The Accuracy of Ultrasound Beyond 14 Weeks to Determine Chorionicity of Twin Pregnancies

Principle Investigator: Dr Zoe Momberg,

Obstetrics and Gynaecology Registrar,

University of Cape Town

Supervisors:

Dr Chantal Stewart

Dr Hetta Van Zyl

Qualifications of Researchers

Dr Momberg (PI): MBChB (UCT), Obstetrics Registrar

Dr Stewart: MBChB, FCOG, MMed (O&G)

Dr van Zyl: MBChB, FCOG

Dr Wainwright: MBChB (UCT), FFPath (Anat Path) (WITS)

ABSTRACT

Background: Determining the chorionicity of twin pregnancies is extremely important as this influences the frequency of surveillance, timing of delivery and management of complications. Monochorionic twins have 2.5 times the perinatal mortality of dichorionic twins, and in the case of a single intra-uterine fetal demise, the surviving twin of a monochorionic pair is at significant risk of neurological damage compared to a dichorionic pregnancy. Chorionicity can be accurately determined before 14 weeks gestation using the lambda or T-sign. After 14 weeks, these ultrasonographic signs become less reliable and the pregnancy may be assumed to be monochorionic for management purposes. The implication of this assumption is that on occasion premature dichorionic fetuses may be delivered unnecessarily. In South Africa, many women have their first antenatal visit after the first trimester or are not scanned by an experienced sonographer until after 14 weeks. There is thus a need for an accurate means to determine chorionicity in the second and third trimesters.

Methods: A diagnostic validity study investigating five ultrasound features to determine chorionicity after 14 weeks gestation was conducted. The number of placental masses, fetal genders, membrane 'take-off' (Lambda or T-sign), dividing membrane thickness and number of layers in the dividing membrane were individually assessed against postpartum histology of the placental dividing membrane (gold standard for chorionicity). Women attending Groote Schuur and Mowbray Maternity Hospitals with twin pregnancies of unknown chorionicity were recruited antenatally and ultrasound features of chorionicity were

scanned for at subsequent visits. After delivery, their placentas were assessed histologically by the UCT Department of Anatomical Pathology.

Results: Two placental masses and discordant fetal genders are accurate in excluding monochorionic twins after 14 weeks. They have a good positive predictive value in diagnosing dichorionicity. However, they are not useful in excluding monochorionicity if they are absent. Membrane thickness was found to be 76% accurate for diagnosing chorionicity (sensitivity 90.9%, specificity 71.4%). Counting the number of layers in the dividing membrane was prone to inter-observer variation. The negative predictive value of four layers for monochorionicity was 100% (95% C.I. 84-100); however, the specificity was only 77% (95% C.I. 58-89) and the positive predictive value for monochorionicity was 60% (95% C.I. 36-80).

Conclusion: Dividing membrane thickness and number of layers in the membrane are promising tests to determine chorionicity after the first trimester but they lack sufficient specificity to be used alone. A diagnostic algorithm to determine the chorionicity of twin pregnancies after 14 weeks is proposed in which over-diagnosis of monochorionic twins is avoided by sequential use of increasingly specific tests.

Contents

Literature Review		Page 1
Methods		Page 11
Results		Page 19
Discussion		Page 33
References		Page 49
Appendices:	Consent	Page 52
	Ethics Approval	Page 57
	GSH Permission	Page 58
	Mowbray Permission	Page 59
	Declaration	Page 60

Aim

To assess the accuracy of ultrasound parameters used to determine chorionicity of twin pregnancies who present for ultrasound after 14 weeks gestation.

Objective

1).To assess whether adding membrane thickness and number of layers in the dividing membrane at ultrasound scan improves the diagnostic accuracy of ultrasound after 14 weeks.

2) Formulation of a diagnostic algorithm, combining multiple ultrasound diagnostic tests to improve the accuracy of determination of chorionicity

LITERATURE REVIEW

Determining the chorionicity of twin pregnancies is important as it has a significant impact on perinatal mortality. Monochorionic Twins (MC) have more than double the risk of singleton pregnancies for perinatal death of at least one twin.[1] If MC twins are diagnosed antenatally, they are followed up more closely by more frequent ultrasound scans and are delivered earlier; at 36-37 weeks gestation as per Royal College of Obstetricians Green Top Guidelines and the UK National Institute of Clinical Excellence.[2,3]

Complications of Monochorionic Twins

The higher incidence of fetal complications in Monochorionic (MC) versus Dichorionic (DC) pregnancies has been well documented. This highlights the need to identify the chorionicity of a pregnancy to plan antenatal surveillance and timing as well as mode of delivery.

Matijevi A et al report that the most common complication is earlier preterm delivery but that MC pregnancies carry a higher perinatal mortality and morbidity.[4] According to Duba, MC twins carry a relative risk of 2,5 times DC twins for perinatal mortality of at least one twin.[1] The higher rate of perinatal mortality mostly occurs before viability (<24 weeks).[5] Velamentous cord insertion is more common in MC pregnancies, causing a greater birth weight discrepancy, and likely placental insufficiency of the smaller twin.[6,7]

Perinatal mortality is nearly twice as common in MC twins as in dichorionics (2.8% versus 1.6%).[8] The surviving twin has a fair prognosis in DC pregnancies, but in MC pregnancies, the survival rate of the remaining twin is much lower. This can largely be attributed to complications of Twin-Twin-Transfusion Syndrome (TTTS), TRAP sequence and selective intrauterine growth restriction.[9] Fichera quotes survival rates of 83,4% for MC survivors and 100% for DC survivors of a single IUFD pregnancy.[10] In addition to mortality, there is a higher rate of neurological damage in the surviving twin of a MC pregnancy.[11] Minakami corroborates these findings with an adverse perinatal outcome (death, cerebral palsy and mental retardation) occurring in 10% of MC pregnancies, compared to 3,7% in DC infants. A growth discordance of more than 25% was associated with all of the adverse outcome babies in MC pregnancies but only with 33% of the damaged babies in DC pregnancies.[12]

Later in gestation, MC pregnancies have higher rates of intra-uterine growth restriction and at delivery: earlier preterm delivery, fetal distress and delivery by Caesarean section.[13]

MC pregnancies also carry the unique risks of cord entanglement, TTTS, Twin Reversed Arterial Perfusion and conjoined twins which do not occur in DC pregnancies.[7,8]

First Trimester Measurement

First trimester sonography has been proven to be very accurate in determining chorionicity. The Glasgow Royal Maternity Hospital quotes sensitivities of 100% and specificities of 99% below 14 weeks.[14] From 5 - 8 weeks gestation, 2 gestational sacs are the best sign of dual chorionicity. From 10-14 weeks the lambda sign is the most commonly used method with a sensitivity and specificity of 97.4% and 100% respectively (when used in combination with number of placentas).[15] Finberg has verified these results and reports an accuracy of 100% for the lambda sign.[16] If the membrane 'take-off' appears as a triangle (twin-peak or Lambda sign); then the pregnancy can be labelled as DC. A 'T sign' at the membrane origin demonstrates a MC pregnancy if it approaches the placenta at a 90 degree angle.[17]

After 15 weeks, the accurate determination of chorionicity declines. According to a study done at the Columbia University Medical Centre, sonography done for chorionicity below 14 weeks has a positive predictive value of 97,8%. This drops to 88,0% after 14 weeks. The negative predictive value for chorionicity is 97,5% before 14 weeks and 94,7% after 14 weeks. This study looked at presence of lambda and T-signs as well as placental location and gender up to 24 weeks gestation.[18]

The problem with these reliable signs is that they are no longer present after 15-16 weeks gestation and may be obscured by fetal parts. Sepulveda et al have demonstrated that the lambda sign becomes progressively more difficult to visualize and will disappear by the 20th week of pregnancy in 7% of DC pregnancies with fused placentas. Presence of the lambda sign is always diagnostic of dichorionicity but its absence after 20 weeks should be interpreted with great caution.[19]

Moon et all at Kwandong University College of Medicine in Seoul report 92,9% accuracy in predicting chorionicity in a twin pregnancy with a single placental mass at 11 -14 weeks gestation.[20] Positive predictive value may be higher for DC pregnancies than MC

pregnancies.[21] 11-14 weeks would appear to be the best time to scan for chorionicity but a 7-9 week scan approaches this accuracy.[21]

Non-useful Sonographic findings to determine chorionicity

Several ultrasound characteristics have been looked at to determine chorionicity and have not been shown to be of any predictive value. These include placental volume which does not depend on chorionicity, but rather fetal number.[22] Rate of placental growth does not vary even between singletons and twins between 11 and 13+ weeks gestation.[22] Twin pregnancies of mono and dichorionicity both have increased rates of congenital abnormalities (2.6%) without a statistical significance between them. [23] However, monozygotic (not monochorionic) twins have a higher incidence of congenital malformations.[24] Growth velocity of the individual foetuses is independent of fetal sex, chorionicity and zygosity.[25] Taylor GM et al also found that although velocity is reduced compared to singletons, there are no significant differences between pregnancies of differing chorionicity.[26]

Ultrasound Factors to look at in 2nd and 3rd Trimester to determine chorionicity

Sex of the fetuses is the first and most important factor as it has the highest predictive value. If the fetuses have differing sexes, dichorionicity can be established with 100% accuracy. With like-sex twins, there is roughly a 50% chance of the pregnancy being MC or DC.[17] There may be difficulty in seeing genitalia due to fetal position during sonar which may require other factors to be looked at or for a repeat scan to be done.

If two placentas are clearly seen on opposite sides of the uterine cavity, the diagnosis of DC can easily be made. However; this only occurs in one third of twin gestations.[17] Two placental sites have a sensitivity of only 32%, but a predictive value of 100% for dichorionicity. A single placental site (fused placentas with vascular connections) or two closely adjacent placentas are difficult to differentiate by scan and this decreases the diagnostic value of placental site evaluation in this situation.

