first trimester and testing for HCG, uE3 and alpha fetoprotein in the second trimester.

Quadruple testing for late antenatal care bookers. It is second trimester test involving a serum biochemistry. It may include uE3 HCG, inhibin A and alpha fetoprotein. This is the best screening strategy for women presenting later than 14 weeks. This may however not be effective enough to meet the recommended screening outcome. Threshold level for risk assessment was categorized as high or low risk and this was achieved by using a cut-off of 1 in 150 at term for first trimester screening strategies and 1 in 200 at term for 2nd trimester screening strategies. This is to ensure measurement of performance and quality assurance. Furthermore, it was suggested that age standardization must be applied to the overall performance for the population screened to give a correct representation of the detection rate and SPR.

In improving the performance of first trimester screening, measurement of fronto-maxillary facial angle has been suggested (Borenstein *et al*., 2008). Down's syndrome is associated with a flat face. This can be assessed by measuring the fronto maxillary facial angle. The fronto maxillary facial angle is seen to be wider in 1st and 2nd trimester in foetuses with down syndrome than in foetuses that are euploid.

Current works suggests that during the 11-13(+6) weeks scan, the nasal bone is absent in 70% of foetuses with trisomy 21 and this percentage differs with ethnicity. Absent nasal bone can also be seen in foetuses with trisomy 13 and 18. It has also been shown that there is no relationship between absent foetal nasal bone and maternal serum PAPP-A or free beta HCG in cases with trisomies 13, 18 or 21. Another serum marker inhibin A has also been seen to improve the efficiency of serum screening for Down's syndrome. A study done using blood specimen from 77 singleton pregnancies with associated Down's syndrome and 385 singleton pregnancies of normal foetuses matched for gestational age, age of mother and time window of storage of the blood specimen, reported raised serum inhibin-A in those pregnancies associated with down's syndrome (Wald *et al*., 1997). However, Alfred in a study done to estimate and compare accuracy of second trimester serum markers for the detection of Down's syndrome, agreed that tests involving two or more markers in combination with maternal age are significantly more sensitive than those involving one marker. They found that the value of combining four or more tests or including inhibin however does not result in any statistically significant improvement.

Another factor that may affect risk estimation is ethnic group. Down's syndrome screening performance is only slightly improved after adjustment of serum markers for ethinic group. It however is of value during screening for open neural tube defect using alpha fetoprotein (Wald *et al*., 1992).

Foetal hemodynamic in second and third trimester foetuses shows that changes in ductus venosus flow velocity waveforms are in keeping with imminent failure of the heart. When it was newly discovered, it was thought that first trimester abnormal ductus venosus flow was an independent and strong marker for detecting Down's syndrome (Timmerman *et al*., 2010). Perfumo *et al*. (2005) also commented that abnormal ductus venosus flow is significantly associated with Down's syndrome. In other however to improve the effectiveness of screening for aneuploidies, doppler studies of ductus venosus have been added to nuchal translucency in screening programmes. Souka *et al*. (2001), Hyett *et al*. (1996, 1995), Zosmer *et al*. (1999) . It has been found that even foetuses with normal nuchal translucency and abnormal first trimester ductus venosus doppler findings may have an adverse outcome hence mid trimester foetal anomaly evaluation with echocardiography and ensuing follow up

is advised (Prefumo *et al*., 2005). Investigation of ductus venosus has been integrated into the first trimester combined test in many centers in a bid to improve sensitivity (Prefumo *et al*., 2005; Nicolaides, 2004). The rationale for this is that many foetuses with Down's syndrome have a blood flow pattern that is not normal (Prefumo *et al*., 2005). Assessment of ductus venosus Doppler findings can be performed by 2 main methods; use of the venous Doppler index by means of a cut off value (semi quantitative) and wave form pattern of velocities which corresponds to atrial systole (a-wave). The first study that showed the added value of DV-PIV as a continuous variable to nuchal translucency measurement alone in first trimester population that were high risk was published in 2010 (Timmerman *et al*., 2010). They also concluded that combining DV-PIV, nuchal translucency and maternal age using logistic regression, can improve the accuracy of screening for chromosomal abnormalities including Down's syndrome in a high risk population. Foetal aneuploidies and abnormal flow in the ductus venosus are clearly associated and heart defects and abnormalities of the great vessels are the most common congenital malformations and are found in 2-8 of every 1000 pregnancy.

The ductus venosus is a trumpet shaped structure that connects the intra abdominal umbilical vein to the inferior vena cava. It allows oxygenated blood bypass the liver from the placenta. It accelerates the blood jet crossing the inferior vena cava directly to the left atrium via the foramen ovale avoiding mixture with blood from the right chamber that is deoxygenated (Ritter *et al*., 2004). The ductus venosus wave form in normal foetuses shows a peak velocity during ventricular systole and diastole. During atrial contraction it shows a nadir (Antolín *et al*., 2001) (Fig. 1).

The sensitivity in the detection rate of Down's syndrome increases to 94% and the likelihood ratio of a negative test decreases to 0.08 (Mavrides *et al*., 2002) when ductus venosus velocimetry is combined with nuchal translucency as compared to using either test alone. It was suggested that the ductus venosus blood flow pattern cannot be used independently to reduce the indication for foetal Karyotyping. Ductus venosus velocimetry is of great importance in counseling of parents in who enlarged nuchal translucency but normal karyotype has been seen in their foetuses. Ductus venosus velocimetry can be used to identify those foetuses with increased risk of adverse outcome and those that will need intensive follow up (Bilardo *et al*., 2001).

Ductus venosus assessment for chromosomal abnormalities in general and cardiac defects: An abnormal atrial contraction velocity is more frequent in foetuses presenting enlarged nuchal translucency than in

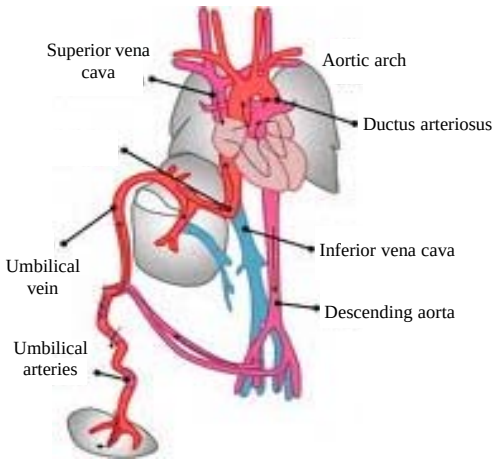

Fig. 1: Foetal circulation (http://php.med.unsw.edu.au)

