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DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY,

UNIVERSITY COLLEGE CORK

Placental Growth Factor;

Potential for its use in twin pregnancy and evaluation of its benefit in singletons with suspected preterm pre-eclampsia

Deirdre Hayes-Ryan

Supervisors

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A thesis submitted to the National University of Ireland, Cork

for the degree of Doctor of Philosophy in Medicine, 2020.

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Glossary

ACOG	American College of Obstetricians & Gynaecologists
AE	Adverse Event
AJOG	American Journal of Obstetrics & Gynaecology
ALT	Alanine Transaminase
AR	Adverse Reaction
ART	Artificial Reproductive Technology
BJOG	British Journal of Obstetrics & Gynaecology
BP	Blood Pressure
CE	Certification Mark
CEAC	Cost Effectiveness Acceptability Curves
CRCT	Cluster randomised controlled trials
CO	Cardiac Output
CRF	Case Report Form
CRF-C	Clinical Research Facility Cork
CTU	Clinical Trials Unit
CUMH	Cork University Maternity Hospital
DBP	Diastolic Blood Pressure
DCDA	Dichorionic Diamniotic
DIC	Disseminated Intravascular Coagulopathy
DIDB	Development International Birth Date

DMC	Data Monitoring Committee
DSR	Data Summary Report
DSUR	Development Safety Update Report
eCRF	electronic Case Record Form
EDC	Electronic Data Capture
EFW	Estimated fetal weight
ELISA	Enzyme linked Immunosorbent assay
EME	Efficacy and Mechanism Evaluation
EudraCT	European Clinical Trials Database
EVT	Extravillous Trophoblast
FDA	Food & Drug Administration
FGR	Fetal Growth Restriction
FiO2	Fraction of Inspired Oxygen
fullPIERS	Pre-eclampsia Integrated Estimate of RiSk
GCP	GCP Good Clinical Practice
GDM	Gestational Diabetes
GDPR	General Date Protection Regulation
HDP	HDP Hypertensive Disorder of Pregnancy
HELLP	Haemolysis, elevated liver enzymes and low platelets
HIPE	Hospital Inpatient Enquiry Scheme
HOMP	Higher Order Multiple Pregnancy

HPO	Healthcare Pricing Office
HRB	Health Research Board
HRBCTN	Health Research Board Clinical Trials Network
HSE	Health Service Executive
ICC	Intraclass correlation coefficient
ICER	The Incremental Cost Effectiveness Ratio
ICH	International Conference for Harmonisation
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
INFANT	Irish Centre for Fetal and Neonatal Translational Research
ISF	Investigator Site File
ISSHP	International Society for the study of Hypertension in Pregnancy
IUGR	Intrauterine Growth Restriction
MCDA	Monochorionic Diamniotic
MCMA	Monochorionic Monoamniotic
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NIMP	Non Investigational Medicinal Product
NNU	Neonatal Unit
NO	Nitrous Oxide

NPEC	National Perinatal Epidemiology Centre
NPV	Negative Predictive Value
OCLA	OCLA Office of Corporate and Legal Affairs
OC	Obstetric Cholestasis
OR	Odds Ratio
PET	Pre-Eclampsia Toxaemia
PlGF	Placental Growth Factor
PIH	Pregnancy Induced Hypertension
PIL	Patient Information Leaflet
PPV	Positive Predictive Value
PSA	Probabilistic sensitivity analysis
PSF	Product Specification File
PV	Pharmacovigilance
PWV	Pulse Wave Velocity
QALYs	Quality Adjusted Life Years
QC	Quality Control
R&D	Research and Development
RCT	Randomised Control Trials
REC	Research Ethics Committee
ROC	Receiver Operating Curve
RSI	Reference Safety Information

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SBP	Systolic Blood Pressure
SGA	Small for Gestational Age
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
SVR	Systematic Vascular Resistance
SWCR	Stepped Wedge Cluster Randomised
TMG	Trial Management Group
TRAP	Twin Reversed Arterial Perfusion
TSC	Trial Steering Committee
TTD	Time to Delivery
TTTS	Twin-Twin Transfusion Syndrome
UCC	University College Cork
UK	United Kingdom
VEGF	Vascular Endothelial Growth Factor

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university. The work, upon which this thesis is based, was carried out in collaboration with a team of researchers and supervisors who are duly acknowledged in the text of the thesis. The library may lend or copy this thesis upon request.

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List of publications and presentations

Publications

- **D Hayes-Ryan,** S Meaney, C Nolan & K O'Donoghue
  An exploration of women's experience of taking part in a randomised controlled trial of a diagnostic test during pregnancy; a qualitative study
  Health Expectations Feb 2020. Volume 23, Pages 75-83. doi:10.1111/hex.12969

- **D Hayes-Ryan**, S Meaney, A Hodnett, M Geisler & K O'Donoghue
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  Acta Obstetricia et Gynecologica Scandinavica Jan 2020. Volume 00, Pages 1–12 doi:10.1111/aogs.13774

- **D Hayes-Ryan**, K Hemming, F Breathnach, A Cotter, D Devane, A Hunter, F M McAuliffe, JJ Morrison, DJ Murphy, A Khashan, B McElroy, A Murphy, E Dempsey, K O'Donoghue & LC Kenny
  PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial Research Study Protocol
  BMJ Open March 2019. Volume 9, Issue 2 doi:10.1136/bmjopen-2018-023562

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  Placental Growth Factor: A review of literature and future applications
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- D Finn, **D Hayes-Ryan**, A Pavel, J M. O'Toole, V Livingstone, G.B. Boylan, L.C. Kenny, E.M. Dempse.
  Clamping the Umbilical Cord in Premature Deliveries (CUPiD): Neuromonitoring in the Immediate Newborn Period in a Randomized, Controlled Trial of Preterm Infants Born at <32 Weeks of Gestation
  The Journal of Paediatrics May 2019. Volume 208, Pages 121–126.e2
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  Antenatal course of Placental Growth Factor; A prospective study
  Pregnancy Outcome BJOG (2019): International Journal of Obstetrics & Gynaecology Volume 126, Issue S1 EP.326, Pages 115-134. doi:10.1111/1471-0528.15636

- **D Hayes-Ryan**, K Ismail, S Meaney, A Cotter & K O'Donoghue
  Antenatal 2D ultrasound measurements of placental surface area and volume and their relationship to gestationally matched PlGF; A prospective study
  Fetal Medicine BJOG (2019): International Journal of Obstetrics & Gynaecology Volume 126, Issue S1 EP.192, Pages 60-86. doi:10.1111/1471-0528.15634

- **D Hayes-Ryan**, S Meaney, A Hodnett & K O'Donoghue
  Retrospective Analysis of Hypertensive Disorders of Pregnancy in a Multiple Pregnancy Cohort
  Maternal Medicine BJOG March 2019: International Journal of Obstetrics & Gynaecology Volume 126, Issue S1 EP.114, Pages 16-59. doi:10.1111/1471-0528.15633

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  Pregnancy Hypertension October 2018. Volume 13, Supplement 1, Pages S141. doi.org/10.1016/j.preghy.2018.08.419

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  Pregnancy Hypertension July 2017, Volume 9, Page 43. doi.org/10.1016/j.preghy.2017.07.093

- **D Hayes-Ryan**, A Totorika, C McCarthy, A Doyle, E Snapes K O'Donoghue & LC Kenny
  Cross sectional study of placental growth factor in multiple pregnancy.
  Pregnancy Hypertension July 2017, Volume 9, Page 44
  doi.org/10.1016/j.preghy.2017.07.093

Presentations

Oral (Local, National and International)

- **D Hayes-Ryan** & K O'Donoghue
  Placental Growth Factor; an overview
  Association of Clinical Biochemists in Ireland Annual Conference Limerick, 9-10th November 2018

- **D Hayes-Ryan,** S Meaney & K O'Donoghue
  A comparative study of two Immunoassays of Placental Growth Factor
  Royal College of Physicians of Ireland Annual William Stokes Competition, Dublin 18th October 2018

- **D Hayes-Ryan,** S Meaney, A Hodnett & K O'Donoghue
  Retrospective Review of Hypertensive Disorders of Pregnancy in Multiple Pregnancy
  International Society of Obstetric Medicine Annual Conference Amsterdam, 6-7th October 2018

- **D Hayes-Ryan**, S Meaney, C McCarthy, LC Kenny & K O'Donoghue
  A comparative study of two Immunoassays of Placental Growth Factor
  International Society of Obstetric Medicine Annual Conference Amsterdam, 6-7th October 2018

- **D Hayes-Ryan,** S Meaney, A Hodnett & K O'Donoghue
  Retrospective Review of Hypertensive Disorders of Pregnancy in Multiple Pregnancy
  South-Southwest Hospital Group Annual Research Conference, University College Cork, 22nd June 2018

- **D Hayes-Ryan**, K Hemming, F Breathnach, A Cotter, D Devane, A Hunter, F M McAuliffe, JJ Morrison, DJ Murphy, A Khashan, B McElroy, A Murphy, E Dempsey, K O'Donoghue & LC Kenny
  PARROT Ireland – Placental growth factor in assessment of women with suspected pre-eclampsia to reduce maternal morbidity– A stepped wedge cluster randomised control trial.
  Bi-Annual Irish Congress of Obstetrics & Gynaecology, Kilkenny 30th November 2017

- **D Hayes-Ryan** & K O'Donoghue
  Placental Growth Factor; potential of its benefit in singletons
  The Irish Centre for Maternal and Child Health Research (INFANT) Annual Research Day, University College Cork, 13th November 2017

- **D Hayes-Ryan**, A Totorika, C McCarthy, A Doyle, E Snapes K O'Donoghue & LC Kenny
  Comparison of placental growth factor immunoassays in a multiple pregnancy cohort.
  The Irish Centre for Maternal and Child Health Research (INFANT) Annual Research Day, University College Cork, 23rd November 2016

- **D Hayes-Ryan**
  Pre-eclampsia; Knowing is half the battle.
  Famelab Ireland Annual Competition, University College Cork, 25th January 2017

- **Poster (National and International)**
  D Hayes-Ryan, S Meaney, C Nolan & K O'Donoghue
  An exploration of womens experience of being involved in research during pregnancy
  Blair Bell Annual Research Meeting, Royal College of Obstetricians & Gynaecologists London, 27-28th February 2020

- **D Hayes-Ryan**, S Meaney, C Nolan & K O'Donoghue
  An exploration of womens experience of being involved in research during pregnancy
  New Horizons Annual Medical Conference, University College Cork, 3rd December 2019

- **D Hayes-Ryan,** S Meaney, C Nolan & K O'Donoghue
  An exploration of womens experience of being involved in research during pregnancy
  Bi-Annual Irish Congress of Obstetrics & Gynaecology, Galway 28th November 2019

- **D Hayes-Ryan**, S Meaney, C Nolan & K O'Donoghue
  A Qualitative Study on Involvement of Pregnant Women in Research
  Annual Royal College of Obstetricians & Gynaecologists World Congress, London 19-19th June 2019

- **D Hayes-Ryan**, K Ismail, S Meaney, A Cotter & K O'Donoghue
  Antenatal course of Placental Growth Factor; A prospective study
  British Maternal and Fetal Medicine Society Annual Conference, Edinburgh 28-29th March 2019

- **D Hayes-Ryan,** K Ismail, S Meaney, A Cotter & K O'Donoghue
  Antenatal 2D ultrasound measurements of placental surface area and volume and their relationship to gestationally matched PlGF; A prospective study.
  British Maternal and Fetal Medicine Society Annual Conference, Edinburgh 28-29th March 2019

- **D Hayes-Ryan,** S Meaney, A Hodnett & K O'Donoghue
  Retrospective Analysis of Hypertensive Disorders of Pregnancy in a Multiple Pregnancy Cohort.
  British Maternal and Fetal Medicine Society Annual Conference, Edinburgh 28-29th March 2019

- **D Hayes-Ryan**, S Meaney & K O'Donoghue
  A prospective comparative study of Placental Growth Factor in a multiple pregnancy cohort.
  The Society for Maternal-Fetal Medicine Annual Conference, Las Vegas USA, 29th Jan to 3rd Feb 2019

- **D Hayes-Ryan**, S Meaney, LC Kenny & K O'Donoghue
  Gestational distribution of placental growth factor in multiple pregnancy; a cross sectional prospective study.
  British Maternal and Fetal Medicine Society Annual Conference, Brighton 19-20th April 2018

- **D Hayes-Ryan,** K Hemming, F Breathnach, A Cotter, D Devane, A Hunter, F M McAuliffe, JJ Morrison, DJ Murphy, A Khashan, B McElroy, A Murphy, E Dempsey, K O'Donoghue & LC Kenny
  PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to Reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial.
  New Horizons Annual Medical Conference, University College Cork, 15th December 2017

- **D Hayes-Ryan,** K McNamara, N Russell, L Kenny & K O' Donoghue
  Maternity Ultrasound in the Republic of Ireland 2016; A Review.
  Bi-Annual Irish Congress of Obstetrics & Gynaecology, Kilkenny 30th November 2017

- **D Hayes-Ryan**, K Hemming, F Breathnach, A Cotter, D Devane, A Hunter, F M McAuliffe, JJ Morrison, DJ Murphy, A Khashan, B McElroy, A Murphy, E Dempsey, K O'Donoghue & LC Kenny
  PARROT Ireland – Placental growth factor in assessment of women with suspected pre-eclampsia to reduce maternal morbidity– A stepped wedge cluster randomised control trial.
  International Society for the Study of Hypertension in Pregnancy Annual Conference, Berlin 6th-9th Sept 2017

- **D Hayes-Ryan,** A Totorika, C McCarthy, A Doyle, E Snapes K O'Donoghue & LC Kenny
  Cross sectional study of placental growth factor in multiple pregnancy.
  International Society for the Study of Hypertension in Pregnancy Annual Conference, Berlin 6th-9th Sept 2017

Awards and Prizes

- “A comparative study of two Immunoassays of Placental Growth Factor”.Shortlisted for the Royal College of Physicians of Ireland William Stokes award 2018.

- “Gestational distribution of placental growth factor in multiple pregnancy; a cross sectional prospective study”
  Recipient of a University College Cork 2018 Travel Bursary for attendance and presentation at British Maternal and Fetal Medicine Society Annual Conference, Brighton 19-20th April 2018.

- “Gestational distribution of placental growth factor in multiple pregnancy; a cross sectional prospective study”
  Shortlisted for Best Poster award at British Maternal and Fetal Medicine Society Annual Conference, Brighton 19-20th April 2018

- “Comparison of placental growth factor immunoassays in a multiple pregnancy cohort”
  Recipient of the 2017 South-Southwest Hospital Group ANU Research Medal

- “Placental Growth Factor; potential of its benefit in singletons with suspected preterm pre-eclampsia”
  Bitesize PhD presentation recipient at the Irish Centre for Maternal and Child Health Research (INFANT) Annual Research Day, University College Cork, 13th November 2017

Preface – How this thesis is structured

This thesis is presented in the form of a publication-based thesis.

Chapter 1, the introduction, gives an overview of hypertensive disorders of pregnancy, multiple pregnancy as well as current understanding and use of placental growth factor. It introduces the gaps in current knowledge relating to placental growth factor and the challenges to conducting research in a pregnant population. I believe it is important for the reader of this thesis to have a thorough understanding of the background to the content of this thesis and the day today challenges faced by clinicians caring for women with disorders of placental dysfunction.

Chapter 2 focuses on hypertensive disorders and twin pregnancy and is made up of one manuscript (Paper 1). This paper was **published in Acta Obstetricia et Gynecologica Scandinavica January 2020**. It was a retrospective review of a large cohort of women delivering a twin pregnancy in a single large tertiary unit, evaluating the implications of hypertensive disorders of pregnancy on both maternal outcomes and perinatal outcomes.

Chapter 3 highlights the origin, structure and function of placental growth factor and its receptors. It also comprises of one manuscript (Paper 2) which was **published in Pregnancy Hypertension March 2018.** This literature review, discusses how the pro-angiogenic/anti-angiogenic synergism of these biomarkers are critical for successful placentation and how an imbalance in

PlGF may be utilised as a diagnostic marker of disease or a potential therapeutic target for adverse pregnancy outcomes.

Chapter 4 focuses on expanding knowledge of placental growth factor in twin pregnancy and is comprised of two papers. The first manuscript (Paper 3) is a comparative study of two immunoassays of maternal placental growth factor, conducted in a twin pregnancy cohort. It highlights the requirements for translating a lab-based test into one appropriate for clinical utility and is **currently under review in the Irish Journal of Medical Science.** The second manuscript (Paper 4) is a prospective study of placental growth factor in twin pregnancy. It compares gestational specific PlGF levels in twin pregnancies complicated by pre-eclampsia/HDP/IUGR to controls and presents a dichorionic twin pregnancy specific reference range for placental growth factor. This manuscript is **currently submitted to BJOG: an international journal of obstetrics and gynaecology.**

Chapter 5 presents the PARROT Ireland randomised controlled trial and is comprised of two papers. The first manuscript (Paper 5) details the methodology of the multi-site stepped wedge randomised controlled trial and is **published in the British Medical Journal Online Open Access March 2019**. The second paper outlines the interim results of the trial and is presented in confidence for the purpose of this thesis.

Chapter 6 identifies barriers and facilitators to pregnant women's participation in clinical research and is comprised of one manuscript (Paper 7) which was

published in Health Expectations August 2019. This qualitative study examines pregnant women's willingness to participate in research while pregnant and explores women's experience about being involved in a clinical trial, specifically a randomised controlled trial, while pregnant.

Chapter 7 is the discussion of this thesis and is presented by themes as follows;

Theme 1; (Chapters 2 and 4) The impact of HDP in the setting of twin pregnancy and the potential of using PlGF as a potential biomarker of HDP/placental dysfunction in twin pregnancy.

Theme 2; (Chapter 3 and 5) An overview of PlGF knowledge and use to date and investigate the impact of adding PlGF to routine clinical investigations of women with suspected preterm pre-eclampsia and a singleton pregnancy.

Theme 3; (Chapter 6) the facilitators and barriers to conducting clinical research in a pregnant population.

Abstract

Hypertensive Disorders of Pregnancy are common and may result in increased maternal and neonatal morbidity and mortality. Twin pregnancies confer an increased risk of development of a hypertensive disorder of pregnancy.

Placental growth factor is an angiogenic protein highly expressed during pregnancy. The pro-angiogenic/anti-angiogenic synergism of PlGF and its receptors is critical for successful placentation in early pregnancy. Circulating maternal levels of placental growth factor correlate well with placental function. Women presenting with suspected pre-eclampsia are currently triaged based on hypertension and dipstick proteinuria. Numerous studies advocate a role for placental growth factor testing as a useful adjunct in the management of women presenting with preterm pre-eclampsia.

Several automated immunoassay platforms to quantify placental growth factor are currently available. Comparative studies of these immunoassays are limited. Current reference values and clinical cut-offs for PlGF were constructed from singleton pregnancy cohorts. Given the larger placental volume present in a twin pregnancy, separate reference ranges are likely required.

Pregnant women are seldom included in randomised controlled trials and their attitudes and experiences of this are not often investigated. Gathering feedback of their experience is paramount for future trial design to facilitate participation.

In this thesis, I reviewed nine years of clinical data in twin pregnancies from a single maternity unit to understand the impact of hypertensive disorders on maternal and neonatal outcomes. I examined cross sectional values from uncomplicated twin pregnancies to assess the potential for using PlGF in this population. I compared the PlGF results obtained from an ELISA to an automated immunoassay, to determine if clinical cut-offs developed for one platform were transferrable to another. I conducted a national multi-site randomised control trial; PARROT Ireland, to evaluate the impact of incorporation of PlGF testing into routine clinical care. Lastly, through one on one interviews with trial participants, I investigated the barriers and facilitators to pregnant women taking part in clinical research.

The data from these studies revealed that maternal age >40 years, nulliparity, conception through use of a donor oocyte, and presence of obstetric cholestasis are all important risk factors for the development of a hypertensive disorder in a twin pregnancy. The incidence of iatrogenic late prematurity and neonatal hypoglycaemia are increased when a hypertensive disorder complicates a twin pregnancy. PlGF levels in twin pregnancy differ significantly between those women with a pregnancy that will later be complicated by pre-eclampsia and those that will not. The difference is present many weeks before clinical signs or symptoms are present, indicating that PlGF has potential to aid diagnosis of pre-eclampsia in twin pregnancies. A dichorionic twin pregnancy specific reference range for PlGF has been developed, which may be utilised for further interventional research on PlGF in twins.

The findings also indicate that PlGF biomarker levels vary significantly between different immunoassay platforms, highlighting the importance of developing validated clinical cut-offs for any automated immunoassay before they can be clinically applied.

The result of the interim analysis from the PARROT Ireland trial is of no significant reduction in either maternal or neonatal morbidity with the integration of point of care PlGF based testing. These are interim results only however and the final results may differ. Should the final trial results demonstrate a positive impact on maternal morbidity, without a negative impact on neonatal morbidity, it would indicate that PlGF testing should be incorporated into routine clinical investigations for women presenting with suspected pre-eclampsia before 37 weeks' gestation.

The final study of the thesis highlights that pregnant women are interested and willing to participate in research. Identifying the correct timepoint and location to approach women, as well as the manner and language used to communicate with them, are key elements in ensuring their participation.

The findings from this thesis, though supportive of the current literature in relation to the potential of PlGF, highlight that there is more research required.

Chapter 1: Introduction

1.1 Hypertensive Disorders of Pregnancy

1.1.1 Definition of Hypertension

Hypertension constitutes raised blood pressure measurements. In the setting of pregnancy; a systolic blood pressure ≥ 140mmHg and/or a diastolic blood pressure ≥ 90mmHg are considered elevated. In order to ensure accuracy and reproducibility, it is important that blood pressure measurements are taken with a woman resting in a sitting position with the arm at the level of the heart using a calibrated aneroid device or an automated machine that has been validated for use in pregnancy (1, 2).

Hypertension in pregnancy can further be defined as mild, moderate or severe dependent on the degree of elevation of blood pressure readings. Most international guidelines on hypertension in pregnancy agree on the following definition of severity (1, 3, 4);

- *Mild Hypertension;* Diastolic blood pressure 90–99mmHg or systolic blood pressure 140–149mmHg
- *Moderate Hypertension*: Diastolic blood pressure 100–109mmHg, systolic blood pressure 150–159mmHg.
- *Severe Hypertension*: Diastolic blood pressure 110mmHg or greater, systolic blood pressure 160mmHg or greater.

1.1.2 Classification of Hypertension

There are two Irish national guidelines on hypertensive disorders in pregnancy (2012 & 2016) (1, 2) as well a NICE guideline (2010)(3). In order to harmonise these guidelines and provide a standardised approach to the classification of the hypertensive disorders of pregnancy (HDP), the International Society for the study of Hypertension in Pregnancy (ISSHP) published updated recommendations in 2018 (4). Essentially all guidelines agree that HDP can be broken down into the following classification groups; chronic hypertension, gestational hypertension and pre-eclampsia. The exact definition for each of these varies slightly with each guideline and is summarised in Table 1.1.

Table 1. 1: Classification of hypertensive disorders of pregnancy. From Irish (1, 2), NICE (3) and ISSHP (4) guidelines.

	ISSHP	**Irish Guidelines**	**NICE Guidelines**
Chronic Hypertension	Hypertension pre dating pregnancy or in first trimester	Hypertension pre dating the pregnancy or appears before 20 weeks' gestation	Hypertension that is present at the booking visit or before 20 weeks or if the woman is already taking antihypertensive medication when referred to maternity services
Gestational Hypertension	New onset hypertension after 20 weeks gestation	New onset hypertension after 20 weeks gestation without any maternal or fetal features of pre-eclampsia that resolves by 3/12 postpartum	New hypertension presenting after 20 weeks without significant Proteinuria

Pre-eclampsia	New onset hypertension after 20 weeks gestation with one/more of the following new onset • Proteinuria • Maternal organ dysfunction • Uteroplacental dysfunction	New onset hypertension after 20 weeks gestation with one/more of the following new onset • Proteinuria • Maternal organ dysfunction • Fetal growth restriction	New hypertension presenting after 20 weeks with significant proteinuria
Severe Pre-eclampsia			Pre-eclampsia with severe hypertension and/or with symptoms, and/or biochemical and/or haematological impairment.
Superimposed Pre-eclampsia	1. Patients with underlying hypertension who develop one/more of the following	When a woman with chronic hypertension or pre-existing proteinuria develops one or more of the systemic features	

	• Proteinuria • Maternal organ dysfunction • Uteroplacental dysfunction	of pre-eclampsia after 20 weeks' gestation
	2. Patients with underlying renal disease +/- proteinuria who develop new onset • Maternal organ dysfunction	

Chronic Hypertension

All guidelines agree on the criteria for diagnosing chronic hypertension; when elevated blood pressure predates the pregnancy or is found to be present prior to 20 weeks' gestation. It's estimated that between 0.2% to 5% of pregnancies are complicated by chronic hypertension and with the rising tide of obesity and the advancing age of the prospective mother at conception this prevalence is likely to increase further in years to come. A number of subtypes of chronic hypertension exist;

Essential Hypertension

Essential hypertension is the commonest form of chronic hypertension, accounting for approximately 90-95% of cases. It is defined as confirmed raised blood pressure either before pregnancy or before 20 completed weeks' gestation without a known cause and hence is considered a diagnosis of exclusion. Normally in pregnancy, generalised vasodilation causes a decrease in peripheral vascular resistance resulting in a physiological drop in blood pressure during the second trimester. Occasionally this drop in blood pressure can obscure pre-existing hypertension, making diagnosis difficult in women whose blood pressure before pregnancy or early in the first trimester is unknown. Essential hypertension can only be diagnosed once a thorough assessment has been performed to eliminate secondary causes of hypertension.

Secondary Hypertension

Secondary hypertension is defined as chronic hypertension occurring secondary to an underlying medical cause. If a woman is identified as having high blood pressure in the first half of pregnancy, it is important she is promptly evaluated for underlying medical causes, as many of these causes have their own implications for pregnancy. Dependent on the cause or medical condition suspected however, complete evaluation may need to be deferred until after delivery if the necessary investigation is not appropriate to conduct during pregnancy.

The commonest underlying medical causes of hypertension are; chronic kidney disease (glomerulonephritis, reflux nephropathy & adult polycystic kidney disease) renal artery stenosis, systemic disease with renal involvement (diabetes mellitus, systemic lupus erythaematosus), endocrine disorders (phaeochromocytoma, Cushing's syndrome & primary hyperaldosteronism) and coarctation of the aorta. In the absence of any of the above conditions, it is likely that a woman with high blood pressure in the first half of pregnancy has essential hypertension.

White Coat Hypertension or Transient Hypertensive Effect

Some women with apparent essential hypertension may actually have white-coat hypertension. This is defined as a raised blood pressure in the presence of a clinical attendant but normal blood pressure otherwise. White-coat effect in early pregnancy is fairly common, partly due to heightened anxiety when attending for medical appointments. Delineation from essential hypertension may be achieved by performing ambulatory or home blood pressure monitoring using a calibrated device, validated for use in pregnancy. Occasionally elevated blood pressure may be due to environmental stimuli such as the pain of labour or. Such a transient hypertensive effect is distinguishable by performing serial blood pressure assessment.

Gestational Hypertension

All guidelines agree on the criteria for diagnosis of gestational hypertension. It is characterised by the new onset of hypertension after 20 weeks' gestation without any maternal or fetal features of pre-eclampsia, followed by return of blood pressure to normal within 3 months post-partum. It is estimated to complicate up to 10% of pregnancies. Some women (up to 25%) initially diagnosed with gestational hypertension will progress to develop pre-eclampsia, hence increased vigilance in this group is good clinical practice. Also, some women diagnosed with gestational hypertension will have persistent blood pressure elevation beyond 12 weeks post-partum and eventually be classified as having chronic hypertension.

Pre-eclampsia

Pre-eclampsia is a multi-system disorder of pregnancy characterised by hypertension and involvement of one or more other organ systems and/or the foetus that occurs after 20 weeks gestation. Raised blood pressure is commonly, but not always, the first clinical sign of pre-eclampsia. Traditionally the presence of significant proteinuria (spot urine protein/creatinine >30 mg/ mmol (0.3mg/mg) or >300mg/day or at least 1g/L (‘2 +’) on dipstick testing) with elevated blood pressure amounted to diagnosis of pre-eclampsia.

Although proteinuria is the most commonly recognised additional feature of pre-eclampsia after hypertension, both ISSHP and Irish guidelines advocate that it is no longer considered mandatory to make the diagnosis. In the absence of proteinuria, the presence of maternal organ dysfunction or fetal growth restriction should be considered sufficient criteria for a diagnosis of pre-eclampsia.

Examples of maternal organ dysfunction that may arise with pre-eclampsia include;

- Renal Insufficiency; serum or plasma creatinine >90µmol/L
- Haematological Involvement; thrombocytopenia <100,000/µL, haemolysis or disseminated intravascular coagulation (DIC)
- Liver Involvement; raised serum transaminases, severe epigastric and/or right upper quadrant pain

- Neurological Involvement; eclampsia, hypereflexia with sustained clonus, persistent new headache, persistent visual disturbances (photopsia, scotomata, cortical blindness, posterior reversible encephalopathy syndrome, retinal vasospasm), Stroke
- Pulmonary Oedema.

ISSHP does not advocate for any clinical distinction between mild and severe pre-eclampsia in usual clinical practice. Instead, it advises all cases of pre-eclampsia should be treated in the knowledge that the condition can change rapidly and that worldwide, pre-eclampsia remains a major cause of maternal mortality.

Superimposed Pre-Eclampsia

It is well documented that women with pre-existing hypertension or renal disease are at increased risk of developing pre-eclampsia in their pregnancies. In recent years the term “superimposed pre-eclampsia” has been coined to describe this particular subset of women. The diagnosis of superimposed pre-eclampsia is often difficult. In women with chronic hypertension after 20 weeks gestation, worsening or accelerated hypertension should increase surveillance for pre-eclampsia but it is not diagnostic. Proteinuria normally increases in pregnancy, so in women with chronic renal disease worsening proteinuria is not diagnostic of pre-eclampsia but warrants close observation. In order to reach a diagnosis of superimposed pre-eclampsia, there needs to be

development of other maternal systemic features of pre-eclampsia or the presence of fetal growth restriction in the setting of chronic hypertension or chronic renal disease. ISSHP includes superimposed pre-eclampsia as a subtype of pre-eclampsia whereas the Irish guidelines categorise it separately. The NICE guideline does not comment on superimposed pre-eclampsia.

1.1.3 **Reducing the risk for development of HDP**

A large number of randomised controlled trials have shown that the antiplatelet agent aspirin may reduce or prevent pre-eclampsia among women at moderate or high risk of developing it (5-7). Meta-analyses have shown a 53% (95% confidence interval 35% to 66%) reduction in relative risk for pre-eclampsia among high risk women when aspirin is commenced prior to 16 weeks' gestation (8, 9). Internationally published clinical practice guidelines strongly recommend that physicians and midwives risk assess in early pregnancy with consideration of administration of aspirin to women at high risk of pre-eclampsia (1, 3, 4). Risk factors that are easily identifiable in early pregnancy increase a womans risk for the subsequent development of pre-eclampsia exist. These include; hypertensive disease during a previous pregnancy, chronic kidney disease, autoimmune disease such as systemic lupus erythematosis or antiphospholipid syndrome, type 1 or type 2 diabetes, chronic hypertension, nulliparity, maternal age 40 years or older, maternal body mass index (BMI) of 35 kg/m2 or more at first visit, family history of pre-

eclampsia and a twin pregnancy (1, 3, 4). A recent systematic review and meta-analysis of 92 cohort studies has validated these risk factors (10). Determination of whom is at increased risk is critical not just for the commencement of aspirin prophylaxis but also for appropriate planning of subsequent antenatal care (11, 12).

1.1.4 **Potential Adverse Outcomes of HDP**

Hypertensive disorders of pregnancy account for nearly 18% of all maternal deaths world-wide, with an estimated 62 000–77 000 deaths per year (13). Women with a pregnancy complicated by hypertension require frequent surveillance by an experienced clinician and potentially multidisciplinary care (3, 4, 14). Chronic hypertension, whether essential or secondary, poses increased risk of pregnancy complications and adverse pregnancy outcomes compared to gestational hypertension (3, 14, 15). There is some evidence that in women with chronic hypertension and end organ damage, tighter blood pressure control is beneficial and therapy should be used to keep systolic blood pressure below 140 mmHg and diastolic blood pressure at 80-90 mmHg (1, 16).

Pre-eclampsia, especially if undiagnosed and left untreated, may progress to a myriad of maternal complications including eclampsia, cerebrovascular accidents, liver rupture, disseminated intravascular coagulation and death (3, 17). Eclampsia, similar to a generalised tonic-clonic seizure, is estimated to

complicate 0.28% of pregnancies in low resource settings with maternal near-miss incidents up to 60 times more frequent in women with eclampsia (18). In developed countries, the incidence of eclampsia and its complications have decreased significantly following the introduction of management guidelines for eclampsia and pre-eclampsia in the last number of years (19, 20). While the latest MBRRACE report shows the maternal death rate from pre-eclampsia and eclampsia continues to be low (0.26 per 100,000 maternities 2014-2016) there is however no evidence of an ongoing decrease in the mortality rate, highlighting this remains an on-going area of concern (21).

Infants of women with a hypertensive disorder of pregnancy, especially chronic hypertension or pre-eclampsia, are at increased risk for complications such as intrauterine growth restriction and stillbirth due to deteriorating placental dysfunction, which often necessitates iatrogenic preterm delivery (1, 4, 16, 17, 19). Preterm birth (<37wks gestation) is one of the leading causes of perinatal morbidity and mortality worldwide, accounting for 75% of cases of perinatal mortality and more than half the cases of long-term morbidity (22, 23). Each year in Ireland, approximately 6.5% of infants are born prematurely (5% for singleton births and 55% for twin births) (24). The 2017 NPEC report identified 345 perinatal deaths, of which 235 were stillbirths (>500g and/or ≥24wks gestation) and 110 were deaths of liveborn infants within the first 7 days of life. Of the 110 early neonatal deaths in normally formed infants, 36 (32.7%) cases were directly attributable to preterm birth (24). Prematurity related perinatal mortality is even higher in low and middle income countries where many public

hospitals have limited access to neonatal intensive care and necessary facilities (25-27).

1.1.5 **Long Term Health Implications**

Pregnancy is a physiological stress test and as such is an effective indicator of potential long-term health issues. Women who develop pre-eclampsia are known to have a 4-7 fold increased risk of long term hypertension, 2-3 fold increased risk of coronary artery disease and 4-7 fold increased risk of chronic renal failure (28, 29). Hypertensive disorders of pregnancy may be considered a natural screening tool for cardiovascular events, enabling cardiovascular risk prevention. It is an ideal opportunity for health care professionals to intervene and counsel women in relation to reducing risk factors such as obesity, cholesterol and smoking (3). All women with previous hypertensive disorders in pregnancy should be encouraged to undergo an annual blood pressure check after pregnancy and regular assessment of other cardiovascular risk factors including serum lipids and blood glucose (1, 28, 29).

Advances in medical care have resulted in improved survival rates for preterm infants over recent decades, however longitudinal studies with follow-up into adulthood are needed to determine if these improvements in neonatal medicine have translated into improved outcomes. These infants have a high prevalence of intellectual disabilities, behavioural, social and emotional problems and learning difficulties that frequently persist past childhood and

into adult life (30). The “developmental origins of adult disease” hypothesis, often called “the Barker hypothesis” has provided clear evidence that pace and pathway of growth, including intrauterine and early neonatal growth, increases a person’s susceptibility to obesity, diabetes, insulin insensitivity, hypertension, and hyperlipidaemia and thereby increases the incidence of later adult complications such as coronary heart disease and stroke (31, 32).

1.2 Twin Pregnancy

1.2.1 Epidemiology

A twin pregnancy, also known as a multiple pregnancy, arises by one of two means. The first type of twinning, known as dizygotic twinning, accounts for approximately 70% of cases of twins. It occurs when superovulation due to increased levels of follicle stimulating hormone results in the release of two oocytes during a single cycle. These separate oocytes are then fertilised by separate sperm. The resultant zygotes are essentially siblings and share no more genetic identity than any other siblings share. Each will have their own placenta and amniotic sac known as a dichorionic, diamniotic twin pregnancy (33-36).

The remaining 30% of cases of twins are monozygotic. These occur when a single oocyte is fertilised by a single sperm and the resultant blastocyst splits in the early days following fertilisation into two separate but genetically identical zygotes (33, 35, 37). Timing of separation is critically important. Early

separation before 72 hours results in separate placenta and separate amniotic sacs; a dichorionic diamniotic pregnancy (DCDA). Separation from day 4-7 results in separate sacs but a shared placenta; a monochorionic diamniotic (MCDA) pregnancy. Separation between day 7-14 is rarer and results a single shared sac as well as a shared placenta; monochorionic monoamniotic (MCMA) (Figure 1) (38, 39).

Occasionally pregnancies with more than two or three fetuses, known as higher order multiple pregnancies, (HOMP) occur. Rarely these may result spontaneously from a blastocyst splitting into more than three parts, resulting in monozygotic quadruplet pregnancy (40, 41). Alternatively, the practice of multiple embryo transfers in assisted reproductive technology (ART) may result in HOMP of differing zygosity (42). Due to the increased maternal and neonatal morbidity risk associated with a HOMP, fetal reduction may be employed in some such cases, resulting in an ongoing twin pregnancy, in countries where this practice is permissible (43, 44).