The inter-fetal or dividing membrane is likely the most valuable sonographic feature to determine chorionicity if the two previous characteristics are ambiguous. The dividing membrane can be assessed for origin or 'membrane take-off', thickness and number of layers.[17] A review article by Shetty quotes accuracy rates of 97% in the third trimester if a composite cascade of ultrasound features is used to determine chorionicity. It is noted that membrane thickness and counting the membrane layers are less reliable than the lambda or T sign.[24]

The thickness of the dividing membrane has been studied for over 20 years. It is becoming more popular as a technique as high resolution ultrasound has become more available, making assessment easier and more accurate.[17] A cut-off of 2mm has been set as the standard: >2mm is classified as DC pregnancy as this membrane would consist of two chorions and two amnions. This is method can be used in 89-90% of twin pregnancy studies. Less than 2mm is classified as MC – only two amnions make up the septum. This has differing accuracies for the different types of pregnancies: accuracy of 82% for MC and 95% for DC gestations. The greater inaccuracy of the membrane thickness in MC pregnancies is due to over-estimation of the membrane thickness. In all cases it is best to zoom in on the membrane to improve accuracy.[17] Bracero et al used a membrane thickness cut-off of 2,2mm and correlated this to fetal outcome and chorionicity. This team got a positive predictive value of 96.6% and found that a thicker membrane was definitely associated with a better fetal outcome.[27] A team working in South Carolina, U.S.A. looked at membrane thickness after 22 weeks gestation and derived a combined accuracy for MC and DC pregnancies. They found a sensitivity and specificity of greater than 91% for the two types of twin pregnancies.[28]

Townsend et al did a retrospective study on known dichorionic pregnancies in the third trimester. They found that a thick membrane was only found in 52% of these pregnancies. [29]

3D multiplanar ultrasound has recently been used to examine membrane thickness between 20 and 35 weeks gestation. A mean thickness of 1,42mm in MC pregnancies and 2,48mm in

DC pregnancies was measured. This led the investigators to deduce that the best membrane thickness cut-off should be 1.8mm to differentiate chorionicity.[30]

The number of layers of the dividing membrane is probably the most accurate way to determine chorionicity after differing sexes and 2 distinct placentas visualised. To improve the accuracy of this method, the sonographer must zoom in on the membrane and it is crucial that the membrane is at a 90 degree angle to the sound wave. This is because an ultrasound transducer has better accuracy in axial resolution.[17] If four layers are seen (amnio-chorion-chorion-amnion), then the pregnancy is said to be DC. If two layers are visualized (amnion-amnion), then the pregnancy is MC. The positive predictive value (PPV) of looking at membrane number for chorionicity is 98,5% and the accuracy approaches 100% in DC gestations.[17] A Mexican study found a PPV for chorionicity of 94,6%. They were unable to assess the membrane in 12,4% of their participants, but were able to do so in all the DC pregnancies.[31]

If no dividing membrane is seen, the pregnancy is then mono-amniotic and therefore monochorionic as well. Looking at membrane characteristics becomes inaccurate in the case of TTTS and a stuck-twin where the donor twin is adherent to the dividing membrane making it difficult to see at all or making it difficult to measure as it is stuck around the smaller twin. This can also occur in cases of severe oligo- or anhydramnios.[17,32] The French conclude that counting the layers of the dividing membrane should be the first line method to determine chorionicity in the 2nd and 3rd trimesters. They used high frequency 10MHz transabdominal ultrasound.[32,33]

Higher rates of birth weight discordance and IUGR have been reported in MC twins[13] even if they do not have TTTS but it would be difficult to establish cut-off criteria for these growth disorders as they also occur in DC pregnancies. The Harris Birthright Research Centre for Fetal Medicine conclude that MC twins have a lower birth weight relative to DC twins and suggest that MC placentation itself has an effect on intrauterine growth.[34]) Of interest is that growth velocity is not influenced by chorionicity.[25,26]

There is a correlation between congenital cardiac malformations and chorionicity: MC twins have higher rates of pulmonary stenosis and endocardial elastosis while the Ebstein malformation is more common in DC pregnancies.[35]

MC twins have a seven fold increase in cerebral white matter lesions relative to their gestational equals who were DC. However, this was only diagnosed postnatally.[36]

Two thirds of twin pregnancies are dizygotic (DZ) and one third are monozygotic (MZ). All DZ pregnancies are dichorionic and one third of MZ pregnancies are also dichorionic (DC), the remainder being monochorionic (MC). It is the chorionicity of the pregnancy and not its zygosity which influences outcomes and therefore management of the pregnancy.[1]

Management of Complications in Twin Pregnancies

In the case of single IUFD in a MC pregnancy, parents may choose to terminate the pregnancy due to the frequency of neurological damage in the surviving twin. If this occurs late in gestation, fetocide of the surviving twin may be preferable before induction. It is obviously of great importance to be certain of chorionicity before offering parents these choices and accuracy of estimation of chorionicity would be essential to guide parents in making this decision.

Chorionicity also has implications for management of fetal anomalies. If a fetal abnormality in one twin is fatal during intra-uterine life; this may cause death of the co-twin or neurological damage.[8] If chorionicity were known, this may prompt pre-term delivery of the normal twin (before the abnormal twin demises) if the pregnancy was MC but the pregnancy could be left to progress until term if it was DC. Alternatively, if fetocide of a severely abnormal twin was requested, intra-cardiac potassium chloride could not be used as it may be circulated to the normal twin via a shared placenta. In MC pregnancies, umbilical cord ligation is used for single twin fetocide.[8] Single twin fetocide can precipitate labour or cause chorioamnionitis, regardless of chorionicity, leading to pre-term delivery or loss of the whole pregnancy. These complications could be avoided if dichorionicity was diagnosed with certainty and no intervention was required.

Screening and diagnosis of genetic abnormalities such as trisomies is complicated by unknown chorionicity. Same-sex twins of unknown chorionicity could be either monozygotic or dizygotic.[8] If one twin had a thickened nuchal translucency and the parents requested an amniocentesis for diagnosis; both fetuses would have to be tested. Performing an amniocentesis on both amniotic sacs would increase the miscarriage rate of this procedure. If this pregnancy was known to be monochorionic, amniocentesis need only be performed on one fetus as this already high risk pregnancy can be assumed to be monozygotic.[37]

Severe IUGR in a single twin is also managed differently depending on chorionicity. In a DC pregnancy, expectant management is prudent as single fetal demise may not affect the normal twin but selective fetocide may precipitate preterm delivery and compromise the entire pregnancy[8]. If the pregnancy is confirmed MC and IUFD is anticipated in a single severe IUGR twin before viability; photocoagulation of the IUGR twin's cord vessels is advisable. This will separate the two placental circulations and therefore prevent acute fetofetal haemorrhage at the time of the expected IUFD. This procedure does place the entire pregnancy at risk. If single severe IUGR is diagnosed after viability; preterm delivery to protect the normally grown twin is advocated.[8]

If TTTS was picked up early by more frequent surveillance of MC twins, laser ablation of placental vascular anastomoses could be performed. Alternatively, amnio drainage or septostomy could be done.

South African Context

In South Africa, many women do not attend antenatal clinics in their first trimester. There are many reasons for this including lack of transport, financial issues and poor education regarding antenatal care. While The WHO recommends booking at or before 16 weeks in

developing countries[38], Myer and Harrison found that many rural women in South Africa did not perceive the need for more than one antenatal visit as pregnancy was not seen as a significant threat to health.[39] The main reason for these women booking was to obtain an antenatal attendance card which enabled them to deliver at a health care facility. Labour was perceived as a time of significant health risk and therefore women wanted biomedical care at this time.[39]

While approximately 94% of pregnant women in South Africa receive some antenatal care, only 27% initiate antenatal care before 20 weeks gestation.[40]

A Cape Metropole West audit performed in 2008 showed that, of 61 women of advanced maternal age delivering at the facility only 19 women (31%) initiated antenatal care within the first 20 weeks of gestation.[41]

Many patients are not aware of the exact gestation of their pregnancy as menstrual calendars or monitoring are not part of basic health education at schools in South Africa. Injectable progesterone contraception is widely used, leading to oligo- or amenorrhoea, and therefore difficulty in determining gestation from last menstrual period (LMP). This results in missed opportunities for early diagnosis of twins and therefore chorionicity. Due to the significant levels of obesity and morbid obesity in our country (56.6% of females according to The South African Demographic and Health Survey conducted in 2002[42], gestation is not easy to assess by symphysis fundal height in these patients and, in a service where early ultrasound is not routine, the problem of early diagnosis of twins is further compounded. Lack of sufficient numbers of trained ultrasound personnel (many general practitioners do the first scan) may also contribute to the lack of early determination of chorionicity.

Currently in the Mowbray Maternity Hospital (MMH) and Groote Schuur Hospital (GSH) ultrasound service, membrane thickness and number of layers in the membrane are not routinely assessed. This means that late estimation of chorionicity is based on fetal sexes, number of placental masses and lambda sign if still present. It is important to determine the accuracy of this current practice and to evaluate if the addition of membrane thickness and number of membrane layers leads to a significant improvement in our setting.

Definition of Terms

Dichorionic Twins (DC): Each fetus has its own chorion and therefore amniotic sac

Monochorionic Twins (MC): Twin fetuses in separate amniotic sacs but share a single chorion.

Late for Chorionicity Ultrasound scan:

For the purposes of this study, after 14 weeks gestation

(After which it may not be possible to see a Lambda or T sign).

METHODS

1. Study Design

This is a Cross-Sectional, Diagnostic Validity Study where post partum histology (macroscopic and microscopic) of placentas is accepted as the gold standard test for chorionicity. [43, 44]

1.1 Study Size

The UCT Bio-Statistics department was consulted to determine the sample size required to power the study for chorionicity. This study has been designed as a validity study comparing ultrasound features of chorionicity to histology which is the accepted gold standard test for chorionicity.

1.2 Study Setting

The study was conducted at Groote Schuur and Mowbray Maternity Hospital ultrasound departments and antenatal clinics.

1.3 Characteristics of Study Population

Patients attending GSH and Mowbray antenatal clinics with twin pregnancies at any gestation above 14 weeks were included. Consecutive twin pregnancies were enrolled during the months of June and early July 2011. Data collection (antenatal scans) were performed between June and September 2011 (4 months). Participants delivered up until December 2011 and placental histology collection was completed by February 2012.

1.4 Recruitment

Participants were recruited while awaiting routine twin ultrasound scans at Groote Schuur Hospital (GSH) and Mowbray Maternity Hospital (MMH) and at antenatal clinics at these hospitals. The principal investigator(P.I.) recruited patients and explained the study to them and subsequently obtained written consent. The P.I. enlisted the help of other Obstetric registrars and nurses working in the antenatal clinics as well as sonographers to help identify patients with twin pregnancies.

1.5 Exclusion criteria

- Chorionicity previously determined by ultrasound before 14 weeks
- Monoamniotic twins (no dividing membrane)
- Pre-existing TTTS or TRAP (Twin reversed arterial perfusion syndrome) if diagnosis certain as these conditions would confirm a MC pregnancy
- Rupture of membranes
- Patients who declined participation in the study or object to placental histology.
- Mothers younger than 18 years old. As per the Declaration of Geneva, only people over the age of 18 years can give informed consent.

2. Data Collection

2.1 Ultrasound Data Collected:

- Number of placentas
- Fetal genders
- Membrane 'take-off' (Lambda or T Sign)
- Thickness of dividing membrane
- Number of layers in dividing membrane

2.2 Membrane Image Acquisition (instructions to sonographers):

- At Mowbray Maternity, only 3.5 megahertz (MHz) ultrasound probes were being used at the time of the study. Groote Schuur Hospital had access to 5 MHz curvilinear probes. Accordingly, images at the two different study sites, were captured by machines of different wave strengths which generated images of differing quality at the respective sites.
- The fetal dividing membrane was to be measured at 90 degrees to the ultrasound beam (angle of insonation) to prevent beam scatter and blurring
- The membrane image was magnified to the point where distinct layers were seen within the membrane.
- Membrane thickness was measured twice, within three centimetres of its insertion into the placenta. The average of these two measurements was then taken.