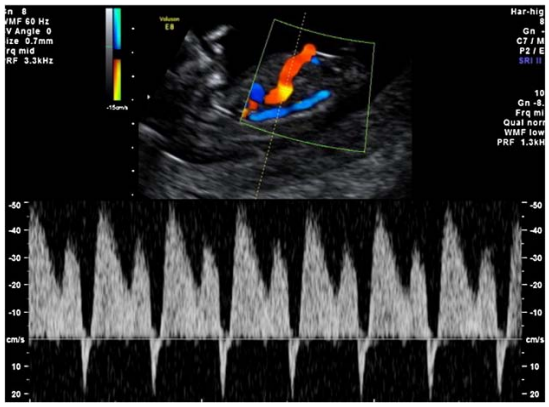

Fig. 2: Normal ductus venosus waveform in a 13 weeks foetus (malone first trimester screening, obstet gynecol in (2003)

those having normal nuchal translucency and in foetuses having the larger nuchal translucency thickness, the probability of having a chromosomal abnormality is greater when an abnormal atrial velocity contraction is seen. The waveforms of the ductus venosus flow were classified as normal if the lowest forward velocity during contraction was positive and abnormal if it was absent or negative (Zoppi *et al*., 2002). Hence, ductus venosus Doppler can predict foetal outcome even in normal karyotype. In 4% of normal pregnancies ductus venosus blood flow was reversed in diastole and the percentage of pregnancies with Down's syndrome that showed reversal of blood flow in diastole was 64% (Maiz *et al*., 2008a). Ductus venosus blood flow can also be measured quantitatively as the ductus venosus pulsatility index. The ductus venosus pulsatility index is the difference between the maximum blood velocity in systole and that at the end of diastole divided by the maximum velocity average over the time of one heartbeat (Fig. 2 and 3).

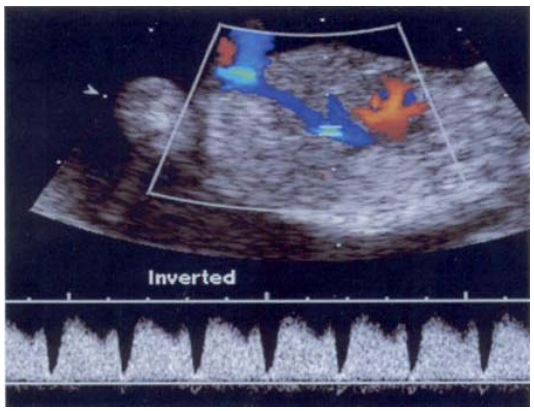

Fig. 3: Reversal of "a" wave in a 12 weeks old foetus (increased nuchal translucency and congenital heart disease Rita Mugra *et al.*)

Maiz *et al.* (2012) in their study done in 2012 where foetal ductus venosus pulsatility index in veins was measured during first trimester screening for foetal abnormalities in singleton pregnancies. They then estimated the performance of screening for aneuploidies by DV-PIV alone and in combination with foetal nuchal translucency thickness and serum free beta-human chorionic gonadotrophin and PAPP-A. It was seen that in screening for trisomy 21 by maternal age, nuchal translucency and biochemistry at a risk cutoff of 1 in 100, a value of 89.7% as detection rate was gotten and 2.74% as false positive rate. When DV-PIV was included, the values were 93.5 detection rate and 1.63% false positive rate. They concluded that DV PIV improves performance of first trimester combined test for chromosomal anomalies. The detection rate of turner syndrome, trisomies 18, 21 and 13 are 100, 92, 98 and 100%, respectively with 3% false positive rate when ductus venosus is added to screening modalities for these chromosomal abnormalities (Maiz *et al.*, 2009). Hence to improve first trimester screening for aneuploidies, Timmerman *et al.* (2010) modeled the added value of DV-PIV screening as a continuous variable to nuchal translucency measurement done in first trimester population with high risk. Ductus venosus is readily identified using the color doppler and its waveform is seen with pulsed Doppler in first trimester.

Ductus venosus assessment for Down's syndrome screening: Between 11-13 weeks, the prevalence of abnormal a wave increases as crown rump length reduces (Maiz *et al.*, 2012). Wald *et al.* (2012) examined the effect of adding ductus venosus blood flow as a categorical variable to the combined and integrated tests in Down's syndrome was examined. In his study, he reiterated that the pulsatility index of the ductus venosus when added to integrated and combined screening tests for Down's syndrome, improved the performance of these tests. Out of 534 consecutive pregnancies, 73% of those with Down's syndrome screened between 10-18 weeks had abnormal DV-PIV (Borrell *et al.*, 2009). In these studies DV assessment was performed for the women in the high risk group suggesting that although ductus venosus assessment may not be included in routine antenatal clinic screening it may be useful as a second line screening method amongst high risk population. They discovered that that effective performance of ductus venosus assessment was related to gestational age. Between 10-13 weeks gestation was considered most effective. No significant difference was found when comparing the efficacy of DVPIV among maternal age groups. In evaluating trisomies and other chromosomal abnormalities, ductus venosus blood flow assessment is invaluable as the detection rate and odds ratio are respectively increased to 76.9% and 74 (95% CI 20-277) for trisomies and 42.9% and 17(95% CI 4-76) for chromosomal abnormalities using the 95th centile as cut off (Borrell *etal.*, 2009). According to the result they got, they suggest that DVPIV should be used as a secondary screening test for those who have been found screen positive for Down's syndrome hence reducing the need for invasive testing. They noticed that ductus venosus assessment does not increase the detection rate achieved using nuchal translucency between 10-16 weeks (Borrell *et al.*, 2005). Using ductus venosus blood flow as a categorical marker is said to be simpler and less discriminatory hence it is sometimes preferred to DVPI. Using the integrated test and ductus venosus as a categorical marker for a detection rate of 90% there is a false positive rate of 1.5% as compared to 1.1% of DVPI (Wald *et al.*, 2012, 1997) (Fig. 4 and 5).

In a study done by Nicolaides *et al.* (2005), they suggested that patient with intermediate risk should be offered further non-invasive testing after the 1st trimester combined screening and suggested that highly sensitive and specific markers like increased impedance to flow in the ductus venosus, absent nasal bone or tricuspid regurgitation should be used. As examining these markers properly will be time consuming and require highly skilled operators, they felt that it is unlikely that examination of these markers will be added to routine first trimester screening but however it may come in handy in re-evaluating intermediate risk patients after the routine first trimester combined screening (Nicolaides *et al.*, 2005). Kagan *et al.* (2010) suggested that a Down's syndrome first trimester screen that will be effective can