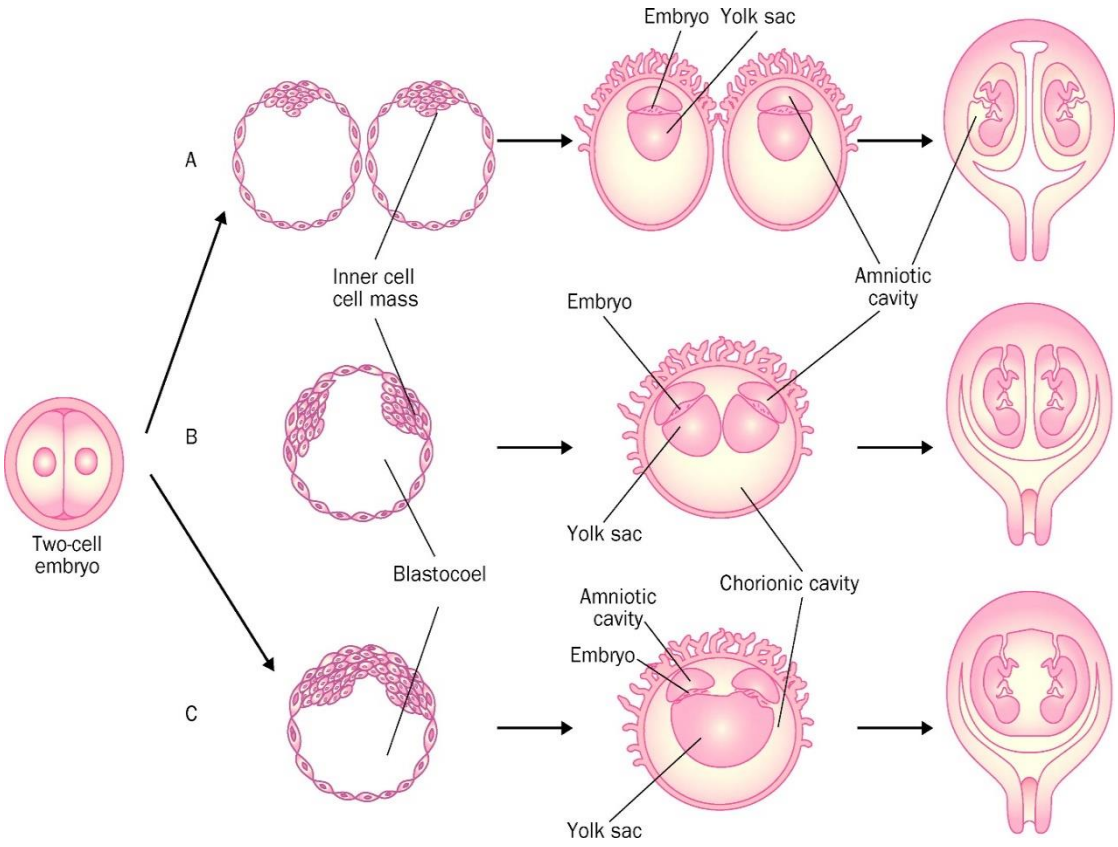

Figure 1. 1: *Monozygotic twin pregnancy showing three types of monozygotic placenta and membranes. A=dichorionic diamniotic pregnancy. B=monochorionic pregnancy. C=monochorionic monoamniotic pregnancy. From The Lancet, J. Hall (33).*

1.2.2 Incidence

Although geographic variation exists, the incidence of twin pregnancy has been consistent throughout the centuries, arising spontaneously approximately one in every 80 pregnancies (33, 45, 46). Over the last four decades, the incidence of twin pregnancy has been steadily rising, with a more pronounced increase in dizygotic twins (37, 47). A shift toward older maternal age at conception, when multifetal gestations are more likely to occur naturally,

and increased use of ART, which is more likely to result in a multifetal gestation are most likely behind these rising rates (48-50). According to Irish data, the number of twins born here has increased from 10.5 per 1,000 live births in 1985, to 19.0 per 1,000 in 2016, with an increase of 22.5% over the last decade alone (51). In the UK, a concerted effort has been made to reduce the high multiple birth rate following IVF. Campaigns such as the “One at a time; better outcomes from fertility treatment” have resulted in a reduction in the multiple birth rate following IVF treatment from 24% in 2008 to 10% in 2017 (52). Currently in the UK, multiple births account for approximately 3% of live births (53) and similar trends are reported in other developed countries (54-56).

Currently no regulations of the Irish ART sector exist; hence no monitoring or audits are in place for the practice of multiple embryo transfer or use of ovulation induction agents (50, 57). Unlike the UK, Irish fertility clinics are not obliged to officially publish their success rates therefore figures on the incidence of multiple births following IVF in Ireland are difficult to ascertain, but estimated to be in the region of 10-20% (51, 58). The Irish ART sector is predominantly privately funded, hence providers may be pressured to perform multiple embryo transfers in order to maximise chances of achieving a pregnancy for their clients (59). A number of European countries that have introduced state funding for ART services have shown a significant reduction in rates of multiple embryo transfer (60, 61).

1.2.3 Potential Adverse Outcomes of Twin Pregnancy

The specific risks of a twin pregnancy correlate well with the underlying chorionicity (53, 62), hence why it is important to identify a twin pregnancy at an early gestation, ideally <14 weeks gestation, when chorionicity can be accurately determined by ultrasound (63). Twin pregnancies overall have a higher risk of adverse perinatal outcomes such as mortality, preterm birth, congenital abnormalities and fetal growth restriction with monozygotic more vulnerable than dizygotic (49, 56, 64-66).

Specifically to monochorionic pregnancies, conditions such as twin-twin transfusion (TTTS) or twin reversed arterial perfusion sequence (TRAP) may arise, while in monoamniotic pregnancies cord entanglement is a significant concern (56, 67). The widespread use of ultrasound in obstetrics has enabled better surveillance and earlier detection of some of these complications (67). Clinical advances such as fetoscophic laser surgery have allowed therapeutic intervention in cases who previously would have had a dismal prognosis (68). A retrospective study in 2016 from three tertiary maternity hospitals in Ireland, reported a reduction in perinatal mortality in twin pregnancies between 1996-2012 owing to reduction in mortality from TTTS during the time period examined (69). Figures from the 2017 NPEC perinatal mortality report highlight an association between perinatal death and twin pregnancies. Twin births accounted for 3.8% of all births in 2017, yet accounted for 12.4% of all perinatal deaths (24). Of the 43 perinatal deaths in twin births, the vast majority (n=29, 67.4%) were in dichorionic pregnancies. Half of these deaths (n=21)

were stillbirths, with aetiology ranging from specific placental conditions (n=8, 38.1%), specific fetal conditions (n=5, 23.8%), major congenital anomalies (n=2, 9.5%) associated obstetric factors (n=1, 4.8%) and unexplained (n=5, 23.8%). The remaining perinatal deaths (n=22) were early neonatal deaths, with the majority (n=12, 54.5%) due to respiratory disorders, most often severe pulmonary immaturity. The remaining early neonatal deaths were due to major congenital anomalies (n=8, 36.4%) and neurological disorders (n=2, 9.1%) (24).

As well as adverse neonatal outcomes, adverse maternal outcomes are also more frequently observed with a twin pregnancy (67, 70). Obstetric complications such as such as anaemia, post-partum haemorrhage, gestational diabetes mellitus (GDM) and obstetric cholestasis (OC) arise more often in a twin pregnancy (56, 71). Despite our knowledge of increased maternal risks, active surveillance for same and dedicated multiple pregnancy clinics led by consultant obstetricians with expertise in management of twin pregnancy, maternal morbidity remains a problem. Figures from the 2017 NPEC severe maternal morbidity report highlight the increased incidence of severe maternal morbidity with a multiple pregnancy, with a rate of 5.76 per 1,000 maternities in singletons to 28.17 per 1,000 maternities in multiples (72).

1.2.4 Hypertensive Disorders of Pregnancy in Twin Pregnancy

Multiple pregnancy confers a 2-3 fold-increased risk for the development of hypertensive disorders of pregnancy (HDP) compared to singletons (73, 74). The incidence of HDP in twin pregnancy varies in the literature from 13-37% (73-77) with some recent studies, focused on comparison of gestationally matched singletons to twins, reporting a nine fold increased risk of pre-eclampsia with twin pregnancy (78). The reason for this increased risk of HDP in twin pregnancy is not fully understood, but is likely due to a combination of maternal risk factors and the presence of a larger placental mass (56, 73).

Maternal age >40 years, nulliparity and conception through use of a donor oocyte are independent risk factors for adverse perinatal outcomes (79-81). They are also well established risk factors for the development of HDP in a pregnancy (3, 53). These risk factors are more likely to be present in women with a twin pregnancy as in developed countries women are now commonly deferring pregnancy to focus on education and career advancement (82). Due to reduced fertility with advancing maternal age, older women often require assistance by ART, and commonly also oocyte donation, in order to achieve a pregnancy (81, 83, 84). The use of oocyte donation confers increased risk of adverse outcomes, in particular in relation to the development of hypertensive disorders of pregnancy (3, 85, 86).

With any type of twin pregnancy, a larger placenta mass is present and hence increased production of placental angiogenic factors (87). Circulating levels of

these angiogenic factors have been shown to correlate with the clinical onset of pre-eclampsia (88, 89) with each two fold elevation in sFlt-1 increasing the risk of development of pre-eclampsia (90). In section 3 of this Introduction, I will expand further on these angiogenic growth factors and their respective receptors while in section 4, I will discuss them in relation to twin pregnancy.

1.2.5 Potential Adverse Outcomes from a Hypertensive Disorder in Twin Pregnancy

When a multiple pregnancy is complicated by a HDP, national and international guidelines and research report a higher risk of adverse pregnancy outcomes compared either to an uncomplicated twin pregnancy or to a singleton pregnancy complicated by a HDP (14, 53, 72, 74, 77). HDP commonly arises due to impaired placentation, hence small for gestational age infants and placental abruption due to placental dysfunction are both more likely to occur (91). Owing to maternal and /or fetal concerns iatrogenic preterm delivery is also more likely and consequently an increased incidence of caesarean delivery is also reported (24, 72, 74, 92). Further driving perinatal morbidity, these complications usually arise earlier in the setting of twin pregnancy with HDP compared to singletons with HDP or to uncomplicated twin pregnancy (75, 78, 93, 94).

1.3 Placental Growth Factor

1.3.1 Background

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family of proteins (95). All VEGF proteins share a similar structure with a distinctive cystine knot (96, 97) characterised by eight spatially conserved cysteines stabilised by a hydrophobic core region (96, 98-100). VEGF-A was discovered in 1989 and two years later PlGF was the second member of the family identified (101-103). This pro-inflammatory factor is produced by trophoblast cells of the placenta and was discovered by an Italian scientist while she was investigating the angiogenic potential of human placental tissue (95).

PlGF can exist in multiple isoforms with four isoforms of human PlGF currently known to exist (104, 105). PlGF-1 and PlGF-2 are thought to be the predominate isoforms and they share almost 88% sequence identity (106). The isoforms differ in terms of their size, number of amino acids, binding and secretion properties (107). VEGF-A and PlGF can also exist as homodimers and heterodimers (PlGF:PlGF, PlGF:VEGF-A, VEGF-A:VEGF-A), with PlGF:VEGF heterodimers displaying fair less mitogenic activity than VEGF homodimers (108, 109) (*Figure 1.2*).

Figure 1. 2: *Depiction of the hetero and homodimer structures in which PlGF and VEGF-A may exist. From Pregnancy Hypertension, D. Hayes-Ryan et al (110).*

All VEGF proteins, including PlGF, must bind and activate a tyrosine kinase receptor in order to be able to function. There are three known homologous receptors they may bind to; Flt-1, Flk-1 or VEGFR3 (111-113). PlGF has been shown to only bind to Flt-1 (114). Flt-1 is non mobile, anchored to the cell wall membrane (115). A very similar receptor known as sFlt-1 also exists, however as it is soluble and non-membrane bound it can circulate freely (116) (Figure 1.3).

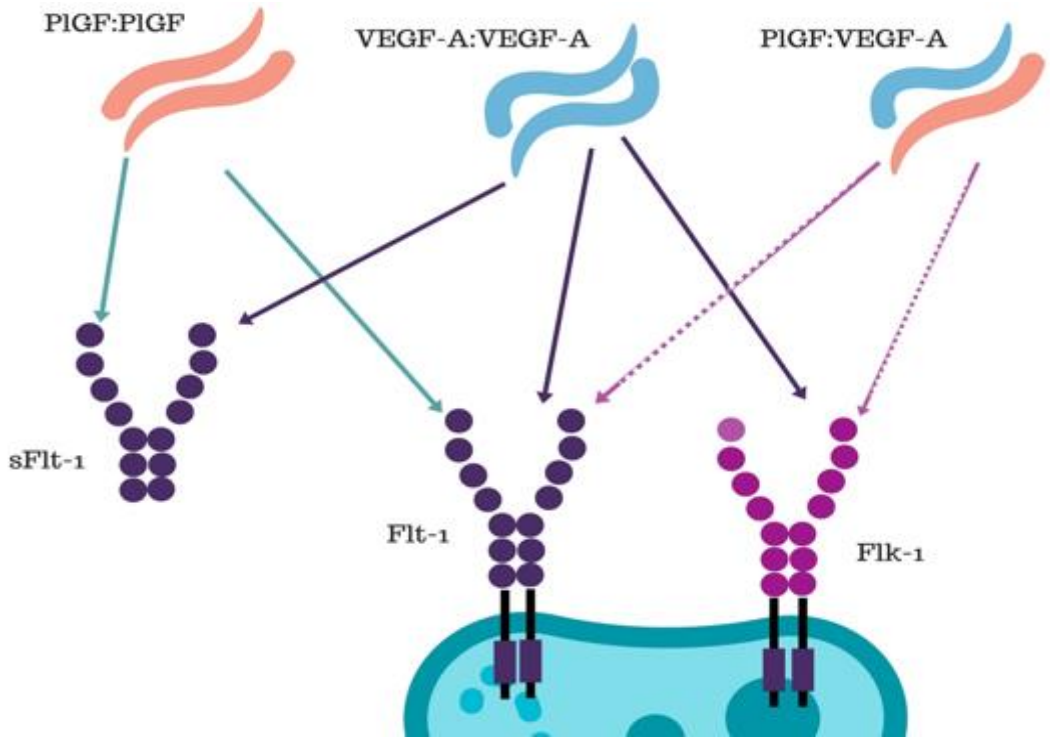

Figure 1. 3: *Depiction of the binding that may occur between PlGF/ VEGF-A proteins and the membrane bound receptors Flt-1 ad Flk-1 and the freely circulating receptor sFlt-1. From Pregnancy Hypertension, D. Hayes-Ryan et al (110).*

Placental growth factor (PlGF) is highly involved in the regulation of angiogenesis under pathological conditions (95). Early studies in mice, bred to be deficient in PlGF, demonstrated they had reduced angiogenic ability when subjected to ischaemia, inflammation or cancer (18). PlGF exerts this angiogenic effect by binding to the anchored membrane receptor Flt-1. This binding activates endothelial cells, macrophages and haematopoietic progenitor cells and displaces VEGF-A from Flt-1, allowing VEGF-A to bind instead to the more potent FDK (109, 117-121). In contrast, sFlt-1 has an anti-angiogenic effect. It binds freely circulating PlGF, essentially neutralising PlGF

effects by reducing the amount of PlGF available to the cell membrane bound Flt-1 (122, 123). The net result of this is vasoconstriction and endothelial dysfunction (124) with levels of sFlt-1 rising under hypoxic conditions in an effort to compensate for impaired oxygenation (125).

1.3.2 Hypertensive Disorders of Pregnancy & Placental Growth Factor

Studies have shown a distinct gestational pattern of PlGF in normal pregnancy corresponding to placenta development (87). PlGF levels rise alongside gestational age, peaking at 32 weeks' gestation when the placenta is developed fully and then decline until term (126, 127). In women whose pregnancies are complicated by pre-eclampsia, the rise and fall of PlGF shows the same pattern but the exact levels of PlGF are much lower throughout all their pregnancy, and extremely low at the time point when pre-eclampsia exists clinically (87-89). Also in women who develop pre-eclampsia there are increased levels of sFlt-1 present, particularly in the early onset form of the disease, weeks prior to the clinical existence of pre-eclampsia (88, 89, 128-132). It is thought that the substantial increase in sFlt-1 levels arise from the endothelial dysfunction that occurs in pre-eclampsia (133, 134). Support for this theory comes from ex vivo studies that show significant damage occurs to endothelial cells when they are exposed to serum from pregnant women with pre-eclampsia compared with controls (133, 135-137).

A three-stage model for the development of pre-eclampsia is now generally accepted; should impaired placentation occur in early pregnancy, it results in a deficiency of PlGF and consequently impaired angiogenesis. This leads to hypoxia and oxidative damage from 20 weeks gestation with low PlGF levels likely to be a good marker of an oxidatively damaged placenta. The hypoxic placenta then releases sFlt-1 in a self-defence bid to induce vasoconstriction and increase oxygen supply. The net result of this is systemic endothelial cell dysfunction and end-organ ischemia, which leads to the classical clinical signs and symptoms of pre-eclampsia (138-140).

In 2016 the UK National Institute for Clinical Excellence (NICE) issued guidance advocating that PlGF testing, combined with routine clinical care, could be used to help rule out pre-eclampsia in singleton pregnancies in women presenting with suspected pre-eclampsia between 20 weeks and 34 weeks plus 6 days of gestation. NICE also recommended that these tests should not yet be used to diagnose pre-eclampsia until further research was available, specifically on how an abnormal PlGF result would affect management decisions regarding timing and gestation of delivery and the outcomes associated with this (141).

1.3.3 Placental Growth Factor Immunoassay platforms

A number of different immunoassay platforms for quantification of PlGF alone or in combination with sFlt-1 in blood plasma or serum are available (141).

Initial laboratory work on the biomarker was conducted using laboratory based immunoassay platforms such as an enzyme-linked immunosorbent assay (ELISA) (87, 142-146). Commercial interest in the biomarker has resulted in a number of companies developing automated tests that allow rapid and easy quantification of PlGF, most of which require significant infrastructure and financial investment for initial set up (147-151). Although ELISAs continue to be available they are predominantly now used for research purposes rather than clinical application.

The Quantikine® Human PlGF Immunoassay (R&D systems)

This plate ELISA has a measureable range of 15.6-1000 pg/ml of PlGF and an assay completion time of 3.5 to 4.5 hours. The pre-coated 96 well polystyrene microplate uses a monoclonal antibody specific for human PlGF. The manufacturers report the ELISA detects PIGF-2 and PIGF-3 isoforms in addition to PlGF-1. The procedure for performing analysis with this platform is in line with any ELISA and is generally performed in duplicate with the average measurement obtained, to aid precision and to minimise the potential for error (152).

ELISA procedure;

I. Standards and samples are loaded onto the plate and any PlGF present is bound by the immobilized antibody

II. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human PlGF is added to the wells

III. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of PlGF bound in the initial step

IV. The colour development is stopped and the intensity of the colour is measured using an automated microplate reader

Triage® PlGF test (Quidel, San Diego)

The Triage® PlGF test (Quidel, San Diego) is currently the only point of care test on the market for measuring PlGF. The CE marked platform involves a single use, point of care, fluorescence immunoassay device. The test procedure involves the addition of several drops of plasma to the sample port on the single use cartridge device. The plasma reacts with the fluorescent antibody conjugates and flows through the test cartridge by capillary action. The presence of a target analyte causes the corresponding fluorescent antibody conjugates to bind to the discrete zone specific to that analyte. The test cartridge is inserted into the meter which is programmed to automatically perform an analysis after the specimen has reacted with the reagents within the cartridge. The analysis is based on the amount of fluorescence the meter detects within a measurement zone on the test device. The results are displayed on the meter screen in approximately 15 minutes and have a measureable range from 12-3000 pg/ml (148). The Triage® immunoassay

uses antibodies against PlGF isoform-1, with some cross-reactivity for isoform-2. Developed initially by Alere Inc. (San Diego, USA) the intellectual property rights were acquired by Quidel Inc. (San Diego, USA) in 2018 and the product is now commercially available again (153).

Elecsys® Immunoassay sFlt-1/PlGF ratio (Roche Diagnostics, Germany)

This immunoassay ratio test quantifies levels of both PlGF and sFlt-1. The ratio is formed by combining the results from two CE-marked sandwich electrochemiluminescence immunoassays (Elecsys® PlGF and Elecsys® sFlt-1 assays), which are compatible with both the Roche Elecsys® and the Cobas e automated analysers. The laboratory information system calculates and reports the sFlt-1/PlGF ratio and the individual assay values. The Elecsys® sFlt-1 immunoassay has a measuring range 10 to 85,000 pg/ml while the Elecsys® PlGF immunoassay has a measuring range 3 to 10,000 pg/ml. The Elecsys® PlGF immunoassay detects free PlGF isoforms as well as that part of PlGF isoforms complexed with sFlt-1. It has a limit of quantitation of 10 pg/ml and manufacturer quoted cross-reactivity with PlGF-2 of< 8%. The turnaround time of test is about 18 minutes (149).

The DELFIA® Xpress PlGF 1-2-3 test (Perkin Elmer, Finland)

This CE-marked fluoroimmunometric sandwich assay is designed to measure free PlGF Isoform-1 in serum samples. It is compatible with a 6000 DELFIA

Xpress random access analyser, has a measureable range from 1.9 to 4,000 pg/ml, a limit of quantitation of 3.3 pg/ml and a test turnaround time of about 30 minutes. The manufacturers quote PlGF-2 and PlGF-3 isoforms cross-reactivity of 28% and 20% (non-glycosylated) respectively (150).

BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio (Thermo-Fisher Scientific)

This assay is formed by combining the results from 2 automated immunofluorescent sandwich assays, the BRAHMS sFlt-1 Kryptor assay (measuring range 22 to 90,000 pg/ml) and the BRAHMS PlGF plus Kryptor assay (measuring range 3.6 to 7,000 pg/ml). The BRAHMS PlGF plus assay is designed to measure the free PlGF-1 isoform with a manufacturer quoted cross-reactivity with PlGF-2 and PlGF-3 isoforms of 13% and 4% respectively. Serum samples are required for use as well as the BRAHMS Kryptor compact plus analyser. The turnaround time for the BRAHMS sFlt-1 Kryptor assay is 9 minutes and the turnaround time for the BRAHMS PlGF plus Kryptor assay is 29 minutes (151)

1.3.4 Inter-immunoassay performance

The Triage® and Elecsys® platforms have been utilised in a number of clinical studies and appropriate clinical cut-offs for PlGF +/- sFlt-1 validated (88, 89,

154-156). The manufactures of both the DELFIA® and the BRAHMS® assays provide reference ranges for each assay in the product instructions, however the companies recommend that individual laboratories should validate these ranges or establish their own reference ranges before clinical use (150, 151). Importantly to note, PlGF values obtained with one automated platform are likely not interchangeable with those from another. Differences in actual PlGF and sFlt-1 values between test platforms, owing to inter-manufacturer immunoassay analytical differences and isoform cross reactivity, may exist, a fact highlighted in a recent comparative study including the Delfia®, Elecsys® and Brahms® platforms (157).

A dearth of information currently exists on comparative performance of commercially available PlGF immunoassay platforms (147, 157, 158). The recently published COMPARE study was the first head-to head comparison of three such platforms; Delfia®, Elecsys® and Triage®. It reported a performance of each, with high negative predictive values, in prediction of delivery within 14 days from testing in women with a singleton pregnancy and suspected preterm pre-eclampsia before 35 weeks' gestation (158). NICE have advocated that at present should PlGF testing be conducted in a clinical setting, either the Triage® or the Elecsys® should be the platforms preferentially used owing to the lack of available information as yet on the DELFIA® and the BRAHMS® platforms. NICE also recommends that further validation studies, head-to-head comparative studies, and cost effective analyses comparing these platforms are performed (141).

1.4 Placental Growth Factor and Twin Pregnacy

1.4.1 Background

Owing to a larger placental mass and use of assisted reproductive therapy (ART), especially use of non-autologous gametes, women with a twin pregnancy are at a two to three fold increased risk of developing pre-eclampsia (159-161). As knowledge on the aetiology of pre-eclampsia expands, the importance of the balance of placental angiogenic factors in its development becomes more apparent (131, 137, 162). As previously described, placental growth factor (PlGF) has shown great promise as a predictor of subsequent preterm pre-eclampsia and overall placental dysfunction in a singleton cohort, with lowered levels of angiogenic PlGF and increased levels of anti-angiogenic sFlt-1 in maternal plasma, weeks prior to the clinical onset of disease (87, 89, 95).

Few studies have examined these angiogenic factors in twin pregnancy, unsurprising given that research in a twin pregnancy cohort is more challenging given that both zygosity and chorionicity need to be considered. Also, given the inherently higher incidence of adverse outcomes in this population, larger numbers of recruits are required in order to obtain a normal sample of the population (163, 164). Prior studies of angiogenic factors in twin pregnancy vary hugely on a wide variety of factors, limiting comparison of results. Numbers of participants, gestational age at time of sampling, immunoassay platform employed and clinical endpoints frequently differ in

each study, not to mention studies often involve pooled results from a number of sites or countries across a variety of time periods (73, 160, 164-173)

1.4.2 Normal Range of Angiogenic Factors in Twin Pregnancy

Initially it was assumed that given the doubling of placental size in a twin pregnancy, that all biomarkers would be present in double the concentration in maternal serum in a twin pregnancy compared to a singleton (174). A 2012 paper by Sanchez et al, demonstrated increased levels of PlGF and its soluble anti-angiogenic receptor sFlt-1 in 61 twin pregnancies compared to 50 matched singletons, using an ELISA immunoassay for quantification of the angiogenic factors (165). The following year a paper by Cowans et al assessing first trimester PlGF concentrations using the DELFIA platform, demonstrated that PlGF levels were 41% higher in 440 dichorionic compared to 116 monochorionic twin pregnancies and 16% higher in monochorionic compared to 116 matched singleton pregnancies (175). A Spanish 2016 study demonstrated a similarity in median PlGF/sFlt-1 ratios, quantified using the ELECYS platform in twins and singletons up until 29 weeks gestation, however a significant lower median PlGF value in twins thereafter (176).

These results highlight that normal reference ranges for singletons cannot simply be doubled for use in the twin population. Individual chorionicity-specific, twin reference ranges, need for to be developed PlGF and its

receptors, to enable further research on the clinical utility of these angiogenic factors in the twin population.

1.4.3 Potential benefit of PlGF use in screening in twin pregnancy

A number of studies have demonstrated the potential use of PlGF in the twin population. A 2011 study, performed using an ELISA immunoassay, examined first trimester levels of circulating angiogenic factors in 61 women with a twin pregnancy. They noted maternal serum sFlt-1 levels to be significantly higher in the pregnancies conceived through assisted reproductive techniques (ART) compared to spontaneous conceptions (165). It is well documented that ART assisted pregnancies are at increased risk of pre-eclampsia development, hence the potential for using these angiogenic factors as a combined first trimester screening tool for pre-eclampsia (177). Indeed a 2012 study by a Canadian group showed PlGF to be a useful predictor of subsequent pre-eclampsia as early as 12 to 18 weeks in twin pregnancies, but not clinically useful enough to be used as a single marker (178).

1.4.4 Potential benefit of PlGF in diagnostics in twin pregnancy

In singletons, PlGF is currently being utilised and evaluated as an adjunct to aid the diagnosis of preterm pre-eclampsia (141, 179). Studies in twins have highlighted the potential for similar clinical utility of PlGF in the twin population.

Powers et al published in 2010 on a series of 234 women with a twin pregnancy that had quantification of circulating PlGF and sFlt-1 samples across their pregnancy using an ELISA immunoassay. The authors reported increased risk for the development of pre-eclampsia with each two fold increase in sFlt-1 (OR 2.18, 95% CI 1.46-3.32) and reduced risk for pre-eclampsia development with each two fold increase in PlGF (OR 0.50 95% CI 0.30-0.82) (90). A study by Droge et al reported on a cohort of 49 women with a twin pregnancy, 18 of whom developed pre-eclampsia. Maternal serum PlGF levels were decreased and sFlt-1 levels increased in the pre-eclampsia cases at time of their presentation compared to the controls with quantification of angiogenic factors performed using the Elecsys platform (166). Another study by Rana et al, again utilising the Elecsys platform, described serum PlGF and sFlt-1 in 79 women with a twin pregnancy presenting with suspected pre-eclampsia in the third trimester (180). Using a clinical outcome of an adverse clinical event in the subsequent fortnight, the angiogenic factors were compared between the two groups. An adverse maternal outcome was defined as the presence of hypertension plus at least one of the following; elevated aspartate aminotransferase or alanine aminotransferase (≥80 U/L), platelet count ≤100 × 109/L, disseminated intravascular coagulation (DIC), abruption (clinical and/or pathological), pulmonary oedema, cerebral haemorrhage, eclampsia, abnormal renal function (creatinine >132.6 µmol/L), or maternal death. An adverse fetal/neonatal outcome included iatrogenic delivery for hypertensive complications of pregnancy, small-for-gestational age birth weight (≤10th percentile for gestational age), abnormal umbilical

artery Doppler (absent or reverse flow), fetal death, and neonatal death (in either twin). Median PlGF was noted to be significantly reduced, while median sFlt-1 was elevated in those that developed an adverse event (n=52). Together, these studies highlight the potential that exists for use of PlGF +/- sFlt-1 as a diagnostic aid for pre-eclampsia in twin pregnancy and as prognostic indicator for its subsequent adverse outcomes.

1.4.5 Cut-offs for clinical utility of angiogenic factors in twin pregnancy

To allow these biomarkers to be utilised clinically in the twin population, it is imperative that relevant cut-offs are developed and validated specifically for this group. This was highlighted in a 2018 study by Saleh et al. The Dutch group compared PlGF and sFlt-1 levels in normotensive and pre-eclamptic singleton and twin pregnancies using the Elecsys platform (181). Previously, a sFlt-1/PlGF ratio cut-off of ≤38, using the Elecsys, has been identified as useful in predicting the one week absence of pre-eclampsia in women with clinical signs/symptoms with a high negative predictive value (88). Twenty-one twins along with 21 matched singletons were included and analysed. All pre-eclamptic singleton pregnancies had a ratio of >38 however only 5 of the 13 women with a pre-eclamptic twin pregnancy had a ratio of >38. Importantly this demonstrates that clinical cut-offs for PlGF +/- sFlt-1 identified for use in singletons are not transferrable to twin pregnancies.

1.4.6 PlGF in Growth Discordant Twin Pregnancy

Birth weight discordance in a twin pregnancy may represent either a physiological variation or a pathological event (67). Significant birth weight discordance (>greater than 25% difference) that arises after the mid trimester is generally the result of uterine inability to equally nurture both placentae (163). Published in 2008, a paper by Nevo *et al* evaluated the similarity in angiogenic imbalance between IUGR and pre-eclamptic pregnancies by examining placental angiogenic factors from IUGR, SGA, pre-eclamptic and control pregnancies in both singletons and twins. The main findings were a similarity in the angiogenic imbalance in both pre-eclamptic and severe IUGR pregnancies compared to SGA and controls, supporting the role of these angiogenic factors in conditions of pathological placental dysfunction and their potential for use as biomarkers of these conditions. Interestingly, a significant difference in angiogenic factors was also noted in twins discordant for growth, with the IUGR twin placenta having much increased expression of sFlt-1 compared to the normally sized co-twin (167).

Following on from this, Ruiz-Sacedon et al published a case controlled study in 2013 with 18 discordant (>20%) and 46 concordant dichorionic twin pregnancies. An ELISA was utilised for quantification of angiogenic factors (VEGF and sFlt-1). Not only did they demonstrate a lower maternal circulating level of angiogenic factors in the discordant twin pregnancies, but examination of fetal umbilical vein samples at delivery demonstrated reduced angiogenic levels in the smaller twin in discordant cases. This study confirmed that an

anti-angiogenic environment exists in mothers and fetuses with growth discordance, potentially enabling these angiogenic factors to be utilised in screening for and monitoring of discordant twin growth (164).

1.4.7 Limitations in current knowledge

Potential exists for PlGF use in twin pregnancy as a diagnostic aid for pre-eclampsia/ placental dysfunction, or as a prognostic aid for adverse outcomes. However; further research is first required to order to provide clarity and allow for its prudent use by clinicians. Reference ranges for PlGF in “normal” twin pregnancy need to be established, with consideration given to potential variations secondary to chorionicity. Following on from this, adequately powered, high quality prospective observational studies of women with a twin pregnancy and suspected pre-eclampsia/placental dysfunction are required in order to develop and validate clinically useful cut-offs for PlGF +/- sFlt-1 in twins. Ideally, studies should examine a number of PlGF platforms at once, in order to maximise efficiency and also to allow for comparison of performance (141). With rates of twin pregnancy rising over the last number of decades, expansion of our current knowledge on these angiogenic factors, especially PlGF, in twin pregnancy is paramount and requires our attention (48, 55, 182, 183).

As discussed in Section 2.5, hypertensive disorders arising in the setting of a multiple pregnancy confer a higher risk of adverse pregnancy outcomes (74,

75, 77). Use of PlGF in twin pregnancy may facilitate earlier identification of hypertensive disorders and prediction of subsequent adverse outcomes, thus enabling optimisation of antenatal care for these patients and careful planning regarding setting, timing and mode of delivery. In the next section of the Introduction, I will discuss the research to date on PlGF as an adjunctive aid for pre-eclampsia diagnosis in singleton pregnancies.

1.5 Placental Growth Factor as a Diagnostic Biomarker

1.5.1 Observational Studies

Over the last number of decades, the concept of using PlGF as a potential diagnostic marker for pre-eclampsia has been extensively examined (88, 89, 132). Studies have shown that as a pregnancy progresses, circulating levels of PlGF correlate directly with placental function (129, 131). Pregnant women with low circulating levels of PlGF are at increased risk for adverse outcomes such as pre-eclampsia, HELLP syndrome, eclampsia, fetal growth restriction, and stillbirth (184, 185). A number of prospective cohort studies have published on clinically relevant cut-offs for PlGF +/- slt-1 using automated testing platforms (89).

PELICAN (2013)

PELICAN was an international, multi-site, blinded observational trial that ran in seven consultant led maternity units throughout the UK and Ireland from January 2011 and February 2012. It was the first and largest prospective study of PlGF in women with suspected pre-eclampsia (89). Over 600 women were enrolled, all >20 weeks gestation and all with signs or symptoms concerning for evolving pre-eclampsia. All had quantification of circulating maternal plasma PlGF performed at the time point of enrolment. Their PlGF results were blinded to their caregivers and their pregnancies continued as per usual hospital care practice pathways. The PlGF immunoassay platform employed for this study was the Triage® PlGF test (Formerly Alere Inc. now Quidel Inc. San Diego). The primary aim of the study was to establish how effective PlGF quantification at presentation to the hospital was at determining the subsequent need for delivery for confirmed pre-eclampsia within 14 days.

Approximately half (n=346; 55%) of those recruited developed pre-eclampsia. Results published in 2013 positively demonstrated that PlGF testing could be beneficial in stratifying care for women presenting with suspected pre-eclampsia between 20 weeks and 34 weeks plus 6 days of gestation. For a test cut-off <100 pg/mL, PlGF alone showed 96% sensitivity (95% CI, 89–99), 56% specificity (95% CI, 49–63), 44% positive predictive value (PPV) (95% CI, 36–52), and 98% negative predictive value (NPV) (95% CI, 93–100) in determining those that would require delivery for a confirmed diagnosis of pre-eclampsia within the next 14 days. The cut-off value of 12 pg/ml yielded lower

sensitivity for identifying women likely to develop pre-eclampsia needing delivery within 14 days of testing. (Table 1.2).

The study reported that using a cut-off of 100 pg/ml had high sensitivity for predicting both preterm pre-eclampsia and also delivery within 14 days of testing *independent* of the pre-eclampsia diagnosis. A cut-off value of 12 pg/ml had poor sensitivity but good specificity for predicting preterm delivery *independent* of the pre-eclampsia diagnosis (Table 1.3). Secondary analysis of an older gestational cohort (n=137) showed the test had poor diagnostic accuracy in the 35-36+6 weeks gestational group. (Table 1.4).

Table 1. 2*: Triage® test accuracy for predicting pre-eclampsia needing delivery within 14 days for women presenting between 20 weeks-34+6 weeks gestation. From BJOG, Duckworth et al (89).*

Test cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
<100 pg/ml	0.96 (0.89 to 0.99)	0.56 (0.49 to 0.63)	0.44 (0.36 to 0.52)	0.98 (0.93 to 1.00)
≥100 pg/ml	0.96 (0.89 to 0.99)	0.56 (0.49 to 0.63)	0.43 (0.36 to 0.51)	0.98 (0.93 to 1.00)
< fifth centile	0.96 (0.89 to 0.99)	0.55 (0.48 to 0.61)	0.43 (0.36 to 0.51)	0.98 (0.93 to 1.00)
< 12 pg/ml	0.63 (0.51 to 0.74)	0.90 (0.85 to 0.94)	0.70 (0.57 to 0.80)	0.87 (0.82 to 0.91)

Table 1. 3: *Triage® test accuracy for predicting preterm delivery or delivery within 14 days, independent of diagnosis of pre-eclampsia for women presenting between 20 weeks-34+6 weeks gestation. From BJOG, Duckworth et al (89).*

Test cut-off	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Preterm pre-eclampsia				
<100 pg/ml	0.90 (0.83 to 0.95)	0.65 (0.58 to 0.73)	0.65 (0.57 to 0.72)	0.90 (0.83 to 0.95)
Delivery within 14 days of testing				
≥100 pg/ml	0.94 (0.87 to 0.98)	0.57 (0.50 to 0.64)	0.47 (0.39 to 0.55)	0.96 (0.91 to 0.99)
Preterm delivery				
< 12 pg/ml	0.44 (0.36 to 0.52)	0.97 (0.93 to 0.99)	0.94 (0.86 to 0.98)	0.62 (0.55 to 0.68)

Table 1. 4: *Triage® test accuracy for predicting pre-eclampsia needing delivery within 14 days for women presenting between 35 weeks-36+6 weeks gestation. From BJOG, Duckworth et al (89).*

Test cut-off	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
< fifth centile	0.70 (0.58 to 0.81)	0.64 (0.52 to 0.75)	0.65 (0.53 to 0.76)	0.69 (0.57 to 0.80)
< 12 pg/ml	0.22 (0.13 to 0.34)	0.91 (0.82 to 0.97)	0.71 (0.48 to 0.89)	0.55 (0.46 to 0.64)

The PELICAN study validated a fixed cut-off value for PlGF of <100 pg/ml for the Triage® platform as having clinical utility, and similar performance to a gestational cut-off of the 5th centile. It also reported a PlGF <100 pg/ml to be a better predictor than all other current commonly used predictive tests of pre-eclampsia, either singly or in combination (blood pressure, urinalysis or biochemical markers) with an area under the ROC curve for low PlGF of 0.87 compared to 0.76 for the next best predictor.