2.3 Pre-Study Education of Sonographers

Prior to data collection of dividing membrane images, the theory of the histological differences between monochorionic and dichorionic pregnancies was discussed with the sonographers as well as sonographic images represented in the literature on this topic.[29,50] For the first ten participants in the study, data acquisition was collected as a group with all sonographers at each hospital and the P.I. present to ensure uniformity of image capturing and adherence to the protocol.

3. Criteria used to classify pregnancies as DC :

- Two distinct placentas at different locations
- Different genders of the fetuses
- Lambda sign
- Dividing membrane containing four layers
  (Two amnions and two chorions).
- Dividing membrane greater than 2mm thick

If it was not possible to obtain all five parameters at the initial scanning visit, this was attempted at the next routine twin scan.

4. Histology Data Collection:

Post delivery, placentas and membranes were examined by the UCT Anatomical Pathology Department and the PI for chorionicity. If, on macroscopic histology the chorionicity was

clear, then this was the final and conclusive test as it is possible to see an intervening membrane between two separate placentas. If, however it was difficult to see if two placentas are lying closely adjacent with no anastomoses or if a single placenta was present (i.e. a single placental mass seen macroscopically); microscopic histology of the dividing membrane was performed in the majority of cases.

5. Patient Management after Data Collection

Our current practice is to assess chorionicity based on the ultrasound parameters we currently look at: fetal genders, number of placental masses and membrane 'take-off' (if still present). Previous studies have attempted to validate membrane thickness and number of layers in the dividing membrane but the vast majority have concluded that these parameters on their own have poor reproducibility. [17,28,29,32] This study did not attempt to re-validate these diagnostic tools. This was not an intervention study and as such, these two new parameters were excluded from the estimation of chorionicity and the participants were managed as per our previous protocols. If there was doubt as to chorionicity, patients were presumed to be MC and managed as such.

As monochorionic pregnancies occur in about one third (33%) of all pregnancies, a minimum sample size of 77 MC pregnancies (the rarer event) and a total number of 231 pregnancies was needed to adequately power the sample. These numbers were arrived at by using a Two-by-Two Table and Epicalc, and using a PPV of 89, 6% (the average of what has been quoted in previous studies, 82% - 97.8%.[18, 27]

A chi squared test was formulated for each ultrasound parameter separately to determine the merits of each test. The accuracies of the tests were then compared. We looked at Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value as well as diagnostic accuracy.

6. Data Management and Analysis

Data was reported on the standard ultrasound report form, was collected and digitalized by the PI. A Chi-Squared test was used to determine the validity of ultrasound findings relative to histology.

ETHICS

Written informed consent was obtained when patients attend antenatal clinic or while waiting for their routine ultrasound (which the participants received regardless of whether participating in the study or not). Participants were therefore not dependent on the study for medical care as they received their routine twin scans even if they declined to be in the study. Consent forms were in English. Afrikaans and Xhosa translators were used for non-English speakers. The study and procedure was explained to the patients verbally; then a written explanation and consent form was given to them. If they were undecided about enrolling in the study, they would still receive their ultrasound scan that day as per standard care and they would then be free to go home and discuss it with their partner, family or friends.

The data was linked to the patient via patient number only and the patients' names were not recorded to ensure confidentiality.

Risks and Benefits of Study

There were no anticipated risks to this study.

Potential Benefits:

By correctly diagnosing a pregnancy as Monochorionic, that pregnancy would directly benefit by more frequent surveillance (scans every 2 weeks rather than every 4 weeks) to detect growth restriction (which is more common) and other complications specific to MC pregnancies. The timing of delivery for MC pregnancies would also be implemented to produce a better outcome as per RCOG guidelines.[2] It would also impact on the management of a single IUFD or fetal anomaly.

By validating late ultrasound scans to determine chorionicity, obstetricians would be able to manage these pregnancies with more confidence in this diagnostic tool. By correctly assigning dichorionicity; this study could help to decrease the frequency of scanning of dichorionic pregnancies. Importantly; it may prevent the incorrect earlier delivery of twins of unknown chorionicity (which are treated as MC to be safe). This is important in a resource-constrained setting with a limited neonatal service which has to accommodate the preterm babies delivered early unnecessarily.

This non-invasive test would help to improve the PPV of ultrasound in our service for chorionicity. In summary, more accurate determination of chorionicity would streamline the management of complicated twin pregnancies and also prevent unnecessary intervention in uncomplicated twin pregnancies.

Alternative to Participation

Patients who declined to participate in this study still received their regular antenatal ultrasound scans looking at fetal growth.

The harm: benefit ratio is very low. This study was considered to have minimal harm as any pregnancy with uncertain chorionicity is assigned MC and currently DC pregnancies are being over investigated or delivered earlier. Therefore our current practice may be harmful and this study could potentially change this.

Patients received the results of this study via SMS notification after histological examination was performed.

Privacy and Confidentiality

Chorionicity of twins was not considered particularly sensitive information, as such; this information would not ordinarily be kept secret from the patient or a patient's partner or family. Chorionicity may become apparent during the twins' childhood as it is closely related to zygosity. Non-identical twins are almost always (99.7%) dichorionic and identical twins are mostly monochorionic (75%).[37] Hospital folder numbers were used to identify patients, as well as date and hospital of delivery. Patient's names were not collected and data was password protected on the PI's computer.

Within one year of the completion and submission of this study for M.Med and assessment has been received, a decision in conjunction with the supervisor about the disposal of this data set will be made. As this data is not very sensitive and the result will be evident to the parents of the children; this data set may be kept as it may be useful for further study of twins. The PI, M.med supervisors (Dr van Zyl, Dr Stewart) and the UCT Department of

Obstetrics and Gynaecology will have access to this data as well as Dr Wainwright from the Department of Anatomical Pathology. The UCT department of Biostatistics will have access to the data in order to give assistance with the interpretation of the findings.

A the End of the Study

At the end of the study, participants were contacted by the PI to receive the histological diagnosis of their pregnancy. The results of this study were presented to the UCT department of Obstetrics and Gynaecology, including the sonographers who work in the maternity health care system. The department of Anatomical Pathology were invited to this presentation.

RESULTS

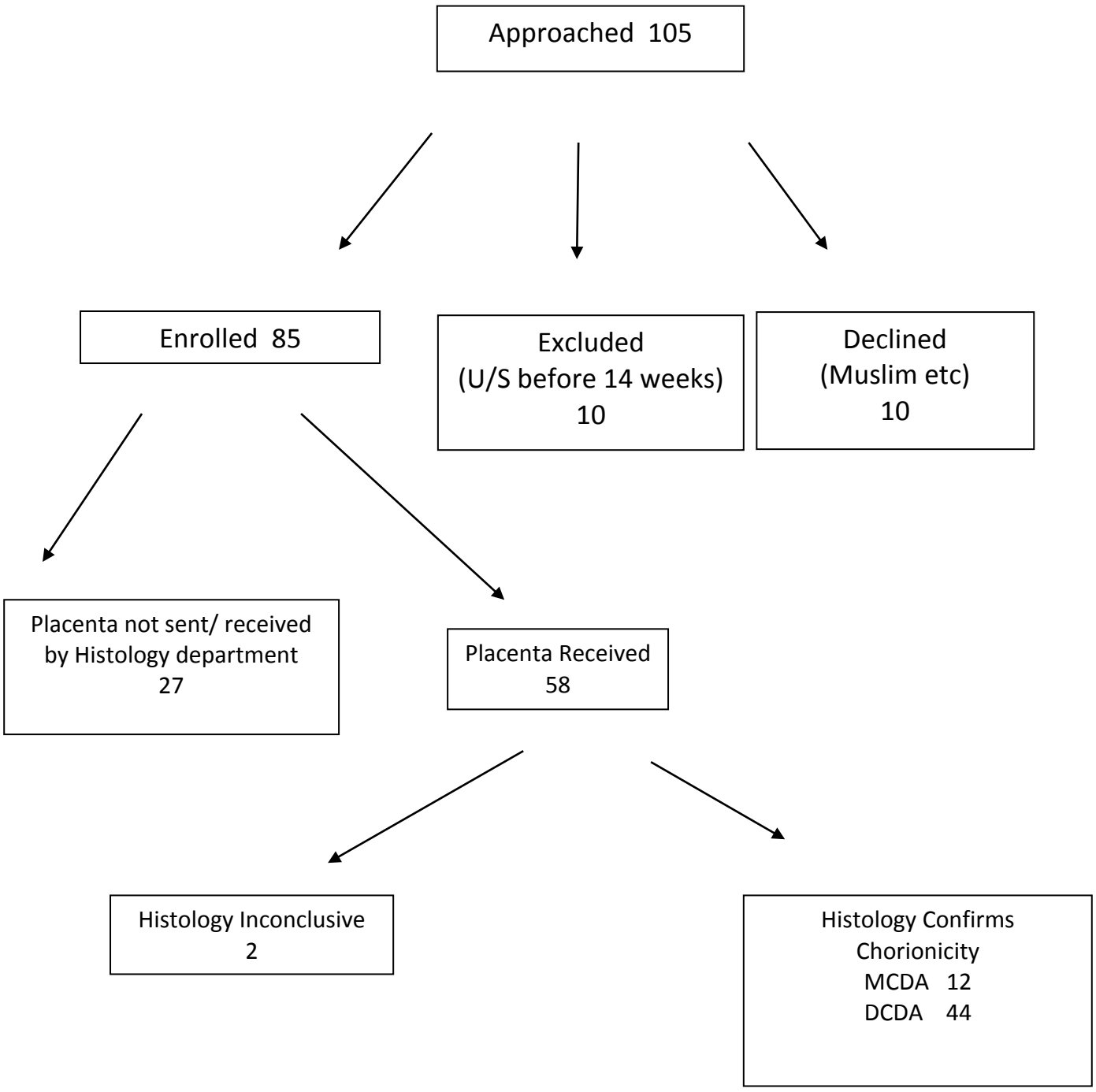
Histology

Of the 85 patients enrolled, 58 sets of placentas were available for histological examination. 2 placental sets had inconclusive histology due to incorrect site of membrane sampling or no area of fused membranes identified. The remaining 56 histology results were compared with the ultrasound characteristics. Histology diagnosed 12 MCDA twin sets and 44 DCDA twins, which is in keeping with the known incidence of MC twin pregnancies.
Unfortunately, not all 5 ultrasound parameters were documented for all of the twin sets, which decreased the sample sizes. In an attempt to keep numbers as significant as possible; each of the parameters was assessed separately.

Analysis

Each of the five ultrasound parameters for chorionicity was analysed separately in relation to the corresponding histology result and a contingency table was formulated. STATA software was then used to perform the analysis. The University of Cape Town Statistical Consultancy Service in the Department of Statistical Science and The Department of Health Sciences Biostatistics Division was consulted for their analysis of the data. The respective sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each parameter.