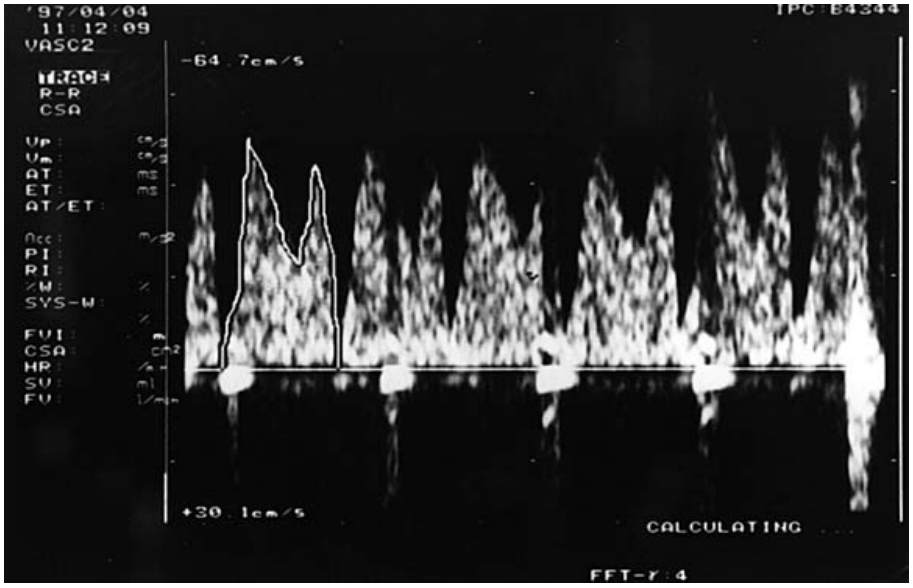

Fig. 4: Waveforms of ductus venosus in a down syndrome foetus showing 'twin peaks" shape but positive velocities during atrial contraction (Abnormal ductus venosus blood flow in trisomy 21 foetuses during early pregnancy (Borrell *et al*., 1998)

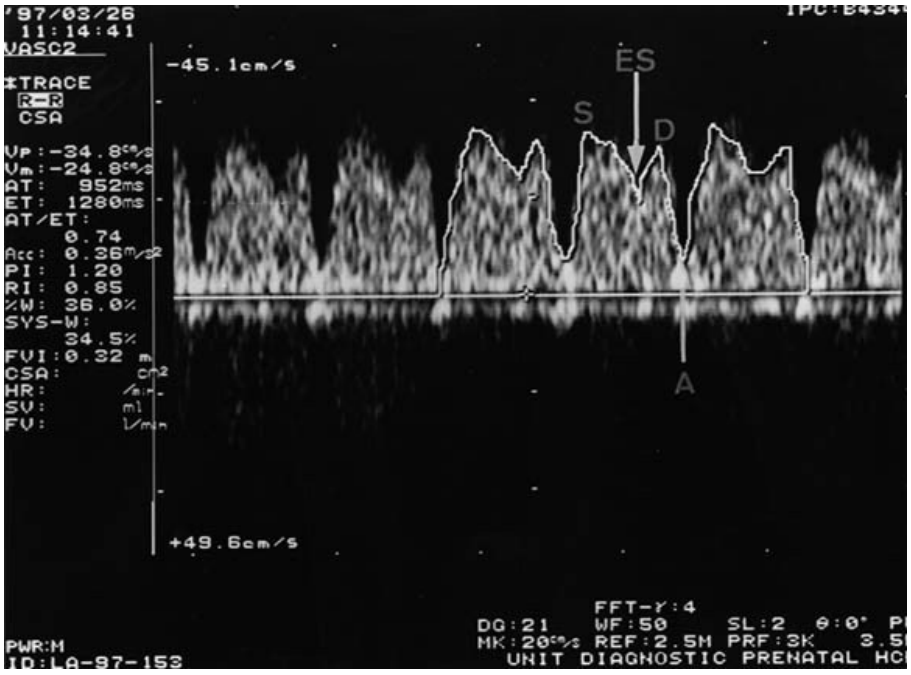

Fig. 5: This shows pulsatility index (pi) for veins = 1.20 in 3-cycle waveform. Systolic (s), end-systolic (es), diastolic (d), and atrial contraction (a) velocities (Adapted from abnormal ductus venosus blood flow in trisomy 21 foetuses during early pregnancy (Borrell *et al*., 1998)

be achieved by a contingent policy in which first stage testing is based on ultrasound examination (nasal bone, nuchal translucency, ductus venosus flow, tricuspid valve) and 2nd stage testing is based on biochemical markers. In their study this policy gave the best performance. In an area serviced by Catalan public health

centre, a study was done by Monoz-Cortes *et al.* (2012) were they proposed first trimester contingency screening involving the use of 2nd trimester sonographic markers (nasal bone, ductus venosus blood flow and tricuspid flow) on patient with intermediate risk as predetermined by first trimester combined test, reduced the screen false positive rate but the detection rate of Down's syndrome was not greatly affected. They however, noticed there was poor compliance of patients as the women among the high intermediate risk group opted for invasive testing.

Certain pitfalls associated with ductus venosus assessment: Borrell *et al.* (2009) examined the possible problems of first trimester ultrasound markers in the detection of foetal abnormality. He said although ultrasound markers are the only once getting detection rate above 50% and undoubtedly the best markers for antenatal detection of foetal aneuploidies, there are some pitfalls associated with their use. For instance ineffective examination of markers which can be due to foetal positions and movements, previous surgical procedures or maternal body conformation or physique (habitus), incorrect assessment or incorrect interpretation which may be due to poor image magnification, venous contamination, bad placement of caliper (nuchal translucency), arterial contamination in tricuspid regurgitation, poor angle of insonation in nasal bone assessment amongst others. Venous contamination in ductus venosus waveforms may present as an abnormal blood flow when it is normal and as a normal blood flow when it is abnormal (Borell *et al.*, 2009; Al-Noor *et al.*, 2016). Many centres have tried to offer ductus venosus screen to pregnant women but they couldn't sustain it because of inadequate skill resources. Moreover, the newly discovered use of free foetal DNA in non-invasive antenatal diagnosis is proving to be such that might make the use of methods like ductus venosus flow, redundant or if it does not ultimately prove to be diagnostic, it could be used in combination with other approaches to improve on sensitivity, specificity and detection rates of screening protocols.

Since 1989, improvements and expansion in antenatal screening have reduced the incidence of Down's syndrome which occurs with increasing age of mothers. The number of prenatal diagnosis of Down's syndrome when comparing older and younger women has stayed quite the same in older women as compared to the astonishing increase seen in younger women. With this in mind, the tendency that we will get huge numbers of Down syndrome births despite new innovations in screening is still very much present hence, proper monitoring is needed to make sure there is proper arrangement for the possible requirements this may pose (National Down's syndrome cytogenetic register).

MATERIALS AND METHODS

Studies were taken from medline, web of science and pubmed using the keywords 'ductus venosus', Down's syndrome, 'chromosomal abnormalities', 'DV' 'screening'.

Eligibility criteria:

- Studies in which the intervention includes screening for Down's syndrome and other foetal aneuploidies in both high, low risk and general antenatal populations
- Studies that provided data on the presence or not of Down's syndrome according to ductus venosus assessment
- Ductus venosus assessment by an experienced sonographer according to Foetal Medicine Foundation guidelines
- Studies were diagnosis of Down's syndrome was determined by chorionic villous sampling or amniocentesis, performed by a specialist, post mortem or post natal physical examination

Exclusion criteria:

- Papers not available in english language
- Papers that do not use human subjects

Extraction of data: For all studies, I recorded the names of authors of the study, country of origin, size of sample, unselected or high risk population, design of the study, mean maternal age at the time of the study, age of gestation when ductus venosus assessment was done and the outcomes. I also recorded the percentage of foetuses where ductus venosus examination was successfully performed.