Of note in the PELICAN study there were seven intrauterine deaths, a higher rate than anticipated in the population of 625 women recruited. Post hoc analysis identified all seven women as having an abnormal PlGF result. At least five of these cases were at gestational ages and birthweights where they would have been reasonably expected to survive had the PlGF result been revealed to clinicians and the women delivered.

Alvarez et al (2014)

This Spanish group conducted a retrospective cohort study on 257 pregnant women, triaged with suspected pre-eclampsia (185). Each had their PlGF: sFlt-1 ratio quantified at time of presentation using the Elecsys® test. Researchers collected outcomes data such as development of pre-eclampsia and reviewed and time between clinical presentation, diagnosis and delivery was calculated. The study showed that the best ratio cut-off to diagnose pre-eclampsia changed according to gestational age: 23 (92.0% sensitivity, 81.1% specificity) and 45 (83.7% sensitivity, 72.6% specificity) for women < 34 and ≥ 34 weeks' gestation, respectively. This highlighted that using gestational

adjusted cut-off values the sFlt-1/PlGF ratio could be used to rule out pre-eclampsia at obstetric triage and to predict imminent delivery with better accuracy than previous accepted cut-offs (Figure 5).

Table 1. 5: *Elecsys® test accuracy for ratio of 85 ratio to diagnose PE using different cut-off values in predicting short-term absence of pre-eclampsia within one week. Taken from "New biomarkers in diagnosis of early onset pre-eclampsia and imminent delivery prognosis" (185)*

Cut-off value	**Pregnancies presenting at <34 weeks gestation**		**Pregnancies presenting at ≥34 weeks gestation**	
	23	85	45	85
	Value (95% CI)	Value (95% CI)	Value (95% CI)	Value (95% CI)
Sensitivity %	92.0 (72.5-98.6)	56.0 (35.3-75.0)	83.7 (69.8-92.2)	51.0 (36.5-65.4)
Specificity %	81.1 (64.3-91.4)	97.3 (84.2-99.9)	72.6 (64.5-79.5)	88.4 (81.7-92.9)

PPV %	76.7 (57.3-89.4)	93.3 (66.0-99.7)	50.6 (39.4-61.8)	59.5 (43.3-74.0)
NPV %	93.8 (77.8-98.9)	76.6 (61.6-87.2)	93.0 (86.2-96.7)	84.3 (77.4-89.5)
Positive likelihood ratio	4.86 (2.47-9.57)	20.7 (2.91-147)	3.05 (2.28-4.09)	4.38 (2.59-7.40)
Negative likelihood ratio	0.10 (0.03-0.38)	0.45 (0.29-0.70)	0.22 (0.12-0.43)	0.55 (0.42-0.74)

PROGNOSIS (2016)

The PROGNOSIS study was a prospective, multicentre, blinded, observational study conducted across 14 countries from 2011 to 2014 (88). Its aim was to derive and validate a ratio of serum sFlt-1 to PlGF that would be predictive of the absence or presence of pre-eclampsia in the short term. It included women with singleton pregnancies from 24 weeks to 36+6 weeks' gestation in whom a clinical suspicion of pre-eclampsia existed. The Elecsys® immunoassay was the platform used to quantify serum levels of PlGF and sFlt-1.

Results published in 2016 reported on a development cohort of over 500 participants and a validation cohort of a further 500 women. It reported a sFlt-1: PlGF ratio ≤ 38 to be clinically important, with a negative predictive value in the subsequent week of 99.3% (95% CI 97.9–99.9) for ruling out pre-eclampsia, with relatively high sensitivity and specificity. The positive predictive value; a diagnosis of pre-eclampsia, eclampsia, or the HELLP syndrome within 4 weeks, was 36.7% (95% CI, 28.4 to 45.7) using the same sFlt-1: PlGF ratio of 38. Post hoc analysis however showed this was still an improvement in prediction compared to the use of clinical variables such as blood pressure and urinalysis alone (Table 1.6).

Table 1. 6*: Elecsys® test accuracy for ratio of <38 in predicting short-term absence of pre-eclampsia within one week. Taken from the PROGNOSIS study results (88)*

	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Rule-out of pre-eclampsia within 1 week	0.86 (0.73-0.94)	0.79 (0.77-0.82)	0.17 (0.12 -0.22)	0.99 (0.98 -1.00)
Rule–in of pre-eclampsia within 4 weeks	0.70 (0.62-0.78)	0.83 (0.81-0.86)	0.39 (0.33-0.45)	0.95 (0.93-0.96)

Interestingly, the same cut off of <38 was predictive of the absence of fetal adverse outcomes within 1 week with a negative predictive value of 99.3% (95% CI, 97.9 to 99.9). This study showed that an sFlt-1: PlGF ratio of 38 or lower could be used to predict the short-term absence of pre-eclampsia and adverse fetal events in women in whom the syndrome is suspected clinically.

PETRA (2016)

The PETRA (Pre-eclampsia Triage by Rapid Assay) Trial was a prospective multicentre observational study performed at 24 North American sites (186). The study aimed to examine the relationship between PlGF and time-to delivery (TTD) among women presenting with signs or symptoms of pre-eclampsia. From November 2010 to January 2012, 753 women with a singleton gestation between 20-34+6 weeks were enrolled. Maternal blood was drawn at enrolment and circulating plasma PlGF measured by a central lab using the Triage® assay. Diagnosis of pre-eclampsia was made according to ACOG guidelines and adjudicators were blinded to the PlGF result.

The study showed that in women with suspected pre-eclampsia < 35+0 weeks, a low PlGF (<100 pg/ml) strongly correlated with the need for preterm delivery independent of a diagnosis of pre-eclampsia or the gestational age at presentation (Figure 1.5). It also showed that a normal PlGF (≥100 pg/ml) predicted pregnancy prolongation, even in patients destined to acquire a diagnosis of pre-eclampsia. Overall, the study suggested that PlGF better

reflected underlying placental pathology than traditional clinical markers of pre-eclampsia.

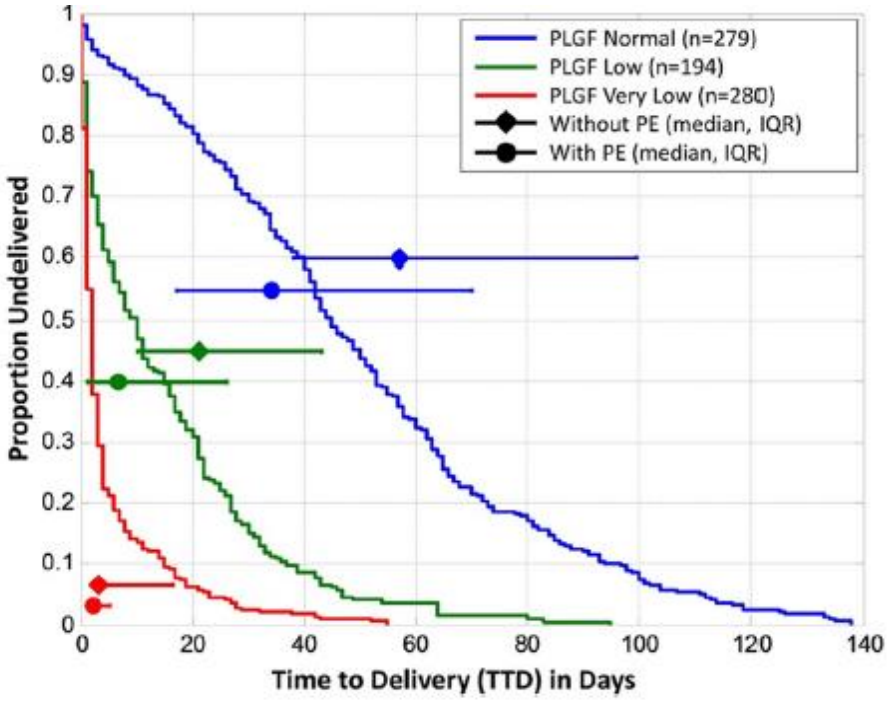

Figure 1. 4: *Triage® test accuracy for time to delivery (TTD) in days in those women with suspected pre-eclampsia at <35 weeks gestational age. From AJOG, Barton et al (186).*

1.5.2 Interventional Studies

As discussed, several prospective cohort studies have demonstrated high sensitivity and a negative predictive value of automated PlGF-based testing in determining the need for delivery in women with suspected pre-eclampsia. In order to get an insight into the impact of integration of PlGF into clinical care

pathways, interventional studies are required. This point was highlighted by NICE in their 2016 guidance on PlGF testing (141).

MAPPLE (2018)

The MAPPLE (The Management of pregnancy complications with PIGF testing) study aimed to examine the clinical implications of introducing PlGF into routine clinical care (155). It prospectively enrolled 397 women with suspected pre-eclampsia or fetal growth restriction at <35 weeks gestation across four maternity units in Europe and Australia between April 2014 and March 2016. PlGF testing was performed as part of usual clinical care using the Triage® PlGF test. Clinicians were made aware of the PlGF result and were expected to adjust care accordingly. Clinical outcomes were compared to the previously discussed PELICAN study where PlGF results were blinded to care givers. Although these cohorts were from different centres and different time periods, it did allow comparison of revealed PlGF testing to blinded PlGF testing for the first time.

The study published in 2018 and reported that when PlGF is revealed perinatal outcomes were significantly altered. Overall, with revealed PlGF testing, there was earlier delivery of patients, (on average by 1.4 weeks), no difference in maternal morbidity and no difference in rates of caesarean delivery. There were significantly fewer perinatal deaths and babies born less than the <10th

centile in the revealed PlGF arm but significantly increased perinatal adverse outcomes (mainly respiratory) due to prematurity.

The MAPPLE study highlighted a concern in relation to PlGF testing; that an abnormal PlGF result may prompt earlier intervention by clinicians, resulting in maternal benefit at the detriment of neonatal outcomes. Hence, the importance of adequately powered, ideally randomised controlled trials, to determine clinical utility and overall cost effectiveness. The net result of this was the randomised controlled UK PARROT trial and the PARROT Ireland trial (179, 187).

PARROT Trials UK and Ireland

In section 6, I will discuss the PARROT Ireland trial. The UK PARROT trial recruited from June 2016 to October 2017 across eleven maternity units throughout the UK. Over a thousand women with singleton pregnancies, between 20 weeks to 36+6 weeks gestation inclusive enrolled, with signs or symptoms suggestive of evolving pre-eclampsia. All had immediate PlGF quantification performed with the result revealed only in those randomised to intervention. The primary outcomes measure was the duration of time required to diagnose confirmed pre-eclampsia using the ISSHP 2014 definition. The trial reported a reduction in time to diagnose preterm pre-eclampsia (from 4.1 to 1.9 days) as well as a reduction in maternal adverse outcomes in those with revealed PlGF testing (188). The similarities and differences of the PARROT

UK trial to the PARROT Ireland trial, along with the differences in results is discussed further in Chapter 5, Paper 6; “PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: Results of Interim Analysis” as well as in Chapter 7, the discussion of this thesis.

1.6 PARROT Ireland

1.6.1 Rationale for the PARROT Ireland trial

Clearly placental growth factor has merit in terms of prediction of preterm pre-eclampsia and overall placental dysfunction however, in order to adequately access its clinical impact before routine adaption into clinical practice, a randomised controlled trial (RCT) should be performed (189). The processes used during the conduct of an RCT minimise the risk of confounding factors influencing the results and therefore a RCT is considered to provide the most reliable evidence on the effectiveness of interventions (190, 191).

The objective of the PARROT Ireland randomised trial is to evaluate the impact of knowledge of PlGF measurement on relevant maternal and neonatal outcomes. By improving risk stratification antenatally, the addition of PlGF measurement to the clinical assessment of women with suspected pre-eclampsia prior to 37 weeks' gestation may reduce associated maternal morbidity through earlier diagnosis and targeted management of women with the disease. However, any intervention in late pregnancy may have an impact

on the fetus hence it is equally important that neonatal outcomes be adequately assessed. Earlier diagnosis of pre-eclampsia may influence obstetricians to expedite delivery and may lead to an increase in neonatal morbidity and mortality secondary to iatrogenic prematurity. However, improved identification of those neonates at highest risk of imminent placental dysfunction may reduce neonatal morbidity by allowing for timely intervention.

1.6.2 My role in PARROT Ireland

When I commenced working as a clinical research fellow in July 2015, funding for PARROT Ireland had been secured from the Health Research Board Clinical Trials Network (HRB-CTN). As one of the few people to have worked on this trial since this time, I have been involved in every aspect of its development, organisation and conduct. I drafted the initial trial protocol and disseminated it for review and feedback among the principal investigators at each participating site. The trial protocol was essential to ensure the feasibility of conducting the trial across seven maternity units, and including details on every aspect of the trial including its design, statistical power, PlGF assessment, clinical management algorithms and outcome measures. Once the finalised initial draft was agreed in 2016, I organised an application for ethical approval to each of the seven ethics committees involved. In mid-2016 a project manager (PM) was hired and together, under the guidance of the lead principal investigator (PI) we worked on a variety of essential components of the trial to enable it to commence. These included; Securing support of the

trial sponsor, development of the electronic trial database, production of necessary trial documents, local site files and master files, appropriate training of all research staff and site initiation visits as each site, development of plan for purchase, use and delivery of consumables and contract negotiations with all parties.

Once the trial was up and running, I continued to work alongside the PM and the trial monitor to ensure there were no operational issues. This included; troubleshooting any day to day issues with database, test equipment or plgf consumables, organisation of meetings for the DMC and TSC, development of reports such as the DSR and trial newsletters, hosting regular teleconferences with researchers as well as training newly appointed researchers. I also spent a week at each site when they transitioned from control to intervention so as to support the research staff locally with the change in procedure as well as providing education and information to clinical staff locally regarding the trial and PlGF itself.

1.6.3 Trial Planning

Trial Sites

The trial was planned to be carried out across the seven largest maternity units in the Ireland of Ireland; The Coombe Women and Infants University Hospital Dublin, Cork University Maternity Hospital, University Maternity Hospital Limerick, The Royal Jubilee Maternity Hospital Belfast, University College

Hospital Galway, The National Maternity Hospital Dublin and The Rotunda Maternity Hospital. Combined, these seven units have an annual birth rate of over 44,000. Some are standalone units while others are co-located alongside general hospitals. All receive referrals from peripheral units of complex pregnant patients requiring specialised expertise and all have a long tradition of clinical research. Further to this, a research collaborative already exists between these seven units; the Health Research Board Mother and Baby Clinical Trial Network Collaborative.

Trial Design

Given that those randomised to the intervention in PARROT Ireland trial would receive a diagnostic test that may have led to their physician altering their on-going management, it was agreed that a cluster design would be best utilised (Figure 1). In a cluster randomised trial, the unit of randomisation is a group/cluster (in our case a maternity hospital) rather than an individual pregnant woman. This type of trial design allows for the intervention to be implemented at a hospital level rather than at an individual patient level. This pragmatic approach is preferential when evaluating an interventional test as it allows a true evaluation of what would happen in reality, should the intervention be introduced into clinical practice. The effectiveness of the intervention is assessed in terms of the outcome for the patient.

Cluster trials are well documented to be beneficial when the intervention is aimed at health care professionals (192, 193). In addition to the cluster randomisation in PARROT Ireland, a stepped wedge approach was also utilised (Figure 1.5). Stepped wedge allows for the phased implementation of an intervention. This approach has many benefits. Firstly; it enables the intervention to be rolled out in a systematic manner. From a practical point of view this is hugely beneficial given the requirement for consumables, equipment and staff education necessary to implement the interventional test at each site. Having all sites, with their wide geographic distribution, commence the interventional test on the same day would be extremely challenging and likely unachievable. Secondly, a stepped wedge design results in all clusters/maternity units receiving the interventional test at some time point during the trial, increasing its acceptability. It was paramount to the success of the trial that all units remained committed to the trial and did not allow the interventional test to be utilised in their unit outside of the research setting (194).

There are some negatives with stepped wedge cluster design; the foremost being fixed time constraints. Given the amount of clusters is fixed and the time duration for each step must be equal, the total trial duration is unable to be altered once the first cluster has transitioned from control to intervention. The result of this is that for trials that under-recruit, extension is not an option. It also results in longer duration trials, which are most susceptible to contamination in terms of external events (195, 196).

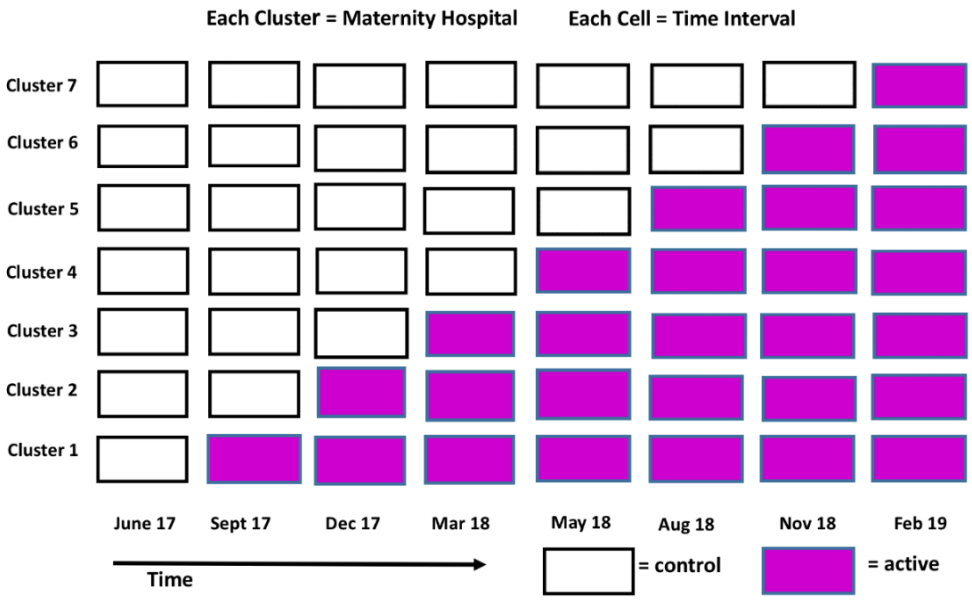

Figure 1. 5: *Stepped Wedge Cluster Randomised Design for PARROT Ireland. From BMJ Open, D Hayes-Ryan et al (179).*

PlGF Platform

Much consideration was given as to which commercial PlGF platform to utilise for the trial. Many companies were interested in being involved in the study however given the limited performance data on many of the commercial platforms we focused our decision only on platforms endorsed by NICE (141). Ultimately, the trial management group (TMG) of which I was part of, choose to utilise the Triage® PlGF test for the trial. The point of care design of the test platform with its quick turnaround time is important for clinical practice, where plans regarding ongoing investigations and location of care need to be decided acutely. Secondly, given the lack of significant infrastructure required for the

Triage® PlGF test, it potentially could be integrated into use in developing countries should the trial prove beneficial.

Trial Documentation

I developed a number of necessary documents prior to the commencement of the trial which were reviewed and approved by the TSC. The trial protocol was the first of these; a detailed plan as to how the project would be run with involvement, input and approval of all the principal investigators and collaborators. Included in the protocol was a power calculation (conducted by the trial statistician), suggested management algorithm based on PlGF results (developed based on the NICE and HSE hypertension guidelines) and a detailed definition of planned outcomes measures. Prior to the launch of the trial, and following feedback from the sponsor and ethical committees, the protocol underwent multiple revisions and was updated from Version 1.0 dated 9th July 2015 to the final Version 9.0 dated 16th November 2017.

Along with the trial protocol, I developed a patient information leaflet (PIL) and consent form. These documents required the use of plain english with enough information to adequately inform the eligible woman regarding the trial but without being over-whelming (197). Again, the PIL/Consent form underwent multiple revisions, following review at trial site ethical committees, until finally Version 4.0 dated 6th November 2017 was agreed upon.

In addition to the above, I developed a substantial number of other trial documents, outlined in Table 1.7 and included in Appendix 1, with the assistance of the project manager. I also wrote a paper detailing the methodology of the trial which has since been published in an international peer reviewed journal (179).

Table 1. 7: *Trial Documentation developed for PARROT Ireland (Appendix 1)*

• Trial Steering Committee Charter	• QC Device Results Log
• Data Monitoring Committee Charter	• QC Samples Results Log
• Trial Monitoring Plan	• PlGF Devices Testing Log
• Definitions List V 2.2	• SOP 002: Personnel Training V 1.0
• Enrolment Log V 2.0	• LAB 001 Sample Handling V 1.0
• Pre-Screening Log V 2.0	• SOP CLIN 003: eCRF Parrot V 2.0
• Documentation of enrollment for Participants Chart V 1.0	• Recruitment Guide v 1.0
• SOP 001: Informed Consent Process V 1.0	

Ethical Approval

As PARROT Ireland was a non-regulated trial, individual approval was required from each ethical committee of all seven hospitals involved. The project manager and I together submitted applications to each ethics committee, some required a written application with multiple copies and others were online electronic applications. In general the committees met on a monthly basis. Three committees (Limerick, Coombe and Rotunda) invited a representative from the trial to present a brief overview in person at their meeting, which I did in 2016. Initially we planned to use a no-consent model for the trial, however many of the ethics committees were uncomfortable with this approach (198). Concerns were also raised over the management algorithm potentially increasing work burden for units and intervention for patients hence “suggested” was added to it to order to facilitate physician flexibility. Replies from each committee were received 1-3 months following meetings and unfortunately, if even one committee did not approve any aspect of the protocol or PIL, all seven had to be approached again with amended documentation. I worked to alter the protocol and PIL in response to the feedback given by the ethics committees, all the while keeping the TSC informed of these changes and ensuring they agreed. Full ethical approval took many months but finally a consensus from all seven committees was achieved in April 2017.

Trial Organisation

A number of groups and committees were established for trial operational purposes, all of which I was heavily involved with either through organisation, activities or duties.

Trial Management Group

The composition of the TMG is shown in Table 1.8. Along with myself it included the lead site principal investigator (initially Professor Kenny and then Dr O Donoghue), the project manager; of which there were four during the course of the trial and the trial monitor; appointed in 2017. The TMG was responsible for the overall day to day management of the trial and we acted on behalf of the sponsor to ensure that all the sponsors' responsibilities were carried out. It was our responsibility as the TMG to confirm all approvals were in place before the start of the trial, maintain the Trial Master File, provide adequate training, study materials and 24-hour advice to the research assistants. We were also responsible for data management, updating collaborators regularly regarding the progress of the study and responding to any questions (e.g. from collaborators) about the trial. The TMG also were required to ensure data security as well as data quality were maintained while observing data protection laws and ensuring the trial was conducted in accordance with the ICH GCP E6 (R2).

Throughout the trial, the TMG planned to meet weekly, to discuss any particular site issues and organise delivery schedules of consumables. We conducted monthly phone meetings with researchers at site to troubleshoot any issues locally, promote the trial and motivate staff. The TMG was responsible for circulating relevant communications and updating the investigator site file (ISF), providing quarterly reports to the DMC, ensuring adequate recruitment occurs throughout the trial, addressing any safety concerns and organising monthly trial steering committee (TSC) meetings. We also prepared for regulatory audits from the trial sponsor UCC (held on 21st/22nd March 2018) and from the HRB quality & regulatory manager (held on 21st Feb 2018) as well as developing annual progress reports for each of the ethics committees, as well as the sponsor and funder.

Table 1. 8: *Composition of the Trial Management Group for PARROT Ireland*

Lead Site-Chief Investigator	Dr Keelin O'Donoghue
Clinical Research Fellow	Dr Deirdre Hayes-Ryan
Site Monitor	Ms Nicolai Murphy
Project Manager	Dr Sharon Kappala

Trial Steering Committee

The Trial Steering Committee (TSC) was established in March 2016. The function of the TSC was to provide overall supervision on behalf of the Trial Sponsor and Trial Funder and to ensure that the trial was conducted in accordance with Good Clinical Practice (GCP) and all relevant local and national regulations and local policies. The composition of the TSC for PARROT Ireland is shown in Table 1.9. Due to the widespread geographical distribution, the TSC met via teleconference. Meetings were frequent leading up to the greenlight for trial commencement and then were held every quarter.

Table 1. 9: *Composition of the Trial Steering Committee for PARROT Ireland*

Chair/Chief Investigator	Dr Keelin O'Donoghue
Principal Investigators	Professor Fionnuala Breathnach Professor Amanda Cotter Professor Declan Devane Professor Alyson Hunter Professor Deirdre Murphy Professor John Morrison Professor Fionnuala McAuliffe
Clinical Research Fellow	Dr Deirdre Hayes Ryan (DHR)
Project Manager	Dr Sharon Kappala (SK)
Site Monitor	Nicolai Murphy (NM)

HRB Network Manager	Dr Elizabeth Tully
Research Manager at RCSI/Rotunda	Meadhbh Aine O' Flaherty
Lay person representative	Elaine Ní Bhraonáin
Neonatologist	Professor Gene Dempsey
Health Economist	Dr Brendan McElroy Dr Aileen Murphy
Sponsor Representative	Dr Muiris Dowling Dr Ruben Keane
Statistician	Dr Karla Hemming Dr Ali Khashan
Quality & Regulatory Affairs Manager	Jackie O'Leary (JOL)

Data Monitoring Committee

The TMG, with the approval of the TSC, organised for the establishment of an independent data monitoring committee (DMC) for PARROT Ireland, to safeguard the interests of the study participants, the investigators and the Sponsor. The composition of the DMC is shown in Table 1.10. I communicated with the DMC and organised their first meeting in 2018, at

which time they ratified their charter. They requested a data summary report (DSR) be sent every quarter to them from the TMG, detailing numerical information on recruitment, maternal and neonatal morbidity, participant withdrawals, deviations and violation so as they can monitor trial progression. They also requested an interim analysis be conducted when half the recruitment target was reached. In line with these requests, I conducted the quarterly DSR and amended the statistical analysis plan (SAP), with the assistance of the trial statisticians, to include an interim analysis.

Throughout the trial, the DMC monitored the overall conduct of the study in relation to the rights, safety and well-being of the participants and were also in place to protect the validity and credibility of the data. Once the interim analysis was conducted and the results available, I organised for the second meeting of the DMC in April 2019 so as these results could be examined and discussed.

Table 1. 10: *Composition of the Data Monitoring Committee for PARROT Ireland.*

Neonatologist/Chair-Person	Prof. Tony Ryan
Obstetrician	Prof. Richard Greene
Midwife	Ms Grainne Meehan
Statistician	Dr Vicky Livingstone

Equipment and Consumables

In order to perform a PlGF test using the Triage® PlGF test, each site required a mini bench top centrifuge and the Triage® PlGF test metre. Following an agreement with Alere®, this equipment was provided to each site on loan for the duration of the trial. Given the outdated infrastructure and space limitations in most of our participating maternity units, identifying and securing a suitable area for this equipment was a challenge. When I visited each unit prior to trial commencement, I discussed potential locations for placing this equipment with the site PI. The location needed to be close to the clinical area where participants would be recruited, but also secure from the public. Both machines required plug point access and the centrifuge required a stable solid surface and could not be moved once it was serviced and balanced. In some units, it was necessary to arrange installation of new shelves or to rearrange the existing layout of clinical areas to facilitate the installation of the equipment, which I co-ordinated with the assistance of the local researcher. Prior to each site transiting to the intervention, I ensured the agreed PlGF test area was ready and the necessary equipment in place and ready to use.

The consumables for the test itself needed to be stored at 4-8C therefore each unit required access to a fridge. I co-ordinated with local researchers to identify suitable fridges in their units. Daily temperature monitoring of these fridges was a necessary quality requirement so I organised for those fridges without temperature gauges to be equipped with separate validated thermometers and for all researchers to keep a daily log of these readings. The PlGF tests

required a monthly additional test known as a control for QC purposes. These controls required storage at -20C, which necessitated use of a monitored medical freezer. Through discussions with local hospital laboratory managers and nearby research centre managers, I succeeded in securing freezer space for the storage of these controls for the duration of the trial.

Research Staffing

As PARROT Ireland is a HRBCTN trial, each site was responsible for their own staff funds and hiring decisions. Some sites already had research staff in place who were experienced both clinically and in terms of previous research exposure. In other sites, staff were newly appointed. Some of these were well seasoned midwives/sonographers while others were recent college graduates from science/epidemiology/psychology backgrounds as there was no requirement to be a registered midwife for the purpose of the trial. Once in post, all research assistants required appropriate training from the TMG before they could be added to the site delegate log. This included GCP/GDPR certification as well as phlebotomy certification and vaccination records. Trial specific training (database, protocol, PIL/Consent) was performed locally at site by myself during the pre-start up visit. For PlGF specific training, all PARROT Ireland researchers attended Cork University Maternity Hospital (CUMH) for a training day on 13th July 2017 which I organised. The training included detailed information of placental growth factor (PlGF) itself as well as

operational information on the point of care Triage® MeterPro device, provided by myself and a representative from the Triage® company.

Green Light

Once ethical approval was in place, staff hired and competently trained, necessary equipment at site and all site start-up visits performed the last outstanding issue prior to green light was the contracts. These contracts were triparty contracts between the sponsor (University College Cork), each site (maternity hospital) and each hospitals university (e.g.; Trinity College Dublin). Although legal input and review from each Hospital/University was required prior to their signing, the process was not too onerous and was achieved in time for a trial start date for all seven sites on 29th June 2017. The official launch of the PARROT Ireland trial was held in CUMH on 9th October 2017 (199) (Figure 1.6).

Figure 1. 6: *Research Assistants of the PARROT Ireland team meeting for PlGF training in CUMH on 9th October 2017. From UCC News (199).*

Ongoing Education

Although approved by each hospital and PI locally, all staff at each site needed information and training regarding the trial. This was provided during my site initiation visits between January and April 2017. Speaking at events such as grand rounds and journal club, I was able to connect with the vast majority of medical personnel locally. However ongoing education was required, especially when a site transitioned over from control to intervention. Hence during each transition week I relocated to the transitioning site so as to support the research staff locally with the change in procedure and to provide on-going information to all staff locally regarding the trial and PlGF itself.

1.7 Research in a Pregnant Population

1.7.1 Background

Pregnant women are classed as a vulnerable group and are significantly under-represented in terms of clinical research studies (200). In order to evaluate safety and efficacy and provide evidence for outcomes, clinical research studies are necessary in the pregnant population.

Pregnancy research has its own unique set of challenges making it more complex and challenging to conduct. Pregnant women are unique in terms of their circulating hormonal profile and drug metabolism not to mention the additional complexity of an unborn fetus to consider in any decisions (201). Pregnant women are at risk for medical complications such as infections and frequently have complex medical co-morbidities often necessitating the use of multiple drugs during pregnancy which may potentially negatively interact or have unknown fetal implications (200).

Historically women of child bearing age have been excluded from clinical trials, particularly Phase 1 trials, owing to safety concerns (202). The legacy of drugs such as thalidomide and diethylstilbestrol in pregnant populations evokes fear in both funders and pregnant women of potential harm to fetuses due to unknown teratogenic effects and adverse pregnancy outcomes (203, 204). Owing to strict ethical policies and a belief from stakeholders that women will not be willing to participate, few clinical trials are designed with the pregnant population in mind. Thus far too often in obstetrics, tests become adopted into

routine clinical practice without robust prior evidence of benefit; examples being the use of antenatal cardiotocography for prediction of intrapartum hypoxia or fetal fibronectin testing for prediction of preterm labour (205, 206).

Our perception of what is ethically permissible or necessary often changes over time. Excluding any specific group from participating in medical research results in a lack of knowledge about the risks and potential benefits of available treatments to them (207). The dearth of information that exists on the safety and biophysical profile for most drugs in pregnancy results in many pregnant women potentially not receiving optimal treatment, with most drugs being used off-label as few have been approved for this population (208).

In recent years, global policies have shifted towards inclusion of all groups in biomedical clinical research. In 1993 a study evaluating gender differences in the clinical evaluation of drugs overturned the previous 1977 FDA policy of exclusion of all women of child bearing age from clinical trials (202). Signed into law in the US in December 2016; the Task Force on Research Specific to Pregnant Women and Lactating Women (PRGLAC) was established to identify gaps in current knowledge and to advise the US Department of Health and Human Services. In addition, in April 2017 the FDA issued ethical guidance on including pregnant women in clinical trials, advocating an informed and balanced approach to gathering data on the use of drugs and biological products during pregnancy through judicious inclusion of pregnant women in clinical trials with careful attention to potential fetal risk. (208).

Over the last decade, pregnancy research has been steadily increasing globally. In Ireland alone there are now two dedicated perinatal research centres; the INFANT Research Centre, affiliated with University College Cork and the Perinatal Research Centre, affiliated with University College Dublin. With the advent of these dedicated centres and the promotion of all aspects of perinatal research, comes recognition of its requirement and importance.

1.7.2 Current knowledge and limitations

In order for a clinical trial to successfully recruit participants, its purpose and design are key elements to its success. Not only must the potential benefits of participation be weighed against any potential risks for the participant, but the physical time and emotional burden of participation must be considered (209). When developing clinical trials, researchers spend much time determining if the proposed design is not only fit for purpose, but will also encourage participation amongst those eligible to take part. Equally, when determining ethical fitness, ethical committees spend many hours debating if the proposed design and conduct of the trial is fundamentally sound. In general, trial designs that involve a huge time commitment either in the form of repeated attendances or completion of tediously long questionnaires or documents are less likely to successful recruit. Similarly, trials that involve risks to the participants; for example administration of relatively unknown medications or interventional procedures are also less likely to succeed. The specific

participant eligibility criteria for a trial is also highly important to consider. For example, participation in a drug trial of an unlicensed medication would likely not be acceptable to most people, however, for those with an advanced cancer and no other treatment options available, participation in such a trial may be the only potential for treatment and therefore warrant careful consideration (210).

In the late 1990's a group from the UK (211) interviewed 18 women who had declined participation in a trial of a prophylactic tocolytics while pregnant. In this double blind randomised controlled trial (RCT), recruitment had been an issue with only 26% of eligible women agreeing to participate. The researchers reported that risk limitation and apprehension about taking a medication while pregnant was a common barrier prohibiting participation. A lack of interest or support from clinical staff in the trial was also reported as a barrier to participation in this study.

A hypothesis exists that pregnant women are reluctant to participate in research due to a variety of reasons. Owing to the lack of inclusion of pregnant women in randomised controlled trials to date, there is little in the literature regarding feedback on the particular motivators and barriers specific to their participation in clinical research (212-215). From the studies that have been performed, some common overlap exists;

The MAGPIE study was a double blind (RCT) of magnesium sulphate for prevention of eclampsia in women with pre-eclampsia, conducted in 33

countries from 1998 to 2001 (216). As part of the follow-on QUOTE study (215), forty women from the UK arm of the trial were invited to share their participation experience, on average 3 years following completion of the trial. QUOTE specifically focused on the women's decision to participate in MAGPIE. The authors identified limited background knowledge of pre-eclampsia existed amongst the women enrolled. They also reported that the decision to take part in the trial was made independently by the pregnant woman herself. Frequently in QUOTE, participants reported confusion and ambiguity when the MAGPIE trial was initially explained to them. They reported the language used and the approach itself to be complicated and the trial information provided to be of very poor quality. The participants of QUOTE also reported that at the time of their enrolment in MAGPIE they did not fully understand that randomisation meant they might not receive the intervention. This may be attributable in part to the approach adopted by the researchers who were recruiting however it must be considered that the timing and setting when these pregnant women were approached may have also contributed given it was recruiting pregnant women with severe pre-eclampsia in a HDU setting.

A 2005 qualitative study by a UK group (213) evaluated 20 women's experiences following their participation in an RCT of antibiotics for preterm labour (217). The interviews were conducted in person, following publication of the trial results, a number of years after participation. The women reported their main motivation to take part in the RCT was the possibility of an improved

outcome for their baby and the opportunity to help others, but only if there was absolutely no risk involved with participation. The women reported the information provided by the research team to be of good quality, however they reported that the stressful nature of the situation when they were approached (in unanticipated preterm labour) affected their ability to absorb the information fully, thus again highlighting the importance of timing and setting when approaching pregnant women about a trial.

A large study by a group of Brazilian researchers in 2017, assessed 208 women 10 weeks following delivery (212). These women had all participated in an ongoing RCT recruiting women with a short cervix in the 2nd trimester and randomising them to either progesterone alone or progesterone with a cervical pessary. A qualitative follow-up questionnaire was conducted via telephone interview. Results showed the main motivators for participation among the women to be firstly a familiarity with the condition being investigated (premature delivery), secondly the potential to possibly improve the outcome of their pregnancy and lastly to access free medicine and healthcare providers. This study highlights that in countries where health care costs during pregnancy are not provided by the state, participation in clinical research may enable those with limited social means greater access to medical care and support but may also influence their decision to participate.

Another 2017 study from a group of researchers from New Zealand (214) interviewed 20 women with a history of allergic disease who had participated in a double blind RCT of a daily pro-biotic during their pregnancy (218). This

study involved the participation of healthy volunteers as opposed to pregnant women with signs/symptoms of a pregnancy complication. Altruism and a sense of civic duty were identified as important motivators for participation and a deep investment in the value of research was present amongst the women. However, time commitment to the trial was perceived as a burden and randomisation to the placebo regarded as a disadvantage. The group also reported equal involvement of both the women and her partner in the decision to take apart in the RCT. This could be attributed to the requirement to take a medication as part of the study protocol (pro-biotic or placebo). Interestingly, despite this RCT involving taking a tablet, interviewed women perceived it as being risk free, as it was a pro-biotic. Researchers reported participants to be highly risk adverse with pregnant women reporting they would not take part in research if they perceived any potential risk to their unborn baby.

Collectively these studies identify that the context, purpose and potential risk of any research are the most important considerations to an eligible pregnant woman when she is considering participation in a trial. The approach and explanation adopted by both researchers and clinicians is also paramount in aiding women's understanding of a research trial. The personality, timing and language used are key factors influencing women's decision to participate and thus key to ensuring recruitment targets for trials are achieved.