Figure 1: DATA COLLECTION:



All 5 ultrasound parameters were completed for only 36 of the twin pregnancies. An additional 49 pregnancies had incomplete ultrasound information. Each parameter assessed separately to maximise sample size.

Concordance of Fetal Gender

Sonographers were able to determine the sex of both fetuses in 49 of the 56 pregnancies with available histology. Fetal sexes were concordant in 36 of the twin sets and discordant in 13 sets (Table 1).

Table 1: Concordance of Fetal Genders

Histology □	**Monochorionic**	**Dichorionic**	**Total**
Concordant Sexes	9	27	36
Discordant Sexes	0	13	13
Total	9	40	49

27 of the 40 DC pregnancies had same sex fetuses (67.5% false positive rate). This means that the specificity of this test (concordance of sexes) for monochorionicity is very low (32.5%). Concordant sexes on scan have a low PPV for monochorionicity of only 25% but this test is still useful in that discordant sexes have a NPV for MC twins of 100%. Discordance of sexes is a more useful feature than concordant sexes when determining chorionicity.

Number of Placentas

Placental number was commented on in 55 of the 56 pregnancies who had a histological diagnosis of chorionicity. One of the MC pregnancies was noted to have 2 separate placentas seen on ultrasound scan. Two thirds (67%) of all the pregnancies scanned appeared to have one placental mass. This indicates the poor reliability of this test (Table 2).

Table 2: Number of Placentas

Histology □	**Monochorionic**	**Dichorionic**	Total
1 Placenta on U/S	10	27	37
2 Placentas on U/S	1	17	18
Total	11	44	55

One placental mass has a high sensitivity for monochorionicity (90.9%) but a very poor specificity of only 38.6%. Nearly 62% of the DC twin sets appeared to have only one placenta. One placental mass is not a useful test as it only has a PPV of 27% for monochorionicity. Two placental masses can however be a useful tool to exclude monochorionicity as this finding has a NPV of 94.4% for MC pregnancies. The finding of two placentas therefore also has a high sensitivity of 94.4% for dichorionicity. The specificity of two placentas being a dichorionic pregnancy is very low (27%) as the majority of DC pregnancies had only one placental mass seen on ultrasound (a high false negative rate for DC).

Lambda Sign after 14 weeks Gestation

Lambda sign was only measured in 30 of the 56 twin sets available for diagnostic comparison. This small sample size makes any deductions likely to be inaccurate. Often, if sonographers commented that 2 placentas were present, no information was given as to whether a lambda figure was seen or not.

There were no lambda signs seen in the histologically confirmed MC twins (Table 3). But there were only 6 MC pregnancies assessed for this characteristic so absence of lambda sign cannot be assumed to be 100% sensitive for monochorionicity as this variable is not adequately powered.

Table 3: Lambda Sign

Histology □	**Monochorionic**	**Dichorionic**	Total
Lambda Sign ABSENT	6	7	13
Lambda Sign PRESENT	0	17	17
Total	6	24	30

The sensitivity of the presence of the lambda sign is 100% for dichorionicity (there were no lambda signs seen in the MC pregnancies). Although the sample size was small, the presence of a lambda sign at any gestation confirmed dichorionicity and can thus be used early in the algorithm to exclude a monochorionic pregnancy. This is however, is not a specific test as seven of the DC pregnancies did not have a lambda sign.

Membrane Thickness:

Membrane thickness was measured in 46 pregnancies which had corresponding histology results. A review of the literature on this measurement quoted 2mm as the most accurate cut-off to distinguish MC from DC pregnancies. [27,17] Twenty six twin sets had a membrane thickness of >2mm and 20 pregnancies had a dividing membrane <2mm. All but one of the MC pregnancies had a membrane thickness of <2mm making this a promising test for monochorionicity (90.9%). Membrane thickness however is less specific for MC twins as 10 of the 35 DC twins also had membranes <2mm. This gives a specificity of 71.4% for this test.

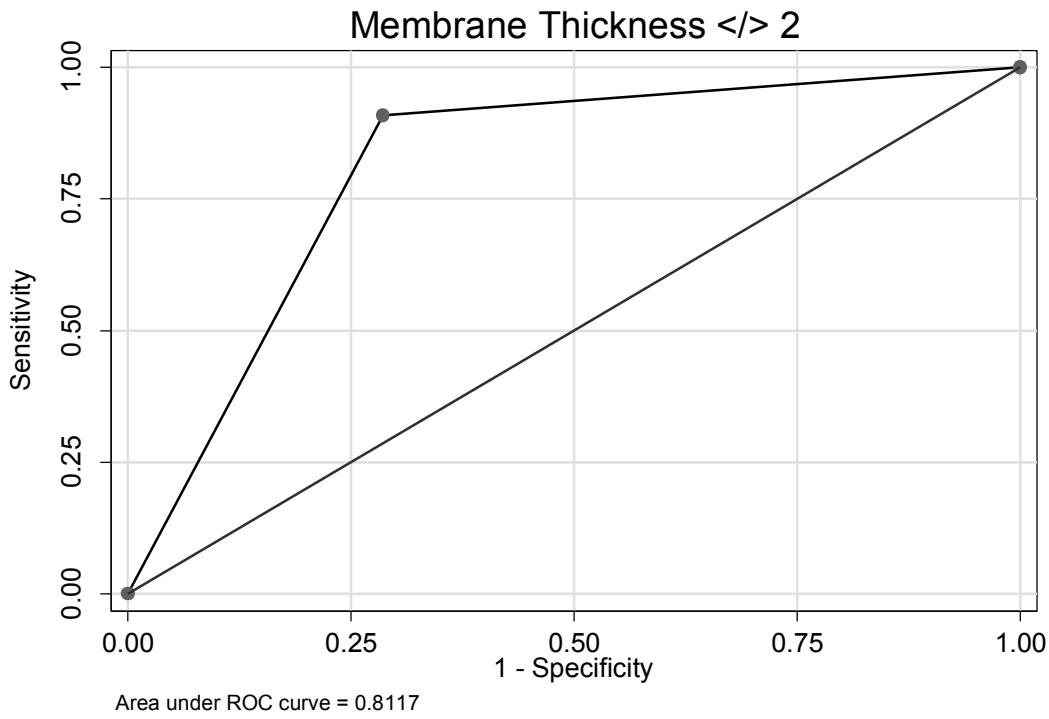
The NPV of a membrane thickness of >2mm is 96.2% for monochorionicity. But the PPV is only 50% for a membrane thickness of <2mm (Table 4).

Table 4: Membrane Thickness

Histology □	**Monochorionic**	**Dichorionic**	Total
< 2mm	10	10	20
> 2mm	1	25	26
Total	11	35	46

To determine the most accurate cut-off point for membrane thickness in predicting chorionicity, a Receiver Operator Characteristic curve (R.O.C) was generated. Initially, we looked at the curve with the discrete cut-off point of 2mm (Graph 1):

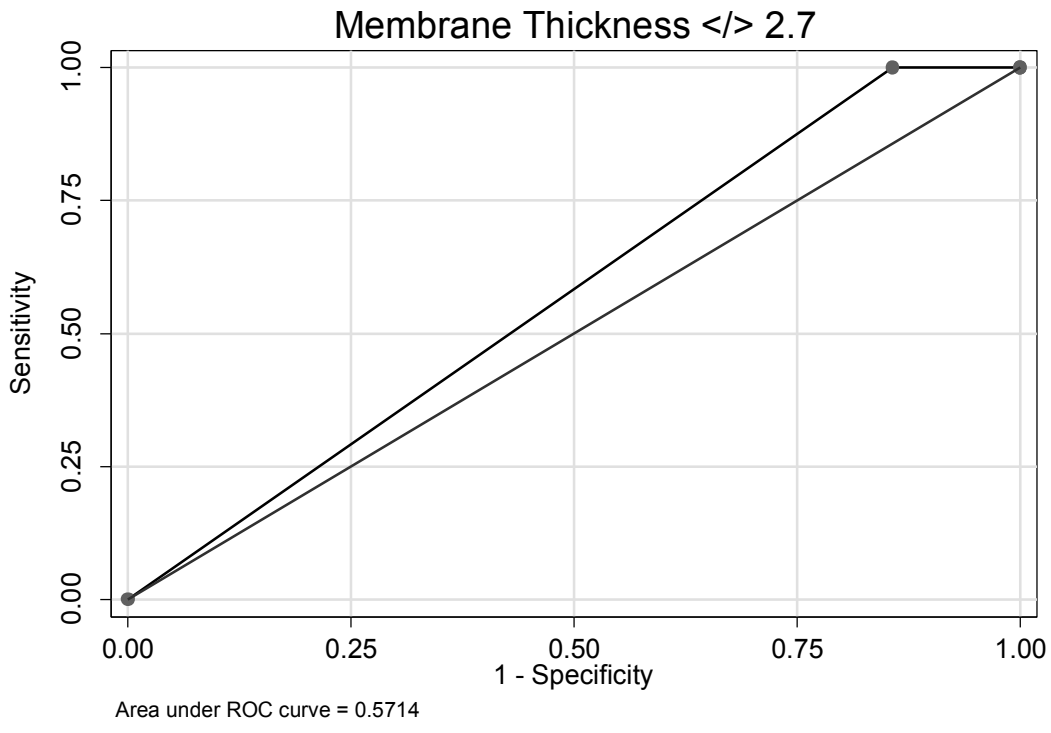
Graph 1. R.O.C curve of Membrane Thickness, 2mm cut-off:



This gives the appearance of an accurate test as the area under the curve is 0.8117. If the membrane thickness is < 2mm, the odds of that pregnancy being monochorionic are 25 times higher than if membrane thickness is > 2mm.