Data regarding detection rates, specificity, positive predictive value, negative predictive value were got in some of the studies. These performance characteristics were tabulated with the aim of summarising the present literature on screening of this form. The statistical difference between detection rates of nuchal translucency and ductus venosus assessment was also calculated and their possible statistical correlation was recorded.

Eligble studies: From the 455 items recovered using electronic search, 417 were rejected after looking through the title and abstract. Full texts of the remaining 38 were screened. Studies that were case reports (4), studies that were editorials (1) (Slack *et al.*, 2006) and studies that did not include screening for Down's syndrome with ductus venosus were removed (5) (Souka *et al.*, 2001; Hyett *et al.*,

1996, 1995; Munoz-Cortes *et al*., 2012; Zosmer *et al*., 1999; Favre *et al*., 2003). Also excluded were studies where they had technical issues concerning doppler examination (4) and studies whose full text could not be accessed (4). At the end 20 studies were eligible for further analysis (Fig. 6).

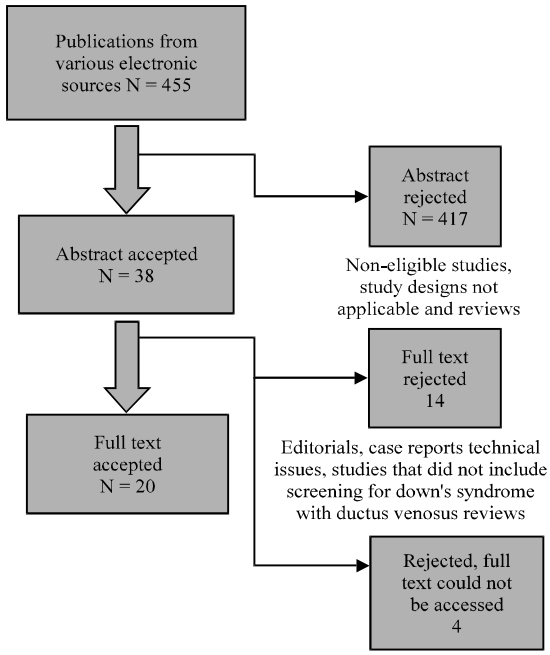

Fig. 6: Flow chart of studies selected and the search strategy used

Study characteristics: Table 1 shows the descriptive characteristics of eligible studies. A total of 131500 foetuses constituted the total population from 20 studies. For analysis of the diagnostic performance of ductus venosus for Down's syndrome screening, 6 studies were included. All the studies were prospective and had been performed in Europe and South America. About 9 studies addressed high risk population (Bilardo *et al*., 2001; Borrell *et al*., 1998; Matias *et al*., 1998; Antonio *et al*., 2001) while the remaining studies addressed the general antenatal population. Sample size had a large variation (92-45060). At the time of examination maternal age mean varied from 32-37 years. Age of gestation ranged from 10-14 weeks in majority of the cases except for Antolin were they did some comparison between results that they got during the optimal gestational age for screening of 10-14 weeks and a later gestational age of 14-16 weeks. They also performed amniocentesis on foetuses 14-16 weeks old. Ductus venosus doppler findings were regarded as abnormal when absent/reversed A wave or increased ductus venosus pulsatility index was noticed. Nuchal translucency was said to be increased when measurements gotten were above the 95th percentile. Some of the studies recorded a failure rate in assessing ductus venosus which ranged from 0.2-3%.

Quality assessment: In assessing the quality of the studies included in this research, I used the QUADAS tool (Whiting *et al*., 2006) (Table 2). In most studies most of the quality criteria were met. In 1 study it was unclear

Table 1: Study characteristics

Researchers	Country of origin	Design	N	Prevalence of down's syndrome (%)	Population	Mean maternal age (years)	Gestational age (weeks)	Measure	Cutoff	Success rate of ductus venosus assessment (%)
Bilardo	Netherland	Prospective cohort	186	19/186; 10	High risk	34	10-17	DV (NT)	Increased PI and absent or reversed flow >95th percentile	86
Borrell *et al*. (1998)	Spain	Prospective cohort	534	11/534; 2	High risk	34	11-12	DV	Increased PI	82
Maiz *et al*. (2012)	Spain	Prospective cohort	45060	202/45060; 0.4	General ANC population	32	11-13	DV	Increased PI	NA
Kagan *et al*. (2010)	United Kingdom	Prospective cohort	19736	122/19736; 0.6	General ANC population	34	11-13	DV (NT)	Absent or reversed flow >95th percentile	NA
Murta *et al*. (2002)	Brazil	Prospective cohort	372	16/374; 4.3	General ANC population	32	10-14	DV (NT)	Absent or reversed flow >95th percentile	99.7
Maiz *et al*. (2008)	United Kingdom	Prospective cohort	10490	45/10490; 0.4	General ANC population	32	11-13	DV	Absent or reversed flow	99.2
Maiz	United Kingdom	Prospective cohort	19800	122/19800; 0.6	General ANC population	34.5	11-13	DV	Absent or reversed flow	NA
Borrell *et al*. (2009)	Spain	Prospective cohort	7250	66/7250; 0.9	General ANC population	32	10-13	DV	Increased PI	96.7
Carmen	Spain	Prospective cohort	8426	34/8426; 0.4	General ANC population	33	10-13	DV	Increased PI	95.3
Borrell *et al*. (2003)	Spain	Prospective cohort	3382	48/3382; 1.4	High risk+ low risk	34	10-14	DV	Increased PI	NA
Timmerman *et al*. (2010)	Netherland	Prospective cohort	479	72/479; 15	High risk	NA	11-13	DV	Increased PI and absent or reversed flow	98

Table 1: Continue

Researchers	Country of origin	Design	N	Prevalence of down's syndrome (%)	Population	Mean maternal age (years)	Gestational age (weeks)	Measure	Cutoff	Success rate of ductus venosus assessment (%)
Antolin *et al.* (2001)	Spain	Prospective cohort	1371	9/1371; 0.7	General ANC population	32	10-16	DV (NT)	Absent or reversed flow >95th percentile	100
Matias *et al.* (1998)	United Kingdom	Prospective cohort	486	38/486; 7.8	High risk	35	10-14	DV (NT)	Increased PI and absent or reversed flow >95th percentile	100
Antonio	Brazil	Prospective cohort	92	7/92; 7.6	High risk	36	12-14	DV	Absent or reversed flow	NA
Toyama *et al.* (2004)	Brazil	Prospective cohort	109	7/109; 6.4	General ANC population	32	11-14	DV	Absent or reversed flow	NA
Prefumo *et al.* (2005)	United Kingdom	Prospective cohort	572	47/572; 8.2	High risk	37	11-14	NT Nasal bones	Increased PI, >95th percentile absent or present	98.5
Mavrides *et al.* (2002)	United Kingdom	Prospective cohort	260	30/260; 11.5	High risk	35	11-14	DV	Increased PI >95th percentile	98.5
Sainz *et al.* (2012)	Spain	Prospective cohort	10452	24/10452; 0.23	General ANC population	32	11-14	DV	Absent or reversed flow	97
Ekelund *et al.* (2012)	Denmark	Prospective cohort	917	23/917; 2.5	High risk population	34	10-14	DV	Absent or reversed flow	91.4
Florganski *et al.* (2013)	Poland	Prospective cohort	1526	21/1526; 1.4	High risk population	37	11-14	DV	Absent or reversed flow	NA