1.7.3 Qualitative studies exploring women's experience of research in pregnancy

The participation of pregnant women in clinical research is paramount in order to advance global knowledge on preventative and treatment options for this specific population. Pregnant women's perspective on the benefits, risks, burdens and experience of taking part in research during pregnancy is crucial in order to facilitate recruitment and retention of future participants. Qualitative research focuses on understanding why things are the way they are in our social world and why people behave in the way that they do (219). For this reason, it is the best method of evaluating pregnant women's experience of being involved in clinical research.

Data collection in qualitative studies usually involves direct interaction with individuals on a one to one basis or in a group setting so is well suited to ascertainment of participants' attitudes or beliefs. One on one interviewing captures not just the words someone says but the intonation of their voice, their mannerisms, their particular style and behaviour and can hence elicit much more information than a word-based assessment can ever adequately convey (220).

Frequently in qualitative research studies, a semi-structured interview style is adopted. This allows the topic under investigation to be approached with open-ended questioning, allowing room for deviation in the discussion by either the interviewer or interviewee should it become apparent that this is necessary

and important. It also allows for on-going analysis by the researcher at each interview, with the integration of any newly identified relevant topics at each subsequent interview until eventually saturation is reached (220).

The use of technology such as audio or video recorders, if consented to by the participants, facilitates reliable recall of information for the researcher for analysis purposes. It also elicits recall of phrases, tone and inflection utilised allowing feelings and meaning to be communicated. Moving on from the interview itself, accurate transcription of the data obtained is important. The same phrase/sentence may have different meanings when stress is placed on different words within. Again recoding of interviews facilitates accurate transcription of data and also aids content analysis and communication of the underlying message being conveyed through behavioural data not just verbal data (221).

Thematic analysis is increasingly now used for analysing qualitative data. Through a careful and systematic approach of a dataset, common themes are identified, giving meaning to a shared set of experiences.

1.8 Summary

Pregnancies complicated by a hypertensive disorder are at increased risk of both maternal and neonatal adverse outcomes. Women with a twin pregnancy have an inherently higher risk of developing a hypertensive disorder in their pregnancy as well as a higher risk of severe maternal morbidity and iatrogenic

prematurity. Rates of twin pregnancy as a direct result of ART are not centrally monitored or subject to any regulations at present in Ireland. Despite the introduction of national clinical guidelines on pregnancy hypertension and on-going education of clinical staff, hypertensive disorders of pregnancy remain an issue. There is often a delay in diagnosis of these conditions and frequently the clinical care provided is lacking.

Angiogenic biomarkers potentially may be a useful adjunct in the diagnosis of placental related disorders such as hypertension and fetal growth restriction. Observational studies to date have shown good correlation between pre-eclampsia and circulating maternal PlGF, with a lowering of PlGF many weeks prior to the onset of clinical identifiable signs. However, concern exists that the introduction of PlGF into clinical care pathways may help diagnosis placental dysfunction/pre-eclampsia earlier but may not actually improve maternal clinical outcomes. Also of concern is that earlier diagnosis may result in earlier intervention by clinicians and with a subsequent rise in iatrogenic prematurity and worsening neonatal outcomes.

Given the research to date on PlGF in preterm singletons, RCTs are now being performed to examine the impact of its clinical use on maternal and neonatal outcomes. Much less information is available in relation to PlGF in twin pregnancy owing to less research on this topic. Potentially PlGF could be a very useful addition to investigations for placental dysfunction in this high risk cohort, however further research in terms of clinically useful cut-offs in twin pregnancy is necessary before its clinical application.

Pregnant women are generally interested in research and amenable to taking part in clinical trials during pregnancy. The approach of the researcher, their demeanour and use of language, are key to engaging pregnant women and gaining their interest and consent for participation.

1.8.1 Thesis Aims

The overall aim of my thesis was to explore and expand on current knowledge of placental growth factor, especially in relation to a twin pregnancy. I also wished to examine the impact of the use of PlGF as a diagnostic test for pre-eclampsia in the preterm singleton population. Lastly, I wished to explore the barriers and facilitators to pregnant women's participation in clinical research.

Overall aims:

- Identify limitations in our current knowledge on PlGF
- Expand on current knowledge of PlGF in twin pregnancy
- Conduct a pragmatic RCT of PlGF as a diagnostic test for preterm pre-eclampsia in a singleton pregnancy cohort
- Explore motivators behind pregnant women's participation in research

In order to achieve these aims, this thesis is comprised of a number of chapters outlined below:

1.8.2 Thesis Outline

Chapter 2: Hypertensive Disorders and Twin Pregnancy

Paper 1: The Maternal and Perinatal Implications of Hypertensive Disorders of Pregnancy in a Multiple Pregnancy cohort

- Highlight the origin, structure and function of Placental Growth Factor and its receptors
- Discuss how the pro-angiogenic/anti-angiogenic synergism of these biomarkers is critical for successful placentation
- Discuss how an imbalance in PlGF may be utilised as a diagnostic marker of disease or a potential therapeutic target for adverse pregnancy outcomes

Chapter 3: Placental Growth Factor

Paper 2: Placental Growth Factor: A review of literature and future applications

Retrospective review of a large cohort of women delivering a twin pregnancy in a single large tertiary unit

- Evaluate the implications of hypertensive disorders of pregnancy on both maternal outcomes
- Evaluate the implications of hypertensive disorders of pregnancy on perinatal outcomes

Chapter 4: Placental Growth Factor and Twin Pregnancy

Paper 3: A comparative study of two immunoassays of maternal placental growth factor

&

Paper 4: A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range

- Develop a dichorionic twin pregnancy specific reference range for placental growth factor
- Compare gestational specific PlGF levels in twin pregnancies complicated by pre-eclampsia or any HDP to controls.
- Examine the similarities and differences in results obtained from two different immunoassays of maternal placental growth factor, highlighting the requirements for translating a lab-based test into one appropriate for clinical utility

Chapter 5: PARROT Ireland Aims

Paper 5: PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial Research Study Protocol

&

Paper 6: PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: Results of Interim Analysis

- By conducting a national multi-site randomised controlled trial evaluate the impact of knowledge of PlGF measurement on both maternal morbidity & neonatal morbidity

Chapter 6: Research in a Pregnant Population Aims

Paper 7: An exploration of women's experience of taking part in a randomised controlled trial of a diagnostic test during pregnancy; a qualitative study

- Identify barriers and facilitators to pregnant women's participation in clinical research
- Examine pregnant women's willingness to participate in research while pregnant
- Explore women's experience about being involved in a clinical trial, specifically a randomised controlled trial, while pregnant.

Chapter 2: Hypertensive Disorders and Twin Pregnancy

Paper 1: The Maternal and Perinatal Implications of Hypertensive Disorders of Pregnancy in a Multiple Pregnancy cohort

Deirdre Hayes-Ryan, Sarah Meaney, Aoibhin Hodnett, Minna Geisler & Keelin O'Donoghue

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2.1 The Maternal and Perinatal Implications of Hypertensive Disorders of Pregnancy in a Multiple Pregnancy cohort

2.1.1 Abstract

Introduction: Hypertensive Disorders of Pregnancy are common and may result in increased maternal and neonatal morbidity and mortality. Multiple pregnancies confer an increased risk of development of a hypertensive disorder of pregnancy. The purpose of this study was to examine a large cohort of women delivering a multiple pregnancy in a single large tertiary unit, and to evaluate the implications of hypertensive disorders of pregnancy on both maternal and perinatal outcomes.

Material and methods: Retrospective study of all twin pregnancies delivered at Cork University Maternity Hospital, Ireland over a 9-year period (2009–2017). The twin pregnancies were divided according to the presence or absence of hypertensive disorder of pregnancy and the two groups compared.

Results: Maternal age >40 years, nulliparity, conception through use of a donor oocyte, and presence of obstetric cholestasis are all risk factors for the development of Hypertensive Disorders of Pregnancy in women with a multiple pregnancy. When a hypertensive disorder complicates a twin pregnancy, it increases the incidence of iatrogenic late prematurity & neonatal hypoglycaemia.

Conclusions: This study is informative for clinicians caring for women with a multiple pregnancy with its relevant data on perinatal outcomes following a diagnosis of hypertensive disorder in pregnancy.

2.1.2 **Introduction**

Variation exists between international bodies on the exact classifications of hypertensive disorders of pregnancy (HDP), but general consensus agrees that they can broadly categorised as follows; chronic hypertension of all causes, gestational (non-proteinuric) hypertension, pre-eclampsia and superimposed pre-eclampsia (4). The potential complications that may occur in a pregnancy affected by a hypertensive disorder directly relate to the underlying pathology of the condition. Late onset gestational hypertension is generally a benign condition with minimal increased risk to mother or baby (222). In contrast, early onset severe pre-eclampsia can confer a risk of morbidity, as well as mortality, especially in developing countries (13).

Multiple pregnancy occurs spontaneously in approximately one in 80 pregnancies (33). Both increasing maternal age and access to assisted reproductive techniques (ART) have had an impact in the number of twin pregnancies globally, with a dramatic increase reported over the past several decades (48, 50). Multiple births account for approximately 3% of live births (53). Figures from the Central Statistics Office show the number of twins born in Ireland has increased from 10.5 per 1,000 live births in 1985, to 18.6 per

1,000 in 2015, with an increase of 22.5% over the last decade alone (51). Similar trends are reported in other countries (55, 56). Multiple pregnancy confers increased perinatal risk, including mortality, preterm birth, congenital abnormalities, fetal growth restriction and twin-twin transfusion syndrome (56). Further, many maternal obstetric morbidities, such as anaemia, haemorrhage, gestational diabetes mellitus and obstetric cholestasis (OC), arise more commonly in the setting of multiple gestation (56, 71). In the latest (2016) annual report from the national perinatal epidemiology centre (NPEC), multiple pregnancy was associated with an almost fourfold increased risk of severe maternal morbidity (223).

Multiple pregnancy is known to be a risk factor for the development of HDP, with a reported incidence ranging from 13-37%, which is 2-3 times higher than that seen in singletons (74). Most studies to date have focused on the risk factors for the development of HDP in a multiple pregnancy cohort (224), but reports on the clinical outcomes when these complications arise are fewer. Studies have suggested that multiple pregnancy complicated by HDP has a higher incidence of adverse pregnancy outcomes, specifically; preterm delivery, small for gestational age infants, caesarean delivery and placental abruption, when compared to HDP in singletons (77). It is reported that when these complications arise, they are likely to occur at an earlier gestation in multiple pregnancy compared to singleton pregnancy, which further increases potential perinatal morbidity (75). The aim of this study was to review a large cohort of women delivering a multiple pregnancy in a single large tertiary unit,

and to evaluate the implications of HDP on both maternal and perinatal outcomes.

2.1.3 **Methods**

This was a retrospective study of all twin pregnancies delivered at Cork University Maternity Hospital (CUMH), Ireland over a 9-year period (2009–2017). All women attending CUMH had a booking appointment and dating ultrasound performed between 11-13 weeks gestation. If the dating scan identified a multiple pregnancy, chorionicity was immediately determined and the women referred to the hospital's dedicated multiple pregnancy clinic. A Maternal-Fetal Medicine (MFM) specialist consultant, experienced in the management of multiple pregnancy, directs the clinic and creates a unique management plan for each patient dependent on their obstetric history and personal preferences. A dedicated midwife sonographer for multiple pregnancy saw all women and performed ultrasounds in accordance to a routine scan schedule as per national guidelines (67) to assess for evolving growth discrepancy or twin-twin transfusion syndrome. In Ireland women have the option of public or private antenatal care. Those attending privately had a consultant obstetrician of their choice review them at each visit and the same Consultant present to oversee the delivery. Those attending the public multiple pregnancy clinic in CUMH were under the care of a single Consultant Obstetrician and their team of non-consultant hospital doctors (NCHD). All

multiple pregnancy births in CUMH are attended by an obstetrician; for public patients this is the Obstetrician assigned to cover the labour ward on the day of the delivery.

Outcome Measures

Maternal obstetric information was obtained from obstetric charts, hospital birth registers, ultrasound reports and laboratory data. Demographic data included; maternal age and body mass index (BMI) at booking, parity, mode of conception (spontaneous conception vs. all ART and type of antenatal care received (public vs. private). Chorionicity was assigned in all twin pregnancies based on ultrasound findings at 11-13 weeks gestation; this was reviewed in charts and after birth for confirmation of scan findings. Gestation at delivery, onset of labour (i.e. spontaneous or induced labour) mode of delivery and indications for induction/assisted delivery were also captured.

Specific maternal outcome measures were; OC (pruritus with deranged liver transaminases (>70 U/L) with elevated fasting serum bile acids (>10mmol/L)), gestational diabetes (diagnosed by oral glucose tolerance test at 24 - 28 weeks' gestation) or presence of a hypertensive disorder of pregnancy; either pre-eclampsia or gestational hypertension. Pre-eclampsia was defined as; "sustained hypertension with systolic BP ≥ 140 or diastolic BP ≥ 90 (on at least two occasions at least 4hrs apart) with significant quantified proteinuria (>300mg protein on 24hr collection or urine protein creatinine ratio

>30mg/mmol or ≥ 3+ on dipstick urinalysis)" occurring after 20 weeks' gestation. Gestational hypertension was defined as "persistent blood pressure readings ≥140/90 mmHg, without associated proteinuria" occurring after 20 weeks' gestation.

Perinatal outcomes were obtained from birth records, neonatal charts and the neonatal intensive care unit (NICU) database. Specific perinatal outcome measures included; Stillbirth of one twin (24 weeks' gestation and/or weighing 500 g or more), suspected SGA (Small for gestational age; defined as an estimated fetal weight less than tenth percentile for gestational age, detected antenatally on ultrasound based on accurate dating) (16), Prematurity (defined as birth occurring at less than 37 weeks' gestation) and the degree of prematurity; very preterm (<32 weeks' gestation), moderately preterm (32+1–33+6 weeks' gestation) and late preterm (34–36+6 weeks' gestation).

Other parameters recorded were; Birth weight (measured in grams), presence of a low Apgar score (defined as <7 at 1 minute or <7 at 5 minutes), NICU admission and the length of stay (days) in the NICU. The possible indications for NICU admission were; prematurity, low birth weight, SGA at birth (birthweight <10th customised centile), suspected infection, poor feeding, apnoea of the newborn, transient tachypnoea of the newborn (TTN), respiratory distress syndrome (RDS), hypothermia, hypoglycaemia or other. Complications in the neonatal period included any of the following; respiratory, haematological, hypoglycaemia, jaundice, respiratory distress syndrome, retinopathy of prematurity or chronic lung disease.

Serious neonatal morbidity was not frequent. A composite measure of perinatal morbidity was chosen and included the following: hypoxic ischaemic encephalopathy of any grade (HIE), necrotising enterocolitis of any grade (NEC), intraventricular haemorrhage grade two or higher (IVHG2) or sepsis (based on positive blood cultures or cerebrospinal fluid).

Statistical Analyses

Initially the entire cohort was examined for trends, and then the twin pregnancies were divided according to the presence or absence of hypertensive disorder of pregnancy and the two groups compared. Descriptive and inferential statistics were employed to analyse the data utilising SPSS version 23. Differences between the hypertensive and non-hypertensive groups were compared using Chi square tests and t-tests where appropriate. Logistic regression analysis was used to assess the impact of HDP on the likelihood of outcomes. Adjusted analysis, for known risk factors for HDP such as maternal BMI >35 Kg/m^2, maternal age >40 years and nulliparity, in accordance with national clinical guidelines were also employed (1). Monochorionic twins are more frequently associated with congenital anomalies as well as having an inherently increased risk of adverse perinatal outcomes when compared with dichorionic twins (225). Therefore, the analysis was then repeated with the monochorionic twin pregnancies removed from the cohort to examine their influence.

Ethical Approval

Ethical Approval for the study was granted by the Cork Research Ethics Committee (ECM 4 (zz) 05/06/18).

2.1.4 **Results**

One thousand five hundred and sixty six women (n=1566) delivered a multiple pregnancy in CUMH from the 1st January 2009 until the 31st December 2017. The majority of the group were dichorionic twin pregnancies (81.2%; n=1271). Almost a fifth of these (17%; n=270) were diagnosed with a Hypertensive Disorder of Pregnancy (HDP). The incidence among dichorionic twin pregnancies was 17% compaired to 15% among the monochorionics. Three thousand one hundred and thirty two neonates (n=3132) were delivered from these pregnancies, with the vast majority (98%; n=3078) of these delivered live born.

Maternal Demographics (Table 2.1)

The maternal and pregnancy characteristics of the twin pregnancies included in the study are shown in Table. When comparing those that developed HDP against those that did not, those that developed a HDP were twice as likely to be greater than 40 years of age (aOR 2.3, 95%CI 1.1-4.69) and more than twice as likely to be nulliparous (aOR 2.6, 95%CI 1.6-4.4). There was no

difference in the incidence of HDP between public and private antenatal care (aOR 2.3, 95%CI 0.8-6.4), BMI >35 Kg/m^2 (aOR 2.0, 95%CI 0.8-5.0) nor with placental chorionicity (aOR 0.8, 95%CI 0.4-1.7). Overall, the incidence of HDP was not increased by the use of ART (aOR 1.4, 95%CI 0.8-2.4). However, when ART was examined by mode of conception, oocyte donation conferred a threefold increased risk of HDP (aOR 2.7, 95%CI 1.4-5.3) while with ovulation induction (any medication without use of IVF) it was eight times more likely to occur (aOR 8.1, 95%CI 2.5-26.5).

Maternal Outcomes (Table 2.1)

Overall, the incidence of gestational diabetes mellitus and OC were 7.9% and 6% respectively. Concomitant gestational diabetes mellitus and OC occurred in just 1% (n=16). Diagnosis of gestational diabetes mellitus did not increase the likelihood of a diagnosis of HDP (aOR 1.1, 95%CI 0.4-2.7) however women with OC were almost four times more likely to develop HDP, even when adjusted for confounding risk factors (aOR 3.7, 95%CI 1.3-10.3). Antenatal steroids for fetal lung maturation were administered to over a quarter of women (28.5%, n=447). When the group was dichotomised by the presence or absence of HDP, those with HDP were almost twice as likely to receive steroids (aOR 1.7, 95%CI 1.0-2.8).

Onset of labour & Mode of Delivery (Table 2.2)

Delivery was by pre-labour caesarean for almost half the cohort (49.6%, n=1553), with the mothers of a third of the babies having a spontaneous onset of labour (30%, n=941). Overall two thirds of the cohort delivered by caesarean section (66.8%, n=2092). No differences were noted in terms of onset of labour, indication for caesarean section or grade of caesarean section when the two groups were compared. Analysis on mode of delivery revealed the HDP group to be three times more likely to undergo an emergency caesarean in labour (aOR 2.9, 95%CI 1.1-3.20) compared to having a spontaneous vaginal delivery. The HDP group was less likely to have a vaginal breech delivery (aOR 0.5, 95%CI 0.3-0.8) or an elective caesarean delivery (aOR 0.5, 95%CI 0.2-0.9). We found that the HDP group were nine times more likely to require an emergency caesarean section for delivery of the second twin (aOR 9.0, 95%CI 1.4-58.9) however it is important to note that the number in this group is very small.

Preterm Delivery (Table 2.3 & Table 2.4)

Over half (57.2%, n=1761) of all live-born twins in the study delivered preterm with the aetiology for the prematurity shown in Table 2.3. Comparing the two groups, those with HDP were more than twice as likely to deliver preterm (aOR 2.5, 95%CI 1.6-3.7). A difference in gestational age between the two groups only existed at 34-36^{+6} weeks gestation with the HDP group almost three times

more likely to deliver then (aOR 2.9, 95%CI 1.9-4.3). Induction of labour was five times more likely (aOR 4.9, 95%CI 2.8-8.7) while emergency pre-labour caesarean delivery was increased fourfold (aOR 3.9, 95%CI 2.3-6.6) in the HDP group with premature delivery compared to controls. Delivery prior to 37 weeks is not uncommon for monochorionic twins. When monochorionic pregnancies were removed and the analysis repeated the conclusions were unchanged (Table 2.4).

Perinatal Outcomes (Table 2.5 & Table 2.6)

Perinatal outcomes for the entire twin pregnancies' study (n=3132) are shown in Table 5. Just over a tenth of cases (12.1%, n=379); were suspected of being small for gestational age on antenatal ultrasound (estimated fetal weight <10th gestational centile using the Hadlock formula). Low birth weight (<2500g) was present in almost half of cases (49%, n=1527) but very low birth weight (<1500g) occurred in very few (7.7%, n=242). Fetal anomaly was present in approximately one in twenty cases (6%, n=190). After birth the incidence of SGA was 2.7% among women with HDP compared to 3.5% among women without HDP. Of those babies born alive (98%, n=3078) admission to the NICU occurred in almost half (42.6%, n=1333), with prematurity (77.6%, n=1027) and low birth weight (<2500g) (59.8%, n=800) being the main reasons for admission. Length of stay was beyond 14 days in almost half of those admitted (48.6%, n=648). Respiratory (37.3%, n=1167) and haematological (28.6%,

n=337) complications were the most commonly occurring in the neonatal period. A composite measure of neonatal morbidity was low (4.6%, n=140).

The incidence of perinatal mortality for the group was low (1.2%, n=39). This figure reflects the combination of intrauterine (1%, n=31) and neonatal (0.9%, n=29) deaths that occurred in the study. The corrected perinatal mortality rate for the entire cohort was 12.3, 5.4 for the dichorionic group and 41.0 for the monochorionic group.

Rates of low Apgar scores, mean birth weight and fetal anomaly did not differ between the two groups (Table 2.5). Low birth weight was four times more frequent in the HDP group compared to the non-HDP group (aOR 3.9, 95%CI 2.3-6.6). Requirement for NICU and length of stay in NCU did not differ either. Indication for admission to the NICU was similar between both groups. HDP was protective against admission for suspected infection (aOR 0.5, 95%CI 0.3-0.9) while apnoea of the newborn was three times more likely in the presence of HDP (aOR 3.0, 95%CI 1.0-8.7). Overall, complications in the neonatal period were similar for both groups with no difference in the composite measure of neonatal morbidity. However significant difference were noted in the incidence of hypoglycaemia with a threefold (aOR 3.3, 95%CI 2.5-7.3) increase respectively seen when HDP was present. Perinatal mortality was not common and although a trend towards lower mortality was present in the HDP, low numbers prevented comparative analysis between the two groups. When monochorionic pregnancies were removed and the analysis repeated the conclusions were unchanged (Table 2.6).

2.1.5 **Discussion**

This study highlights that nulliparity, maternal age over 40 years, conception though use of donor oocyte and presence of OC are important risk factors for the development of HDP with a multiple pregnancy. It also outlines that when HDP does complicate a multiple pregnancy, it more commonly results in iatrogenic late premature delivery and neonatal hypoglycaemia. Almost a fifth of our twin cohort developed a HDP. This rate is in keeping with the higher incidence of HDP quoted in the literature for multiples; usually in the order of a 3 fold increased risk for pre-eclampsia alone. This rate may even be an underestimation, with a recent study comparing incidence of pre-eclampsia in gestationally matched singletons to twins, all at <37 weeks, reporting a nine fold increased risk of pre-eclampsia in twins (78). The reason behind this increased risk of HDP in multiple pregnancy is not fully understood, but is likely due to a combination of maternal risk factors and the presence of a larger placental mass; which may predispose to an angiogenic imbalance. Circulating angiogenic factors, such as placental growth factor (PlGF) and its soluble receptor sFlt-1 are well described as being significantly altered in women prior to the clinical onset of pre-eclampsia (88, 89). With a larger placenta comes higher levels of these circulating angiogenic factors, and studies have shown increased risk of pre-eclampsia with each for each two fold elevation in sFlt-1 (90). Prediction of pre-eclampsia using these angiogenic factors is a topical presently however their utility in aiding

diagnoses of pre-eclampsia in multiple pregnancy warrants further research (179, 188).

Maternal age >40 years, nulliparity and conception through use of a donor oocyte are all well-established risk factors for HDP in both singleton and multiple pregnancies (3). Over the last number of decades, use of assisted reproductive therapy (ART) has increased and pregnancy through use of donor oocyte is now common (86). The popular media portrayal of ART is that it is a viable risk free option for achieving pregnancy at any age with little recognition is given to the increased risks associated with pregnancy at an older age or through use of ART (83).

The higher incidence of HDP in our cohort in those conceiving through ovulation induction needs to be interpreted with caution as the numbers are small (n=22) and when adjusted the confidence interval is very large. We did observe significantly more HDP when conception arose through use of a donor oocyte which is well described by other studies in both singletons and multiples (70, 85). It is worth noting that mode of conception in our cohort is self-reported by women at their booking visit and hence may indeed be under reported. The high rate of twins proves the Irish ART sector needs regulation and protocols aimed at minimising the risk of multiple pregnancy should be advocated (57). Internationally, countries which have had success increasing their rates of single embryo transfer such as Belgium, Norway and Denmark have generous IVF state funding (60). Introduction of state funding, and consequently access

to multiple fresh and frozen IVF cycles, was also seen to radically reduce the rate of multiple embryo transfer province in Quebec (61).

Our study found no difference in the incidence of HDP between either monochorionic or dichorionic twin pregnancies. The relationship between chorionicity and HDP is unclear and sometimes conflicting. A study in 2016 showed dichorionicity to be an independent risk factor for the development of pre-eclampsia but not gestational hypertension (226) while other studies have reported no association between chorionicity and hypertensive disease (224). Ideally, we would like to examine our cohort further by comparing chorionicity against type of HDP present but a limitation of our study is that this is not possible from our data.

A number of studies have reported higher incidence of hypertensive disease with increased maternal BMI and the protective effect against this seen with the use of aspirin (10). Practice on use of aspirin in our unit did not change during the time-period of this review. Women with pre-existing medical co-morbidities (such as hypertension, renal disease, lupus or antiphospholipid syndrome) or concomitant moderate risk factors (IVF, oocyte donation, maternal age >40 years, BMI >30 Kg/m^2 or previous poor obstetric history) were prescribed aspirin 75mg once daily from their booking visit in line with national guidelines (1). Unfortunately, a significant limitation of the study is the lack of data on use of aspirin in women in our cohort, limiting analysis on the influence of this factor in our population.

Our study reports an almost fourfold likelihood of OC in multiple pregnancies complicated by HDP. This predilection for the development of pre-eclampsia with OC is a relatively new concept, with the majority of studies to date reporting on singleton pregnancies (71, 227). Some research has suggested that the pathological mechanisms causing hepatic impairment in women with pre-eclampsia may predispose to cholestasis (228). Further research is warranted to assess if it is the same mechanism responsible in cases of multiple pregnancy. With this evolving knowledge of the predisposition for HDP in those with OC, it is reasonable for clinicians to consider increased surveillance for early sings of HDP in women with a multiple pregnancy diagnosed with OC.

The overall incidence of vaginal breech birth was an interesting finding of the study. Since publication of the Term Breech trial almost twenty years ago, (229) vaginal breech birth in high income countries generally only occurs in cases of a second twin delivery or premature delivery. Our findings illustrate that for every vaginal twin birth that occurred, 1 out of 5 second twins were delivered breech.

HDP is recognised as arising on a background of placental dysfunction, thus those with HDP may have a reduced capacity for placental function when labouring and may require expedited delivery (230). This is a potential explanation for the increased likelihood of caesarean section in labour and caesarean section for second twin that was noted in women with HDP in our cohort. Although a higher incidence of preterm delivery was noted in the HDP

group, differences in gestational age at delivery was only significant at later preterm gestation (34-36+6 weeks). This likely reflects an overall later onset of HDP in our cohort which is in contrast to many studies that have reported HDP to arise earlier in multiples than in singletons (75, 224, 231). It is well described that early onset pre-eclampsia (<34 weeks) is more severe and frequently results in poorer perinatal outcomes (232, 233). A limitation of our study is the lack of knowledge of gestational age at time of diagnosis of HDP.

Given the population under review it is not surprising a high incidence of administration of antenatal steroids for fetal lung maturation was present however the increased incidence of administration when HDP was present reflects the increased risk of premature delivery it confers. The prematurity noted in the HDP group of our cohort was iatrogenic in nature. Other studies have reported similar findings (74, 77), highlighting the potential for fetal and/or maternal morbidity and requirement for medical intervention when HDP complicates a multiple pregnancy. The higher incidence of low birth weight babies with HDP correlates with the higher rates of pre-maturity seen while the similar rates of very low birth weight babies supports the prematurity arising at a later gestational age.

Overall the perinatal outcomes between the HDP groups were largely comparable. A composite measure of perinatal morbidity did not show any difference between the two groups, a similar finding to other researchers (234). We noted an increased incidence of neonatal hypoglycaemia in the infants born to women with a HDP. A number of papers have reported an association

between the administration of antenatal steroids and neonatal hypoglycaemia (235, 236). Timing of antenatal steroids is important with late administration of steroids (≥34 weeks) associated with a 2-fold higher risk of neonatal hypoglycaemia compared to early administration (<34 weeks) (237). Potentially this may be the cause of the hypoglycaemia noted however neonatal hypoglycaemia has also been linked with maternal administration of beta-blockers in late pregnancy (238). Labetalol, a beta-blocker, is the first line anti-hypertensive medication utilised in our unit. Use of labetalol or another anti-hypertensive and the gestational age when administration of antenatal steroids occurred, are data points unfortunately not recorded for our cohort. This is a limiting factor of our study however it highlights a potential area for future prospective research.

Presence of HDP conferred a protective effect against admission to the NICU for reasons of suspected infection. Infection in multiples most usually arises in the case of premature spontaneous onset of labour (239). Given that prematurity in the HDP group in our cohort was iatrogenic, it would be in keeping with less likelihood of infection. A retrospective study by a group of Irish researchers focusing on multiple pregnancies from 1996-2012 has shown a reduction in overall perinatal mortality with multiple pregnancy over the 17 year period examined (69). In our study a trend towards higher incidence of perinatal mortality in the non-hypertensive group was noted, however the numbers are too small to allow a meaningful comparison. A US retrospective study of over 250,000 multiple pregnancies published in 2014 postulated

gestational hypertension to be beneficial for fetal survival in twin pregnancies, but advocated further prospective studies be conducted to assess for confounding variables (240).

Limitations of this study are included in our discussion. In summary the absence of some population data (eg; type of HDP diagnosed, use of aspirin or anti-hypertensive agents or women transferred in from a peripheral unit), given that it was conducted retrospectively, limits analysis and comparison to other published work. Ideally, to fully appreciate the impact of HDP in multiple pregnancy it would be sensible to compare our outcomes to those of singleton pregnancies affected by HDP in women in our unit during the same time-period. Unfortunately, we do not have this information available to us and it is not feasible to obtain, therefore we must rely on the published work of others for comparative purposes. The strength of this work is that it is a collective of nine years' worth of obstetric data from a tertiary maternity unit, with over 8000 deliveries annually and availability of a large neonatal outcome database. The unit also has a dedicated multiple pregnancy clinic, which has been advocated in order to harmonise management and reduce morbidity for multiple pregnancy (53, 241).

2.1.6 **Conclusion**

In summary, we examined a large cohort of women with a multiple pregnancy to look at the incidence of HDP and their impact on perinatal outcomes.

Women must be appropriately counselled by healthcare professionals in relation to potentially increased risks when embarking on a pregnancy at an advanced age, especially through use of ART and/or donor oocyte. It is imperative that women who do conceive multiples, especially if they are older or have medical co-morbidities, are risk assessed and appropriately referred antenatally for more intense maternal and fetal screening and surveillance. Early recognition of complications allows referral to an experienced clinician, ideally within a dedicated multiple pregnancy clinic, and appropriate management employed. Should a hypertensive disorder occur in a twin pregnancy, it is encouraging from this study to note perinatal outcomes overall were good. This information may be useful when counselling patients antenatally.

Table 2. 1a: *Maternal characteristics and antenatal complications according to presence of hypertensive disorder of pregnancy (HDP).*

Maternal Characteristics	**Total population (n = 1566)[b]**	**HDP (n = 270) [b]**	**No HDP (n = 1296) [b]**	**Odds Ratio (95% CI)**	**aOdds Ratio[a] (95% CI)**
Age Range (years)	17-51	18-51	17-51	-	-
Maternal Age >35 years	766 (48.9)	142 (52.6)	624 (48.1)	1.18 (0.9-1.5)	-
Maternal Age >40 years	173 (11)	43 (15.9)	130 (10)	1.6 (1.1-2.4)	2.3 (1.1-4.6)
BMI (Kg/m^2) >35 (n=486)	31 (6.3)	8 (25.8)	23 (74.1)	1.97 (0.85-4.58)	2.0 (0.8-5.0)
Antenatal care					
Public	1106 (70.6)	204 (75.6)	902 (69.6)	1.3 (0.9-1.7)	2.3 (0.8-6.4)
Private	460 (29.4)	66 (24.2)	394 (30.4)	Ref (1.0)	Ref (1.0)
Parity					
Nulliparous	658 (42)	147 (54.4)	511 (39.4)	1.8 (1.3-2.3)	2.6 (1.6-4.4)
Multiparous	908 (58)	123 (45.6)	785 (60.6)	Ref (1.0)	Ref (1.0)
Chorionicity					
Monochorionic	295 (18.8)	45 (16.7)	250 (19.3)	0.8 (0.5-1.1)	0.8 (0.4-1.7)
Dichorionic	1271 (81.2)	225 (83.3)	1046 (80.7)	Ref (1.0)	Ref (1.0)

Mode of conception					
Spontaneous	1082 (69.1)	164 (60.7)	918 (70.8)	Ref (1.0)	Ref (1.0)
Assisted Reproductive Therapy (ART)	484 (30.9)	106 (39.3)	378 (29.2)	1.5 (1.1-2.0)	1.4 (0.8-2.4)
Mode of ART				Ref (1.0)[c]	Ref (1.0) [c]
Ovulation Induction	22 (1.4)	4 (1.5)	18 (1.4)	1.2 (0.6-2.7)	8.1 (2.5-26.5)
Intrauterine Insemination	37 (2.4)	5 (1.9)	32 (2.5)	0.9 (0.4-1.7)	2.1 (0.6-7.0)
In Vitro Fertilisation	351(22.4)	71 (26.3)	280 (21.6)	1.4 (1.1.1.8)	0.9 (0.6-1.5)
Donor oocyte	65 (4.2)	26 (9.6)	39 (3)	3.7 (2.5-5.3)	2.7 (1.4-5.3)
Antenatal complications				Ref (1.0)[d]	Ref (1.0) [d]
GDM	123 (7.9)	23 (8.5)	100 (7.7)	0.9 (0.5-1.6)	1.1 (0.4-2.7)
OC	94 (6)	34 (12.6)	60 (4.6)	2.7 (1.6-4.4)	3.7 (1.3-10.3)
GDM & OC	16 (1)	7 (2.6)	9 (0.7)	4.0 (1.4-10.9)	0.8 (0.09-8.2)
Antenatal Steroids	447 (28.5)	99 (36.7)	348 (26.9)	1.5 (1.1-2.0)	1.7 (1.0-2.8)

[a]Adjusted Odds Ratio (for BMI >35 Kg/m^2, Maternal Age >40 and Nulliparity). Results demonstrating significant differences are highlighted in bold.
[b]Values are shown in *n* (%) unless otherwise stated
[c]Reference is Spontaneous Conception.
[d]Reference is no Antenatal Complication
HDP, hypertensive disorder of pregnancy; GDM, Gestational Diabetes; OC, Obstetric Cholestasis

Table 2.1b: *Maternal characteristics and antenatal complications by chorionicity.*

Maternal Characteristics	Total population (n = 1566)*	Dichorionic (n=1271)	Monochorionic (n=295)
Age Range (years)	17-51	17-51	17-45
Maternal Age >35 years	766 (48.9)	659 (51.8)	107 (36.3)
Maternal Age >40 years	173 (11)	159 (12.5)	14 (4.7)
BMI (Kg/m^2) >35 (n=486)	31 (6.3)	28 (2.2)	3 (1)
Antenatal care			
Public	1106 (70.6)	884 (69.6)	222 (75.3)
Private	460 (29.4)	387 (30.4)	73 (24.7)
Parity			
Nulliparous	658 (42)	540 (42.5)	118 (40)
Multiparous	908 (58)	731 (57.5)	177 (60)
Mode of conception			
Spontaneous	1082 (69.1)	818 (64.4)	264 (89.5)
ART[b]	484 (30.9)	453 (35.6)	31 (10.5)
Mode of ART			
Ovulation Induction	22 (1.4)	19 (1.5)	3 (1)
Intrauterine Insemination	37 (2.4)	35 (2.8)	2 (0.7)
IVF[c]	351(22.4)	332 (26.1)	19 (6.4)
Donor oocyte	65 (4.2)	63 (5)	2 (0.7)
Antenatal complications			
GDM[f]	123 (7.9)	98 (7.7)	10 (3.4)
OC[g]	94 (6)	63 (5)	15 (5.1)
GDM & OC	16 (1)	13 (1)	3 (1)
Antenatal Steroids	447 (28.5)	330 (26)	117 (39.7)

Table 2. 2: *Mode of delivery*

	Total population (n = 3132) [b]	**HDP (n = 541)** [b]	**No HDP (n = 2591)** [b]	**Odds Ratio (95% CI)**	**aOdds Ratio** [a] **(95% CI)**
Onset of labour					
Spontaneous	941 (30)	144 (26.6)	797 (30.8)	Ref (1.0)	Ref (1.0)
Induced	638 (20.4)	104 (19.2)	532 (20.5)	1.0 (0.8-1.4)	1.5 (0.9-2.6)
CS pre labour	1553 (49.6)	293 (54.2)	1260 (48.6)	1.2 (1.0-1.6)	1.4 (0.9-2.2)
Mode of delivery					
Vaginal delivery	1040 (33.2)	156 (28.8)	884 (34.1)	Ref (1.0)	Ref (1.0)
Caesarean Section	2092 (66.8)	385 (71.2)	1707 (65.9)	1.2 (1.0-1.5)	0.9 (0.6-1.3)
Detailed Mode of delivery					
Spontaneous vaginal delivery	691 (22.1)	93 (17.2)	598 (23.2)	Ref (1.0)	Ref (1.0)
Operative Vaginal	249 (8)	49 (9.1)	200 (7.7)	1.5 (1.0-2.3)	0.6 (0.3-1.3)
Vaginal Breech	100 (3.2)	14 (2.6)	86 (3.3)	0.9 (0.6-1.2)	0.5 (0.3-0.8)
Elective CS	1086 (34.7)	135 (25)	951 (36.7)	1.3 (0.9-1.7)	0.5 (0.2-0.9)
Emergency CS in labour	523 (16.7)	88 (16.3)	435 (16.8)	1.0 (0.5-1.9)	0.3 (0.07-1.5)
Emergency CS pre labour	469 (15)	159 (29.4)	310 (12)	3.2 (2.4-4.4)	2.9 (1.1 -3.2)
Emergency CS for 2nd Twin	14 (0.4)	3 (0.6)	11 (0.4)	1.7 (0.4-6.4)	9.0 (1.4 -58.9)

Grade of Caesarean Section[c]					
Grade 1	59 (2.8)	12 (3.1)	47 (2.8)	1.7 (0.8-3.3)	2.3 (0.8-6.6)
Grade 2	576 (27.5)	142 (36.9)	434 (25.4)	2.2 (1.7-2.8)	1.6 (0.9-2.7)
Grade 3	357 (17.1)	90 (23.4)	267 (15.6)	2.3 (1.7-3.0)	1.3 (0.7-2.4)
Grade 4	1100 (52.6)	141 (36.6)	959 (56.2)	Ref (1.0)	Ref (1.0)
Indication for CS					
Elective[d]	601 (19.2)	79 (20.5)	522 (30.6)	Ref (1.0)	Ref (1.0)
Fetal Complications[e]	697 (19.4)	130 (33.8)	477 (27.9)	1.8 (1.3-2.4)	0.8 (0.4-1.8)
Maternal Complications	150 (4.8)	77 (20)	73 (4.3)	6.9 (4.6-10.3)	1.9 (0.8-4.5)
Non-reassuring CTG	107 (3.4)	15 (3.9)	92 (5.4)	1.0 (0.5-1.9)	0.1 (0.01-1.1)
Previous CS	163 (5.2)	16 (4.2)	147 (8.6)	0.7 (0.4-1.2)	0.1 (0.03-0.7)
Non-vertex presentation	366 (11.7)	50 (13)	315 (18.5)	1.0 (0.7-1.5)	0.9 (0.4-1.8)
Intrauterine death	19 (0.6)	2 (0.5)	17 (1)	0.7 (0.1-3.4)	0.0 (-)
Failure to progress	80 (2.6)	16 (4.2)	64 (3.7)	1.6 (0.9-3.0)	0.7 (0.2-2.1)

[a]Adjusted Odds Ratio (for BMI >35, Maternal Age >40 and Nulliparity). Results demonstrating significant differences are highlighted in bold.[b]Values are shown in *n* (%) unless otherwise stated. [c]Grade of Caesarean Section is defined as per the RCOG Classification of Urgency of Caesarean Section 2011.