If the cut-off measurement for dividing membrane thickness is increased to 2.7mm, clearly the sensitivity and NPV for monochorionicity will increase but the specificity and PPV greatly decrease to 14.3% and 26.8% respectively; making this cut-off less accurate at diagnosing chorionicity. This is illustrated by a R.O.C curve with a smaller area under the curve of 0.571 as shown below (Graph 2):

Graph 2. R.O.C. curve of Membrane Thickness, 2,7mm as cut-off:

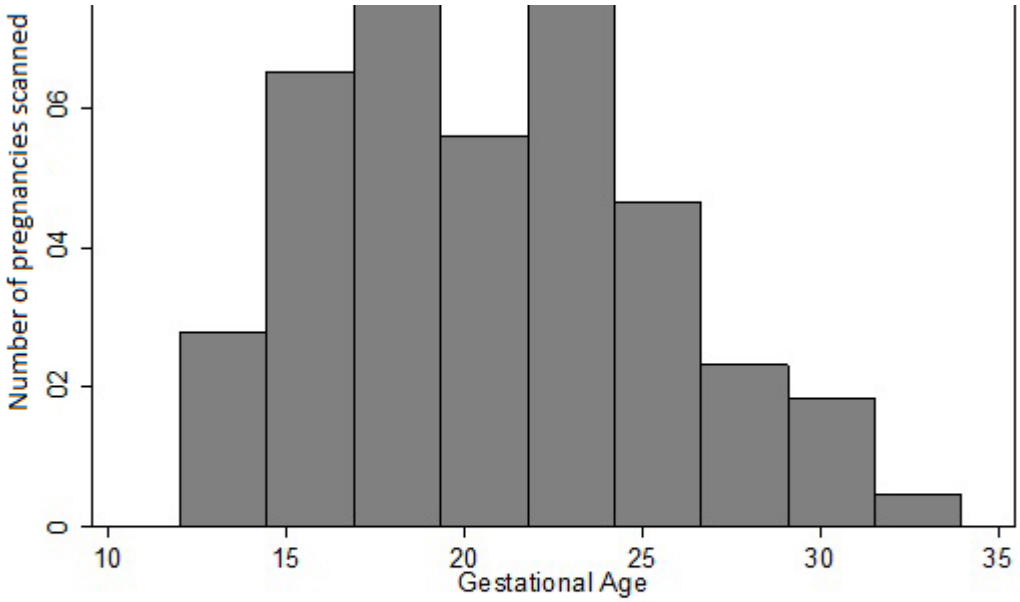


If R.O.C areas are used to compare the accuracy of different tests, the A.U.C 's are compared. A R.O.C with an area close to 0.5 is a worthless test as these results have a 50:50 chance of being correct. Higher R.O.C areas indicate more accurate tests and A.U.C's of greater than 0.8 would indicate a reliable test.

The Impact of Gestational Age on Accuracy of Membrane Thickness:

Most of the scans were done between 15 and 25 weeks gestation, very few (only 8) had a chorionicity scan done in the third trimester. Below is a graph depicting the distribution of scans across the gestational ages (Graph 3).

Graph 3. Distribution of scans by Gestational Age (in weeks):



As only 8 of the 85 scans were done in the third trimester, it would not be useful to compare 2nd trimester to 3rd trimester scans for accuracy as the data set is insufficiently powered to analyse these two groups separately.

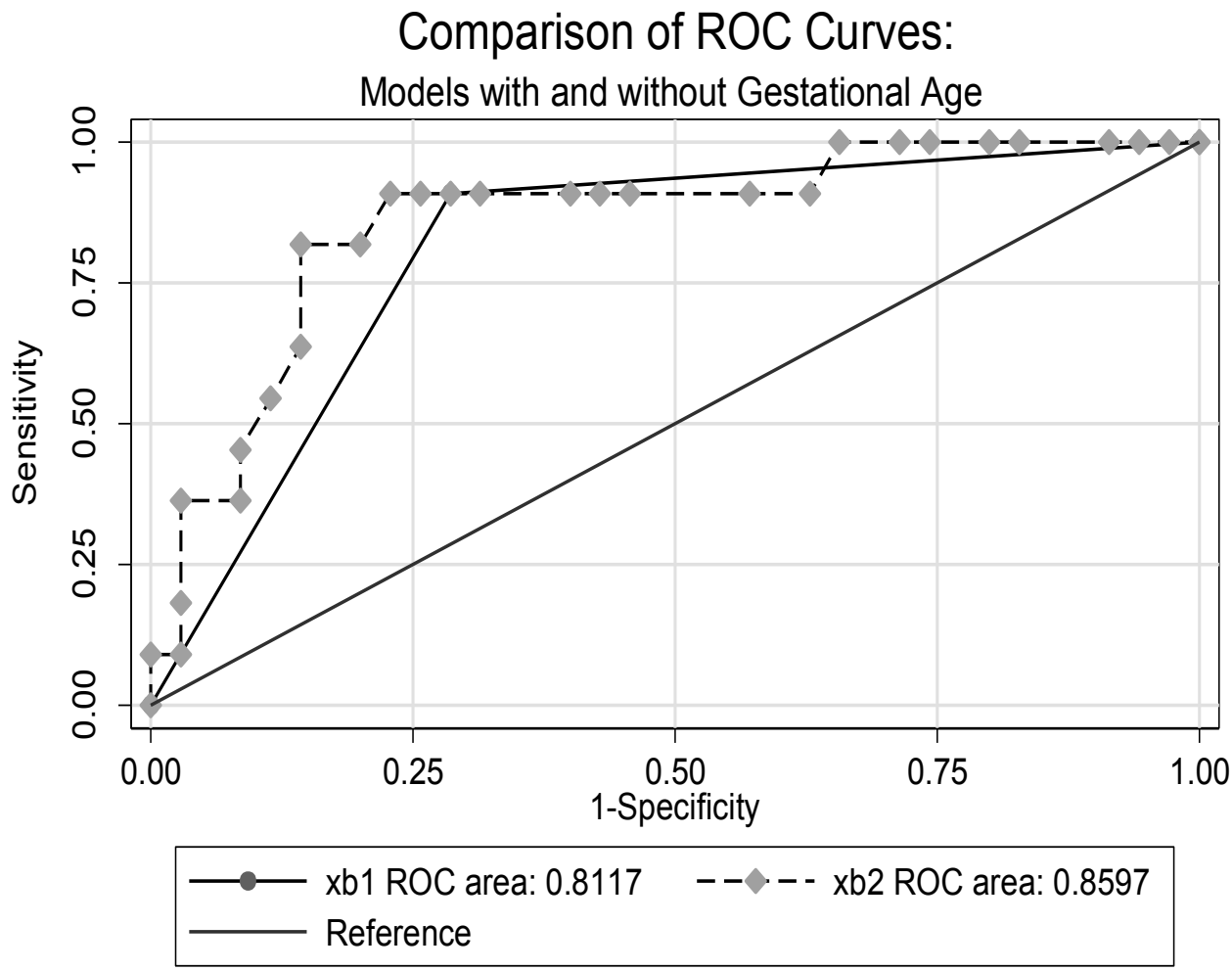
To determine if gestational age at the time of the chorionicity scan had an impact on the accuracy of membrane thickness measurement; a logistic regression analysis was performed. The findings were that gestational age did not significantly alter the accuracy of the membrane thickness as a diagnostic tool.

Several multivariate analysis tests were performed to analyse the interaction of membrane thickness, gestational age and the histology result (true chorionicity). The Z-test p-value for membrane thickness alone and histology was 0.004 ($p < 0,05$ is significant), with confidence

intervals 1.093 – 5.574. This shows that there is a strong relationship between membrane thickness and the histological chorionicity.

The membrane effect or membrane coefficient remains constant (3.219) whether gestational age was added into the model or not, confirming that gestational age has no influence on the relationship between membrane thickness accuracy and the histological outcome in this series. ROC curves taking gestational age into account also showed no change in accuracy of the test (Graph 4).

Graph 4:



Xb1 = Accuracy of Membrane Thickness alone

Xb2 = Accuracy of Membrane Thickness taking Gestational Age into account

Difference in area under the curve (Prob >chi2 = 0.1709) i.e. Not significant

Number of Layers in the Dividing Membrane:

Thirty five sets of twins had the number of layers in their dividing membrane counted as well as histological confirmation of chorionicity. Technical difficulties at Mowbray Maternity Hospital (insufficient magnification and ultrasound wave frequency i.e. only 3.5 MHz) led to the inability to acquire membrane images with the adequate spacial resolution to count the fine layers of the dividing membrane . As the sonographers were unable to interpret these membrane images, only patients seen at the tertiary hospital (with higher resolution ultrasound machines) were able to have the number of the layers in the dividing membrane counted.

As the dividing membrane is only a few hundred cells thick; it was very important to have a high signal to noise ratio to clarify the separate layers. Only high frequency ultrasound machines (5 - 10 MHz machines and greater) were able to delineate the layers which could then be assessed by the sonograghers.

Fifteen pregnancies had dividing membranes containing only 2 layers and 20 pregnancies had 4 layered dividing membranes (Table 5). Where 3 layers were seen, this was presumed to be a 4 layer membrane.

Table 5: Layers in Dividing Membrane

Histology	**Monochorionic**	**Dichorionic**	Total
2 Layers	9	6	15
4 Layers	0	20	20
Total	9	26	35

The sensitivity of 2 membrane layers for monochorionicity is 100% but it is however only 76.9% specific as 6 of the 26 DC pregnancies had only 2 layers visualized on scan. There were no monochorionic pregnancies diagnosed as having 4 membrane layers. This is however a very small sample.

The negative predictive value of seeing 4 layers in the membrane is 100% for monochorionicity which also means that it is 100% sensitive for DC. The limitation of this test is that the PPV for seeing 2 layers is only 60% for MC pregnancies. Once again it is demonstrated that these ultrasound characteristics have greater negative predictive value for monochorionicity than positive.

This test has a R.O.C. curve area of 0.885; describing it as an accurate test when considering it's negative predictive value. It is a weak test when assessing it's positive predictive value in isolation. The limitation of this test is that it is only available at GSH where higher frequency ultrasound machines can perform it.

Discussion

Twin pregnancies have at least double the complication rate compared to singletons[8] and place a larger burden on health care services both antenatally and in neonatal high care units. They require a higher rate of surveillance to predict and hopefully prevent these complications.[8] The incidence of twin pregnancies is increased with advanced maternal age, multiparity and use of Assisted Reproductive Technology.[45]

Twin pregnancies are prone to numerous complications; including an increased miscarriage rate, preterm labour, discordant growth of one twin, twin-twin transfusion syndrome and fetal abnormalities.

Monochorionic twins have twice the perinatal mortality of dichorionic twins (2.8% and 1.6% respectively).[8] This is mostly attributable to vascular anastomoses within the shared placenta which are present in 96% of MC pregnancies. TTTS is the main cause of excess mortality in MC pregnancies and is absent in DC pregnancies. Between 10 and 24 weeks, TTTS causes a six fold increase in fetal loss compared to DC pregnancies.[46]

The determination of chorionicity is critical in many of these conditions, not only because conditions like TTTS are confined to monochorionic pregnancies, but also because management may differ depending on chorionicity. Uncertain chorionicity makes management and maternal counselling in twin pregnancies problematic. The management dilemma is most apparent when a single IUFD occurs in a pregnancy of undetermined chorionicity. A surviving twin in a DCDA pregnancy has a good prognosis where as a MCDA twin survivor has a 17% chance of demising.[11] Where counselling becomes particularly important is the situation where the MC twin survives but is at high risk of neurological injury.[11] Obstetricians managing these pregnancies should ideally be able to quantify the risks for the parents so that they are able to make informed decisions about further management of the pregnancy.