NT: Nuchal Translucency; N: Population; ANC: Anti Natal Care; NA: Not Available; PI: Pulsatility Index; DV: Ductus Venoses

Table 2: QUADAS tool for the evaluation of the quality of the studies included in this review

Researchers	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Bilardo	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Borrell	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Kagan *et al.* (2010)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Murta *et al.* (2002)	Yes	Yes	Yes	Yes	U	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Maiz *et al.* 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Maiz *et al.* (2009)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Borrell (2009)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Carmen	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Borrell (2003)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Timmerman *et al.* (2010)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Antolin *et al.* (2001)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Matias (1998)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Antonio	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Toyama *et al.* (2004)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Prefumo *et al.* (2005)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mavrides *et al.* (2002)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sainz *et al.* (2012)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ekelund *et al.* (2012)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Florganski *et al.* (2012)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

N; No, U; Unclear1: Archetypal patients selection, 2: Clearly described criteria for selection; 3: Target condition rightly classified by standard reference; 4: Reference standard and test time period short enough; 5: Verification of sample collected; 6: Reference standard similar for every patients; 7: Reference standard not dependent of present test; 8: present test sufficiently explained in details; 9: Reference standard sufficiently explained in details; 10: Independent interpretation of present test; 11: Reference standard explained individually; 12: When test results were interpreted same clinical data available; 13: Report of intermediate test; 14: Study withdrawal properly explained.

if the sample received was verified. In addition, detailed description on how the specialized scan was performed is not available in some studies.

RESULTS

Result summary of the studies reviewed: Bilardo *et al.* (2001) in their study of 186 foetuses, found the sensitivity of an absent A wave or abnormal DVPIV to be 65% for chromosomal abnormalities. They found a significant correlation between nuchal translucency and Ductus Venosus Pulsatility Index Velocity (DVPIV).

Borrell *et al.* (2003) saw that DVPIV was raised in 75% of cases (high risk cases) in their study. They studied 1644 pregnancies at increased risk in addition to 1718 pregnancies at smaller risk for aneuploidies. In this study, they also saw that 81% of Down's syndrome foetuses had increased nuchal translucency and 71% when nuchal translucency referrals were omitted.

Maiz *et al.* (2012) found a bimodal distribution of DVPIV with a dominant crown rump length dependent part in euploid pregnancies and this accounted for about 97% of Caucasian cases and 93% of Afro Caribbean cases. In pregnancies with chromosomal anomalies, the

part that was dominant was the crown rump length independent distribution and this accounted for about 85% of trisomies 21, 18 and 70% of cases of trisomy 13. Using maternal age, nuchal translucency and biochemical tests in screening for trisomy 21 the sensitivity was 89.7%. With the addition of DVPIV, the sensitivity was 93.5% with a FPR (false positive rate) of 2.74 and 1.63% respectively for risk cut off of 1 in 100. The study was carried out in 45060 pregnancies.

Toyama *et al.* (2004) observed that for chromosomal abnormality negative predictive value, positive predictive value, specificity and detection rate using ductus venosus assessment were 99.3, 31.3, 96.9 and 68.2%, respectively. For abnormal nuchal translucency the values were 99.7, 11.9, 86.9 and 86.4%, respectively.

Mavrides *et al.* (2002) studied 256 pregnancies. The sensitivities of ductus venosus velocimetry alone were 58.7%, abnormal Nuchal Translucency (NT) alone was 80.4% and NT in addition to ductus venosus assessment in screening for trisomy 21 was 93.5%. The likelihood ratios of abnormal karyotype with ductus venosus velocimetry alone, nuchal translucency alone and nuchal translucency combined with ductus venosus were 9.83, 3.33 and 3.48 accordingly.

Antolin *et al.* (2001) studied 1371 pregnancies in which the detection rate, odds ratio, specificity, negative predictive value and positive predictive value for aneuploidies were 65, 41(95% CI 16-108), 95.7, 99.5 and 18.3%, respectively. The cut off for pulsatility index was 95th centile.

Antonio studied 92 pregnancies of which 12 were found to have chromosomal anomalies. Of this 12, 7 were positive for trisomy 21. They also found that ductus venosus alone, nuchal translucency alone or maternal age alone all showed low levels of sensitivity (41.67-58.33%) and poor positive predictive value (10-45.45%). When ductus venosus, maternal age nuchal translucency were combined they got 100% sensitivity; 14.2% positive predictive value, negative predictive value of a 100 and 6.49% specificity.

Carmen were able to assess ductus venosus successfully in 95.3% of the total 8842 pregnancies studied. They showed that detection rate increases from 85-94% when ductus venosus assessment is added to the combined tests for a fixed screen positive rate of 5% and they also showed that using a fixed detection rate of 85%, the number of invasive tests reduces from 3.7-3.2%.

Kagan *et al.* (2010) studied 19736 pregnancies and they found that they got the best performance when they used contingency testing whereby their first stage tests consisted of maternal age, nuchal translucency and ductus venosus or tricuspid flow. Biochemical testing of only patients with a risk that is between 1 in 51-1 in 1000 came after. When they used ultrasound examination as first phase screening they got a better result than when they used biochemical tests first. Murta *et al.* (2002) found that of the 29 foetuses with chromosomal abnormalities, 27 were correctly picked up by ductus venosus assessment (93.1%). In the foetuses that were normal, 1.7% still had abnormal ductus venosus profiles. They got a negative predictive value and positive predictive value of 99.4, 81.8% and with a specificity of 98.3%.

Maiz *et al.* (2009), noticed reversed A wave in 66.4%, 58.3, 55.0, 75.0% of Down's syndrome, 18, 13 and Turner syndrome foetuses and 3.2% of euploid foetuses. When they added ductus venosus assessment to the first trimester screening, they were able to detect 100% of trisomy 18 foetuses (FPR 3%).

Borrell *et al.* (2009) discovered that as a single marker ductus venosus pulsatility index had a sensitivity of 62% (FPR5%). When the detection rate was 90%, the inclusion of ductus venosus assessment decreased the combined test false positive rate to 4.6 from 8.5% and decreased that for integrated tests from 2.0-1.1%. They also accordingly recorded decreased foetal losses due to diagnostic procedures and it was cost effective.