[d]Elective/ Previous CS/Non-vertex presentation .

[e]Fetal Complications/Non reassuring CTG/ Intrauterine death/Failure to progress.

HDP, hypertensive disorder of pregnancy; CS, cesarean section; CTG, cardiotocography.

Table 2. 3: *Preterm delivery entire cohort*

	Total population (n= 3076)[c]	**HDP (n = 540)[b]**	**No HDP (n = 2536) [b]**	**Odds Ratio (95% CI)**	**aOdds Ratio[a] (95% CI)**
Preterm Live Birth (<37 weeks)				Ref (1.0) [d]	Ref (1.0) [d]
	1761 (57.2)	388 (71.9)	1373 (54.1)	2.1 (1.7-2.6)	2.5 (1.6-3.7)
Reason for preterm birth				Ref (1.0) [d]	Ref (1.0) [d]
Spontaneous	677 (38.6)	99 (26.1)	578 (42.1)	1.2 (0.9-1.6)	1.2 (0.7-2.1)
Induction	206 (11.8)	64 (16.8)	142 (10.3)	3.2 (2.3-4.5)	4.9 (2.8-8.7)
Elective CS	361 (20.6)	95 (25)	266 (19.4)	2.5 (1.9-3.4)	1.4 (0.7-2.8)
Emergency CS (pre labour)	509 (29)	122 (32.1)	387 (28.2)	2.2 (1.7-2.9)	3.9 (2.3-6.6)
Gestation at delivery (weeks)					
≥37	1314 (42.8)	152 (28.1)	1162 (45.9)	Ref (1.0)	Ref (1.0)
34-36+6	1242 (40.4)	303 (56.1)	939 (37)	2.5 (2.0-3.1)	2.9 (1.9-4.4)
32+1 -33+6	234 (7.6)	44 (8.1)	190 (7.5)	1.8 (1.2-2.6)	1.8 (0.8-3.9)
<32	286 (9.3)	41 (7.6)	245 (9.7)	1.3 (0.9-1.9)	1.3 (0.6-2.8)

[a]Adjusted Odds Ratio (for BMI >35 Kg/m^2, Maternal Age >40 and Nulliparity). Results demonstrating significant differences are highlighted in bold.
[b]Values are shown in *n* (%) unless otherwise stated
[c]56 cases of Miscarriage/Stillbirth were removed leaving 3076 livebirths
[d] Reference used is delivery at/after 37 weeks gestation
HDP, hypertensive disorder of pregnancy; CS, cesarean section.

Table 2. 4: *Preterm delivery dichorionic twins only*

	Total population (n= 2519) [c]	HDP (n = 451) [b]	No HDP (n = 2092) [b]	Odds Ratio (95% CI)	aOdds Ratio[a] (95% CI)
Preterm Live Birth (<37 weeks)				Ref (1.0) [d]	Ref (1.0) [d]
	1316 (52.2)	308 (68.4)	1008 (48.7)	2.2 (1.8-2.8)	2.3 (1.5-3.6)
Reason for preterm birth				Ref (1.0) [d]	Ref (1.0) [d]
Spontaneous	544 (41.5)	79 (26.2)	465 (46.1)	1.2 (0.9-1.6)	1.0 (0.5-1.9)
Induction	134 (10.2)	44 (14.6)	90 (8.9)	3.5 (2.3-5.2)	4.4 (2.3-8.3)
Elective CS	261 (19.9)	73 (24.2)	188 (18.7)	2.7 (2.0-3.8)	1.1 (0.5-2.5)
Emergency CS (pre labour)	371 (28.3)	106 (35.1)	265 (26.3)	2.8 (2.1-3.8)	4.9 (2.8-8.6)
Gestation at delivery (weeks)					
≥37	1201 (47.7)	142 (31.6)	1059 (51.2)	Ref (1.0)	Ref (1.0)
34-36+6	964 (38.3)	245 (54.4)	719 (34.8)	2.5 (2.0-3.2)	2.8 (1.8-4.3)
32+1 -33+6	163 (6.5)	34 (7.6)	129 (6.2)	2.0 (1.3-3.0)	1.8 (0.8-4.3)
<32	191 (7.6)	29 (6.4)	162 (7.7)	1.3 (0.9-2.1)	0.7 (0.2-2.1)

[a]Adjusted Odds Ratio (for BMI >35 Kg/m^2, Maternal Age >40 and Nulliparity). Results demonstrating significant differences are highlighted in bold.[b]Values are shown in *n* (%) unless otherwise stated
[c]24 cases of Miscarriage/Stillbirth were removed leaving 2519 livebirths
[d] Reference used is delivery at/after 37 weeks gestation.
HDP, hypertensive disorder of pregnancy; CS, cesarean section.

Table 2. 5: *Perinatal outcomes entire cohort*

	Total population (n = 3132) [b]	**HDP (n = 541)** [b]	**No HDP (n = 2591)** [b]	**Odds Ratio (95% CI)**	**aOdds Ratio[a] (95% CI)**
SGA suspected antenatally	379 (12.1)	62 (11.5)	317 (12.2)	0.9 (0.7-1.2)	1.4 (0.9-2.2)
Low Apgar Score					
1 minute <7	360 (11.7)	73 (13.5)	287 (11.3)	1.2 (0.9-1.6)	0.9 (0.3-2.7)
5 minute <7	89 (2.9)	11 (2)	78 (3.1)	0.7 (0.3-1.2)	1.1 (0.6-1.9)
LBW[c]	1537 (49.1)	300 (55.5)	1237 (48.3)	1.3 (1.1-1.6)	1.6 (1.1-2.4)
VLBW[d]	242 (7.7)	41 (7.6)	201 (7.9)	0.9 (0.7-1.4)	0.9 (0.4-1.8)
Fetal Anomaly[e]	190 (6.1)	28 (5.2)	162 (6.3)	0.8 (0.5-1.2)	0.7 (0.3-1.5)
Perinatal Death					
IUD	31 (1)	1 (0.2)	30 (1.2)	-	-
NND	29 (0.9)	1 (0.2)	28 (1.1)	-	-
Overall Mortality	60 (1.9)	2 (0.4)	58 (2.2)	-	-
Corrected Mortality	39 (1.2)	2 (0.4)	37 (1.4)	-	-
Corrected Perinatal Mortality Rate	12.3	3.7	14.1	-	-

NICU Admission	1332 (42.5)	266 (49.2)	1067 (41.2)	1.4 (1.1-1.7)	1.2 (0.8-1.8)
Length of stay in NICU					
>48 hours	1144 (36.5)	227 (85.3)	917 (85.9)	0.9 (0.6-1.4)	0.7 (0.4-1.6)
>14 days	648 (48.6)	113 (42.5)	535 (50.1)	0.7 (0.6-0.9)	0.7 (0.4-1.2)
Indication for NICU					
Prematurity	1037 (77.6)	217 (81.6)	820 (76.6)	1.3 (0.9-1.9)	0.8 (0.4-1.4)
Low Birth Weight	800 (59.8)	158 (59.4)	642 (60)	0.9 (0.7-1.3)	0.9 (0.5-1.6)
SGA at birth	107 (8)	15 (5.6)	92 (8.6)	0.6 (0.4-1.1)	0.5 (0.2-1.4)
Infection	553 (41.4)	83 (31.2)	470 (44)	0.6 (0.4-0.8)	0.5 (0.3-0.9)
Poor Feeding	101 (7.6)	14 (5.3)	87 (8.1)	0.6 (0.4-1.1)	0.7 (0.2-3.6)
Apnoea of newborn	50 (1.6)	12 (4.5)	38 (3.6)	1.3 (0.7-2.5)	3.0 (1.0-8.7)
TTN	208 (15.6)	42 (15.8)	166 (15.5)	1.0 (0.7-1.5)	1.3 (0.7-2.5)
RDS	532 (39.8)	84 (31.6)	448 (41.9)	0.6 (0.5-0.8)	1.1 (0.6-1.9)
Hypothermia	35 (2.6)	10 (3.8)	25 (2.3)	1.6 (0.8-3.4)	2.8 (0.8-9.4)
Hypoglycaemia	51 (3.8)	13 (4.9)	38 (3.6)	1.4 (0.7-2.6)	2.2 (0.9-5.5)
Other	199 (14.9)	31 (11.7)	168 (15.7)	0.7 (0.5-1.1)	0.3 (0.1-0.9)
Neonatal Complications	1167 (37.3)	224 (41.5)	943 (37.2)	1.2 (0.9-1.4)	1.1 (0.7-1.5)

Respiratory	337 (28.6)	64 (28.2)	273 (28.7)	0.9 (0.7-1.3)	1.4 (0.8 -2.7)
Haematological	116 (9.9)	22 (9.7)	94 (9.9)	0.9 (0.6-1.6)	1.5 (0.6-3.8)
Hypoglycaemia	219 (18.6)	56 (24.7)	163 (17.1)	1.6 (1.1-2.2)	3.3 (1.5-7.3)
Jaundice	480 (40.7)	95 (41.9)	385 (40.5)	1.1 (0.8-1.4)	1.0 (0.5 -2.9)
Phototherapy	397 (33.7)	73 (32.2)	324 (34.1)	0.9 (0.7-1.3)	1.0 (0.5-2.0)
RDS	201 (17.1)	36 (15.9)	165 (17.4)	0.9 (0.6-1.3)	0.8 (0.4-1.7)
ROP	15 (1.3)	3 (1.3)	12 (1.3)	1.0 (0.3-3.7)	1.9 (0.4-10.5)
CLD	25 (2.1)	2 (0.9)	23 (2.4)	0.4 (0.1-1.5)	1.0 (0.1-8.9)
Neonatal Morbidity Composite	140 (4.6)	20 (3.7)	120 (4.7)	1.3 (0.8-2.1)	1.4 (0.6-3.3)
Grade 2 or higher IVH	31 (2.6)	4 (1.8)	27 (2.8)	0.6 (0.2-1.8)	0.3 (0.1-2.7)
NEC	29 (2.5)	1 (0.4)	28 (2.9)	0.1 (0.1-1.1)	-
HIE	9 (0.8)	2 (0.9)	7 (0.7)	1.2 (0.3-5.8)	-
Sepsis	99 (8.4)	14 (6.2)	85 (8.9)	0.7 (0.4-1.2)	0.7 (0.3-2.1)

[a]Adjusted Odds Ratio (for BMI >35 Kg/m2, Maternal Age >40 and Nulliparity. Results demonstrating significant differences are highlighted in bold.
[b]Values are shown in *n* (%) unless otherwise stated
[c]Low Birth Weight (<2500g),
[d]Very Low Birth Weight (<1500g),
[e]Fetal Anomaly includes all anomalies except patent ductus arteriosus (PDA) in a premature infant,
HDP, hypertensive disorder of pregnancy; SGA, small for gestational age; IUD, Intrauterine Fetal Demise; NND, Neonatal Death; NICU, Neonatal Intensive Care Unit; TTN, Transient tachypnoea of the newborn; RDS, Respiratory Distress syndrome; ROP, Retinopathy of prematurity; CLD, Chronic Lung Disease; IVH, Intraventricular Haemorrhage; NEC, Necrotising enterocolitis; HIE, Hypoxic Ischaemic Encephalaloapthy.

Table 2. 6: *Perinatal outcomes dichorionic only.*

	Total population (n = 2543) [b]	**HDP (n = 451)** [b]	**No HDP (n = 2092)** [b]	**Odds Ratio (95% CI)**	**aOdds Ratio** [a] **(95% CI)**
SGA suspected antenatally	287 (11.3)	47 (10.4)	240 (11.5)	0.9 (0.6-1.3)	1.4 (0.9-2.1)
Low Apgar Score					
1 minute <7	274 (10.8)	62 (13.8)	212 (10.2)	1.4 (1.0-1.9)	1.2 (0.7-2.2)
5 minute <7	60 (2.4)	8 (1.8)	52 (2.5)	0.7 (0.3-1.5)	1.0 (0.3-3.5)
LBW[c]	1163 (45.7)	242 (53.7)	921 (44.3)	1.5 (1.2-1.8)	1.8 (1.2-2.6)
VLBW[d]	147 (5.8)	32 (7.1)	115 (5.5)	1.3 (0.9-2.0)	0.8 (0.3-1.9)
Fetal Anomaly[e]	137 (5.4)	26 (5.8)	111 (5.3)	1.1 (0.7-1.7)	0.8 (0.3-1.8)
Perinatal Death					
IUD	11 (0.4)	1 (0.2)	10 (0.5)		
NND	16 (0.6)	0	16 (0.8)		
Overall Mortality	27 (1.0)	1 (0.2)	26 (1.2)		
Corrected Mortality	14 (0.5)	1 (0.2)	13 (0.6)		
Corrected Perinatal Mortality Rate	5.4	2.2	6.2		
NICU Admission	1025 (40.3)	214 (20.9)	811 (79.1)	1.4 (1.2-1.8)	1.2 (0.8-1.8)

Length of stay in NICU					
>48 hours	877 (34.5)	183 (85.5)	694 (85.5)	0.9 (0.6-1.4)	0.7 (0.3-1.5)
>14 days	472 (18.6)	89 (41.6)	383 (47.2)	0.7 (0.6-0.9)	0.7 (0.4-1.3)
Indication for NICU					
Prematurity	769 (30.2)	170 (79.4)	599 (73.7)	1.3 (0.9-1.9)	0.7 (0.4-1.4)
Low Birth Weight	583 (22.9)	120 (56.1)	463 (56.9)	0.9 (0.7-1.3)	0.8 (0.4-1.5)
SGA at birth	75 (2.9)	11 (5.1)	64 (7.9)	0.6 (0.4-1.1)	0.6 (0.2-1.7)
Infection	440 (17.3)	75 (35)	365 (45)	0.6 (0.4-0.8)	0.5 (0.2-0.9)
Poor Feeding	71 (2.8)	10 (4.7)	61 (7.5)	0.6 (0.4-1.1)	-
Apnoea of newborn	37 (1.5)	9 (4.2)	28 (3.4)	1.3 (0.7-2.5)	-
TTN	179 (7)	36 (16.8)	143 (17.6)	1.0 (0.7-1.5)	0.9 (0.5-1.9)
RDS	388 (15.3)	64 (29.9)	324 (39.9)	0.6 (0.5-0.8)	0.8 (0.4-1.6)
Hypothermia	29 (1.1)	9 (4.2)	20 (2.5)	1.6 (0.8-3.4)	3.9 (1.1-14.1)
Hypoglycaemia	42 (1.7)	12 (5.6)	30 (3.7)	1.4 (0.7-2.6)	2.6 (1.0-6.9)
Other	141 (15.5)	27 (12.6)	1141 (14)	0.7 (0.5-1.1)	0.4 (0.1-1.2)
Neonatal Complications	891 (35.4)	181 (40.2)	710 (34.3)	1.3 (1.0-1.6)	1.1 (0.7-1.6)
Respiratory	253 (28.2)	53 (29)	200 (28)	1.0 (0.7-1.5)	1.2 (0.6-2.5)
Haematological	72 (8)	16 (8.7)	56 (7.9)	1.1 (0.6-2.0)	1.3 (0.4-4.3)
Hypoglycaemia	153 (17.1)	41 (22.4)	112 (15.7)	1.6 (1.1-2.3)	2.6 (1.0-6.8)

Jaundice	359 (40)	72 (39.3)	287 (40.2)	0.9 (0.7-1.3)	0.8 (0.4-1.7)
Phototherapy	297 (33.1)	57 (31.1)	240 (33.6)	0.9 (0.6-1.3)	1.1 (0.5-2.3)
RDS	149 (16.6)	29 (15.8)	120 (16.8)	0.9 (0.6-1.5)	0.6 (0.3-1.6)
ROP	7 (0.8)	1 (0.5)	6 (0.8)	0.6 (0.1-5.4)	-
CLD	12 (1.3)	1 (0.5)	11 (1.5)	0.4 (0.1-2.7)	-
Neonatal Morbidity Composite	98 (3.9)	18 (4)	80 (3.9)	0.9 (0.6-1.6)	1.3 (0.5-3.2)
Grade 2 or higher IVH	22 (2.5)	4 (2.2)	18 (2.5)	0.9 (0.3-2.6)	0.3 (0.1-3.6)
NEC	13 (1.4)	1 (0.5)	12 (1.7)	0.3 (0.1-2.5)	-
HIE	6 (0.7)	1 (0.5)	5 (0.7)	0.8 (0.1-6.7)	-
Sepsis	74 (8.2)	13 (7.1)	61 (8.5)	0.8 (0.4-1.5)	0.7 (0.2-2.2)

[a]Adjusted Odds Ratio (for BMI >35 Kg/m2, Maternal Age >40 and Nulliparity. Results demonstrating significant differences are highlighted in bold.

[b]Values are shown in *n* (%) unless otherwise stated.

[c]Low Birth Weight (<2500g).

[d]Very Low Birth Weight (<1500g).

[e]Fetal Anomaly includes all anomalies except patent ductus arteriosus (PDA) in a premature infant.

HDP, hypertensive disorder of pregnancy; SGA, small for gestational age; IUD, Intrauterine Fetal Demise; NND, Neonatal Death; NICU, Neonatal Intensive Care Unit; TTN, Transient tachypnoea of the newborn; RDS, Respiratory Distress syndrome; ROP, Retinopathy of prematurity; CLD, Chronic Lung Disease; IVH, Intraventricular Haemorrhage; NEC, Necrotising enterocolitis; HIE, Hypoxic Ischaemic Encephalaloapthy.

Table 2.7: *Perinatal outcomes monochorionic only*

	Total population (n = 589)*	**HDP (n = 90)***	**No HDP (n = 499)***
SGA[b] suspected antenatally	92 (15.6)	15 (16.7)	77 (15.4)
Low Apgar Score			
1 minute <7	86 (14.6)	11 (12.2)	75 (15)
5 minute <7	29 (4.9)	3 (3.3)	26 (5.2)
LBW[c]	374 (63.4)	58 (64.4)	316 (63.3)
VLBW[d]	95 (16.1)	9 (10)	86 (17.2)
Fetal Anomaly[e]	53 (8.9)		
Perinatal Death			
IUD[f]	20 (3.4)	0	20 (4)
NND[g]	13 (2.2)	1 (1.1)	12 (2.4)
Overall Mortality	33 (5.6)	1 (1.1)	32 (6.4)
Corrected Mortality	25 (4.2)	0	25 (5)
Corrected Perinatal Mortality Rate	42.4	-	50.1
NICU[h] Admission	307 (52.1)	52 (57.8)	255 (51.1)
Length of stay in NICU			
>48 hours	267 (45.3)	44 (48.9)	223 (44.7)

>14 days	176 (29.8)	24 (26.7)	152 (30.5)
Indication for NICU			
Prematurity	268 (45.5)	47 (52.2)	221 (44.3)
Low Birth Weight	217 (45.8)	38 (42.2)	179 (35.9)
SGA[b] at birth	32 (5.4)	4 (4.4)	28 (5.6)
Infection	113 (19.2)	8 (8.9)	105 (21)
Poor Feeding	30 (5.1)	4 (4.4)	26 (5.2)
Apnoea of newborn	13 (2.2)	3 (3.3)	10 (2)
TTN[i]	29 (4.9)	6 (6.7)	23 (4.6)
RDS[j]	144 (24.4)	20 (22.2)	124 (24.8)
Hypothermia	6 (1)	1 (1.1)	5 (1)
Hypoglycaemia	9 (1.5)	1 (1.1)	8 (1.6)
Other	58 (9.8)	4 (4.4)	54 (10.8)
Neonatal Complications	276 (46.8)	43 (47.8)	233 (46.7)
Respiratory	84 (14.3)	11 (12.2)	73 (14.6)
Haematological	44 (7.5)	6 (6.7)	38 (7.6)
Hypoglycaemia	66 (11.2)	15 (16.7)	51 (10.2)
Jaundice	121 (20.5)	23 (25.6)	98 (19.6)
Phototherapy	100 (16.9)	16 (17.8)	84 (16.8)

RDS[j]	52 (8.8)	7 (7.8)	45 (9)
ROP[k]	8 (1.4)	2 (2.2)	6 (1.2)
CLD[l]	13 (2.2)	1 (1.1)	12 (2.4)
Neonatal Morbidity Composite	42 (7.1)	2 (2.2)	40 (8)
Grade 2 or higher IVH[m]	9 (1.5)	0	9 (1.8)
NEC[n]	16 (2.7)	0	16 (3.2)
HIE[o]	3 (0.5)	1 (1.1)	2 (0.4)
Sepsis	25 (4.2)	1 (1.1)	24 (4.8)

[*]Values are shown in *n* (%) unless otherwise stated

[a]Adjusted Odds Ratio (for BMI >35 Kg/m2, Maternal Age >40 and Nulliparity. Results demonstrating significant differences are highlighted in bold.

[b]Values are shown in *n* (%) unless otherwise stated.

[c]Low Birth Weight (<2500g).

[d]Very Low Birth Weight (<1500g).

Chapter 3: Placental Growth Factor

Paper 2: Placental Growth Factor: A review of literature and future applications

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3.1 Placental Growth Factor: A review of literature and future applications

3.1.1 Abstract

Placental growth factor is an angiogenic protein, highly expressed during pregnancy, which correlates well with placental function. In this review, we highlight the origin, structure and function of Placental Growth Factor and its receptors. We discuss how their pro-angiogenic/anti-angiogenic synergism is critical for successful placentation and how their imbalance may be utilised as a diagnostic marker of disease or a potential therapeutic target for adverse pregnancy outcomes.

3.1.2 Introduction

Discovery, Structure & Function

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family of proteins. Crystallography resolution at 2.0 Å resolution shows PlGF to have a three-dimensional structure comprising of 149–amino-acids. Comparison with that of VEGF-A shows a remarkable similarity between the two proteins, with 53% sequence identity between amino acids from positions 39-132 of PlGF and amino acids from positions 38-131 of VEGF-A (96, 97). PlGF was the second member of the vascular endothelial growth factor (VEGF) family identified, VEGF-A having been described in 1989 (101-

103). PlGF, like all proteins of the VEGF family, is a secreted dimeric glycoprotein with a distinctive cystine knot. This knot is characterised by a common motif of eight spatially conserved cysteines, which are involved in intra and inter molecular disulfide bonds (98, 99). The two monomers are oriented side-by-side and head-to-tail and held together by one interchain disulfide bond. The dimeric structure is also stabilised by a hydrophobic core region (96, 100). Its discovery is credited to Italian scientist Dr. Maria Graziella Persico who first described PlGF in 1991. She identified it while investigating the angiogenic potential of human term placental tissue which is why this protein was termed “the placental growth factor”. (95). In 1993 the location of the human PlGF gene on chromosome 14q24 was isolated and reported to consist of seven exons spanning 13.7 kb (242).

PlGF can exist in multiple isoforms due to alternate splicing encoded by the human PlGF gene. Four isoforms of human PlGF are described, PlGF-1, PlGF-2, PlGF-3, and PlGF-4 composed of 131, 152, 203 and 224 amino acids respectively. PlGF-1 and PlGF-2 are believed to be the major isoforms (104, 105, 121, 242, 243). Apart from in size, the PlGF isoforms differ in terms of both their secretion properties and their binding affinities. PlGF-1 and PlGF-3 are non-heparin binding diffusible isoforms while PlGF-2 and PlGF-4 have additional (highly basic 21 amino acids) heparin binding domains (105, 106, 242). Both VEGF-A and PlGF can exist as homodimers and heterodimers (PlGF:PlGF, PlGF:VEGF-A, VEGF-A:VEGF-A), with PlGF:VEGF heterodimers displaying 20-50-fold less mitogenic activity than VEGF homodimers (Figure 1) (108, 109).

Initial studies conducted in healthy mice in the 1990's reported a lack of PlGF did not appear to confer any negative impact on vascular development. However, when mice deficient in PlGF (knockout) were subjected to pathological conditions such as ischaemia, inflammation or cancer, they demonstrated severely impaired angiogenic ability. Their inability to adapt and compensate to these pathological conditions highlighted the role of PlGF in pathological angiogenesis (117).

Receptors

All members of the VEGF family bind and activate one of the following three homologous tyrosine kinase receptors: VEGFR1 (also called fms-like-tyrosine-kinase receptor/**Flt-1**) VEGFR2 (also called **Flk-1**/KDR**)** or VEGFR3. Each of these receptors has a similar structure; a tyrosine kinase extracellular seven Ig-like domain connected to an intracellular tyrosine kinase domain via a single transmembrane helix (111-113). Binding induces the mitogenic action of the cell with KDR being 10 fold stronger than Flt-1 in this regard (244, 245). VEGF-A can bind to either Flt-1 or KDR (246). Despite the structural similarity with VEGF-A, PlGF has been shown to only bind to Flt-1, but it does so with a higher affinity than VEGF-A (114). In 1996, a non-membrane bound soluble receptor known as soluble VEGFR-1 (**sFlt-1**) was identified. This endogenous protein, synthesised by placental cells amongst others, arises from alternative splicing of Flt-1. It retains structural similarity to Flt-1 except that it lacks its transmembrane helix and tyrosine kinase intracellular domain, meaning that it can circulate freely (116). Levels of sFlt-1 rise under hypoxic conditions (125). Circulating sFlt-1 binds PlGF and VEGF-A resulting in reduced levels of these

proteins available to the anchored cell membrane receptors Flt-1 and Flk-1 (Figure 1).

3.1.3 **Function**

PlGF exerts its angiogenic effects by both direct and indirect mechanisms, inducing receptor dimerisation and phosphorylation. PlGF directly activates endothelial cells, macrophages and haematopoietic progenitor cells by binding to the membrane receptor anchored Flt-1, and in doing so may increase the sensitivity of the cell to VEGF-A. PlGF acts indirectly by displacing VEGF-A from Flt-1, allowing VEGF-A to bind instead to the more potent FDK. Lastly, by forming a heterodimer with VEGF in mutually expressed cells, PlGF antagonises the angiogenic action of VEGF. (109, 117-121) sFlt-1 has anti-angiogenic potential, binding and neutralising PlGF and VEGF in the circulation, reducing their bio-availability and thus interaction with the cell membrane bound Flt-1 and Flk-1 (122, 123). In vitro effects of sFlt-1 include vasoconstriction and endothelial dysfunction (124).

Pre-eclampsia

As far back as the late 1980's it had been hypothesised that a circulating factor existed in pre-eclampsia, which was responsible for the widespread endothelial dysfunction observed (134). Vasculogenesis and angiogenesis are two essential components in development of the utero-placental circulatory interface in early pregnancy. It has been proposed that early placentation in

utero occurs in a relatively hypoxic environment with a partial pressure of oxygen as low as 18 mm. At approximately 10 weeks gestation, an increase in the partial pressure of oxygen up to 60 mm occurs, triggering the proliferation of the cytotrophoblast. This process facilitates invasion of uterine spiral arteries of the decidua and myometrium by the cytotrophoblast, allowing these vessels to become functionally capable of supplying the high volumes of well-oxygenated blood necessary for nourishing a growing fetus (247, 248). Should this rise in oxygen pressure fail to occur, the hypoxic environment persists, compromising the invasion of the cytotrophoblast. This leads to impaired placentation and results in inadequate placental perfusion, hypoxia and potential clinical fetal and maternal manifestations: impaired fetal growth and pre-eclampsia later in pregnancy (133, 135).

Studies investigating this circulating factor hypothesis reported significant damage occurring in cultured human umbilical vein endothelial cells (HUVECs) exposed to serum from pregnant women with pre-eclampsia compared with controls (133, 135, 136).

Following the discovery of PlGF and its receptors, reports of increased levels of sFlt-1 and reduced levels of PlGF and VEGF in pre-eclamptic cases compared to controls were published (128-131). Higher levels of sFlt-1 have also been reported in; first versus second pregnancies, multiples versus singletons, molar and trisomy 13 affected pregnancies, all of these being well established independent risk factors for development of pre-eclampsia (137). Patients receiving VEGF antagonists for cancer treatment may develop hypertension and proteinuria, confirming the role of VEGF/PlGF blockade in

endothelial cell dysfunction (249, 250). In the early 2000's, a number of publications reported circulating levels of sFlt-1 and PlGF to be altered several weeks before the clinical onset of disease in pre-eclampsia. They also showed correlation of these angiogenic factors with the severity of disease (87, 132). Hypoxia alone is enough to trigger sFlt-1 over expression by the placenta, in a self-defence type response, to VEGF-A produced by maternal decidual cells (140). A three stage disorder has now been proposed for pre-eclampsia. Initially in early pregnancy from approximately week 8-18 abnormal remodelling of the spiral arterioles and trophoblast invasion occurs due to a deficiency in the pro-angiogenic PlGF and VEGF (Stage 1). The net result of this is impaired placental perfusion, which in turn leads to hypoxia and oxidative damage from 20 weeks gestation (Stage 2). The pathological placenta then induces apoptosis, inflammation, and releases anti-angiogenic factors (sFlt1) and other inflammatory agents such as cytokines in a bid to induce vasoconstriction and increase oxygen supply to the hypoxic placenta. The net result of the release of these factors is systemic endothelial cell dysfunction and end-organ ischemia, which leads to the classical clinical signs and symptoms of pre-eclampsia (Stage 3) which may occur from as early as 20 weeks gestation (138, 139).

3.1.4 **Application**

In the last 15 years, the concept of using sFlt-1 or PlGF, either singly or in combination, as a potential screening tool or diagnostic marker for pre-eclampsia has been explored. Screening for pre-eclampsia appears to make

sense. The ability to stratify women in early (11-13 weeks gestation) pregnancy as high risk and appropriately tailor antenatal care has huge clinical and economic benefits. Currently a huge amount of our antenatal resources are targeted towards routine clinic visits including measurement of blood pressure and urinalysis in women at low or moderate risk for pre-eclampsia. An effective early screening test would enable those at low risk to be identified, and stratified to community based care, with less frequent hospital review. Simultaneously, the early identification of women at risk of pre-eclampsia would enable their antenatal care to be appropriately tailored and hospital resources to be focused to them. In order to be effective, a screening test must demonstrate both clinical and health economic benefits. Clinically relevant outcomes would include both maternal and neonatal morbidity. A health economic model analysis should assess the cost-effectiveness of a screening strategy relative to no screening at all (251).

The recently published ASPRE trial showed a reduction of more than 60% in rates of preterm pre-eclampsia when aspirin is commenced prior to 14 weeks in women at high risk of same (177). Identification of who exactly is high risk is paramount. The ASPRE trial screened over 25,000 women between 11-13 weeks gestation and dichotomised them to high (> 1 in 100) or low risk (<1 in 100) of preterm pre-eclampsia. The predictive model used incorporated maternal serum PlGF, pregnancy associated plasma protein–A (PAPP-A), mean arterial pressure (MAP) and uterine artery pulsatility index (UtA-PI) as well as maternal factors. The authors reported a detection rate of 76.7% for preterm pre-eclampsia with a false positive rate (FPR) of 10%. This compares to a detection rate of just 39% using the traditional approach based on

maternal characteristics and medical history alone recommended by leading international bodies The National Institute for Health and Clinical Excellence (NICE) and the Americana College of Obstetricians and Gynaecologists (ACOG). This study highlights the potential utility of PlGF as part of combined early pregnancy screening. However prior to the introduction of any screening test, both external validation and a health economic analyses are necessary, to confirm utility and reproducibility.

The main use of PlGF in pregnancy currently is in short term prediction of time to delivery in women with suspected pre-eclampsia. Having an effective diagnostic test for pre-eclampsia would eliminate protracted hospitalisations of women and allow resources to be better utilised. A systematic review in 2015 evaluated trials on placental growth factor (alone or in combination with sFlit-1) as an aid to the assessment of women with suspected pre-eclampsia (147). Four prospective (cohort) studies were identified and examined. Meta-analysis was not possible because the studies employed different outcome measures, test cut-off points and gestational periods. The PELICAN study showed that the PlGF test alone had a very high accuracy for predicting pre-eclampsia requiring delivery within 14 days for women presenting with suspected pre-eclampsia between 20 - 35 weeks of gestation. For a test cut-off <100 pg/mL, PlGF alone showed 96% sensitivity (95% CI, 89–99), 56% specificity (95% CI, 49–63), 44% positive predictive value (PPV) (95% CI, 36–52), and 98% negative predictive value (NPV) (95% CI, 93–100) (89). The PROGNOSIS study evaluated whether the sFlt-1:PlGF ratio is predictive of the short-term absence or presence of pre-eclampsia in women with suspicion of pre-eclampsia between 24 and 36+6 weeks of pregnancy. It

reported a sFlt-1:PlGF ratio ≤ 38 had a NPV in the subsequent week of 99.3% (95% confidence interval [CI] 97.9–99.9). In 2016 NICE published guidance on the use of PlGF testing based on this review. The Triage PlGF test and the Elecsys immunoassay sFlt-1:PlGF ratio, used with standard clinical assessment and subsequent clinical follow-up, were recommended to help rule-out pre-eclampsia in women presenting with suspected pre-eclampsia between 20 weeks and 34 weeks plus 6 days of gestation.

NICE recommended that these tests should not yet be used to diagnose pre-eclampsia until further research is available, specifically on how an abnormal PlGF result would affect management decisions regarding timing and gestation of delivery and the outcomes associated with this (141). Interventional studies were recommended to confirm the clinical utility of the results to date. Some smaller cohort studies (MAPPLE) with unblinded PlGF testing have reported a lowering of gestational age at delivery and an increase in neonatal prematurity related morbidity. This highlights the importance of conducting appropriately powered trials before PlGF testing is routinely adapted into clinical practice. (252) A number of randomised controlled trials are currently ongoing, the UK PARROT trial and the PARROT Ireland trial among these, with results expected in 2019 (253).

The prospect of using PlGF as a therapeutic agent has also begun to be considered. Recent studies in mice have demonstrated that administration of VEGF early in pregnancy prevents the development of pre-eclampsia (254) and reduction in circulating sFlt-1 alleviated pre-eclampsia like symptoms again in a mouse model (255). A pilot study on the safety and efficacy of

therapeutic apheresis for preterm pre-eclampsia was conducted on 11 pregnant women with pre-eclampsia ranging from 23 to 32 weeks gestation. A reduction in levels of circulating sFlt-1 was achieved with combinations of single or multiple plasma apheresis. Overall a prolongation of pregnancy without major adverse maternal or fetal consequences was seen (162). A case controlled prospective study is currently ongoing, collecting maternal plasma and serum from patients with both pre-eclampsia and normal pregnancy for in vitro validation of new therapeutics based on extra-corporal removal of sFlt-1 (APHERESE) (256). Also currently recruiting is an interventional trial of a medical apheresis device for Flt-1 in pre-eclamptic women (SAVE) (257).

These studies suggest the potential utility of early pro-angiogenic therapies in treating pre-eclampsia in the future.

Assays

A variety of different assays are now commercially available for quantification of PlGF alone or in combination with sFlt-1; Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test, The Quantikine Human PlGF Immunoassay (R&D systems) and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio. Most of these are laboratory based and require significant infrastructure available to use while the Triage PlGF test is point of care and could be easily integrated to antenatal clinical care algorithms in developing countries. Importantly to note, the normal reference values of PlGF obtained with one platform may not be interchangeable with others. Validation studies, head-to-head comparative

studies, and cost effective analyses comparing these platforms are required as the performance and costs of these may differ between assays.