If a lethal abnormality is discovered in one twin of a MC pregnancy; fetocide should only be performed by cord occlusion or diathermy.[47] If intracardiac potassium chloride is used for single fetal reduction, the co-twin is exposed to the potassium via the placental anastomoses resulting in loss of both fetuses.[48]

Severe IUGR in a single twin is also managed differently depending on chorionicity. In a DC pregnancy, expectant management is prudent as single fetal demise may not affect the normal twin but selective fetocide may precipitate preterm delivery and compromise the entire pregnancy.[8] If the pregnancy is confirmed MC and IUFD is anticipated in a single severe IUGR twin before viability; photocoagulation of the IUGR twin's cord vessels is advisable. This will separate the two placental circulations and therefore prevent acute fetofetal haemorrhage at the time of the expected IUFD. This procedure does place the entire pregnancy at risk. If single severe IUGR is diagnosed after viability; preterm delivery to protect the normally grown twin is advocated.[8]

Optimal timing of delivery for the different types of twin pregnancies also differs. Many obstetricians err on the side of caution and treat all pregnancies of unknown chorionicity as MC so as to avoid any complications which may arise in this high risk group. These pregnancies will then be delivered earlier with the inherent risk of complications of prematurity.

In the original planning of this study, the sample size needed was calculated to be 231 to sufficiently power it to accurately identify monochorionic (MC) pregnancies as they are the rarer outcome (occurring in approximately one third of all twin pregnancies). For this study to be adequately powered, a minimum of 77 monochorionic pregnancies would have to have been diagnosed from the sample population.

Due to time and financial constraints, only 58 pregnancies had a histological diagnosis and of these, 12 were MC. The challenge in the interpretation of this study was evaluating whether or not the results obtained were statistically significant. Due to very few twin pregnancies being recruited in the third trimester, the study could not look at third trimester accuracy separately to the second trimester.

Statistical methods such as p values and were employed to justify the conclusions when reasonable accuracy was found.

Data Summary

Placental number appears to be a poor test to confirm monochorionicity with a very high false positive rate for MC if only one placental mass is seen. The main value of placental number lies in the predictive value of two placentas for dichorionicity. It overall sensitivity and specificity are low. This correlates with the literature which reports a sensitivity of 23% but a specificity of 100% for dichorionicity.[49]

The lambda sign remains useful if it is still present beyond 14 weeks. We know that as gestation advances, the lambda sign is likely to disappear as the amniotic sacs expand and compress the membrane take-off area adjacent to the placentas. This data corresponds with existing literature that indicates that the accuracy of the lambda sign decreases with advancing gestational age.[18] The Columbia University Medical Centre reports a PPV of the lambda sign of 88% after 14 weeks.[18] This study conducted at UCT only found a PPV of 46% but with a NPV of 100% compared to a 94.7% NPV at Columbia University. The sample size in the American study was 410. This implies that the accuracy of this test may in fact be better than demonstrated in the small sample size in the South African Study.

Discordance of fetal gender is very useful to exclude MC twins as discordant sexes are a proxy measurement of dizygosity. MC twins are almost always monozygotic (99,7%).[37] Therefore if the sexes are discordant, we can assume dichorionicity (DC) by virtue of the two fetuses being dizygotic.

Concordant fetal genders may be present in monochorionic or dichorionic pregnancies. Prior to this study all concordant fetuses with a single placental mass and no lambda sign were assumed to be MC and managed as such. Literature on chorionicity does not quote the accuracy of concordant sexes alone to determine chorionicity. Discordant sexes are quoted as 100% accurate for dichorionicity which is what this study found. Previous expert opinion comments that concordant sexes have a 50-50 chance of being DC or MC.[17, 24] This study

found a diagnostic accuracy of 45% which concurs with this. This could be due to the small sample size.

The apparent value of the three diagnostic tests mentioned above (i.e. discordance of fetal sexes, number of placental masses and presence of lambda sign) lies in their negative predictive value (NPV) for monochorionicity and their use in excluding MC pregnancies. None of the tests singularly are valuable in confirming monochorionicity but we can confidently use them to exclude monochorionicity as a negative result is very specific for dichorionicity.

These tests appear to be weighted towards diagnosing dichorionicity: they are highly accurate at diagnosing DC pregnancies but do not have reciprocal accuracy at diagnosing or excluding monochorionics. For example, discordant fetal genders are 100% specific for dichorionicity despite concordant genders being only 32.5% specific for monochorionicity. The same can be noted for the lambda sign; it can confirm DC pregnancies with 100% sensitivity but absence of the lambda can neither confirm nor exclude monochorionics.

They are still valuable as sensitive tests which can be used to decrease the number of pregnancies which will need further ultrasound parameters to confirm monochorionicity but they have poor specificity for monochorionicity as an outcome.

Membrane thickness appears to be a promising test to add to our current practice. It has reasonable sensitivity and specificity. Importantly, it is not influenced by gestational age; making it a useful tool into the third trimester. If the membrane thickness is <2mm, the odds ratio of the pregnancy being MC is 25. If the dividing membrane is > 2mm, the accuracy of this test is not as high. Previous literature demonstrated an accuracy of 82% for MC[17] where as this study found a comparable accuracy of 76%. Bracero demonstrated a 96.6% PPV for DC pregnancies with a cut-off of 2mm. Although that cohort was only 44 pregnancies; the gestational age range was 12-40 weeks (mean GA 26 weeks).[27] The difference in results between these two studies can be explained by the small sample size in both (44 and 46 pregnancies).

Townsend found that in a cohort of 75 twin sets; the sensitivity for DC drops to 52% in the third trimester[29] where as this study proved with logistic regression that GA did not decrease the accuracy of membrane thickness measurement. If membrane thickness was modelled against the outcome of histology, a strong correlation was found (P > I z I = 0.002). Adding gestational age as a categorical variable and again as a coefficient (two different tests), it was proven that the relationship between membrane thickness and chorionicity was not altered. This demonstrates that gestational age did not have an impact on membrane thickness.

Counting the number of layers in the dividing membrane was limited to our tertiary hospital. As this membrane is only a couple of hundred cells thick; high resolution ultrasound machines were necessary for adequate image quality to allow visual interpretation of the image. Although the negative predictive value of 4 layers was 100% (95%C.I. 83.89-100); the specificity is not very high (77%)(95%C.I. 57.95-88.97) , giving a PPV for MC of only 60% with a wide confidence interval (35.75-80.18). D'Alton found a PPV for DC to be 100% but a PPV for MC to be 94%.[50]

The cohort in D'Alton's study was 69 consecutive twin pregnancies compared to only 35 in this study. Again, neither of these studies is sufficiently powered by sample size to determine chorionicity of monochorionic twins. This can explain the discordance of results in the smaller population (monochorionics) which were only 18 in D'Alton's study and 9 in this study.

In formulating a stepwise algorithm, it is important to rank tests and perform them sequentially, starting with tests of high sensitivity but low specificity and gradually moving to tests of higher specificity. In this way, imperfect or less accurate tests can be combined to improve diagnostic accuracy. Determining the correct sequence of these tests is essential to make the diagnosis. The tests in this study all had high sensitivity but disappointing specificity. Table 6 ranks the tests in order of ascending specificity.

Table 6: Ultrasound Parameters Ranked by Specificity

		SENSITIVITY	SPECIFICITY	PPV	NPV
1	Concordance of Sexes	100	32.5	25	100
2	Number of Placentas	90.9	38.6	27	94.4
3	Lambda Sign	100	70.8	46.2	100
4	Membrane Thickness (2mm)	90.9	71.4	50	96.2
5	Layers in Dividing Membrane	100	76.9	60	100

Another way to rank these tests would be to look at Diagnostic Accuracy (D.A.):

$$D.A. = \frac{\text{True Positives + True Negatives}}{\text{Total Number in Study}}$$

Table 7: DIAGNOSTIC VALIDITY COMPARISION

		D. A.
1	Fetal Gender	44.9
2	# of Placentas	49.09
3	Lambda Sign	76.67
4	Membrane Thickness	76.09
5	# of Layers	82.86

If the tests are performed in sequence of ascending diagnostic accuracy, we may be able to improve of the highest score of accuracy (82.86% for number of layers in the dividing membrane) as the confidence intervals are still large (67.32-91.9) due to limited study size. If we compare table 6 and 7, the ranking of tests is virtually the same.

There is a large improvement in both specificity and accuracy of the third, fourth and fifth tests compared to concordance of fetal gender and number of placentas.

Ranking the Lambda sign test in the algorithm is problematic. As it has the smallest sample size (n=30) and was not performed if two separate placentas were seen. It has a slightly better diagnostic accuracy than membrane thickness (76.67 versus 76.09) but a wider confidence interval (59.07-88.21 versus 62.06-86.09)

Interpretation

Previous literature on this topic has always had small sample sizes which have not been statistically powered by study population size (n= 27 to n=100).[27,28,29,31,32,50,51,52] P values have been widely employed to validate these studies. Very few studies quoted confidence intervals.

Most of the previous literature focuses on a single diagnostic test e.g. membrane thickness or layers in the dividing membrane and none have attempted a statistically based comparison of diagnostic ultrasound tests. Monteagudo et al devised a diagnostic algorithm based on 'years of experience in examining multifetal pregnancies' but without numerical justification for the sequence of tests recommended.[17] While this algorithm is based on previous studies of accuracy, there is no validation of the sequence that the author has chosen to use. A review article on the sonographic diagnosis of chorionicity by Shetty and Smith discusses the accuracy of each parameter individually.[24] There are no other studies comparing all five ultrasound parameters as discussed in this study.

Examination of 'classical' tests for chorionicity (e.g. fetal genders, number of placentas and lambda sign) show that while they have very high negative predictive value, they are very non-specific with extremely poor positive predictive values for monochorionicity. Fetal gender and number of placental masses have diagnostic accuracies of only 45% and 49% respectively.

This study has found similar accuracies of membrane thickness and number of layers in dividing membrane to those that have been reported previously. Some variation in the accuracies can be accounted for by the small sample sizes in all studies in this field. The value of adding these two tests is that they greatly improve specificity, especially if added after the three 'classical' tests (prevalence influences sensitivity) and therefore decrease the number of pregnancies that are over-diagnosed and treated as monochorionic. The sensitivities of these tests are 76% and 82.8% respectively.

It may be more appropriate to call all of these tests screening tests as we were not able to demonstrate a test with a specificity of greater than 77% in our audit. Ideally, a diagnostic test should be close to 95% sensitive and 95% specific. Although employing these tests will still mean that we over-diagnose MC pregnancies; the number that we are over-diagnosing will be reduced. As the rarer, more dangerous condition, this may be considered acceptable.

The ultimate aim would be to develop a composite diagnostic algorithm to improve the specificity (and therefore accuracy) of all 5 parameters combined. Determining selection bias in sequential tests is difficult to quantify and to compensate for, making the formulation of the algorithm imprecise. Another way to solve this problem would be to perform all five tests and to allocate a score for each test result. The total score would give an indication of the overall assessment of chorionicity for that pregnancy.

Strengths and Limitations

Lack of sample size was the major limiting factor in interpreting this study. Statistical tools were utilized extensively to assess significance. . P values and confidence intervals were used where possible.