Timermman *et al.* (2010) studied a total of 445 foetuses with risk for Down's syndrome. They had abnormal ductus venosus findings in 426 of cases. The chromosomal abnormalities odds were raised by 4.2% for every MoM increment in ductus venosus pulsatility index velocity after adjustment for maternal age and nuchal translucency. The part below the receiver operative characteristics curve was 0.79 for predicting chromosomal anomalies. They then concluded that in a population having increase risk in the first trimester for Down's syndrome, using logistic regression model of maternal age, DV-PIV and nuchal translucency will enhance the accurateness of screening for Down's syndrome.

Prefumo *et al.* (2005) were able to study 572 foetuses that were undergoing chorionic villus sampling having a risk for trisomy 21 >1 in 300. The likelihood ratio for trisomy 21 was 7.05(95%CI 4.27-11.04) for ductus venosus assessment abnormality and for nasal bones not present, it was 6.42 CI 3.86-10.67 and here it was concluded that trisomy 21 is greatly associated with a first trimester ductus venosus flow abnormality and nasal bone hypoplasia.

Florjanski *et al.* (2012) studied 1526 singleton pregnancies including those at high risk for aneuploidies. They compared combined test with the addition of ductus venosus alongside combined test alone and found increased sensitivity when ductus venosus is added to combined test (92%) false positive rate 2.4% as compared to combined test alone (84%) false positive rate 0.4%.

Ekelund *et al.* (2012) studied 917 pregnant women. 894 of these women were grouped as euploid and 23 women were grouped as trisomy 21. They noticed that screen positive rate significantly reduced from 48.3-17.7% ($p<0.001$). Using a contingent screening strategy, they however found no statistically significant difference between using the combined test and contingent screening tests which involves the inclusion of ductus venosus or nasal bone evaluation.

Sainz *et al.* (2012) performed combined tests on 10452 pregnancies and secondary ultrasound marker assessment on 1017 cases. They used the combined test for first stage testing and the ultrasound markers (nasal bone presence, tricuspid regurgitation and ductus venosus flow) as a second stage contingent test. The contingent test and the combined test had a sensitivity of 70.8% (95% CI; 52.6-88.9) (17/24) having a false positive rate of 2% (95% CI; 1.7-2.3) (220/10408) and 83% (95% 67.9-98) (20/24) having a false positive rate of 3% (95% CI; 2-7-3.3) (316/10430), accordingly. They concluded that though they were able to bring down the false positive rate using contingent test, the contingent test sensitivity was not good enough for it to be used as a Down's syndrome screening method.

Borrell *et al.* (1998) studied 534 foetuses and found 11 with Down's syndrome. Out of which 73% had increased DVPIV They concluded that abnormal DVPIV was discovered in a good number of trisomy 21 foetuses.

Matias *et al.* (1998) after studying 586 foetuses found 63 with chromosomal defects (38 of which had trisomy 21). 90.5% of the cases with chromosomal abnormalities had abnormal ductus venosus flow. About 3.1% of the foetuses that were normal had abnormal ductus venosus flow. They concluded that ductus venosus assessment in high risk pregnancies should be considered in other to reduce the demand for invasive testing.

DISCUSSION

Ductus venosus flow in pregnancy that have normal foetuses: For early screening for foetal structural and chromosomal abnormalities, the 11-13 weeks scan has become popular and widely accepted (Slack *et al.*, 2006). The assessment of ductus venosus is usually done at this time. Studies have indicated that the effective performance of ductus venosus flow evaluation is related to gestational age (Kagan *et al.*, 2010).

In normal pregnancy the ductus venosus flow is usually forward although reversed or absent flow maybe seen in some normal pregnancy (3.9% of the total number of foetuses with normal karyotype as shown in Table 3). In normal pregnancy, Pulsatility Index Velocity (PIV) decreases as gestation increases. In this study, Bace-Budecka, the mean PIV ranged from 1.09 in the first trimester to 0.96 in the second trimester. It is therefore paramount that ductus venosus flow be assessed at the proper time during gestation to allow for accurate results. Ethnicity has also been seen to influence ultrasound markers of aneuploidies in the first trimester and hence should also be taken into consideration (Spencer *et al.* 2005; Collado *et al.*, 2005).

Table 3: Reports on the incidence of ductus venosus flow abnormality in the first trimester in normal foetuses and foetuses with trisomy 21

		Abnormal ductus venosus flow in	
Researchers	N	Normal features (%)	Trisomy 21 foetuses (%)
Bilardo *et al.* (2001)	186	39/186; 21	57/63; 91
Borrel	3382	NA	36/48; 75
Kagan *et al.* (2010)	19736	622/19736; 3.25	81/122; 66.4
Murta *et al.* (2002)	372	7/343; 2.0%	18/18; 100
Maiz *et al.* (2008)	10490	458/10490; 4.4	28/45; 62.1
Maiz	19800	633/19614; 2.2	18/122; 66.4
Borrel *et al.* (2009)	7250	NA	38/66; 58
Borrell *et al.* (2003)	3382	162/3249; 5.0	36/48; 75
Timmerman *et al.* (2010)	479	178/306; 58.2	57/72; 92.2
Antolin *et al.* (2001)	942	39/911; 4.3	5/7; 71.4
Matias *et al.* (1998)	486	13/423; 3.1	30/33; 90
Antonio *et al.* (2008)	92	6/77; 7.8	5/10; 41.7
Toyama *et al.* (2004)	1097	69/1075; 6.4	5/7; 71.4
Prefumo *et al.* (2005)	572	27/497; 5.2	18/47; 38.3
Mavrides *et al.* (2002)	260	7/156; 4.5	27/30; 90
Florganski *et al.* (2012)	1526	110; 1484; 7.4	29/42; 69
Total	70034	2362/58547; 4.0	551/782; 70.5

N population

Ductus venosus flow in assessing foetal aneuploidies: It is common to have abnormal ductus venosus flow in foetuses with chromosomal abnormalities. This is represented in the studies included in this review. Using the combined data in these studies abnormal ductus venosus flow is seen in about 4% of normal foetuses (euploid foetuses). On the other hand, 70.5% of Down's syndrome foetuses have ductus venosus flow abnormality (Table 3).