Outside Pregnancy

PlGF is more than just a pregnancy specific biomarker. Although originally identified in the placenta, PlGF is also expressed in heart, lung, thyroid, adipose tissue and skeletal muscle. Its absence impairs angiogenesis and arteriogenesis during tumour growth and heart, limb and ocular ischaemia (117, 258-263). Patients with sickle cell disease are noted to have increased levels of PlGF, expressed by bone marrow erythroid cells under hypoxic conditions, with levels of PlGF correlating with degree of disease activity. An association between PlGF and β-thalassaemia has also been reported, with levels of PlGF positively correlating with other markers of haemolysis such as lactate dehydrogenase, uric acid and reticulocyte counts in this group (264-266). Whether PlGF may act as a biomarker of disease activity or have a role in potential targeted therapies in patients with haemaglobinopathies remains the subject of ongoing research (267). More recently, PlGF has been identified as a possible contributor in haematologic malignancies, with both PlGF and sFlt-1 expression increased in samples from patients with chronic myeloid leukaemia (CML), acute myeloid leukaemia (AML) and acute lymphoid leukaemia (ALL). This is in contrast to the increased PlGF and reduced sFlt-1 expression seen in patients with pre-eclampsia. The exact mechanism of action of the angiogenic PlGF and anti-angiogenic sFlt-1 in these malignancies is as yet not fully understood, but the potential of targeted anti-PlGF therapy

is being explored (268-270). A recent study on thyroid carcinoma found significantly higher levels of PlGF in metastatic disease, suggesting that antagonising PlGF in this setting may be a promising therapy to suppress cancer metastasis (271). In the pathological models studied, the absence of PlGF impairs the associated inflammation and angiogenesis and confers a general reduction in pathological changes (272).

3.1.5 **Conclusion**

PlGF plays an integral role in the development of a normal pregnancy and aberrations in PlGF concentrations are associated with adverse pregnancy outcomes, in particular pre-eclampsia. The universal integration of PlGF into antenatal assessment of women with suspected preterm pre-eclampsia is dependent on results of current RCTs. The role of PlGF as a biomarker of disease outside of pregnancy shows promise while its potential as a possible therapeutic for pre-eclampsia and some malignancies warrants further research.

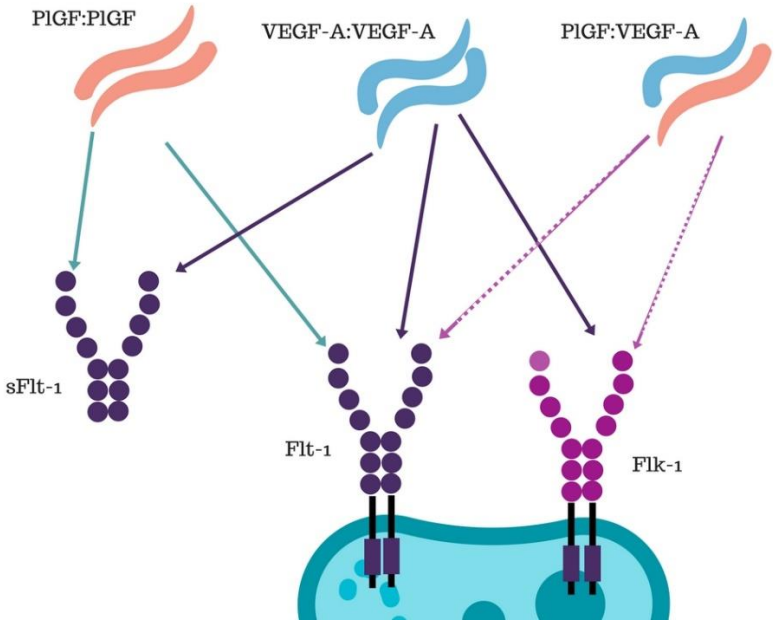

Figure 3. 1: *PlGF and VEGF hetero and homodimer protein structures and their respective membrane bound receptors Flt-1 ad Flk-1 and the freely circulating receptor sFlt-1*

Chapter 4: Placental Growth Factor and Twin Pregnancy

Paper 3: A comparative study of two immunoassays of maternal placental growth factor

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Submitted to Irish Journal of Medical Science 10th December 2019

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Paper 4: A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range

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4.1 A comparative study of two immunoassays of maternal placental growth factor

4.1.1 Abstract

Background*:* Circulating maternal levels of placental growth factor correlate well with placental function and numerous studies advocate its role to help rule-out preterm pre-eclampsia. A number of automated immunoassay platforms to quantify placental growth factor are currently available. The aim of this study was to compare the results obtained from an ELISA to an automated immunoassay of maternal placental growth factor in a twin pregnancy cohort.

Methods: Prospective study conducted in a single large tertiary maternity unit over a two year period. Consenting pregnant women with a twin pregnancy, across a variety of gestations, had a single blood sample taken at one time point only during their pregnancy. The plasma was initially biobanked and then later analysed in batches using both immunoassays.

Results: Although the placental growth factor values of the two immunoassays correlated well, the actual results obtained were significantly different. Poor concordance between the two immunoassays was present, with one assay recording 36 cases as <100 pg/ml whereas the second immunoassay identified only 4 as <100 pg/ml.

Conclusion*:* Biomarker levels may vary significantly between different immunoassay platforms, highlighting the importance of developing validated clinical cut-offs for any automated immunoassay before its clinical application.

These differences need to be understood to facilitate clinical utility given that placental growth factor testing is likely to be introduced into widespread clinical practice.

4.1.2 **Introduction**

Placental growth factor (PlGF) is an angiogenic protein and a member of the vascular endothelial growth factor (VEGF) family (96). Like all proteins of the VEGF family, PlGF is a secreted dimeric glycoprotein with a distinctive cystine knot (97). Both PlGF and VEGF can exist as homodimers or heterodimers (109). They each contain two monomers, oriented side-by-side and head-to-tail, held together by one interchain disulfide bond (98). PlGF was the second member of the VEGF family identified in 1991 by an Italian scientist Dr Maria Graziella Persico who identified it while investigating the angiogenic potential of human term placental tissue; hence the name “the placental growth factor” (95). PlGF binds to an anchored cell membrane receptor, FLT-1, inducing angiogenesis and is an important requirement for successful placentation in early pregnancy (121, 243).

Over the last number of decades, the concept of using PlGF as a potential diagnostic marker for pre-eclampsia has been extensively examined (88, 89, 132). Studies have shown that as a pregnancy progresses, circulating levels of PlGF correlate directly with placental function (129, 131). Pregnant women with low circulating levels of PlGF are at increased risk for adverse outcomes such as pre-eclampsia, HELLP syndrome, eclampsia, fetal growth restriction, and stillbirth (184).

As with any newly identified biomarker, initial studies employed laboratory based immunoassays, such as an ELISA, to quantify circulating maternal PlGF (87, 142). Over time, owing to a plethora of studies demonstrating the potential for PlGF as an adjunct to clinical care, interest in this angiogenic biomarker grew exponentially (273-275). Studies involving PlGF have now transitioned from laboratory based to a clinical setting and from observational to interventional (88, 155, 179, 188). Coinciding with this translation from laboratory to clinical, commercial interest in PlGF grew and automated immunoassay platforms that allow rapid and easy quantification of PlGF were developed (141).

The purpose of this study was to compare the results obtained from two different immunoassays of maternal placental growth factor; one a laboratory based manual assay and the other a point of care-automated assay, in a twin pregnancy cohort. We aimed to examine the similarities and differences between the immunoassays and highlight the requirements for translating a lab-based test into one appropriate for clinical utility, necessary for the development of any biomarker.

4.1.3 Methods

Setting and Design

This study was conducted in a single large maternity hospital in Ireland with over 8000 deliveries per annum. The study was a secondary sub-group analysis of samples collected as part of a prospective cross-sectional study of PlGF in twin pregnancy. Ethical approval was granted from a national research ethics committee (ECM 3 (PPP) 19/05/15). From July 2015 to December 2017, women that were over 20 weeks gestation attending the hospital's dedicated twin pregnancy clinic were approached to participate in the study. If recruited to the study, a 3ml ethylenediaminetetraacetic acid (EDTA) blood sample was taken, centrifuged, divided into aliquots and the plasma biobanked at -80C within 3 hours of sampling. All sampling, processing and biobanking was carried out within the same building. Women had venepuncture performed at one random time point only. PlGF testing is not part of routine clinical care and was not performed separately in this population.

Anonymised clinical and demographic data pertaining to the participant was recorded in the study database. Relevant clinical outcomes, such as the development of pre-eclampsia (defined as; "sustained hypertension with systolic BP ≥ 140 or diastolic BP ≥ 90 (on at least two occasions at least 4hrs apart) with significant quantified proteinuria (>300mg protein on 24hr collection or urine protein creatinine ratio >30mg/mmol or ≥ 3+ on dipstick urinalysis)" occurring after 20 weeks' gestation) were recorded from the clinical notes following delivery and discharge from hospital.

Platforms

Biobanked samples were analysed for circulating levels of PlGF using two immunoassays; the lab based Quantikine® ELISA PlGF kit (R&D Systems, USA) and the point of care Triage® PlGF Test (Quidel, San Diego). Both immunoassays were performed as per manufacturer's instructions.

The ELISA® assay uses a monoclonal antibody specific for human PlGF that has been pre-coated onto a 96 well polystyrene microplate. The colour development was measured using a Thermo Fisher VarioSkan microplate reader. All samples were analysed in duplicate using this platform. The ELISA® reports a measureable range of 15.6-1000 pg/ml of PlGF, with an assay completion time of 3.5 to 4.5 hours. The Intra-Assay Precision (coefficient of variation) on EDTA plasma controls at concentrations of 54.3 and 658 pg/mL is 7% and 5.6%, while the Inter-Assay Precision at concentrations of 55 and 724 pg/ml are 11.8% and 10.9% respectively (152).

The Triage® PlGF Test (Quidel, San Diego) is a CE marked platform and involves a single use, point of care, fluorescence immunoassay device. The lateral flow assay uses a fluorescently conjugated antibody that binds to host PlGF and is automatically analysed once the cartridge is inserted into the metre. The results are displayed on the metre screen in approximately 15 minutes and have a measureable range from 12-3000 pg/ml. The assay has a total precision on plasma controls; at concentrations of 85.2 and 1300 pg/mL is 12.8% and 13.2% (148). For the purposes of this study any result obtained <12 pg/ml was allocated the value of 10 pg/ml. Given the Triage® is a one-

step protocol, a single analyse of samples was recommended by the supplier. sufficient.

Statistics

Descriptive statistics were employed to examine the maternal demographics and PlGF distribution in the cohort. A Bland Altman plot was used to compare PlGF results and Cohen's Kappa was calculated to provide a measure of agreement between the two tests. Analysis were undertaken with SPSS Version 24 and STATA V.12.

4.1.4 **Results**

Demographics

The majority of eligible women approached to take part in the trial consented, resulting in one hundred and seventy eight women with a twin pregnancy included in this cohort study (Table 1). In Chapter 2 of this thesis, detailed demographics pertaining to the twin pregnancy population in our unit has been described. As this study was conducted among this population, a similar demographic distribution is apparent. Maternal age ranged from 20-46 years, Body Mass Index (BMI) ranged from 19-45 Kg/m^2 and almost half the group (n=81; 45.5%) were nulliparous. Ethnicity was very homogenous with the vast majority (92.1%, n=164) Caucasian. Almost a third of the group (30.1%, n=52) had conceived following some form of assisted reproductive therapy (ART).

The majority of these were through In Vitro Fertilisation (IVF) with either their own oocyte (17.4%, n=31) or through use of a donor oocyte (9%, n=16). The majority of the group (84.3%, n=150) was a dichorionic twin pregnancy. Very few concomitant medical conditions that would predispose to placental dysfunction existed within the cohort such as chronic renal disease or essential hypertension (2%, n=4). Of the multiparous women, very few had previous pregnancies complicated by intrauterine growth restriction (3.4%, n=6) or pre-eclampsia (3.9%, n=7) which would also predispose to placental dysfunction. There was good representation of each gestational age category among the cohort (Table 2).

PlGF Distribution

The distribution of PlGF using each of the two assays was first examined. The range of PlGF using the Triage® was narrower given the limits of the platform; 10-3000 pg/ml with a median of 297 pg/ml. PlGF levels using the ELISA® assay ranged from 52-4720 pg/ml with a median of 537 pg/ml.

Correlation

In laboratory analysis studies such as this, it is common to need to assess the level of agreement between two methods of measurement. Before these checks of agreement can be performed, the level of correlation between the two methods of measurement must first be assessed. Using a Spearman's rho test, a strong positive correlation was seen between the PlGF results from the

two assays examined (r=0.88, n=178, p <0.001) (Figure 4.1 a & 4.1 b). This strong correlation indicated that a PlGF result that measured low using one platform also measured low in the other platform. However, correlation studies only assess the relationship between the variables and not the differences between them. Therefore, a Bland Altman plot was used to evaluate the mean difference between the PlGF results from the two assays and estimate their level of agreement (Figure 4.2 a & 4.2 b). The Bland Altman plot identified a mean difference (238.1 pg/ml) that is clinically relevant, highlighting that the PlGF results obtained from the two assays are not interchangeable. In addition, a simple linear regression was calculated to predict the results from the Triage® based on the results from the ELISA®, b = 0.41, t(178) = 6.0, p < 0.001. Combined, these results indicate that there is proportional bias and the two assays are systematically producing different results.

Comparison

The Triage® is intended for use in women with a singleton pregnancy presenting at ≥ 20 weeks gestation with suspected preterm pre-eclampsia. Previous studies using this platform have validated a cut-off of >100 pg/ml as having a high negative predictive value for requirement for delivery due to pre-eclampsia in the subsequent 14 days (89). In our cohort, the Triage® identified 36 cases with a PlGF of <100 pg/ml whereas the ELISA® identified only four as <100 pg/ml. Concordance between the results of the two immunoassays was examined and a kappa value of 0.17 obtained, indicating a very poor level of agreement between the two assays (Table 4.3).

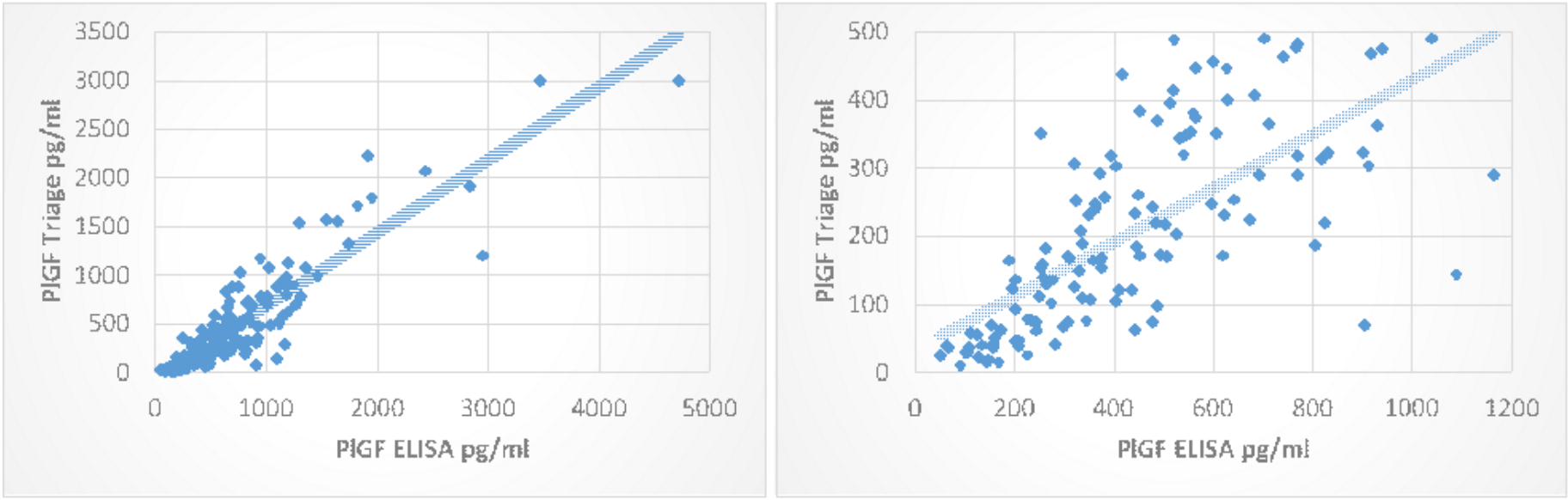

Figure 4. 1a & 4.1b: *Correlation of the PlGF results between the two assays*

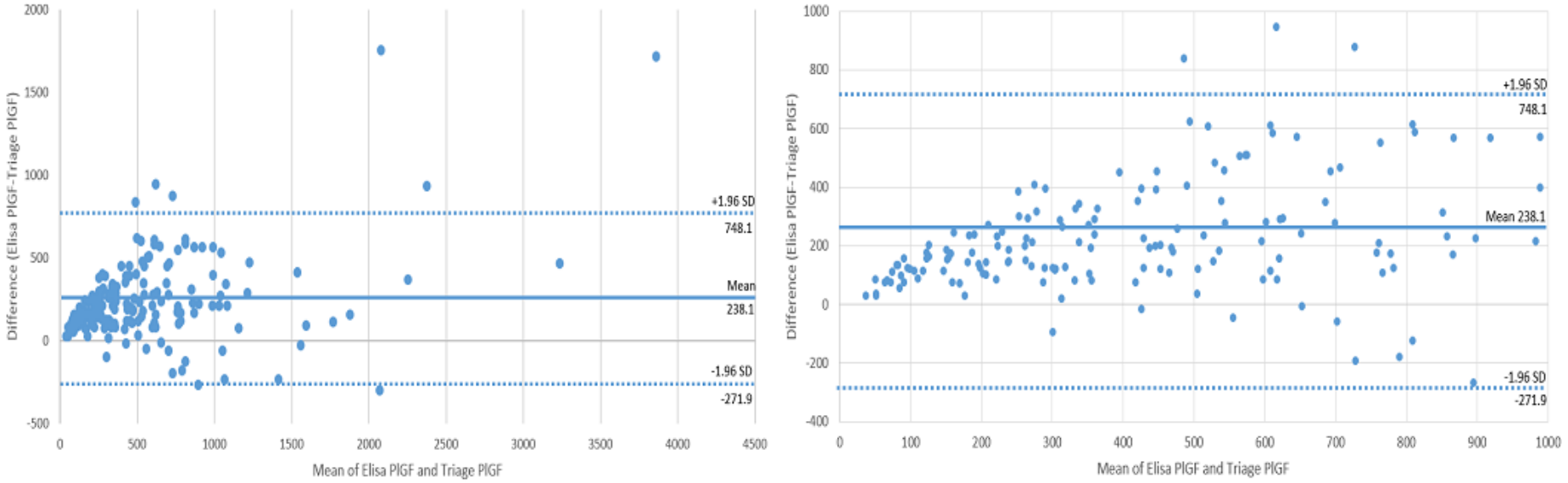

Figure 4. 2a & 4.2b: *A Bland Altman plot demonstrating contrasting patterns of agreement and proportional bias for the two platforms*

4.1.5 **Discussion**

Scientists are continuously identifying new biomarkers with potential for improving screening, diagnosis and monitoring of diseases. The journey from laboratory identification to their clinical application can be lengthy, requires prudence, cautious interpretation and suitable clinical application. This study shows that although there is good correlation between the laboratory and point of care immunoassays examined, there is a significant difference in the results in terms of both the range and in the PlGF values obtained. This crucially highlights that clinical cut-offs developed and validated using one biomarker immunoassay are not transferrable to another immunoassay for the same biomarker. A very recent publication, comparing three different automated PlGF immunoassays (Brahms®, Elecsys® and Delfia®) has also shown that PlGF values obtained on automated immunoassay platforms are manufacturer specific, not interchangeable and require separate validation (157).

The difference in PlGF values obtained may possibly be explained by the use of different antibodies in each assay and their cross reactivity with different PlGF isoforms. PlGF can exist in multiple isoforms due to alternate splicing encoded by the human PlGF gene (104). At least four different isoforms of human PlGF are known to exist, differing in the number of amino acids present (105). PlGF-1 and PlGF-2 are believed to be the major isoforms and share 88% sequence identity (106). PlGF isoforms differ in terms of their size, their secretion properties and their binding affinities (121). The manufacturers report that the Triage® (Quidel, San Diego) immunoassay predominantly measures PlGF Isoform-1 (153), while the ELISA (R&D Systems, USA) assay

detects PlGF-2 and PlGF-3 isoforms in addition to PlGF-1 (276). Other potential causes for the difference in PlGF values seen are; the variation in material used for calibrating, different matrix effects and the potential for hook effects at high concentrations.

Currently four platforms for quantification of PlGF are commercially available, with comparable negative predictive values for preterm pre-eclampsia, in singleton pregnancy (158). Automated platforms are more suitable for use in a clinical setting than a laborious plate ELISA (147, 152). A recently published randomised controlled trial of integration of PlGF testing into clinical care, has shown benefit, with a reduction in time to diagnosis of preterm pre-eclampsia as well as a subsequent reduction in maternal morbidity (188). On foot of this the National Health Service (NHS) has endorsed PlGF testing in maternity units in the UK (187). Given that PlGF testing is poised to be integrated into clinical practice, it is imperative that clinicians and stakeholders are informed and aware of the differences between automated immunoassay platforms cut-offs when considering which company and product to utilise locally.

Our study was not designed to assess clinical performance of the assays in diagnosing pre-eclampsia. Participants in our study were not recruited at a time when a clinical suspicion of pre-eclampsia was present. Our cohort included only women with a twin pregnancy. A clinically meaningful cut-off for PlGF in twin pregnancy is not yet established and likely differs substantially to that in singletons given the larger placental volumes present (90, 160, 165, 166, 168). A PlGF cut-off validated for the Triage® (was utilised in our study (89), a clinically useful cut-off for PlGF using the ELISA® has not been

established given the development of automated immunoassay platforms more suitable for clinical utility.

Important limitations of our study are the lack of inclusion of additional automated PlGF immunoassays for comparison, as advocated by NICE (141). The homogeneity of our cohort, with a primarily Caucasian population from a single centre, may be considered a minor limitation. The strengths of this study are that it includes a large number of pregnancies across a variety of gestational time points, so is a good representation of the pregnant population. Adherence in our unit to a high international biobanking standard, and specimen analysis within three years of biobanking, ensures good quality plasma samples with minimum protein denaturation (277-280).

Dichotomisation of our cohort into < or ≥ 100 pg/ml PlGF appears to demonstrate a better performance of the Triage® compared to the ELISA® , in terms of prediction of subsequent pre-eclampsia. However, given a cut-off specific to the Triage® was utilised, it further illustrates the importance of using an appropriate validated cut-off specific to the immunoassay rather than superiority of the Triage® over the ELISA®. Communication of this key point is an essential component of translational research (281-283).

4.1.6 **Conclusion**

This study highlights the variation that may exist in PlGF levels between immunoassay platforms. Appropriate clinical cut-offs must be developed and validated for each automated immunoassay to facilitate clinical utility. In order

to preserve research integrity, the transition from laboratory to clinical use of biomarkers needs to be appropriate by both scientists and clinicians, with recognition given to differences in biomarker assays and specific cut-offs.

Table 4. 1: *Maternal Demographics of the Cohort (n=178)*

Maternal Age	20 to 46 years (median 34.1)
BMI	19-45 Kg/m^2 (median 25.4)
Nulliparous	45.5% (n=81)
Caucasian	92.1% (n=164)
Assisted conception	30.1% (n=52)
Method of Assisted Conception	
Ovulation Induction	1.1% (n=2)
Intra Uterine Insemination	1.7% (n=3)
In Vitro Fertilisation with own oocyte	17.4% (n=31)
In Vitro Fertilisation with donor oocyte	9.2% (n=16)
Maternal Chronic Renal Disease or Chronic Hypertension	2.3% (n=4)
History of IUGR* in a previous pregnancy (multiparous women only, n=97)	6.2% (n=6)
History of PET in a previous pregnancy** (multiparous women only n=97)	7.2% (n=7)
Dichorionic Placenta	84.3% (n=150)

*IUGR; Intrauterine Growth Restriction, **PET; Pre-eclampsia

Table 4. 2: *Gestational age at sampling*

Gestational Age Interval at Sampling (weeks)	n (%)	Mean Gestational Age (weeks)
20-20+6	21 (11.8%)	20
21-24+6	56 (31.5%)	21
25-28+6	35 (19.7%)	27
29-32+6	38 (21.3%)	31
33-36+6	28 (15.7%)	35

Table 4. 3: *Concordance of PlGF between the two platforms*

		Diagnosis using ELISA		Total
		<100 pg/ml	**≥100 pg/ml**	
Diagnosis using Triage®	**<100 pg/ml**	4	32	36
	≥100 pg/ml	0	142	142
Total		4	174	178
Kappa = 0.17 p-value < 0.001				

4.2 A prospective study of placental growth factor in twin pregnancy and development of a dichorionic twin pregnancy specific reference range

4.2.1 Abstract

Introduction: Circulating maternal levels of placental growth factor (PlGF) correlate well with placental function and used as an adjunct, aid the diagnosis of preterm pre-eclampsia. Current reference values were constructed from singleton pregnancy cohorts. Given the larger placental volume present in a twin pregnancy, separate reference ranges are required. The aim of this study was twofold; to develop a dichorionic twin pregnancy specific reference range for placental growth factor, and to compare gestational specific placental growth factor levels in twin pregnancies later complicated by pre-eclampsia, hypertensive disorder of pregnancy or fetal growth restriction to controls.

Methods*:* Prospective study conducted in a single large tertiary maternity unit over a two year period. Consenting pregnant women, across a variety of gestations, had a single blood sample taken at one time point only during their pregnancy. The plasma was initially biobanked and PlGF was measured later in batches using the point of care Triage® PlGF test.

Results: PlGF levels in uncomplicated dichorionic twin pregnancies were significantly lower in the women who later developed pre-eclampsia than in the controls at all gestational intervals. In those that later developed any HDP, median PlGF was lower only in those recruited before 24 weeks' gestation

while in infants with a customised birthweight below the 3rd centile, PlGF was lower only in those sampled after 24 weeks' gestation.

Conclusion: PlGF levels in twin pregnancy differ significantly between those women with a pregnancy that will later be complicated by pre-eclampsia and those that will not. This difference is present many weeks before clinical signs or symptoms of disease are present. Using cross sectional values from uncomplicated twin pregnancies, we have developed a dichorionic twin pregnancy specific reference range for PlGF.

4.2.2 **Introduction**

Pre-eclampsia is a common complication of pregnancy characterised by new onset hypertension and either proteinuria or other maternal organ dysfunction after 20 weeks' gestation (4). Along with other hypertensive disorders of pregnancy (HDP), it is a major contributor to maternal and neonatal morbidity and mortality (13, 27). Potentially serious maternal morbidity may arise in the form of seizures, cerebral haemorrhage, renal failure, liver rupture and disseminated intravascular coagulation (17). The only definitive treatment for pre-eclampsia is removal of the placenta, often resulting in iatrogenic preterm delivery and subsequent fetal morbidity (284). Women with a twin pregnancy are at a two to three fold increased risk of developing pre-eclampsia, possibly due to a combination of larger placental mass and use of assisted reproductive therapy (ART), especially use of non-autologous gametes (35, 161, 285). Rates of twin pregnancy have risen over the last number of decades globally (48, 51, 54)

Although the exact aetiology of pre-eclampsia is not fully understood, a growing body of evidence suggests that an imbalance of angiogenic factors of placental origin play a crucial role in its development (128, 286-289). Placental growth factor (PlGF) is an angiogenic protein and a member of the vascular endothelial growth factor family (95). Studies in singleton pregnancies have shown lowered levels of PlGF and increased levels of its soluble receptor sFlt-1 in maternal plasma, weeks prior to the clinical onset of pre-eclampsia (87, 132). The UK National Institute for Clinical Excellence (NICE) advocates PlGF testing, combined with routine clinical care, to help rule out preterm pre-eclampsia in singleton pregnancies (141). A number of international randomised control trials (RCTs) are currently on-going, investigating the clinical impact of the integration of PlGF into clinical care pathways (253). The first of these, the UK PARROT study, demonstrated a reduction in time taken to diagnosis pre-eclampsia and reduced maternal morbidity when PlGF is integrated into clinical care algorithms (290).

Few studies to date have evaluated the levels of circulating angiogenic factors during twin pregnancy. In those that have been described, huge variations exist in; the primary outcome (i.e pre-eclampsia, fetal growth restriction or other adverse clinical outcome); the definition/classification of the primary outcome; the gestational age at time of sampling; and the immunoassay used for quantification. (73, 164, 167-173). The aim of this study was twofold; to develop a dichorionic twin pregnancy specific reference range for PlGF and secondly to compare gestational specific PlGF levels in twin pregnancies complicated by pre-eclampsia, any hypertensive disorder of pregnancy (HDP) or fetal growth restriction to controls.

4.2.3 Materials & Methods

Setting and Design

This study was conducted in a single maternity hospital in Ireland with over 8000 deliveries per annum. The study was a prospective cross-sectional cohort study of PlGF in twin pregnancy. From the start of July 2015 to the end of December 2017, women attending the hospital's dedicated twin pregnancy clinic were approached to participate in the study. Any woman with an uncomplicated twin pregnancy from 12+0-36+6 weeks' gestation inclusive, without signs/symptoms or a diagnosis of pre-eclampsia was eligible for inclusion. Those with complications such as a known congenital anomaly in either baby, severe early onset growth restriction or twin-to-twin transfusion syndrome (TTTS) were excluded from recruitment. Following informed patient consent, a 3ml ethylenediaminetetraacetic acid (EDTA) blood sample was taken, centrifuged, divided into aliquots and the plasma biobanked at -80C within 3 hours of sampling. All sampling, processing and biobanking was carried out within the same building according to previously published Standard Operating Procedures (SOPs) (291). Women had venepuncture performed at one random gestational time point only. Clinically relevant outcome data such as the diagnosis of any HDP (chronic hypertension, gestational hypertension, pre-eclampsia or superimposed pre-eclampsia) and infant birthweights were taken from medical notes following delivery. Anonymised clinical and demographic data pertaining to the participant and their offspring were recorded in the study database. For our study, the NICE definitions of hypertensive disorders of pregnancy were utilised (3).Fetal

growth restriction was calculated based on actual birthweight, gestation at birth, fetal gender and maternal ethnicity, parity and BMI using the Gestation Related Optimal Weight (GROW) centile calculator (292).

Placental Growth Factor Immunoassay

Biobanked plasma samples were analysed in batches for circulating levels of PlGF using a point of care immunoassay; the Triage® PlGF test (Quidel Inc., San Diego). This test is not routinely available in the hospital for clinical use. It was purchased by our research centre for the purpose of this study. The test manufacturers had no part in the study design, conduct, analysis or manuscript development. The immunoassay was performed as per manufacturer's instructions, in a single freeze thaw cycle to minimise protein denaturation. The results are displayed on the meter screen in approximately 15 minutes and have a measurable range from 12-3000 pg/ml. The Triage® has a reported measurable range from 12-3000 pg/ml. The manufacturers report total precision on plasma controls at concentrations of 85.2 and 1300 pg/mL as 12.8% and 13.2% respectively. For the purposes of this study, any result obtained <12 pg/ml was allocated the value of 10 pg/ml.

Statistics

SPSS Version 23 and Stata 15 were used to analyse the data.

Part 1: Descriptive statistics were employed to examine the baseline maternal demographics, clinical outcomes and the PlGF distribution in the cohort.

Initially all abnormal cases as well as all monochorionic twin pregnancies were removed, in order to facilitate development of a reference range for PlGF in an uncomplicated dichorionic twin pregnancy. Abnormal cases included women where a stillbirth was diagnosed in either of the twins, as well as women who later developed any form of HDP, or women who developed fetal growth restriction resulting in both twins having a customised birthweight of less than the 3rd centile. The remaining women were divided into five groups dependent on weeks' gestational age at recruitment; 12-20^{+6}, 21-24^{+6}, 25-28^{+6}, 29-32^{+6} and 33-36^{+6}. PlGF ranges for each gestational group were calculated. Log PIGF was modelled as a function of gestational age at recruitment using restricted cubic spline regression with heterogeneous variance. Results were used to estimate the 5th and 95th centile of PIGF as a function of gestational age to develop a reference range for PlGF in uncomplicated dichorionic twin pregnancies.

Part 2: To examine the effect of hypertensive disorders and placental dysfunction on PlGF, the entire cohort including abnormal cases, was divided into 2 groups based on the woman's gestational age at time of her enrolment to the study and hence sampling of maternal plasma PlGF; <24 weeks' gestation and ≥ 24 weeks' gestation. This gestational cut-off was employed as pregnancy related hypertensive complications are unusual prior to this timepoint and also it equated well with the median of the cohort. The groups were stratified by presence of pre-eclampsia, HDP or customised fetal birthweight <3rd centile and PIGF level in the two groups were compared using a non-parametric Wilcoxon rank-sum test. A proportional odds models, with robust variance, was used to compare PIGF levels in two groups after

adjusting for ART, oocyte donation and maternal age >35. Twins were analysed as clusters in the multivariate models.

Ethics

Ethical approval was granted from a national research ethics committee (ECM 3 (PPP) 19/05/15).

4.2.4 **Results**

In total, 275 women with a twin pregnancy were recruited. There were no withdrawals or losses to follow up. Three women (1% of the cohort) had a stillbirth occur in one of the twins while in 4.7% (n=12) of women, an anomaly of one or both twins was diagnosed. Given with twin pregnancy there is differing placental volumes present dependent on chorionicity, circulating levels of PlGF may also vary in line with chorionicity. We found that PlGF was lower in monochorionic twin pregnancy (data not shown) but given the high incidence of complications as well as the small numbers present (n=40) in this subgroup, further analysis was not possible. We limited our analysis to dichorionic cases only for development of the reference range.

Part 1:

Reference Range Demographics

Removal of those with an abnormal pregnancy outcome (pre-eclampsia or HDP in the mother, stillbirth of either twin or where both twins had a customised birthweight of <3rd centile at delivery), or a monochorionic pregnancy left 173 women with an uneventful dichorionic twin pregnancy for inclusion in the reference range analysis (Figure 1: Flowchart). Median maternal age was 34 years, booking BMI was <30Kg/m^2 for the majority (81.5%; n=141) and most were Caucasian (93.6%; n=162). Over half of the group were multiparous (56.1%; n=97), just over a third (35.1.1%; n=60) had conceived the twin pregnancy with use of ART. All women with pre-existing renal disease or essential hypertension developed superimposed pre-eclampsia in their pregnancies and hence were not included in the reference range cohort (Table 4.4). Comparison of participant characteristics between each gestational group showed no significant difference in enrolment characteristics (Table 4.5).

Reference Range Development

The distributions of PlGF concentrations and the 5th-95th centiles within each gestational age (GA) interval were calculated (Table 4.6). With progressing gestational age the median PlGF was seen to rise, simultaneously to the development and maturation of the placentae, and then steadily decrease towards term. In the GA intervals studied, median PlGF concentration peaked in the 25-28+6 gestation interval. (Figure 4.4). Using quantile regression

analysis, the lowest acceptable PlGF value for each gestational age was calculated and is presented. These data provide a valid reference range for PlGF in a normal dichorionic twin pregnancy (Figure 4.4). Removal of those women where both twins had a customised birthweight <3rd centile did not alter the reference range significantly (Figure 4.5).

Part 2:

Comparison of Gestational PlGF

The second aim of this study was to compare gestational PlGF in twin pregnancies complicated by pre-eclampsia, HDP or customised birthweight of both twins <3rd centile, to controls. To this end, the entire cohort (n=275) was divided into 2 groups based on the woman's gestational age at time of her enrolment to the study and hence gestational age at time of sampling of maternal plasma PlGF; <24 weeks' gestation and ≥ 24 weeks' gestation. Just under half the cohort (43.6%; n=120) were recruited at <24 weeks' gestation with the remainder (56.4%, n=155) recruited at ≥ 24 gestational weeks. The groups were then stratified by presence of pre-eclampsia, HDP or customised birthweight <3rd centile for both infants.

Demographics of Entire Cohort

The maternal age of the study group ranged from 20 to 50 years, with 134 women (48.7%) aged >35 years at booking (Table 4.7). The majority of the cohort had a Body Mass Index (BMI) of <30Kg/m^2 at booking (78.9%; n=217)

and were of Caucasian ethnicity (93.8%; n=258). Just under half the cohort were nulliparous (46.9%; n=129). The majority of the group were dichorionic twin pregnancies (81.5%; n=224) and approximately two thirds (65.5%; n=180) of the population studied had conceived the twin pregnancy spontaneously. Where assisted reproductive therapy (ART) was utilised, almost a fifth (17.1%; n=47) had conceived through the assistance of In Vitro Fertilisation (IVF) and a large proportion of these using a donor oocyte (12%; n=33). There was a small number of women with pre-existing renal disease or hypertension (1.8%; n=5). The two gestational groups were well matched, with no differences seen in BMI <30, ethnicity, parity or chorionicity. However, there were significantly more women with ART assisted pregnancies (40.2%; n=48 v 27.8%; n=42, p=0.04), oocyte donation (18.5%; n=22 v 7.3%; n=11, p=0.009) and those with a maternal age >35 years (57.5%; n=69 v 41.9%; n=65, p=0.01) sampled in the <24 weeks' gestational group compared to the ≥ 24 weeks group (Table 4.7).

Clinical Outcomes

Overall, the incidence of a subsequent diagnosis of HDP was 15.3% (n=42) and 11.3% (n=31) developed pre-eclampsia (Table 4.8). Of the 532 infants with maternal BMI information available, 11.8% (n=65) who had a customised birthweight <3rd centile with both twins <3rd customised birthweight in eleven cases. Gestation at delivery ranged from 23 to 38 weeks' gestation, with two thirds of the cohort delivered via Caesarean section (66.5%, n=183). Preterm delivery at <35 weeks occurred in almost a fifth of the cohort (17.8%, n=49)

and in over half of cases was iatrogenic (59.2%, n=20). Preterm delivery at <32 weeks was less common (6.9%, n=19), and again half of cases were iatrogenic (47.4%, n=9). There were no significant differences between the two gestational groups in terms of incidence of HDP or pre-eclampsia, nor were there any differences in preterm delivery or mode of delivery.