R.O.C. curves have not been used in this area of fetal medicine diagnosis before and proved to be a useful tool to determine cut-off points and evaluate the influence of gestational age. Logistic regression analysis has not been used in previous literature on this topic to determine the effect of gestational age on the accuracy of chorionicity diagnosis. It is quoted widely that chorionicity diagnosis becomes problematic after 14 weeks due to the physiologic disappearance of the lambda sign as well as the two placental masses growing towards each other sometimes giving the appearance of a single mass. What this study demonstrates is that membrane thickness and number of layers show promise into the third trimester i.e. these tests can be used at all gestations.

It appears that counting the layers in the dividing membrane may help in the diagnosis of chorionicity in the second and third trimesters of twin pregnancies.

As this study is a diagnostic validity study, it also performs the function of an audit of the current diagnostic and management practices of twin pregnancies in our unit. During this study, all pregnancies with one placental mass and concordant genders (n=36) were managed as MC. In fact only 12 pregnancies in this sample were confirmed MC on histology.

This study enables us to decrease our area of diagnostic uncertainty and to decrease the number of twins being managed as MC. This has a significant impact if we consider that the current management of twins of unknown chorionicity in our unit is to presume an MC pregnancy. This necessitates more frequent visits, more frequent scans and earlier delivery (at 36 weeks) than DC pregnancies as per the UK National Institute of Clinical Excellence guidelines.[3] These practises all have resource implications as well as implications for the pregnancy itself. The major limitation of this study was clearly the sample size. Due to time

constraints and lack of research assistants to help recruit mothers, enrolment of more patients was not feasible. All participants were recruited by the principle investigator.

A main factor making interpretation difficult is that sonographers did not measure all five ultrasound features despite this being a prospective study. This made data sets incomplete for each pregnancy and made the formulation of the algorithm less accurate. Sonographers often used their own diagnostic reasoning to decide on the chorionicity of the pregnancy and when they decided there was enough evidence to diagnose chorionicity, the study protocol was violated. This occurred despite pre-study education. This accounts for the small numbers of patients with all ultrasound parameters measured as well as a histology result.

In the case of the lambda sign, this was never commented on if two placental masses were seen, leading to a sample size of only 30 for this category. This also adds selection bias into this test and therefore influences the true accuracy. This test specifically had the greatest influence of bias, making ranking of the tests problematic. Of the four other parameters, results were not recorded only if it was impossible to see the parameter sonographically. This would not be considered bias as poor visualization of a parameter would be random.

At Mowbray Maternity Hospital, it was frequently not possible to measure the number of layers in the dividing membrane. As High power magnification and a minimum of 5 MHz ultrasound wave strength was required to see the layers separately. . This meant that sonographers were not confident to definitively report on this parameter in most cases at Mowbray Maternity. The data regarding layers in the dividing membrane were obtained at Groote Schuur Hospital where there was more input from senior sonographers and more powerful ultrasound equipment was available. This demonstrates the point of referring cases of uncertain chorionicity for fetal medicine assessment if all of the other four parameters are non-conclusive.

Twenty four placentas did not reach the anatomical pathology laboratory despite education of fellow registrars, medical officers and midwives who were delivering these pregnancies.

Recommendations

Measuring membrane thickness in all twin pregnancies with single placental mass, no Lambda sign and concordant fetal genders (and referral to count the layers in the dividing membrane at GSH if needed) will help to decrease the number of pregnancies incorrectly diagnosed as MC. Performing these extra tests will still over-diagnose monochorionicity as their specificity is not very high but their high NPV (for monochorionicity) will ensure that no MC pregnancies are missed.

If we look at this small sample, only 12 of the 56 pregnancies were confirmed MC. If we use concordance of fetal gender and a single placenta to diagnose monochorionicity; 36 or 37 of these pregnancies will be labelled as MC and subsequently receive 2 weekly ultrasound scans and earlier delivery. If membrane thickness testing is added, the number of pregnancies labelled as MC drops to 20. If the layers is the membrane can be visualized, the pregnancies that would have been labelled as MC is only 15.

Still 3 pregnancies were over diagnosed as MC but the number is greatly reduced by 24 or 25. If we can decrease the pregnancies we manage as MC, these high risk pregnancies can receive more intensive surveillance and management in a resource constrained environment.

By decreasing the number of pregnancies of unknown chorionicity, we will be decreasing the number of pregnancies we manage as MC. This decreases the scan frequency of these pregnancies from 2 weekly to 4 weekly. We can also counsel mothers with a single IUFD or severe fetal anomaly more accurately and not terminate a presumed MC pregnancy with single IUFD. Importantly, we will not be delivering these presumed monochorionics at 36 weeks which are still at risk of the complications of prematurity.

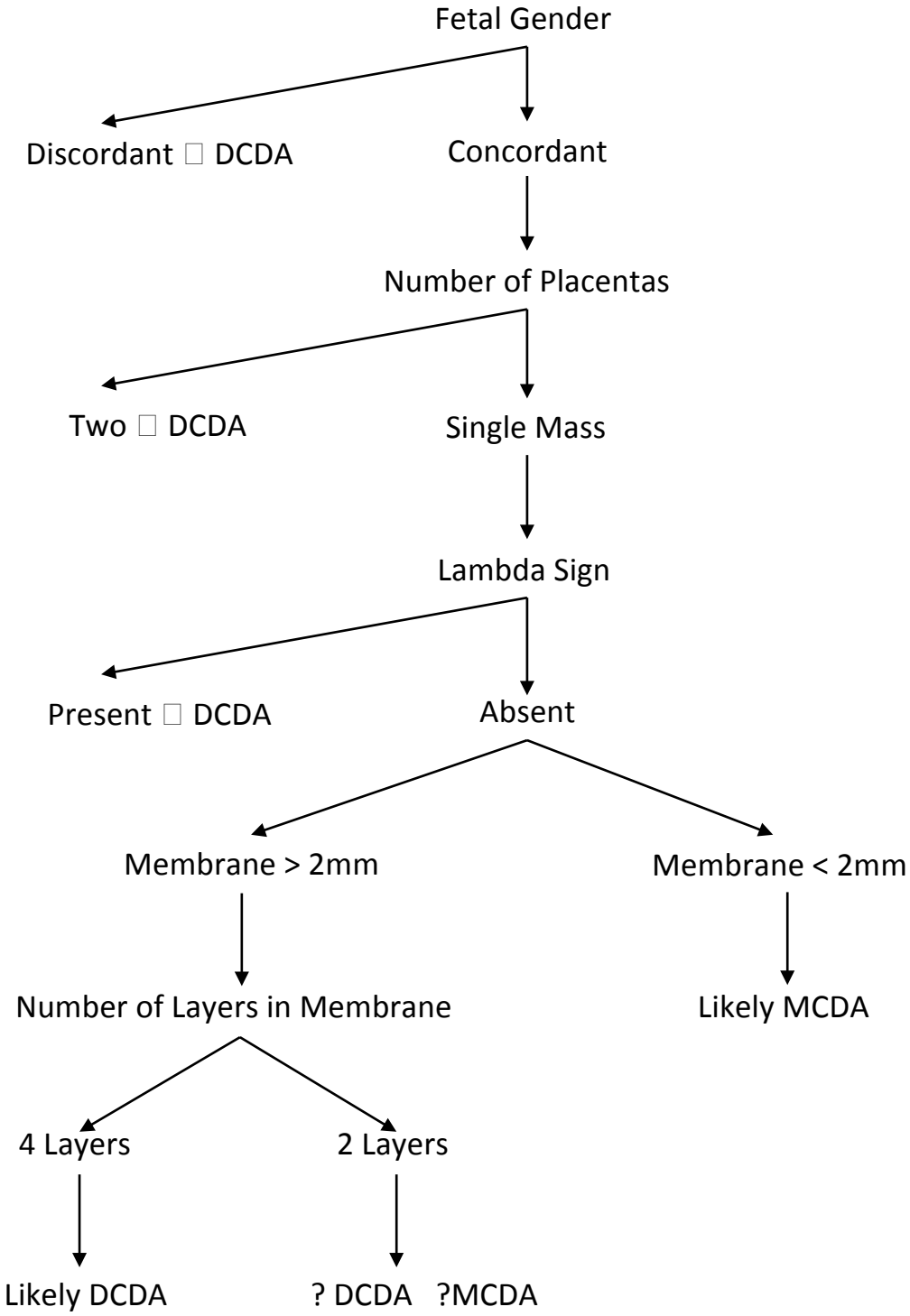
This research is valuable in our setting where many women do not book in the first trimester. The factor that makes this research valuable; that women in our service are not commonly scanned in the first trimester, also causes the problem of uncertain gestation.

If the membrane thickness is measured in all future twin pregnancies at MMH and GSH; this will help to establish a larger data base that can be audited annually to determine if our accuracy is improving with ultrasonographer experience. The learning curve must be taken into account for this M.med study as the sonographers had not been performing these measurements prior to the data collection period.
A prospective trial with sufficient sample size; powered to detect a minimum specificity of 89% would be ideal .This would require a total of 240 twin sets and a minimum of 77 MC twins to validate these tests sufficiently.

An alternative method of validating these measurements would be to construct a meta-analysis from all the studies on this topic. A literature review on second and third trimester ultrasound studies to determine chorionicity of twin pregnancies found 7 studies with 316 pregnancies. If this study is included in the analysis, the analysis should be adequately powered to determine the cumulative accuracy of membrane thickness and number of layers in the dividing membrane.

The most significant result of this study is the proposal to use a diagnostic algorithm to decrease diagnostic uncertainty. Ideally, the sequence of the algorithm should start with tests that have the highest NPV and progressively perform tests with higher accuracy. The aim of looking at all five parameters on a single twin set would be to have a high specificity without missing any MC pregnancies.

PROPOSED DIAGNOSTIC ALGORITHM:



It would be logical to start the algorithm with parameters which are likely to regress as gestation advances. Two separate placentas may grow to form a single mass early in the second trimester. The lambda sign may still be visible up to 20 weeks but is quite rare after this gestation.[19] In this way, a maximal amount of information about the pregnancy can be collected.

Gender is possible to determine throughout pregnancy and if it is not possible to determine due to fetal position, it can be reassessed at a subsequent scan. If the fetal genders are clearly discordant and easily visible, it may not be necessary to perform the other parameters. While discordance of gender can accurately exclude monochorionicity, a large number of twin pregnancies have concordant sexes, causing diagnostic dilemma. This is where the addition of membrane thickness and layers in the dividing membrane has a role to play in the diagnostic process.

If unable to determine number of layers in dividing membrane or incongruent findings e.g. membrane >2mm but 2 layers in dividing membrane; twin pregnancies can be referred to a Fetal Medicine unit for further scanning.