The detection rate of trisomies 21, 13 and 18 are 98%, 100 and 92%, respectively when ductus venosus assessment is added to the screening modalities (Maiz *et al.*, 2009). Ductus venosus assessment for detection of aneuploidy has reported rates ranging from 38.3-95% (Prefumo *et al.*, 2005; Murta *et al.*, 2002) with false positive rates ranging from 2-7 (Table 4). Kagan *et al.* (2010) reported detection rates as high as 96% (Table 6). Antonio recorded sensitivity of 100%. This large variability in detection rate, may be due to differences in ascertainment as the population studied ranged from high risk highly selected population with about 25% aneuploidy rate and 49% increased nuchal translucency rates (Bilardo *et al.*, 2001) to a normal

Table 4: Detection rate of foetal aneuploidy including Down's syndrome by means of ductus venosus measurement when compared to nuchal translucency

Researchers	N	Aneuploidy rate	DV measured	Gestational age (weeks)	DR	FPR (%)	DR (NT) (%)	FPR (NT) (%)	Correlation between NT and DV assessment	Statistical difference in detection rates of DV and NT (95% CI)
Borrell *et al.* (2003)	3382	2.7	PIV	10-14	36/48 (75)	5	81	5	yes	p = 0.025
Bilardo *et al.* (2001)	186	13	PIV+EDV	10-17	57/63 (91)	3	94	33	yes	p = 0.010
Antolin *et al.* (2001)	1371	1.5	PIV	10-16	13/20 (65)	4	75	6	no	p = 0.046
Mavrides *et al.* (2002)	260	18	EDV	11-12	27/46 (59)	7	80	24	yes	p = 0.097
Murta *et al.* (2002)	372	8	EDV	10-14	27/29 (93)	2	79	5	yes	p = 0.052
Toyama *et al.* (2004)	109	NA	PIV	11-14	15/22 (68)	6	86	14	yes	p = 0.075
Prefumo *et al.* (2005)	572	NA	PIV	11-14	18/47 (38)	5	87	50	yes	p = 0.241

N population; DV: Ductus Venosus; NT: Nuchal Translucency; PIV: Pulsatility Index Velocity; DR: Detection rate; EDV: End Diastolic Velocity; FPR: False Positive Rate

Table 5: Diagnostic performance of Ductus venosus assessment in screening for Down's syndrome

Researchers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Timmerman *et al.* (2010)	79.0	51.0	24.0	93.0
Bilardo *et al.* (2001)	63.0	79.0	NA	NA
Antolin *et al.* (2001)	76.9	95.7	14.7	99.8
Mavrides *et al.* (2002)	58.7	94.1	69.0	90.9
Murta *et al.* (2002)	93.0	98.3	81.8	99.4
Prefumo *et al.* (2005)	41.5	94.4	54.8	90.8

PPV (positive Predictive Value; NPV (Negative Predictive Value)

general antenatal population with 1.5% aneuploidy rates and 6% increased nuchal translucency rates (Antolin *et al.*, 2001) (Table 4). When ductus venosus was used as a secondary screening method (Bilardo *et al.*, 2001), 59-70% of aneuploid foetuses were identified, reselecting 7-21% of pregnancies that were chromosomally normal. In contrast, when ductus venosus was used as an initial screening method, sensitivity was as high as 65-93% (FPR 2-5%) (Table 4). All studies in Table 4 record detection rates for nuchal translucency which are better than detection rate for ductus venosus measurement except for Murta *et al.* (2002) and Mavrides *et al.* (2002) who recorded detection rates for ductus venosus as 93 and 90%, respectively. However, it is of note that the false positive rates for ductus venosus measure are generally lower than those for nuchal translucency. In Table 5, although ductus venosus seem to have a fair level of sensitivity, it appears to be highly specific. Murta *et al.* (2002) records a specificity of 98.3%.

Ductus venosus and other sonographic markers: Although, ductus venosus flow assessment is not popular as a single marker for Down's syndrome, it has found its place in combination with other markers. It is not an independent marker for Down's syndrome as formerly thought. It however improves the effectiveness of screening for Down's syndrome when combined with other markers. In ductus venosus measurement, skill and technicality of the procedure is a major limitation for its incorporation as a first line screening test. Operator experience is hugely paramount in qualitative assessment of ductus venosus. Movement as little as maternal breathing can displace the sample gate and cause misdiagnosis of ductus venosus waveforms (Toyama *et al.*, 2004). Studies have proven that sonographers experienced in the 11-13 weeks scan need a learning curve of 80 examinations to have adequate skill in ductus venosus measurement (Toyama *et al.*, 2004; Maiz *et al.*, 2008 b; Braithwaite *et al.*, 1996). In the 7 studies in Table 4, doppler studies of ductus venosus are compared with nuchal translucency in screening for foetal aneuploidies including Down's syndrome. In these studies, both screening methods are used independently. However to improve the effectiveness of screening for Down's syndrome, ductus venosus assessment has been added to nuchal translucency in screening programmes with great success (Mavrides *et al.*, 2002; Murta *et al.*, 2002; Braithwaite *et al.*, 1996; Carmen *et al.*, 2009) showed a statistically significant positive correlation between Ductus Venosus Pulsatility Index Velocity (DVPIV) and nuchal translucency seen in Down's Syndrome pregnancies (r = 0.284). So also does all the studies on Table 4 except for Antolin *et al.* (2001). Antolin *et al.* (2001) suggested that nuchal translucency can be used as first line screening so as to keep its high detection rates and ductus venosus should be used as second line test in order to decrease the false positive rates and also bring down the need of invasive testing to <1%. They however, did not find any significant correlation between nuchal translucency and ductus venosus assessment. This could be due to the fact that the pregnancies evaluated were comparatively small. They had a total of 20 cases of chromosomal abnormalities. Of the 20 cases, 14 were between the gestational age of 10-14 weeks and 9 had trisomy 21.

In the event of a normal nuchal translucency and abnormal ductus venosus doppler finding, mid trimester foetal anomaly examination plus echocardiography and follow-up are indicated as such foetuses have been seen to have adverse outcomes (Prefumo *et al.*, 2005).

Ductus venosus and other markers in general: When measured alongside, PAPP-A, free Beta HCG and nuchal translucency, logistic regression analysis have shown that ductus venosus assessment contributes independently to predict chromosomal anomalies (Comas *et al.*, 2009; Maiz *et al.*, 2009, 2008; Borrell *et al.*, 2009, 1998). So also, logistic regression studies have

Table 6: Table showing the improvement of detection rates when ductus venosus is added to other screening protocols

					DV+Test			
Researchers	Test	Population studied	Specificity (%)	Detection rate (%)	Type of DV	Cut off	Specificity (%)	Detection rate (%)
Borrell *et al.* (2003)	NT	General	95.0	75.0	DVPIV	NA	95.0	65.0
Carmen *et al.* (2007)	NT PAPP-A HCG	General	35.0	85.3	DVPIV	1:270	56.0	88.2
Kagan *et al.* (2010)	Combined test	General	97.7	89.0	DVPIV	1:100	75.0	96.0
Mavrides *et al.* (2002)	NT	High risk	76.0	80.4	DVPIV	1:400	73.0	93.5
Maiz *et al.* (2012)	Combined test	General	97.3	89.7	DVPIV	1:100	98.4	93.5
Prefumo *et al.* (2005)	NT Nasal bone	High risk	95.0	36.2	Absent/Reversed a wave	1:300	99.6	21.3
Florganski *et al.* (2013)	Combined test	High risk	92.8	84.8	Absent/Reversed a wave	1:100	97.4	92.8
Sainz *et al.* (2012)	Combined test	General	NA	83.0	Absent/Reversed a wave	1:100	NA	70.8

DV: Ductus Venosus; DVPIV: Ductus Venosus Pulsatility Index Velocity; NT: Nuchal Translucency; PAPP-A: Pregnancy-Associated Plasma Protein A; MA: Maternal Age; HCG: Human Chorionic Gonatrophins; BCH: Biochemistry

shown that combining DVPIV, maternal age and nuchal translucency can enhance the screening accuracy for Down's syndrome amongst other chromosomal abnormalities in high risk population (Borrell *et al.*, 2005).