Comparison of PlGF

The median PlGF was 230.5 pg/mL when sampling occurred at <24 weeks and 276 pg/mL when sampling was ≥24 weeks. Following stratification by subsequent diagnosis of pre-eclampsia, HDP or customised birthweight <3rd centile in both twins, a Wilcoxon Rank Sum test revealed PlGF levels were significantly lower in the women who later developed pre-eclampsia than in the controls *(153 pg/ml vs. 247 pg/ml, p = 0.04, 99.8 pg/ml vs. 304 pg/m, p = 0.01)* independent of gestation at sampling. Adjusting for the higher incidence of ART, oocyte donation and maternal age >35 in this group, the association between PlGF and the later development of pre-eclampsia remained significant in the ≥24 group only. In those that subsequently developed any HDP, PlGF was lower in the <24 weeks group only (*150 pg/ml vs 250 pg/ml, p = 0.02)* and was unaffected by adjustment for confounders. In those that subsequently had either twin born at a birthweight <3rd customised centile, PlGF was only lower in the group recruited >24 weeks (170 pg/ml vs 304 pg/ml, p=0.04), and again was unaffected by adjustment for con-founders (Tables 4.12, 4.13 & 4.14).

4.2.5 **Discussion**

This study shows that maternal plasma PlGF in twin pregnancy follows the same gestational pattern as described in singletons (126, 127); a steady rise corresponding with development of the placenta, peaking slightly earlier at approximately 28 weeks' gestation, and then declining thereafter. It also shows that maternal plasma PlGF is significantly lower in twin pregnancies that will later develop pre-eclampsia but not other HDP, independent of gestational age at time of sampling of PlGF, compared to controls.

To our knowledge, this is the largest prospective study of PlGF in twin pregnancy from a single site. This allows us to describe the twin pregnancy specific distribution of gestational PlGF, as well as develop a dichorionic specific reference range for PlGF in twin pregnancy, which has not been previously described. This is also the only study to date examining PlGF in twin pregnancies specifically using the Triage® PlGF test. The Triage® PlGF test is currently the only point of care test on the market for measuring PlGF, is CE marked and has been endorsed by NICE for use in further research (141).

Previous studies of angiogenic factors in twin pregnancy have had limited numbers of participants, varied gestations at quantification, varied outcome measures and often involve pooled results from a number of sites or countries across a variety of time periods (160, 165, 166). Often these studies require shipment of specimens to laboratories in other countries, which may affect the quality of samples. In contrast, all of the laboratory analysis in our study was

performed on site, by a single researcher, in a single freeze thaw cycle, to minimise the chance of protein denaturation.

A Spanish study in 2011 examined first trimester levels of circulating angiogenic factors in 61 women with a twin pregnancy (165). Using a R&D systems immunoassay, they reported higher serum concentrations of both PlGF and sFlt-1 in twins compared to matched singletons. They also reported maternal serum sFlt-1 levels were higher in twin pregnancies conceived through ART compared to spontaneous twin conceptions, supporting the well-accepted concept that ART pregnancies are at increased risk of pre-eclampsia development.

A study from Boston in 2012 (160) described 79 women with a twin pregnancy presenting with suspected pre-eclampsia in the third trimester. Serum PlGF and sFlt-1 from the women was quantified using the Roche Elecsys immunoassay Ratio test. The outcome measure utilised was the diagnosis of an adverse clinical event in the subsequent fortnight, of which 52 women met the criteria. The authors reported median PlGF was significantly reduced, while median sFlt-1 was elevated in those that did develop an adverse event indicating that these angiogenic factors have potential utility as prognostic indicators in twin pregnancies with suspected pre-eclampsia.

A German group in 2014 published on a small cohort of 49 women with a twin pregnancy, 18 of which developed pre-eclampsia. Maternal serum PlGF and sFlt-1 was quantified again using the Roche Elecsys immunoassay Ratio test. The researchers reported PlGF levels were decreased and sFlt-1 levels increased in the pre-eclampsia cases at time of presentation with pre-

eclampsia symptoms compared to the twin controls, indicating the potential for integration of angiogenic factors into clinical care pathways for investigation of suspected pre-eclampsia in twin pregnancy (166).

Clearly, potential exists for use of PlGF and sFlt-1 as biomarkers for prediction of pre-eclampsia in twin pregnancies. However, before these biomarkers are introduced into clinical use for twins, it is important that relevant cut-offs are developed and validated specifically for this group. Several large prospective observational studies have published on clinically relevant cut-offs for use in singletons. The PROGNOSIS study, using the Roche Elecsys immunoassay Ratio test in 550 women with suspected pre-eclampsia, reported a sFlt-1:PlGF ratio of ≤38 as having a negative predictive value for pre-eclampsia in singletons in the subsequent 7 days of 99.3% (88). The PELICAN study, using the Triage® PlGF test in 625 women with suspected pre-eclampsia, reported a PlGF of >100 pg/ml as having a 98% negative predictive value for pre-eclampsia in the subsequent 14 days in singletons presenting at < 35 weeks' gestation (89).

A 2018 Dutch study compared PlGF and sFlt-1 levels in normotensive and pre-eclamptic singleton and twin pregnancies using the Roche Elecsys immunoassay Ratio test (181). Numbers were small, with only 22 twin pregnancies included. Again, differences in serum sFlt-1 and PlGF levels were noted in the normotensive twins compared to the matched singletons and in the pre-eclamptic twin cases compared to the twin controls. Importantly, they demonstrated that the previously defined sFlt-1/PlGF ratio cut-off of ≤38 for predicting short-term absence of pre-eclampsia in singleton pregnancies is not applicable to twin pregnancies. Importantly this demonstrates that established

reference ranges for PlGF/sFlt-1 in singletons are not transferrable to twin or higher order multiple pregnancies. This highlights the need for quality prospective observational studies of women with twin pregnancy presenting with suspected pre-eclampsia, in order to develop and validate clinically useful cut-offs for PlGF/sFlt-1 in twins.

We recognise there are limitations to our study specifically the use of a customised birthweight centile not specific to twin pregnancy and the exclusion of cases where only both twins were <3rd customised centile. This choice was pragmatic given our numbers however we recognise that reduced placental volume in either twin may affect the circulating maternal PlGF levels. Normal twin growth patterns are the subject of much debate with differing opinion as to which is the most appropriate growth curve to use in clinical practice (293, 294). Concerns exist that twin specific growth charts, adjusted to reflect the smallness of twins compared to singletons, may not identify growth restricted twins with underlying placental pathology, thereby resulting in increased perinatal morbidity (295). There is no consensus as to whether fetal growth charts should be customised by factors such as ethnicity, height, weight and parity or not and there is also no agreement regarding which is the most appropriate growth calculator to use (296-301).

A second limitation of the study was single sampling of participants. Serial sampling of maternal PlGF may have provided a much more robust, informative account of PlGF distribution. However, it would have deterred many women from and given that participation was truly altruistic, a single timepoint only approach was adopted. Our population is largely homogenous; white Caucasian and non-obese, which potentially limits extrapolation to

minority ethnic groups. Although a large number of women with a twin pregnancy were enrolled, we do not have sufficient power at present to develop a monochorionic twin pregnancy specific reference range, although it would be possible to expand on the study and add to our numbers in the future to achieve this. An additional limitation of our study is the use of only one automated commercial platform for quantification of PlGF rather than on multiple commercially available platforms such as the DELFIA Xpress PlGF 1-2-3 test, Brahms Kryptor and the Roche Elecsys ratio test, as advocated by NICE (141). Comparative studies performed in singleton pregnancies have shown similar performance of all three platforms in ability to rule out pre-eclampsia (158). As sufficient plasma remains biobanked in our site, this is an area for potential future research subject to funding and ethical approval.

4.2.6 **Conclusion**

We have shown that PlGF levels in twin pregnancy differ significantly between those pregnancies that later will be complicated by pre-eclampsia and those that will not. This difference is present many weeks before clinical signs or symptoms of disease are present. We provide a valid overall reference range for PlGF in a normal twin pregnancy and specifically in a normal dichorionic twin pregnancy. With further research, PlGF has potential as an adjunct to clinical care as a predictor of evolving pre-eclampsia and/or adverse clinical outcomes in twin pregnancy.

Table 4. 4: *Demographics of the dichorionic reference range cohort of participants at recruitment (n=173)*

Patient Characteristics at Recruitment (n=173)	**Mean (SD) / % (n)**
Age (years)	34.0 +/- 4.9
BMI < 30 (Kg/M^2)	81.5 (141)
Caucasian Ethnicity	93.6 (162)
Multiparous	56.1 (97)
Fertility Assisted Conception	35.1 (60)
Ovulation Induction	1.7 (3)
Intra Uterine Insemination	1.7 (3)
In Vitro Fertilisation	20.2 (35)
In Vitro Fertilisation with Oocyte Donation	11.0 (19)
Maternal Morbidity at Recruitment	
Pre-existing Renal Disease or Essential Hypertension	0 (0)
Previous PE[A]	2.9 (5)
Previous IUGR[B]	3.5 (6)

[A]Pre-eclampsia

[B]Intra-Uterine Growth Restriction

Table 4. 5: *Characteristics of reference range cohort of participants at recruitment divided by gestational age interval (n=173)*

Patient Characteristics at Recruitment	Total Cohort (n=173)	12-20+6 (n=35)	21-24+6 (n=40)	25-28+6 (n=42)	29-32+6 (n=35)	33-36+6 (n=21)
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Age (years) mean (SD)	34.0 +/- 4.9	36.1 +/-4.9	33.4+/-5.5	34.2 +/- 4.6	32.9 +/-4.9	33.4 +/- 4.1
BMI < 30 (Kg/M^2)	81.5 (141)	76.5 (26)	87.5 (35)	87.8 (36)	85.3 (29)	78.9 (15)
Causasian Ethnicity	93.6 (162)	97.1 (34)	95.0 (38)	97.6 (41)	82.9 (29)	95.2 (20)
Multiparous	56.1 (97)	57.1 (20)	50.0 (20)	50 (21)	68.6 (24)	57.1 (12)
Fertility Assisted Conception	34.7 (60)	42.9 (15)	35.0 (14)	40.5 (17)	22.9 (8)	28.6 (6)
Previous PE[A]	2.9 (5)	2.9 (1)	-	-	8.6 (3)	4.8 (1)
Previous IUGR[B]	3.5 (6)	2.9 (1)	-	-	5.7 (2)	14.3 (3)

[A]Pre-eclampsia [B]Intra-Uterine Growth Restriction

Table 4. 6: *Normal Reference Range percentiles of PlGF by gestational age interval quantified using the Triage® PlGF test (n=173)*

Gestational Age Interval (weeks)	Number (n)	Mean Gestational Age	Percentile of PlGF (pg/mL)						
			5th	10th	25th	50th	75th	90th	95th
12-20+6	35	17.2	11	14.9	57.2	154	260	556.80	748.6
21-24+6	40	21.9	69.3	139.3	257.8	410.0	773.3	1016.9.8	1177.0
25-28+6	42	26.9	67.5	138.6	278.3	501.0	1072.5	1717.0	2250.5
29-32+6	35	30.8	34.2	46.2	102	305	708	1426	1972
33-36+6	21	34.8	26.1	32.2	56.6	72.4	150.5	335.2	669.7

Table 4. 7: *Demographics of entire cohort of participants at recruitment (n=275) [A] Pre-eclampsia [B] Intra-uterine Growth Restriction*

Patient Characteristics at enrolment	**Entire Cohort** *(n=275)* **% (n)**	**Gestation at recruitment (< 24weeks)** *(n=120)* **% (n)**	**Gestation at recruitment (≥ 24 weeks)** *(n=155)* **% (n)**	**p-value**
Maternal Age Range (years)	20-50	20-50	21-50	
Median Age	34	35	33	
Maternal Age ≥ 35	48.7 (134)	57.5 (69)	41.9 (65)	**0.01**
BMI < 30 (Kg/M^2)	78.9 (217)	74.1 (89)	85.3 (128)	0.1
Causasian Ethnicity	93.8 (258)	95 (114)	92.9 (144)	0.64
Multiparous	53.1 (146)	51.7 (62)	54.2 (84)	0.76
Fertility Assisted Conception	32.7 (90)	40 (48)	27.8 (42)	**0.04**
IVF	17.1 (47)	19.2 (23)	15.5 (24)	0.52
IVF with Egg Donation	12 (33)	18.5 (22)	7.3 (11)	**0.009**
Maternal Co-Morbidities:				
Renal Disease or Essential HTN	1.8 (5)	-	-	
Previous PE[A]	3.3 (9)	1.7 (2)	4.5 (7)	0.33
Previous IUGR[B]	3.3 (9)	1.7 (2)	4.5 (7)	0.33
Chorionicity				
Dichorionic	81.5 (224)	81.7 (98)	81.3 (126)	1.00
Monochorionic	18.5 (51)	18.3 (22)	18.7 (29)	

Table 4. 8: *Clinical Outcomes of entire group and subdivided by gestation at recruitment of <24 weeks or ≥ 24 weeks (n=275)*

Clinical Outcomes	Entire Cohort *(n=275)* % (n)	Gestation at recruitment (< 24weeks) *(n=120)* % (n)	Gestation at recruitment (≥ 24 weeks) *(n=155)* % (n)	p-value
HDP[A]	15.3 (42)	17.5 (21)	13.5 (21)	0.46
PE[B]	11.3 (31)	12.5 (15)	10.3 (16)	0.70
Delivery Gestation Range (weeks)	23-38	23-38	28-38	-
Delivery Type				
Caesarean	66.5 (183)	65.8 (79)	67.1 (104)	0.92
Vaginal	33.5 (92)	34.2 (41)	32.9 (51)	
Delivery <35 weeks	17.8 (49)	18.3 (22)	17.4 (27)	0.97
Spontaneous	3.6 (10)	6.6 (8)	7.7 (12)	
Iatrogenic	3.2 (9)	11.6 (14)	9.7 (15)	0.77
Delivery <32 weeks	6.9 (19)	10 (12)	4.5 (7)	0.12
Spontaneous	3.6 (10)	5 (6)	2.6 (4)	
Iatrogenic	3.2 (9)	5 (6)	1.9 (3)	1

[A] Hypertensive Disorder of Pregnancy [B] Pre-eclampsia

Table 4. 9: *PlGF by gestational group at enrolment in twin pregnancies complicated by [A] Pre-eclampsia (de novo or superimposed) compared to those that were not, quantified using the Triage® PlGF test (n=275)*

Gestation at recruitment (weeks)	**Median** (IQR) **PlGF pg/mL (n=275)**	**Median** (IQR) **PlGF PE[A] present pg/mL (n=31)**	**Median** (IQR) **PlGF PE not present pg/mL (n=244)**	**p-value[1]**	**p-value[2]**
<24	230.5 (79.4-437.8)	153 (54-224)	247 (81-489)	*0.01*	0.06
≥24	276 (71.6-577)	99.8 (24-273)	304 (73-652)	*0.02*	*0.03*

1. based on a Wilcoxon Rank Sum test
2. based on a proportional odds model that adjusted for ART, oocyte donation and maternal age >35.

Table 4. 10: *PlGF by gestational group at enrolment in twin pregnancies complicated by [B] Hypertensive Disorder of Pregnancy compared to those that were not, quantified using the Triage® PlGF test (n=275)*

Gestation at recruitment (weeks)	Median (IQR) PlGF pg/mL (n=275)	Median (IQR) PlGF HDPB present pg/mL (n=42)	Median (IQR) PlGF HDP not present pg/mL (n=233)	p-value1	p-value2
<24	230.5 (79.4-437.8)	150 (45-229)	250 (84-490)	0.001	0.03
≥24	276 (71.6-577)	123 (32-425)	304 (73-598)	0.09	0.09

1. based on a Wilcoxon Rank Sum test

based on a proportional odds model that adjusted for ART, oocyte donation and maternal age >35.

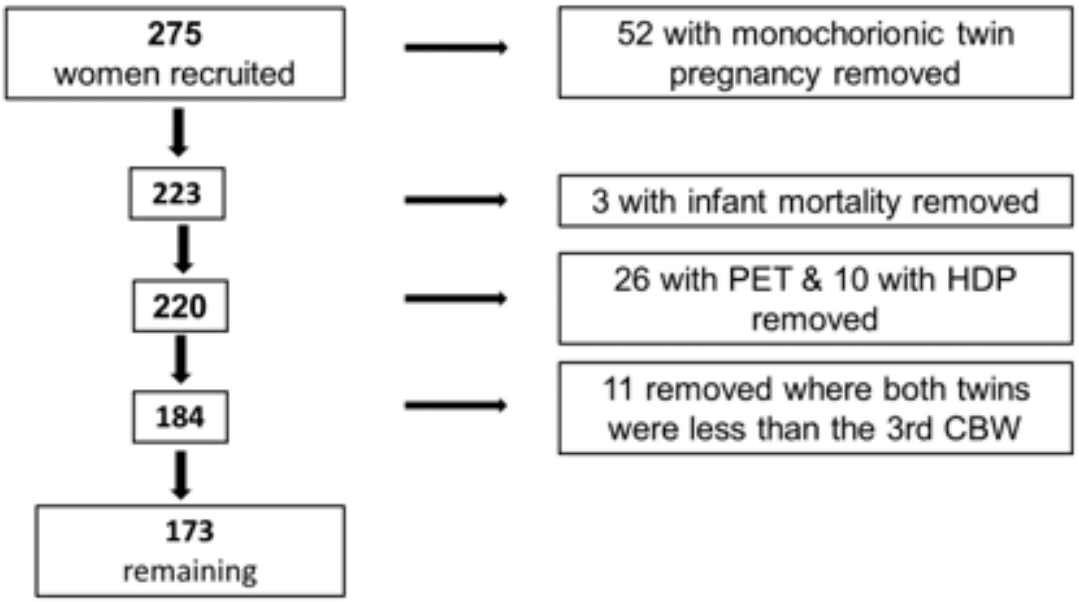

Figure 4. 3: *Flowchart of recruited women*

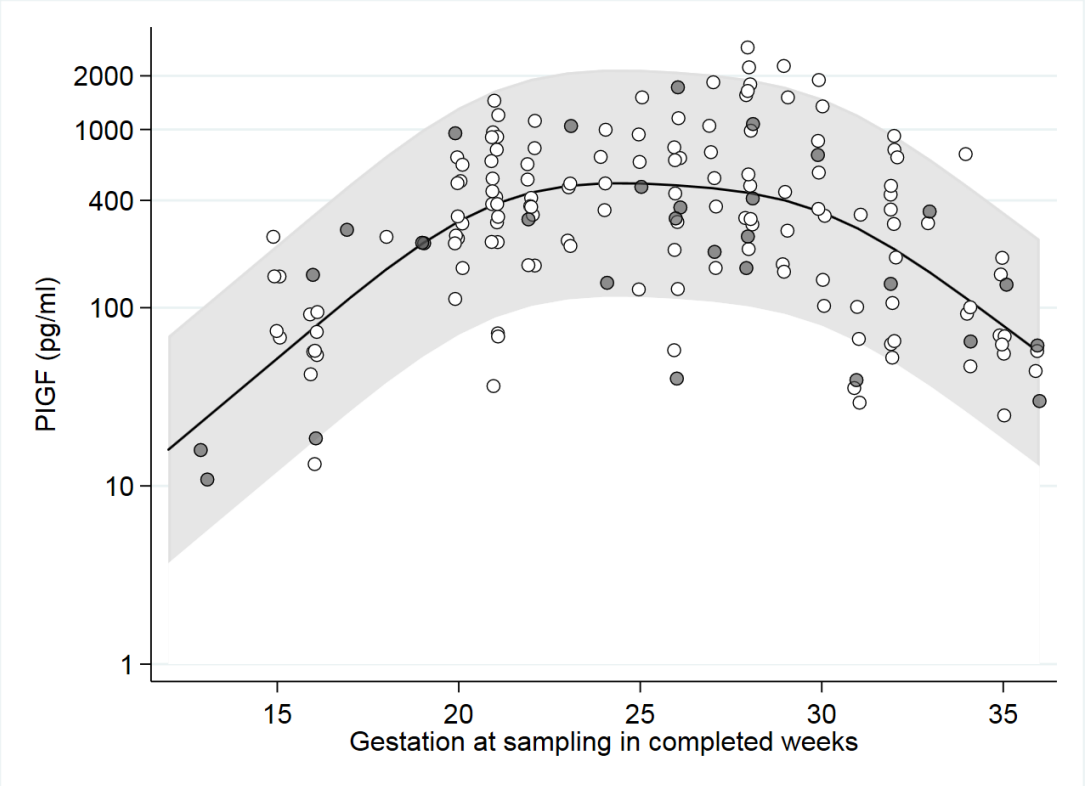

Figure 4. 4: *Scatter plot of gestational PlGF for the uncomplicated dichorionic twin pregnancy cohort. Shaded area represents the reference range from the 5th to 95th percentiles.* ***Grey dots represent the cases where birthweight is <3rd customised centile.***

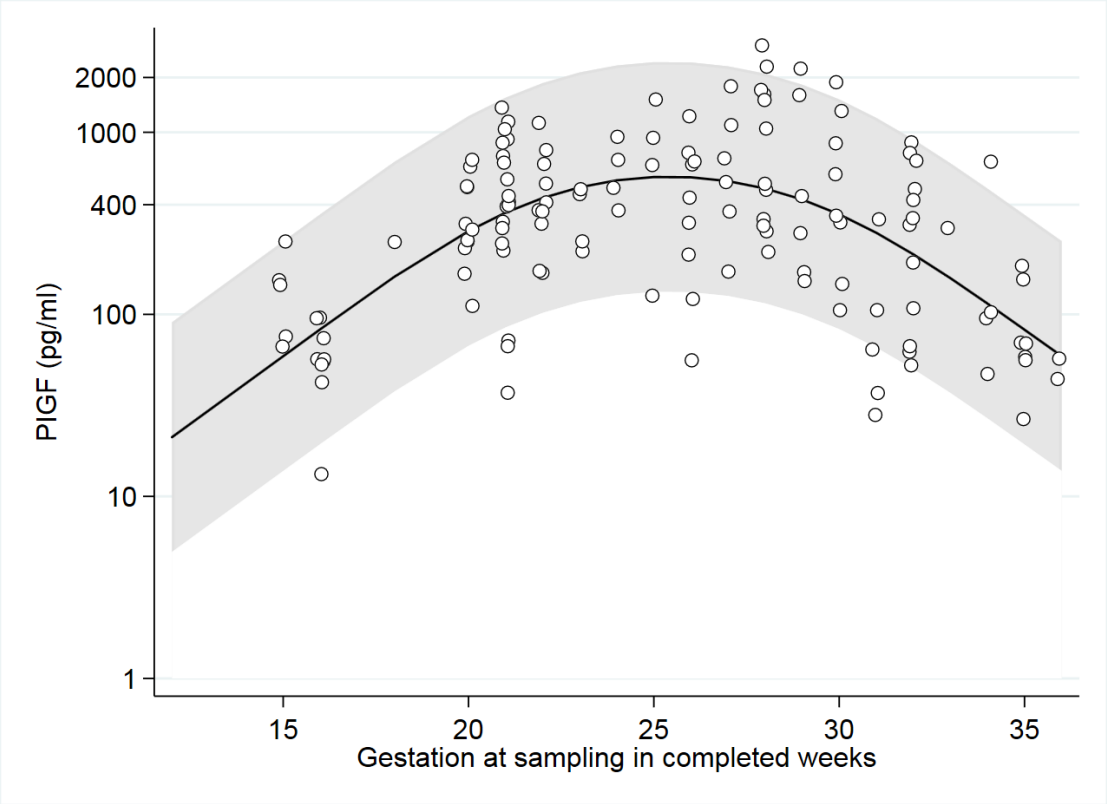

Figure 4. 5: *Scatter plot of gestational PlGF for the uncomplicated dichorionic twin pregnancy cohort without those with a customised birthweight <3rd centile (n=173). Shaded area represents the reference range from the 5th to 95th percentiles.*

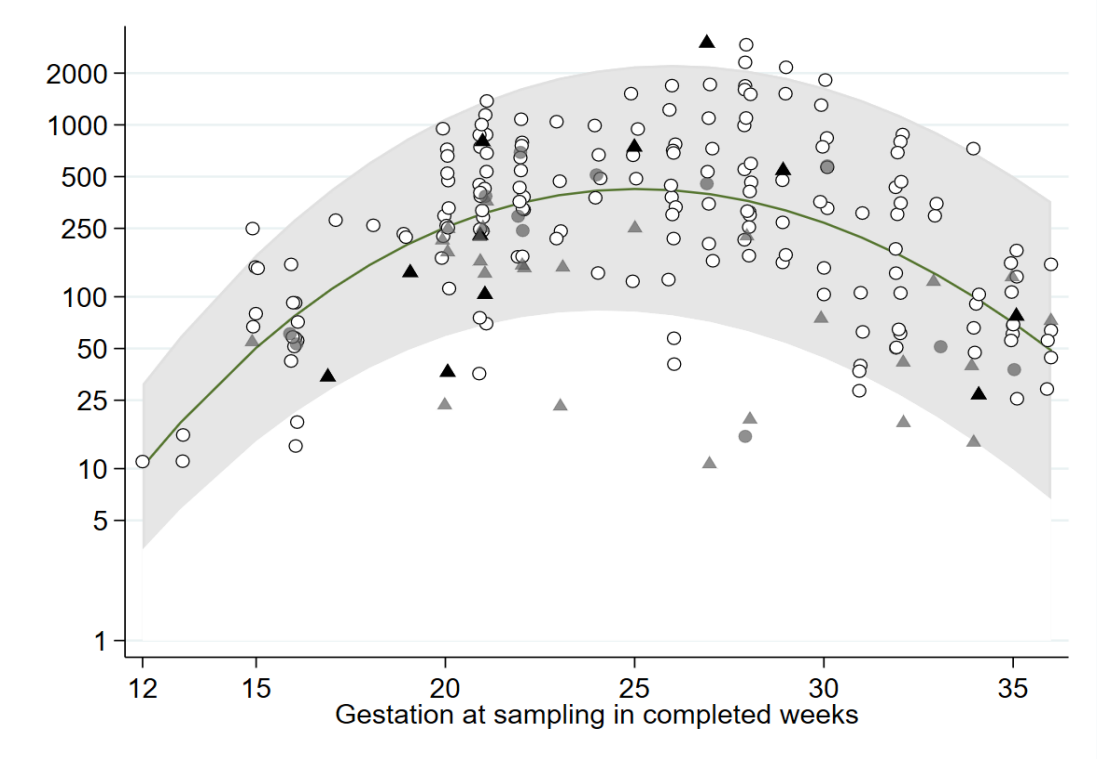

Figure 4.6: *Scatter plot of gestational PlGF. Shaded area represents the reference range from the 5th to 95th percentiles (n=222) ○ uncomplicated dichorionic twin pregnancy cohort, ▲HDP and PET present, ▲ HDP present, ● both neonates <3rd CBW*

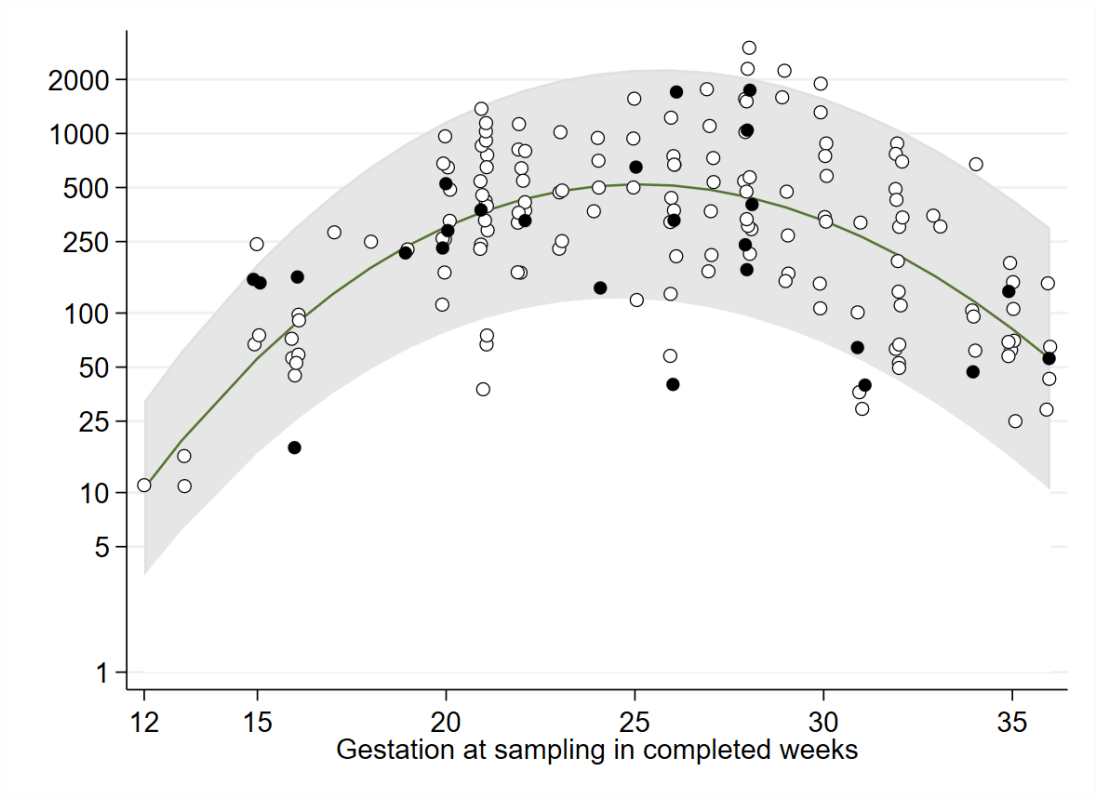

Figure 4.7: *Scatter plot of gestational PlGF in dichorionic twin pregnancy. Shaded area represents the reference range from the 5th to 95th percentiles ○ uncomplicated dichorionic twin pregnancy cohort (n=147) ● growth discordance of >20% EFW (n=25, with smaller twin having EFW <10th centile)*

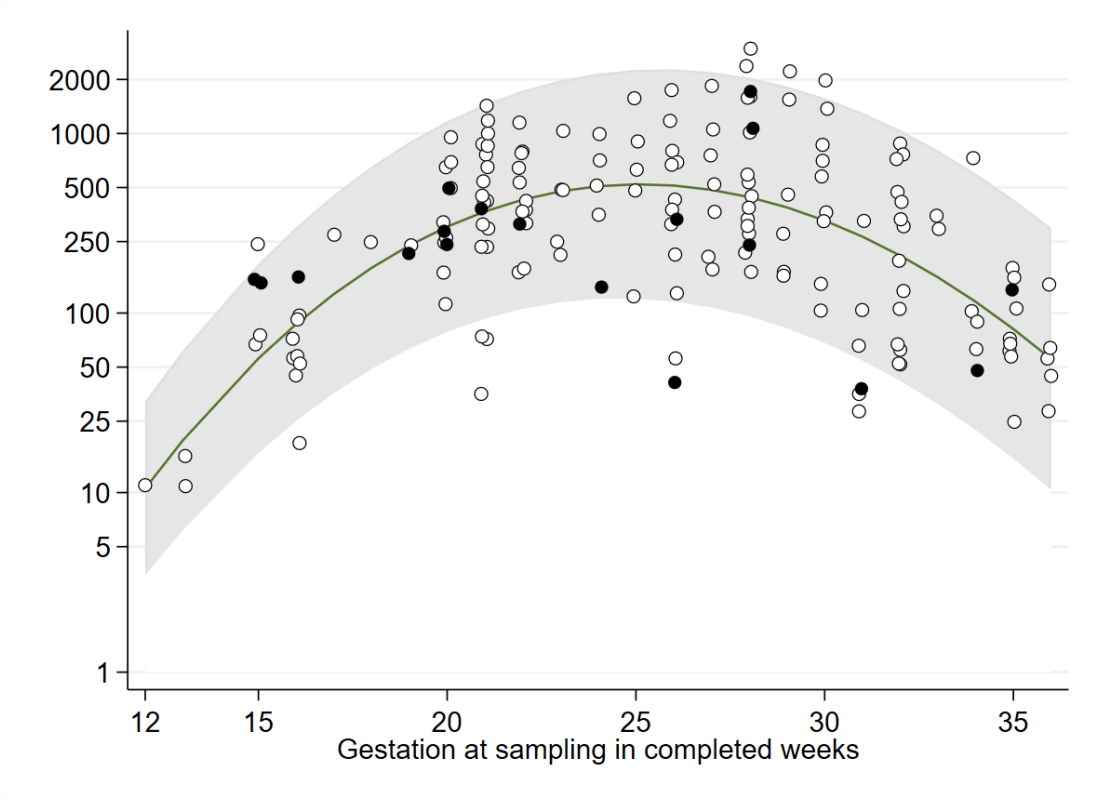

Figure 4.8: *Scatter plot of gestational PlGF in dichorionic twin pregnancy. Shaded area represents the reference range from the 5th to 95th percentiles ○ uncomplicated dichorionic twin pregnancy cohort (n=154) ● growth restriction of >25% EFW (n=15, with smaller twin having EFW <10th centile)*

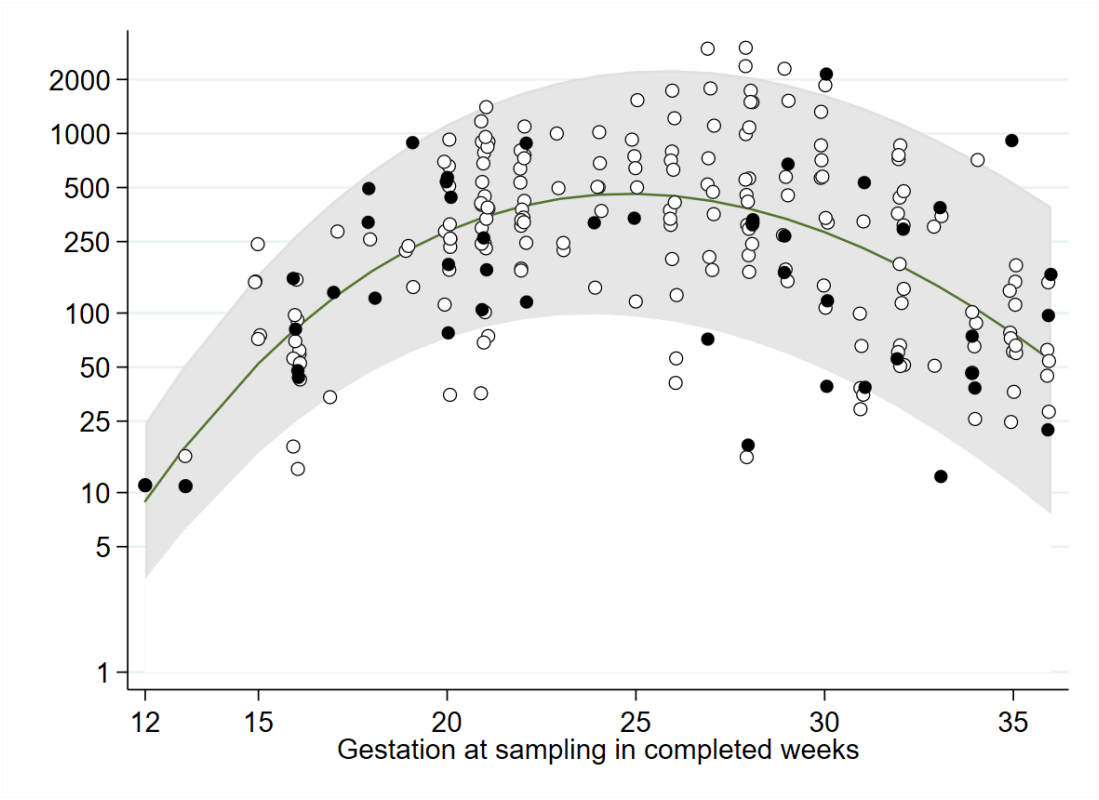

Figure 4.9: *Scatter plot of gestational PlGF. Shaded area represents the reference range from the 5th to 95th percentiles ○ uncomplicated dichorionic twin pregnancy cohort (n=195) ● uncomplicated monochorionic twin pregnancy cohort (n=46)*

Chapter 5: PARROT Ireland

Paper 5: PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial Research Study Protocol

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Paper 6: PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: Results of Interim Analysis

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Submitted in confidence to the Data Monitoring Committee of PARROT Ireland 10th December 2018

5.1 PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial Research Study Protocol

5.1.1 Abstract

Introduction

Women presenting with suspected pre-eclampsia are currently triaged on the basis of hypertension and dipstick proteinuria. This may result in significant false positive and negative diagnoses resulting in increased morbidity or unnecessary intervention. Recent data suggests that placental growth factor testing may be a useful adjunct in the management of women presenting with preterm pre-eclampsia. The primary objective of this trial is to determine if the addition of placental growth factor testing to the current clinical assessment of women with suspected preterm pre-eclampsia, is beneficial for both mothers and babies.

Methods and Analysis

This is a multicentre, stepped wedge cluster, randomised trial aiming to recruit 4000 women presenting with symptoms suggestive of preterm pre-eclampsia between 20 and 36+6 weeks' gestation. The intervention of an unblinded point of care test, performed at enrolment, will quantify maternal levels of circulating

plasma placental growth factor. The intervention will be rolled out sequentially, based on randomisation, in the seven largest maternity units on the island of Ireland. Primary outcome is a composite outcome of maternal morbidity (derived from the modified fullPIERS model). To ensure we are not reducing maternal morbidity at the expense of earlier delivery and worse neonatal outcomes, we have established a co-primary outcome which will examine the effect of the intervention on neonatal morbidity, assessed using a composite neonatal score. Secondary analyses will examine further clinical outcomes (such as mode of delivery, antenatal detection of growth restriction and use of antihypertensive agents) as well as a health economic analysis, of incorporation of placental growth factor testing into routine care.