Conclusion

Adding membrane thickness and number of layers in the dividing membrane into the diagnostic process will help to decrease the number of twins of unknown chorionicity. This will help us to avoid managing pregnancies as MC unnecessarily, importantly preventing earlier preterm deliveries and the incorrect management of complications such as growth discordance, discordance of fetal anomalies and a single IUFD.

Although this study was underpowered in sample size, we can validate these tests by combining these results with other studies to improve cohort size. Gestational age does not seem to significantly affect the accuracy of these tests.

These findings concur with the previous literature on the accuracy of these tests and it is recommended that they are incorporated into our current practice.

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CONSENT:

USING ULTRASOUND SCAN TO DECIDE IF TWINS ARE MONOCHORIONIC OR DICHORIONIC

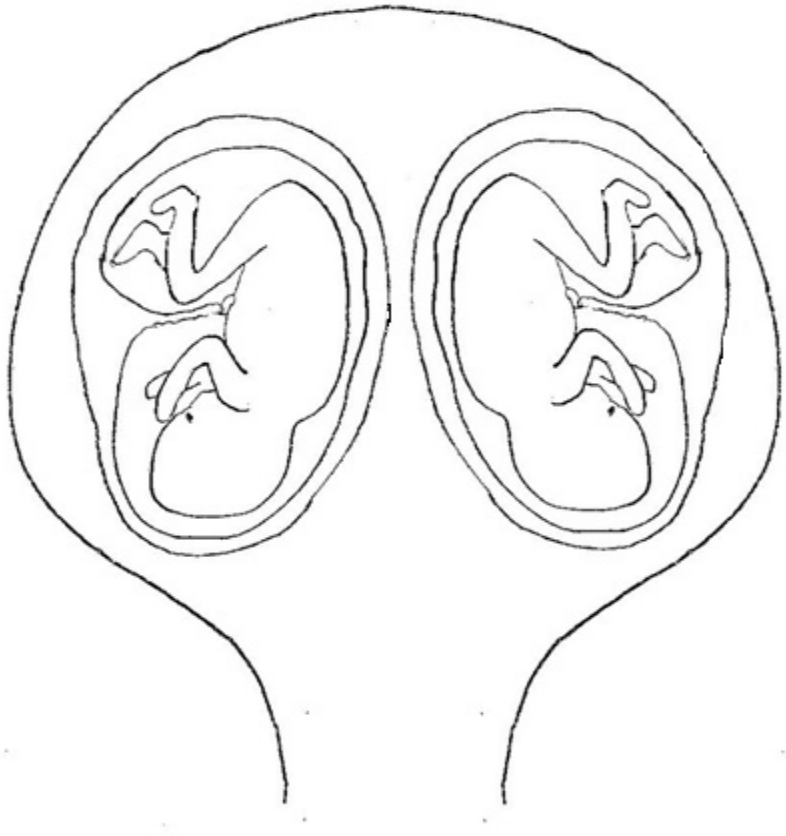
When twins are inside the womb, they can either have 2 separate placentas (afterbirths) or share 1 placenta.

Twins that share 1 placenta are usually Identical Twins and will always look the same. This is called a Monochorionic pregnancy. The other type of twins that have separate placentas are in 2 separate sacs and are called Dichorionic twins.

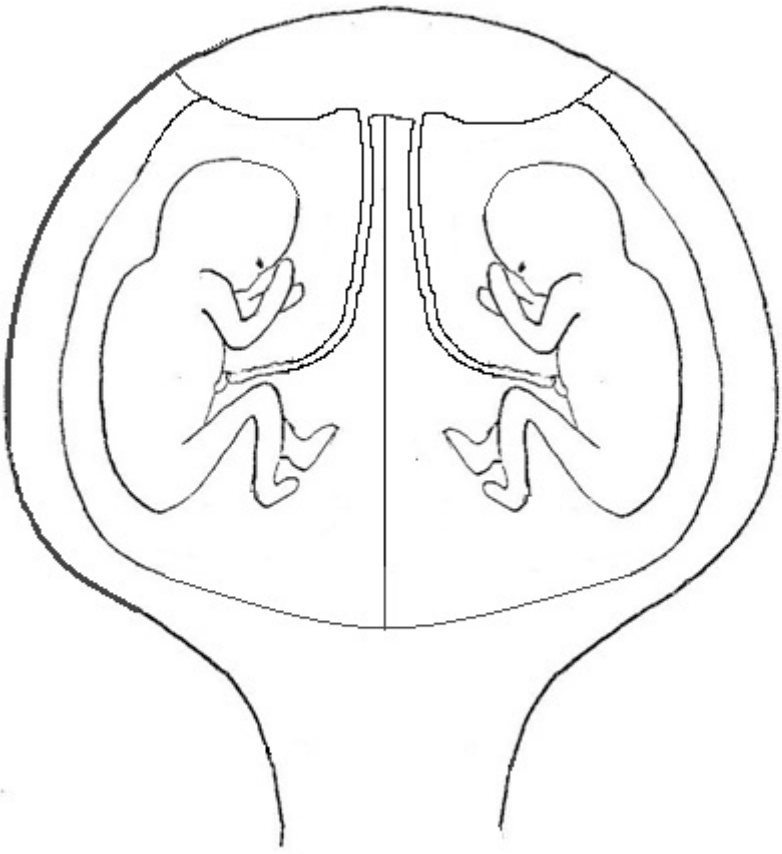
It is important to know if they share 1 placenta because we need to watch them more closely and maybe deliver them a bit earlier. There can be more problems during the pregnancy if there is only 1 placenta.

When we scan the babies for the first time after 14 weeks (3 and a half months) of pregnancy, it is difficult to tell if there are 1 or 2 placentas.

DICHORIONIC TWINS HAVE 2 SEPARATE PLACENTAS AND SACS



IDENTICAL TWINS SHARING ONE PLACENTA



I, Dr Momberg, and the University of Cape Town Department of Obstetrics (Maternity) would like to do a study to see if we can do scans after 14 weeks of pregnancy to decide if your twins share 1 placenta or have 2 separate placentas.

How the study works:

- You would attend your normal antenatal clinic visits as usual and receive all of your scheduled scans as if you weren't in a study.
- During one of your scans, we would look at 2 more things during the scan: how thick the sac wall is between the babies, and how many layers there are in that sac wall. This might take a little bit longer during the scan but it shouldn't be more than 10- 15 minutes more time.
- We will then try to guess if the babies share a placenta or have separate placentas and will tell you our GUESS if you would like to know.
- If you choose not to be in the study, you will still get all your scans as before and will still come to your normal clinic appointments.

- When you have delivered the babies, we would like to take the placentas (afterbirths) to the laboratory and look at them under a microscope to see if our guess on the scan was right.
- After we've looked at them in the laboratory, the placentas will then be thrown away (incinerated), unless you would like them back for burial.
- We will not be able to return the placentas within 24hrs for burial
- Normally, placentas that are not studied are also thrown away (incinerated) by the hospital.

- You will not be given any money for being part of this trial and will not be given transport money to come to your normal antenatal visits.

- I will be able to tell you the results or our laboratory investigation if you would like to know.
  You can contact me for the results (about 2 weeks after the delivery of your babies) on my cell phone number:
  Dr Momberg:

CONSENT

I, _____

Agree to be part of the study looking at the number of placentas in my twin pregnancy and agree to let the placentas be taken and examined in a laboratory under a microscope to help confirm what was seen on the ultrasound scans.

I understand what the ultrasound scans will be looking for and agree to the afterbirths being discarded after the examination in the same way that the hospital usually discards afterbirths.

This study has been explained to me by Dr Momberg who will be running the study. She will be available to answer any further questions that I have.

Signed: _____

Date: _____

At: _____

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • Facsimile [021] 406 6411
email: shuretta.thomas@uct.ac.za

24 May 2011

Sent via Internal mail & Email

HREC REF: 234/2011

Dr Z MOMBERG,
OBSTETRICS & GYNAECOLOGY
H-45
OMB

Dear Dr MOMBERG,

PROJECT TITLE: ACCURACY OF ULRASOUND IN THE SECOND AND THIRD TRIMESTER TO DETERMINE CHORIONICITY IN TWIN PREGNANCIES

Thank you for submitting your new study to the Faculty of Health Sciences Human Research Ethics Committee

It is a pleasure to inform you that the Ethics Committee has formally approved the above-mentioned study.

Approval is granted until 28 May 2012

Please submit an annual progress report (FHS018) if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

A/PROF MARC BLOCKMAN

CHAIRPERSON, FHS HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB0001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

DEPARTMENT of HEALTH
Provincial Government of the Western Cape

GROOTE SCHUUR HOSPITAL
Bpatel@pgwc.gov.za
Tel.: 021-404-6288 Fax: 021-404-6125
Private Bag, Observatory, 7935
www.capegateway.gov.za

REFERENCE: Zoe Momberg/Chantal Stewart/Hetta van Zyl
ENQUIRIES: **Dr Bhavna Patel**

Dr Zoe Momberg
Registrar: Obs & Gynae
H-Floor – Old Main Building

E-mail: zoe.momberg@gmail.com

Dear Dr Momberg

RESEARCH: The Accuracy of Ultrasound in the Second and Third Trimester to Determine Chorionicity of Twin Pregnancies

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research.

Please note the following:

a) Your research may not interfere with normal patient care
b) Hospital staff may not be asked to assist with the research.
c) No hospital consumables and stationary may be used.
d) No patient folders may be removed from the premises or be inaccessible.
e) Please introduce yourself to the person in charge of an area before commencing.

I would like to wish you every success with the project.

Yours sincerely

DR BHAVNA PATEL
SENIOR MANAGER: MEDICAL SERVICES
Date: 7th June 2011

Groote Schuur Hospital
Private Bag,
Observatory, 7935
Telephone : 021 404-9111

Study Approval from Mowbray Maternity Hospital:

Dear Dr Momberg,

Thank you for submitting your "Accuracy of Ultrasound in the Second and Third trimester to determine Chorionicity in Twin Pregnancies" to the Mowbray Maternity Hospital Research Committee for review.

This serves to inform you that this was reviewed at our meeting on 28th June 2011. This study was formally approved to be done on women pregnant with twins attending at Mowbray Maternity Hospital antenatal clinic. Permission has therefore been granted for you to do the study within the time frame set by the UCT HREC at our institution.

Yours faithfully,

Lucy Linley acting chairman Research Committee, Mowbray Maternity Hospital

Lucy Linley
Neonatologist-in-Charge
Mowbray Maternity Hospital
(Div Neonatal Medicine
School of Child and Adolescent Health
University of Cape Town)

Tel 27216595562/4
Cell 27825643265
Fax 27216852991

DECLARATION OF ORIGINAL WORK

I hereby declare that the dissertation 'The accuracy of ultrasound in the 2nd and 3rd trimesters to determine chorionicity of twin pregnancies ' is my original work (except where acknowledgements indicate otherwise).

Candidate : Dr Zoe Momberg

Signature: Signed by candidate

Date: 25/09/12

Supervisor: Dr Chantal Stewart

Signature:

Date: 25/9/12.