Ductus venosus flow assessment has also been integrated into the combined test in many centres in other to improve sensitivity (Prefumo *et al.*, 2005; Borrell *et al.*, 2005). The addition of DVPIV to nuchal translucency, PAPP-A and Beta HCG in a study done by Carmen *et al.* (2009) (Table 6) increased the specificity from 35-56% and the detection rate from 85.3-88.2% and significantly reduced false positive rates from 6.5-4.4% ($p<0.001$). Maiz *et al.* (2012) in a similar study found an improvement in detection rate from 89.7-93.5% on addition of DVPIV to nuchal translucency, PAPP-A and Beta HCG with false positive rates reduced from 2.74-1.63%. A study by Ghaffari *et al.* (2012) found that when they added ductus venosus, nasal bone and tricuspid flow to the combined tests, they had an increase in detection rate from 93.8% FPR 4.8-100% detection rate (FPR 3.4%).

In all the studies in Table 6, the diagnostic performance of the various screening methods is improved on addition of ductus venosus assessment, except for Borrell *et al.* (2003) where they recorded a reduction in detection rate when ductus venosus was added to nuchal translucency assessment however they recorded a negative predictive value and positive predictive value of 99.4 and 32%, respectively on addition of ductus venosus to nuchal translucency assessment (Table 6). Ekelund *et al.* (2012) found a decrease in screen positive rate when they added ductus venosus and tricuspid flow assessment to combined tests. They however did not find any significant difference in both test strategies.

Various studies have examined the different ways ductus venosus assessment can come in handy in screening for Down's syndrome. Nicolaides *et al.* (2005) suggested that highly sensitive and specific markers like ductus venosus, nasal bone and tricuspid regurgitation should be used following first trimester combined screening for patients with intermediate risk. They acknowledged that adding these markers in routine first trimester screening might be unlikely due to the fact that accessing these markers require highly skilled personnel and also is time consuming. Kagan *et al.* (2010) suggested a policy where by ultrasound examination and other sonographic examinations are done as first stage testing and biochemical markers as second stage testing. In their study, this gave the best performance. Carmen *et al.* (2009) saw that they could avoid 432 invasive tests in their group population by adding ductus venosus to their previous screening strategy. They were able to reduce false positive rates from 8.6-3.0%.

Ductus venosus assessment (cost implication): Borrell *et al.* (2009) examined the cost of adding DVPIV measurement to screening programmes. They found that the costs of programmes based on using the combined test are reduced with the inclusion of DVPIV. For a 90% rate in detection, the programme cost decreases from 3.6 million pounds to 2.9 million pounds for every 100,000 women screened and from 17800-14200 pounds for every pregnancy with Down's syndrome diagnosed, this is as a result of the fact that a reduced number of invasive test for diagnosis were necessary to reach the same detection rate. Using the integrated test for comparison, they found little consequence on the cost of the programme with screening using the integrated test without or with addition of DVPIV.

New innovations in screening tests for Down's syndrome: Quake and Lo (2008), gave a report that MPS from maternal plasma could be used for detection of Down's syndrome. This new finding of the use of cell-free-foetal DNA stands a chance of competing with

ductus venosus assessment and other ultrasonographic markers in a place in the routine screening for trisomy 21. Its sensitivity and specificity are very close to 100%. However, problems such as cost and success in sequencing might create restrictions in its use. In addition, it is necessary to measure foetal DNA percentage by using DNA or genetic methylation markers in all samples prior to detection. This is to reduce the possibility of false negative results as a consequence of too little foetal DNA concentration. It may also be difficult to use in obese women as obesity has a significant association with lower cff DNA. Furthermore, the confined placental mosaicism may be a cause of possible false positives. There is also an issue with its use in twin pregnancies especially with twins discordant for trisomies. Studies comparing its efficacy with that of ductus venosus are needed. Its use in practical clinical situations should also be evaluated.

CONCLUSION

The practicality of ductus venosus blood flow studies as a primary screening tool has met with various criticisms. Its drawbacks have been outlined by Hecher (2001). The need for experienced sonographers, cost, strict methodologies and time are some of the reasons given for its impracticality. However, we have been able to bring to light that in the long run, ductus venosus assessment for Down's syndrome screening is actually cost effective (Toyama *et al*., 2004) and from most studies in this review (Antolín *et al*., 2001; Bilardo *et al*., 2001; Mavrides *et al*., 2002; Borrell *et al*., 2003; Hecher, 2001) the maximum time for a ductus venosus assessment is about 5 min with a few cases needing more time. Moreover, the long wait for biochemical laboratory result is eliminated and no bloodletting involved. In addition it is a one off screen test which does not need a second antenatal visit to conclude on results. Sonographers already doing 11-13 weeks anomaly scan could be trained within a short period of time for ductus venosus assessment. When added first trimester sonographic aneuploidy markers are introduced, they can prevent the need for second trimester chromosomal abnormality screening (Zoppi *et al*., 2002).

Ductus venosus assessment can be used in counselling parents in whom enlarged nuchal translucency has been noted in their foetuses but have normal karyotype. It can be used to identify those with increased risk of adverse outcome (Bilardo *et al*., 2001), even in foetuses with normal karyotype (Borrell *et al*., 2005). It can be used to identify foetuses with cardiac defects not related to Down's syndrome. In some of the studies examined in this review, ductus venosus was assessed in women that were in the high risk group. This may suggest that although ductus venosus assessment may not be included in routine antenatal care screening protocols, it is definitely helpful as a second line screen amongst high risk population. The drawback of this though is that the effectiveness of ductus venosus assessment will be dependent on the first line screen as the false negative rate of the first step will affect the overall efficacy results.

The establishment of national recommendations for Down's syndrome screening have been expected for a long time as regards the least possible standard for women who choose to embark on Down's syndrome screening during their pregnancy. Considering the resources necessary to support the development of a screening programme based on ultrasound and biochemical evaluation, it is going to be difficult to incorporate ductus venosus assessment in routine screening within the cost restricted national health system. It is therefore left to the policy makers to look at the long term benefits. In my opinion, gathering from the discussions above, I believe ductus venosus screening test should be added to routine screening for Down's syndrome. Its benefits in reducing the rates of invasive testing outweigh its potential difficulties. It should however not be used alone. Its use alongside nuchal translucency tests alone and also the combined test is highly recommended.

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