First trimester prediction of adverse events in monochorionic twins using first trimester ultrasound and biomarkers: The OMMIT study

Dr Fiona L Mackie, Dr Katie Morris, Professor Mark Kilby

Introduction

We are currently unable to predict which monochorionic (MC) twins will develop complications (e.g. twin-twin transfusion syndrome (TTTS), selective fetal growth restriction (sFGR), antenatal growth discordance, single intrauterine fetal demise (sIUFD), twin anaemia polycythaemia sequence (TAPS)) or how severe they will be. There is some evidence of prodromal features present in 'at risk' MC twin pregnancies at first trimester ultrasound examination (11 and 13+6 weeks). These have been described as: a) discordance in fetal nuchal translucency (NT) thickness (Kagan et al, 2007; El Kateb A et al, 2007) and b) discordance in fetal crown rump length (CRL) measurements (El Kateb A et al, 2007). Our group has reported the following maternal biomarkers changes: a significant increase in maternal serum alpha-fetoprotein (αFP) and β-hCG, and a decrease in maternal angiogenic activity in early onset TTTS with increased sFlt-1/PlGF ratio (Fox CE et al 2010). However, we do not know if these markers have a predictive ability.

Progress

We are in the process of performing an international, multicentre, retrospective cohort study using fetal ultrasound biometry and maternal serum samples taken at $11\text{-}13^{+6}$ weeks, from MCDA twin pregnancies booked at 32 hospitals within the West Midlands and North Thames, and the Royal Prince Alfred Hospital, Sydney. The variables were: crown-rump length, nuchal translucency, β-hCG, PAPP-A, patient characteristics, and aFP, PlGF and sFlt-1.

I have collected the outcome data and ultrasound measurements for 185 MCDA twin pregnancies. The included women had a median maternal age of 31 (range 27-34 years), booking BMI of 24.0 (21.1-28.1), and 12/171 (7.0%) were current smokers. 84 pregnancies developed at least one complication (45.4% 84/185) including: sFGR 19/185 (10.3%), both FGR 2/185 (1.1%), antenatal growth discordance 38/185 (20.5%), birthweight discordance 43/185 (23.2%), TTTS 20/185 (10.8%), sIUFD 9/185 (4.8%), and both IUFD 8/185 (4.3%). 29.7% (55/185) had a pre-term birth <34 weeks. I have also performed ELISAs on the 185 maternal serum samples to measure αFP, PlGF and sFlt-1. Alongside the retrospective study I am currently recruiting to the prospective arm of the OMMIT study whereby I take serial blood samples at 12, 16 and 20 weeks from women pregnant with MC twins who are acting as the uncomplicated cohort, as well as blood samples and amniotic fluid samples from women undergoing fetoscopic laser ablation (FLA) for TTTS pre- and post FLA. On 10 of

these serum samples (5 from normal MC twin pregnancies and 5 from TTTS pregnancies) which have been matched for gestational age, maternal age and maternal BMI as closely as possible, I have extracted microRNA, and microRNA expression is currently being analysed by real-time quantitative PCR with the aim of identifying changes between the 2 groups which may aid pathophysiological knowledge and provide potential biomarkers in the future.

Problems encountered

The number of samples eligible for inclusion was lower than initially anticipated. However, since submitting the application for the award, we agreed a collaboration with Professor Jon Hyett at the Royal Prince Alfred Hospital in Sydney, and were able to include their samples as well. The cost of analysing the initial markers of αFP, PlGF, VEGF-D and Ang-2 was more than previously calculated due to them being unable to be performed at Birmingham Women's Hospital as previously planned. Therefore Ang-2 was not analysed, and sFlt-2 instead of VEGF-D was analysed, following results from our systematic review and meta-analysis.

Presentations/abstracts/publications/Prizes

1) Mackie FL. 'Optimising Management in Monochorionic Twins' (OMMIT) study. I was an invited speaker to 'The Gynaecology Club' Annual Meeting, Birmingham, March 2016.

2) Mackie FL, Morris RK, Kilby MD. The prediction, diagnosis and management of complications in monochorionic pregnancies: the OMMIT (Optimal Management of Monochorionic Twins) study. *BMC Pregnancy Childbirth* 2016 (submitted, under peer review)

3) Mackie FL, Dunn WB, Allwood JW, van Mieghem T, Morris RK, Fox CE, Kilby MD. Metabolomic changes in amniotic fluid from twin-twin transfusion syndrome recipient twins in relation to treatment and cardiovascular risk. Oral presentation at International Society of Ultrasound in Obstetrics and Gynaecology World Congress, Rome, September 2016. Presented by F Mackie and winner of best free communication presentation in category prize.

4) Mackie FL, Hall M, Hyett J, Mills I, Riley R, Morris RK, Kilby MD. First trimester prediction of adverse events in monochorionic diamniotic twins: The OMMIT study. Submitted as an 'either' oral or poster presentation to BMFMS Annual Conference, Amsterdam, March 2017.

Future direction of work

The database of the patient outcome data, ultrasound measurement and serum analytes is in the process of being cleaned and coded as we decided to leave it as long as possible to collect as much outcome data as possible. We will then construct ROC curves to demonstrate predictive ability of the

variables, individually and in combination, and perform multiple logistic regression analysis to identify independent risk factors. This will be done with the help of Professor Richard Riley's team at Keele University.

Following the results of the retrospective cohort, we will validate the results in a prospective cohort to which I am currently recruiting from multiples antenatal clinic at Birmingham Women's Hospital.