5.1.2 **Introduction**

Pre-eclampsia is characterised by hypertension and proteinuria, complicates 2-8% of pregnancies, and is associated with significant maternal and neonatal morbidity and mortality (17). Currently women who present with suspected pre-eclampsia are triaged on the basis of hypertension and dipstick proteinuria. Both of these clinical endpoints are subject to observer error and poor test accuracy, with false positive and negative diagnoses of pre-eclampsia occurring in clinical practice (302-305) Current biochemical tests are imperfect at stratifying women for more intensive surveillance as they only identify advanced disease where there is already marked end-organ damage (17). While biomarkers and imaging techniques have been evaluated for

improving detection, none have adequate sensitivity and/or specificity for the diagnosis of pre-eclampsia (306).

Placental growth factor (PlGF) belongs to the vascular endothelial growth factor (VEGF) family and represents a key regulator of angiogenic events in pathological conditions (95). PlGF exerts its biological function through the binding and activation of the receptor Flt-1 (111, 244). In pre-eclampsia, it is thought that endothelial dysfunction leads to an increased level of a circulating decoy receptor, known as soluble Flt-1, (sFlt-1), a soluble receptor for both vascular endothelial growth factor type A (VEGF-A) and PlGF (133). Circulating levels of sFlt-1 are increased in pre-eclampsia and particularly in the early onset form of the disease, resulting in reduced levels of free VEGF-A and PlGF in the maternal circulation. Thus, the endothelial dysfunction observed in pre-eclampsia may be due to excess neutralisation of VEGF-A and PlGF by circulating sFlt-1. Levine et al. showed that in normal pregnancy, PlGF levels track the development of the placenta, peaking at about 32 weeks' gestation when the placenta is developed fully and then declining until delivery (87). However, in pre-eclampsia, this rise and fall is considerably lower throughout pregnancy, and levels are strikingly lower when the condition presents clinically.

The PELICAN study was the first and largest prospective evaluation of PlGF in women presenting with suspected pre-eclampsia (89). This blinded observational cohort study was conducted in seven consultant-led maternity units in the UK and Ireland between January 2011 and February 2012. It enrolled women being investigated for suspected pre-eclampsia, quantified their plasma PlGF using a point of care device, the Alere Triage PlGF test ®,

but did not reveal the result to their clinician. The study found that a PlGF value <100 pg/ml, in women presenting prior to 35 completed weeks' gestation had a negative predictive value of 98% (95% CI, 93 to 99.5) and a positive predictive value of 44% (95% CI, 36 to 52) in determining those that would require delivery for a confirmed diagnosis of pre-eclampsia within the next 14 days. The study reported a PlGF <100 pg/ml to be a better predictor than all other current commonly used predictive tests of pre-eclampsia, either singly or in combination (blood pressure, urinalysis or biochemical markers) with an area under the ROC curve for low PlGF of 0.87 compared to 0.76 for the next best predictor.

The PROGNOSIS study was a prospective, multicentre, blinded, observational study conducted in 14 countries from 2011 to 2014 (88). Its aim was to derive and validate a ratio of serum sFlt-1 to PlGF that would be predictive of the absence or presence of pre-eclampsia in the short term. It included women with singleton pregnancies from 24 weeks to 36+6 weeks' gestation in whom a clinical suspicion of pre-eclampsia existed. The Elecsys immunoassay was used to quantify levels of PlGF and sFlt-1. The development cohort of over 500 participants identified a sFlt-1:PlGF ratio of 38 as having an important predictive value. The subsequent validation cohort, again with over 500 participants, reported a negative predictive value of 99.3% (95% CI 97.9–99.9) for ruling out pre-eclampsia within one week. Interestingly, the same cut off of 38 was predictive of the absence of fetal adverse outcomes within 1 week; negative predictive value of 99.3% [95% CI, 97.9 to 99.9]. The study showed that an sFlt-1: PlGF ratio of 38 or lower can be used to predict the short-term absence of pre-eclampsia and adverse fetal events in women

in whom the syndrome is suspected clinically (88). The positive predictive value; a diagnosis of pre-eclampsia, eclampsia, or the HELLP syndrome within 4 weeks, was 36.7% (95% CI, 28.4 to 45.7) using the same sFlt-1: PlGF ratio of 38. Post hoc analysis however showed this was still an improvement in prediction compared to the use of clinical variables such as blood pressure and urinalysis alone.

NICE (The National Institute for Health and Clinical Excellence, UK) has recently published guidance on incorporation of PlGF testing, in addition to clinical assessment, in women presenting with suspected pre-eclampsia from 20-34^{+6} weeks' gestation. It advises that the Triage PlGF test or Elecsys immunoassay sFlt-1/PlGF ratio test may be used, in combination with clinical assessment, to "rule-out" pre-eclampsia in this group of women. However, it advises that these tests should not yet be used to diagnose pre-eclampsia until further research is available, specifically on how an abnormal PlGF result would affect management decisions regarding timing and gestation of delivery and the outcomes associated with this (141).

The objective of this randomised trial is to evaluate the impact of knowledge of PlGF measurement on clinically relevant outcomes. We hypothesise that adding PlGF measurement to current clinical assessment of women with suspected pre-eclampsia prior to 37 weeks' gestation will reduce associated maternal morbidity through improved risk stratification, earlier diagnosis and targeted management of women with the disease. Any intervention in late pregnancy may have an impact on the fetus. On the one hand, earlier diagnosis of pre-eclampsia may precipitate earlier delivery and lead to an increase in neonatal morbidity and mortality secondary to iatrogenic

prematurity. Conversely, improved identification of those neonates at highest risk of imminent placental dysfunction may reduce neonatal morbidity by allowing for timely intervention. It is therefore imperative that full evaluation of both potential benefit and harm is conducted before PlGF testing is implemented routinely into clinical practice. If this trial demonstrates a beneficial impact on maternal morbidity and/or neonatal morbidity, alongside a favourable health economic assessment, then there would be a strong case for incorporating PlGF testing into routine clinical investigations for women presenting with suspected pre-eclampsia before 37 weeks' gestation in a wide variety of healthcare settings.

5.1.3 **Methods**

Study Design

PARROT Ireland is a multi-centre, stepped wedge cluster-controlled trial of PlGF measurement in women presenting with suspected pre-eclampsia from 20 weeks and prior to 37 weeks' gestation. As implementation of a diagnostic test may alter physician management, a cluster design was chosen rather than individual randomisation. This allows for a change in management to occur at a hospital rather than at an individual woman level, which is preferable in trials involving a diagnostic test and allows the clinical influence of the additional test to be evaluated in a pragmatic fashion (192). Each maternity hospital acts as a cluster. All clusters commenced the trial in the control arm and in turn, each cluster transitions at random from the control to the intervention at pre-specified time points. Once a cluster has changed over to the intervention, it

continues as such for the remainder of the trial so that by the end of the trial all clusters will be in the intervention arm (Figure 5.1). A stepped wedge design was chosen so as to increase the social acceptability of the trial to the 7 hospitals (the stake holders /decision makers in all of the hospitals expressed a desire to participate in a trial in which they were guaranteed to get the intervention); and because a trial with just 7 clusters risks baseline imbalance in a parallel design.

The trial will continue for a period of twenty-two months, and with seven clusters the interval between transitions is approximately three months in duration. A restricted method of randomisation was used to provide a balance in total (expected) number of observations across intervention and control periods (details below) (307-309). There is a short transition period of one week whenever a new cluster transitions from control to the intervention. Data collected during this transition period will not be included in any analysis of outcomes. Recruitment will stop on a pre-specified fixed date in late April 2019 and the study will end when the last recruited participant and neonate are discharged and all outcome data collected.

Setting & Participants

The trial is being conducted within the Health Research Board Mother and Baby Clinical Trial Network Collaborative. The Coombe Women and Infants University Hospital Dublin, Cork University Maternity Hospital, University Maternity Hospital Limerick, The Royal Jubilee Maternity Hospital Belfast, University College Hospital Galway, The National Maternity Hospital Dublin

and The Rotunda Maternity Hospital Dublin are the seven largest consultant-led maternity units on the island of Ireland. Combined, they have over 44,000 births annually, representing over half of the country's total annual births. Women attending these maternity units who present with suspected preterm pre-eclampsia are eligible for inclusion in this trial. Detailed inclusion and exclusion criteria are described (Table 5.1 & 5.2).

Table 5. 1: *Inclusion Criteria for Parrot Ireland*

Pregnant women between 20+0 and 36+6 weeks of gestation (inclusive) with a;

- Singleton pregnancy
  - Aged 18 years or over
  - Able to give informed consent.
  - Presenting with suspected pre-eclampsia: (one or more of the following).
  - Hypertension
  - Dipstick proteinuria
  - Headache
  - Visual disturbances
  - Epigastric or right upper quadrant pain
  - Increasing oedema
  - Suspected fetal growth restriction

If the healthcare provider deems hat the woman requires further valuation for possible pre-eclampsia

Table 5. 2: *Exclusion Criteria for PARROT Ireland*

Confirmed pre-eclampsia at point of enrolment;

"sustained hypertension with systolic BP ≥ 140 or diastolic BP ≥ 90 on at least two occasions at least 4hrs apart) with significant quantified proteinuria (>300mg protein on 24hr collection or urine protein creatinine ratio >30mg/mmol) or abnormal pre-eclampsia bloods"

- ≥37 weeks gestation
- Multiple pregnancy
- Abnormal pre-eclampsia bloods (new onset reduced number of platelets or deranged liver function/renal function tests, identified during routine care prior to enrolment and not attributable to anything other than pre-eclampsia).
- Decision regarding imminent delivery already made
- Lethal fetal abnormality present
- Previous participation in PELICAN trial in a prior pregnancy
- Participation in a conflicting trial at the same time as PARROT Ireland.
- Plan to use off protocol PIGF testing.

Randomisation

The trial statisticians for the study developed a randomisation sequence for site transition from control to intervention; however, the order of site transitioning is concealed from sites and principal investigators until 12 weeks prior to the sites transition date. An allocation sequence was randomly selected (i.e. a cross-over order for the 7 clusters) from a set of random sequences constrained so that the sum of the total cluster sizes in the intervention status was similar to the total sum of the cluster sizes in the control status. Similar was defined to be a difference in the total sums exposed to intervention and control statuses being no different than the expected middle 25th percentile range of differences. To implement this, 10,000 simulations of possible (unique) allocation sequences were performed. From this, the difference in number exposed to intervention and control for each sequence was determined. An allocation sequence was then selected at random from those falling within the middle 25th percentile range of differences (307, 308, 310).

Control

Eligible women are approached and provided with detailed information about the trial, both verbally and written, by a trained researcher. Eligibility is determined by review of symptoms and signs at the time of presentation to the maternity hospital by the local researcher. Participants are not aware of their maternity hospitals current randomisation prior to their enrolment on the trial. Informed consent is obtained in accordance with ICH - GCP guidelines (311).

Once an eligible woman has given written informed consent for inclusion in the study, her maternity hospitals current group allocation is revealed (Figure 5.2). Participants enrolled in the control arm receive usual hospital care as per National guidelines; these are Health Service Executive/Institute of Obstetrics and Gynaecology Irish guidelines for those in the Republic or the NICE guidelines for those in Northern Ireland (Figure 5.3a and 5.3b) (3, 14). Eligible women who are approached but who decline to participate in the trial will continue to receive usual hospital care.

Intervention

Participants enrolled in the intervention arm have their plasma PlGF quantified in addition to routine hospital investigations. The PlGF result is made immediately available to the participants clinical team and documented clearly in the participant's medical notes. A suggested further management algorithm is provided to the clinician based on both the degree of hypertension present and the PlGF result. (Figure 5.4). This algorithm advocates increased frequency of review for those participants identified as having an abnormal PlGF result. The final decision regarding frequency of review remains with the treating clinician. If 4 weeks or more pass and the participant re-presents with symptoms suggestive of pre-eclampsia, a repeat PlGF quantification may be performed as long as the inclusion/exclusion criteria are still satisfied. In certain sites the option of plasma Biobanking will be available. Participants will be consented separately for this. For those who give consent, a portion of the specimen taken will be used to measure the level of PlGF in the plasma and

the remainder of the sample will be stored in University College Cork Biobanking facility.

PlGF Quantification

Maternal plasma PlGF quantification is performed on an ethylenediaminetetraacetic acid (EDTA) venous blood sample obtained in the standard fashion. Plasma is obtained through centrifugation and the sample is then processed immediately using a CE marked validated point of care platform; the automated Triage® Meterpro (ALERE San Diego, CA). Each hospital has the necessary equipment in situ and appropriately trained researchers in place, to perform this test as per manufacturer's guidelines. The PlGF measurement is reported as the absolute value in pg/ml within 30 minutes of commencing processing of the sample. All samples taken will be analysed without delay by the researcher after venepuncture has occurred and in accordance with manufacturers instructions. The Triage© PlGF test platform and consumables necessary to perform testing are brought to the cluster just at the point of transition to intervention. It is therefore not available at site for use while the site is in the control arm.

Patient and Public Involvement

Patients/ public were not involved in the development of this trial.

Outcome Measure

Primary Outcome Measure

To evaluate if the intervention is beneficial to both women and their babies and more importantly to ensure it is not harmful to either, the study has two equally important co-primary outcome measures. These are maternal morbidity and neonatal morbidity. For maternal morbidity assessment, an adaption of the fullPIERS score is used (Table 5.3). The definition of hepatic dysfunction is based on ALT rather than INR, requirement for ICU admission is included as well as the presences of severe hypertension. Severe systolic hypertension is an independent risk factor for stroke in pregnancy and in high resource settings uncontrolled hypertension is the main cause of death in women with pre-eclampsia. (312-314) The interval from diagnosis of pre-eclampsia to delivery is not a suitable outcome measure to use, as we are aware that knowledge of PlGF result may alter clinician management and expedite delivery (252). For neonatal morbidity assessment, babies are dichotomised into having or not having identified neonatal morbidity by means of a composite neonatal score (Table 5.4). In order to avoid subjectivity in the diagnosis of morbidity, the majority of components of the neonatal composite score are objective measures; pH < 7.2, positive cultures, admission to NICU. We acknowledge that some subjectivity can arise with staging of disease

hence why all stages of each disease will be captured and will comprise the composite outcome; NEC Stage 1-3, IVH Grade 1-4 and ROP Stage 1-5. Neonatal outcomes and morbidity will be captured from local case note review, as documented by the treating neonatologist. In cases where any uncertainty is present, the researcher will discuss the case with the local PI and or the trial clinical fellow and a consensus will be reached.

Table 5. 3: *Components of the Maternal Morbidity Composite Score*

- Confirmed placental abruption
- Intensive Care Admission
- CNS compromise; Generalized tonic clonic seizure due to eclampsia, GCS <13, cerebral haemorrhage/ infarct, cortical blindness, retinal detachment, Transient ischaemic attack, reversible ischaemic neurological deficit
- Cardiorespiratory compromise; myocardial ischaemia/ infarction, SpO2 <90%, >50% FiO2 for >1hr, intubation (other than for Caesarean section), pulmonary oedema, need for positive inotrope support
- Haematological compromise; transfusion of any blood product, platelet count <100 x 109/l;
- Liver compromise; hepatic dysfunction (ALT or AST >70 IU/L, haematoma, rupture;
- Kidney compromise; acute renal insufficiency (creatinine >150 micromol/l); hemodialysis
- Severe hypertension (systolic BP ≥ 160 mmHg on at least one occasion)

Table 5. 4: *Components of the Neonatal Morbidity Composite Score*

- Perinatal death or death before hospital discharge
- NICU admission for ≥48 hrs.
- Birthweight ≤ 5th customised centile*
- Apgar score <7 at 5 minutes
- Umbilical artery acidosis at birth (cord pH <7.2)
- Admission to neonatal unit
- Respiratory distress syndrome
- Interventricular haemorrhage
- Retinopathy of prematurity
- Confirmed infection (confirmed on blood or CSF cultures)
- Necrotising enterocolitis

*Customised birth weight at delivery is calculated using the GROW centile

Secondary outcome measure

Secondary outcomes include each component of the primary outcome reported individually as well as further maternal and neonatal assessments such as mode of delivery and use of antihypertensive agents (Table 5.5 & 5.6). Fetal growth restriction, identified on antenatal ultrasound, has been included as a secondary outcome measure of neonatal morbidity. As PlGF correlates well with placental dysfunction it may be able to differentiate between those babies with pathological growth restriction rather than constitutional growth restriction and hence improve neonatal outcomes.

Table 5. 5: *Secondary Outcomes -Maternal*

- Final diagnosis of hypertensive disorder of pregnancy (*Chronic HTN, Gestational HTN or pre-eclampsia*)
- Gestation at diagnosis of pre-eclampsia
- use of 1 or more antihypertensive drugs
- Instrumental Delivery (V*entouse or Forceps*)
- Severe hypertension (systolic BP ≥ 160 mmHg on at least one occasion)
- Maternal morbidity by fullPIERS model
    - Confirmed placental abruption
    - Intensive care admission
    - Central Nervous System Compromise
    - Cardiorespiratory Compromise
    - Haematological Compromise
    - Liver Compromise
    - Kidney Compromise
- Progression to severe pre-eclampsia as defined by ACOG practice bulletin
    - Systolic BP ≥ 160mmHG or diastolic BP ≥ 110mmHG on 2 occasions at least 4 hours apart while the patient is on bed rest (unless antihypertensive therapy is initiated before this time)
    - Thrombocytopenia (Platelet count <100 x 109/L)
    - Impaired liver function as indicated by abnormally elevated blood concentrations of liver enzymes (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by an alternative diagnoses, or both.
    - Progressive renal insufficiency (serum creatinine concentration greater than 1.1mg/dL (150 µmol/L) or a doubling of the serum creatinine concentration in the absence of other renal disease)
    - Pulmonary oedema
    - New onset cerebral or visual disturbances
- Elective delivery: induction of labour or Caesarean section
- Caesarean section: emergency and elective

Table 5. 6: *Secondary Outcomes -Neonatal*

- Fetal growth restriction identified on antenatal ultrasound*

(Estimated Fetal Weight and/or abdominal circumference <10th customised centile, abnormality in umbilical artery doppler velocity or reduced level of amniotic fluid)

- Gestation at delivery
- Perinatal death or death before hospital discharge
- Admission to NICU
- NICU admission for ≥48 hours
- Birthweight ≤ 5th customised centile
- Apgar score <7 at 5 minutes
- Umbilical artery acidosis at birth (*arterial cord pH <7.2*)
- Respiratory distress syndrome
- Interventricular haemorrhage
- Retinopathy of prematurity
- Confirmed infection (confirmed on blood or CSF cultures)
- Necrotising enterocolitis

*Antenatal detection of Fetal Growth restriction is based on formal ultrasound assessment of fetal biometry using the Hadlock formula.

A separate health economic evaluation is assessing the intervention's economic impact. This is achieved through the use of participant quality of life (QoL) questionnaires (EQ-5D & SF-36), (315, 316) a specially designed study specific participant costing questionnaire and by assessment of costs to the health service of community based/ inpatient/day case care, through chart review at discharge (317-319)

Data collection

Trial data captured locally at site by researchers are transmitted securely using an electronic clinical record form (eCRF) to a specific database developed by MedSciNet. Baseline demographic data, QoL questionnaires and the PlGF result are entered live to the eCRF at point of recruitment. The full eCRF is completed after discharge from the maternity hospital post-delivery, and includes neonatal and maternal medical outcome, costing questionnaire & repeat QoL questionnaires. All data entered to the eCRF is pseudo-anonymised with each participant identified by a unique study number. The identifier key is kept separately locally at site in a secure location. The data system is built to the same security and confidentiality standards as those of hospital electronic health records. The data at each participating centre are handled in accordance with local regulatory legislation and Ethics Committee approval. A detailed description of schedule and timing of data collection is provided (Table 5.7).

Table 5. 7: *SPIRIT Flow Diagram for Schedule of events in PARROT Ireland*

	On presentation with suspected PET Between 20+0 and 36+6 weeks	**From enrollment to discharge post delivery**		**Discharge post delivery**	
	In-person visit	Chart	In-person visit	Chart	In-person completed
Randomisation-Institutional level	X				
Inclusion/Exclusion	X				
Informed Consent	X				
Demographics		X[a]			
History, Comorbidities		X[a]			
Con Medications		X[a]		X	
Physical Measurements		X[a]			
Clinical readings		X[a]			
PlGF[b] measurement	X		X[c]		
Biobank sample[d]	X				
Fetal assessments				X	
Prenatal admissions				X	

Table 5. 7: *(Continuation) SPIRIT Flow Diagram for Schedule of events in PARROT Ireland*

	On presentation with suspected PET Between 20+0 and 36+6 weeks	**From enrollment to discharge post delivery**		**Discharge post delivery**	
	In-person visit	Chart	In-person visit	Chart	In-person completed
Maternal PET bloods				X	
Newborn data				X	
Neonatal outcome				X	
Maternal outcome				X	
Complications				X	
Postnatal admissions				X	
Clinical Management				X	
Final Outcomes				X	
EQ-5D, SF-36	X				X
Costing questionnaire					X
In person visits	X		X[c]		

[a] May be captured in chart review or in consultation with participant at any time following enrolment.[b] PlGF testing depends on Institutional randomisation allocation. [c] PlGF testing will be repeated if readmission for suspected pre-eclampsia. May be repeated more than once. No more often than 4 weekly. [d] Only at biobanking sites

Sample Size

The sample size was fixed by the number of sites and the study duration. It is anticipated that the total sample size will be in the region of 4000 participants; split across 7 clusters and the 8 time periods in the design (equivalent to a cluster-period size of about 71). With a sample size of 4000 and using a two-sided type I error rate of 0.025 (to allow for two co-primary outcomes), we determined the power to detect a 7% reduction in maternal morbidity (relative risk reduction of 20%) from 35% to 28% in the intervention i.e. 'active' group (based on a reported rate of adverse maternal outcome in the region of 35% in the PELICAN trial) (89). This is assuming an ICC in the region of 0.01; but also consider Sensitivity to a range of ICC values between 0.005 and 0.05. The second co-primary outcome is adverse neonatal outcomes. Due to scarcity of information on the ICC, the same ICC as for the maternal outcome is assumed. Current rates of adverse events are around 10%. We determine power to detect an absolute change in neonatal adverse outcomes of 6%.

To allow for the longitudinal nature of the trial, where correlations may differ between observations in the same cluster-period; and those measured in different cluster periods, we incorporate cluster-auto correlations (CAC). There is little information to support likely values for the CAC, so we are guided by values in the literature and explore sensitivity across a range of values (0.64, 0.80 and 0.96). (320, 321)

The power has been estimated using an online RShiny App. (322, 323) We have not included transition periods in the calculation but given the transition periods are just one week in length, this is not expected to significantly affect

power. There has been no allowance for varying cluster sizes as this is currently not something which is technically possible in a stepped wedge study. Sample size calculations were performed assuming linear mixed models with categorical effects for time; random cluster and random cluster by period effects. (310) Under these assumptions, we constructed power curves, which reveal that under most anticipated scenarios the trial will have in the region of 80% power (Figures 5.5 & 5.6). (195, 321)

Data Analysis

Clinical Outcome

The primary aim of the study is to evaluate whether there is a difference in the two composite outcomes before and after exposure to the intervention. There will be no double counting of outcomes, individuals not events will be presented for the composite . Mixed effects regression models will be used to allow for the clustering within sites. Calendar time will also be adjusted for since the intervention is sequentially rolled-out both by including fixed categorical time effects and random cluster by categorical time effects (195).

The primary estimate of the treatment effects will therefore be cluster and time adjusted. Time adjustment is essential, as it is a stepped wedge trial. Log Poisson regression models with robust variance estimation (to allow for misspecification of binomial errors) will be used so as to allow estimates of relative risks (324); to estimate risk differences corresponding Binomial models with log links will be fitted. Secondary analysis will adjust for individual and cluster level covariates. In the first instance, comparative estimates of

differences between groups will be adjusted for variables used in the randomisation procedure (eg; site, time and hospital size). Further, more fully adjusted analyses, will also be performed. These more fully adjusted analyses will adjust for gestational age at recruitment, maternal age, smoking status, maternal BMI, public versus private obstetric care and maternal co-morbities such as Chronic Renal Disease, SLE/APS & Diabetes. It will also adjust for hospital size (< or >5000 deliveries/annum). Categorised continuous variables (e.g. age) will be treated as continuous variables in this adjustment. If covariate adjustment is not practical, unadjusted estimates will be produced and it will be made clear in the output why this occurred (e.g. not possible due to low event rate lack of model convergence). Null hypotheses and analyses for secondary outcomes take a similar form to that for the primary outcome, and where outcomes are not binary, analysis will be using the generalized linear mixed model. Transformations will be performed where data are markedly not normally distributed. For the analysis adjusted for covariates and for the secondary outcomes (unadjusted) multiple imputation methods will be used if the proportion of missing data is more than about 5%, and this multiple imputation will also allow for the clustered and temporal nature of the trial. It is not expected that there will be any missing data in the primary outcome; as it will be assumed that if the outcome is present then it will be recorded and if it is not recorded we will assume it is absent. This is a standard and realistic assumption. Results will be presented as adjusted risk ratios with confidence intervals (CI) and risk differences to allow full appreciation of clinical effect. To allow for the two primary outcomes, we will follow good practice and adjust for

this multiplicity using a Bonferroni correction and so report 97.5% confidence intervals.

For secondary continuous outcomes mean differences will be reported and 99% confidence intervals for secondary outcomes. We will report latent intra-cluster correlations for all outcomes, along with 95% confidence intervals. Pre-specified subgroup analysis will be undertaken on the primary outcome based on women presenting <35 weeks' gestation versus >35 weeks' gestation; size of unit and final confirmed diagnosis. The stepped wedge trial design will also allow investigation of treatment effect heterogeneity across clusters and time. These exploratory analyses will be reported using 99% confidence intervals. Analysis will be conducted by intention to treat and sites will be considered exposed to the intervention post randomised cross-over date.

Health Economic Outcome

The economic evaluation will be informed by a decision analytical model, which will be designed and constructed for the study to reflect the maternal and fetal pathway and health states. Employing a decision analytical model allows for the extrapolation of existing data and the opportunity to systematically synthesise evidence from various sources. Primary data on maternal health outcomes will be available from the study with the distribution of EQ-5D-5L & SF-3F6 questionnaires which will inform the estimation of Quality Adjusted Life Years (QALYs). Neonatal outcomes will be informed by secondary sources. A systematic literature review will be conducted to identify QOL/utilities (or proxies for same) associated with neonate outcomes which

will be incorporated into the decision analytical model to estimate QALYs. Primary data on resource utilisation will be collected using the costing questionnaire (Appendix 2). The costs and effects of the intervention and comparator will be compared to estimate an incremental cost effectiveness ratio in a Cost Utility Analysis. To address parameter and structural uncertainties, a probabilistic sensitivity analysis (PSA) will be performed.

Trial Management

Day to day running of the trial will be coordinated by the Trial Management Group (TMG). The TMG consist of the lead site investigator plus the project manager and the clinical fellow. The TMG will act on behalf of the Sponsor and will be responsible to the Trial Steering Committee (TSC) to ensure that all Sponsors' responsibilities are carried out. The TSC is comprised of all Principal Investigators as well as the TMG, sponsor, HRB and representatives from Statistics, economics, neonatology, laboratory and a lay person. The role of the TSC is to provide overall supervision of the trial. In particular, the TSC will concentrate on the progress of the trial, adherence to the protocol, participant safety and consideration of new information.

Data Monitoring

To provide protection for study participants an independent data monitoring committee (DMC) has been appointed for this trial. The DMC comprises of 4 members who are not involved with any other aspect of the trial. They include

an Obstetrician, a neonatologist, a statistician and a midwife. The DMC met and ratified their charter and have advised that all serious adverse events such as stillbirth/neonatal death or profound maternal morbidity in the Intervention arm of the study be reported to them immediately. The DMC will receive regular updates on the progress of the trial every quarter from the trail management group (TMG). The purpose of these updates is for the DMC to; 1) ensure the quality of data collection 2) ensure that the intervention is being rolled out according to the randomisation plan 3) monitor balance between arms to monitor for potential selection biases and 4) ensure PlGF testing is not overwhelmingly better or worse than no PlGF testing with respect to maternal morbidity with neonatal morbidity. Once outcomes for 1500 participants are available, an interim analysis will be conducted and reviewed by the DMC. The interim analysis will report on the co-primary outcomes, follow the same methods as those of the primary analysis, and examine if there is proof beyond reasonable doubt that one particular intervention is definitely indicated or definitely contra-indicated in terms of a net difference of a major endpoint. There will be no formal stopping criteria put in place, but the DMC will be guided by the knowledge that proof beyond reasonable doubt cannot be specified precisely, but a difference of at least three standard deviations in an interim analysis of the primary outcome would be consistent with strong level of evidence. No allowance for this interim analysis has been made in power calculations. There will be no stopping of the trial for futility as the study will be underpowered to detect small effects.

5.1.4 **Discussion**

Based on previous experience during the PELICAN study, an analysis of success criteria and barriers to our proposed study was conducted. Potential barriers include the overestimation of (i) identification of eligible women by the research team, (ii) primary outcome event rate (iii) and retention / attrition i.e. gaining outcomes data on all women included.

A recruitment feasibility audit conducted in Cork University Maternity Hospital (CUMH) over the course of a typical week in July 2016 identified 21 women who would be eligible for inclusion in the PARROT Ireland study. This would equate to almost 1100 women per annum in CUMH, approximately 13% of its annual delivery rate. This is in keeping with the quoted 10% incidence of hypertensive disorders of pregnancy (HDP) in the population (27). It is anticipated that over the 22 month duration of the study across the 7 hospitals approximately 10,486 women will meet the study inclusion criteria (13% of the combined annual delivery rate), and of these 4,000 will be recruited into this trial (approximately 38% of those eligible). As inclusion in the trial will be optional and require informed consent from participants, not all eligible women in each unit will be included. Projected inclusion rates will be apparent via a dedicated MedSciNet database pre-programmed, available online and contemporaneously updated, allowing prompt action to intervene when not optimal. A conservative requirement of <50% of all eligible women to be recruited in order to reach targets has deliberately been chosen and successful recruitment of the same population in the PELICAN study is reassuring. As with any study we may get a higher or lower incidence of the

primary outcome of interest than anticipated. We should get an early indication of this at the interim analysis.

As participation in the trial does not require any extra attendances/input from the participant for the remainder of the pregnancy, it is likely that retention of participants will not be an issue. Similarly, the data outcome to assess for maternal and neonatal morbidity can be readily obtained post-delivery following discharge of the participant from their stored medical records locally at each unit. However, in order to fully examine the health economic outcomes there exists a reliance on the return of completed questionnaires by the participant post-delivery. To minimise attrition rates, the researcher at each site will endeavour to meet with each participant post-delivery prior to their discharge and encourage them to complete the health economic questionnaires. In the PELICAN study only 1% of the cohort were lost to follow up. The risk of incomplete data collection of outcomes in studies such as this is more relevant if women deliver in a different unit to that which they are recruited in to the trial. However, all seven clusters in our trial are large tertiary referral units and patient transfer during pregnancy is rare. We are therefore confident that the likely rate of loss to follow up will be similar and in the order of 1%.

There are a number of advantages with the use of stepped wedge design. It allows a phased implementation of the intervention, which is preferable when commencement in all clusters simultaneously would be challenging. As all clusters ultimately receive the intervention, it increases willingness of the clusters to partake in the trial. We acknowledge that seven clusters is a small number of clusters and this is an important limitation of the study. Mostly this

is a limitation because it will mean that the findings have questionable generalisability. But, if these clusters are representative then the findings may still be generalizable in part. The other limitation that seven clusters brings about is questionable internal reliability. However, because all of the clusters receive both the intervention and control condition, the clusters serve as their own controls. Not only does this lessen the impact of chance imbalance but it also increases the power of the study (particularly so when the ICC is large, as is the case here). The study does only have in the region of 80% power and should parameters such as the ICC be very different to that which we have assumed, then it is correct that the study might be underpowered. To ensure that this is properly accounted for at the analysis stage, we will report appropriate CIs around all point estimates, so the impact of any impression is properly reported.

Another potential limitation worth noting is the slightly different management algorithm for one cluster, Belfast, in the control arm. The Belfast control arm algorithm is taken directly from the NICE Hypertension in Pregnancy guidelines. All other clusters are using an algorithm taken from the HSE Guidelines for Hypertension in Pregnancy. The two are essentially the same except the HSE algorithm also includes a recommendation for a fetal ultrasound in cases where the participant is <34 weeks gestation. It is not anticipated that the difference in these algorithms should have any bearing on the overall trial results. We will conduct a sensitivity analysis with the Belfast site removed and see if the result remains consistent.

Ideally PlGF testing should be performed for all participants enrolled in the study, with blinding of the result for those in the control arm. This would allow

for test performance statistics to be performed. Unfortunately, testing of control participants will not be conducted in our trial, which is a notable limitation of the study.

The primary aim of the PARROT Ireland trial is to establish the effectiveness of revealed plasma PlGF measurement in reducing maternal morbidity (with assessment of neonatal safety in parallel) in women presenting with suspected pre-eclampsia prior to 37 weeks' gestation. Should the trial show a reduction in maternal morbidity without an increase in neonatal morbidity, or indeed a reduction in neonatal morbidity with no change in maternal morbidity, it would provide a strong argument for its incorporation into routine obstetric practice. The long-term aim of the trial is to demonstrate if PlGF measurement enables appropriate antenatal stratification of women presenting with suspected pre-eclampsia.

Avoiding unnecessary hospital admission would be both clinically and economically beneficial. In contrast, those at increased risk of imminent adverse events, identified by an abnormal PlGF result, would have hospital resources re-directed to them. We anticipate that this trial will provide a definitive result on the benefits of PlGF testing which will act to influence international clinical practice.

A separate RCT, also entitled “PARROT”, has completed recruitment in the United Kingdom since the end of 2017. Although recruiting a similar population of women and using the same PlGF platform, the primary outcome measure for the two RCT’s is different, with the UK PARROT trial focusing on time from

enrolment to diagnosis. Both studies are using the same electronic clinical record forms developed by MedSciNet and thus will have a large cross-over of data. The advantage of having these two similar RCT's conducted almost simultaneously is that robust information on the impact of incorporation of PlGF into clinical care will be generated. In addition the potential exists for a collaborative project such as an individual participant data meta-analyses in the future.

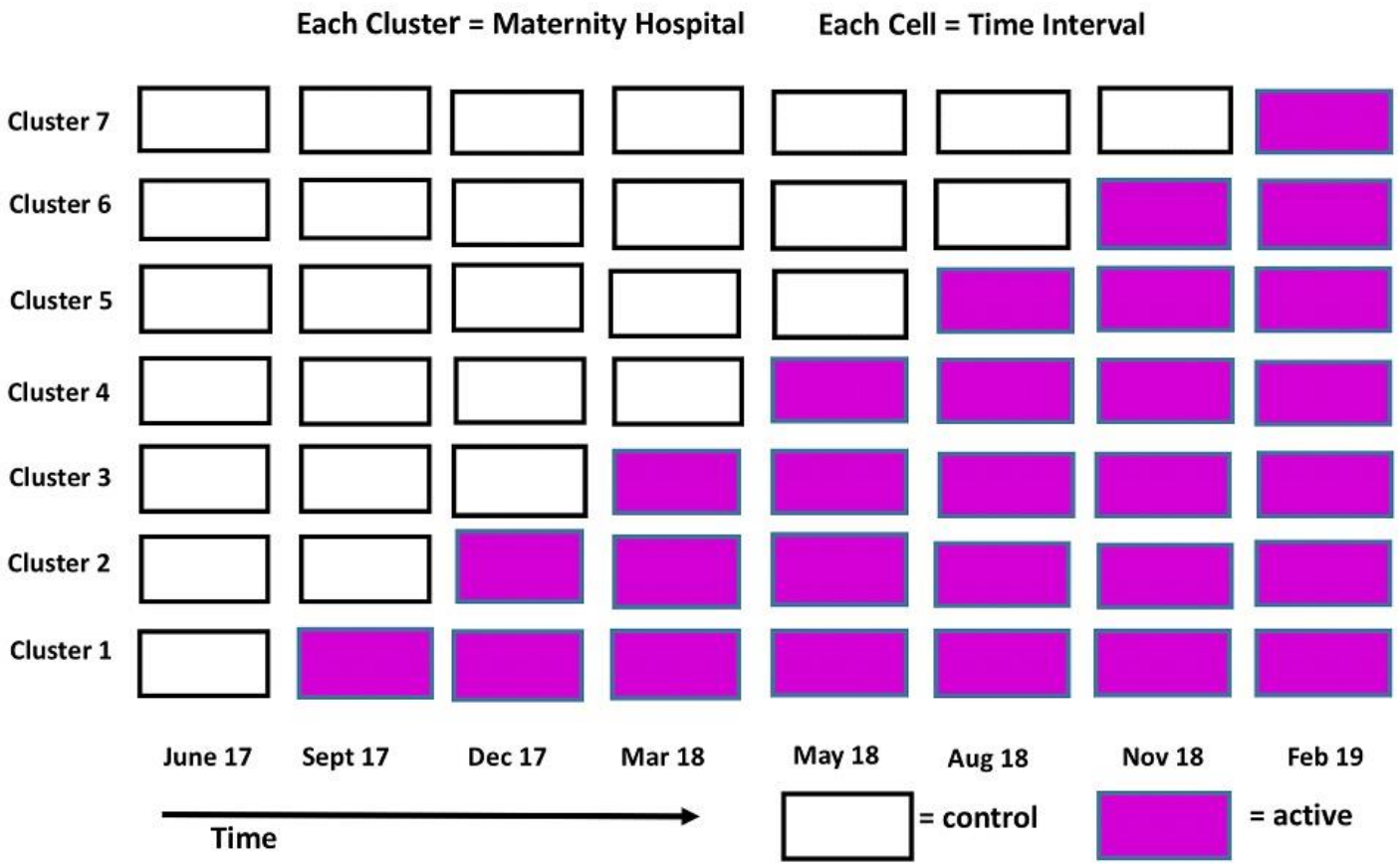


Figure 5. 1: *Stepped Wedge Cluster Randomised Design for PARROT Ireland*

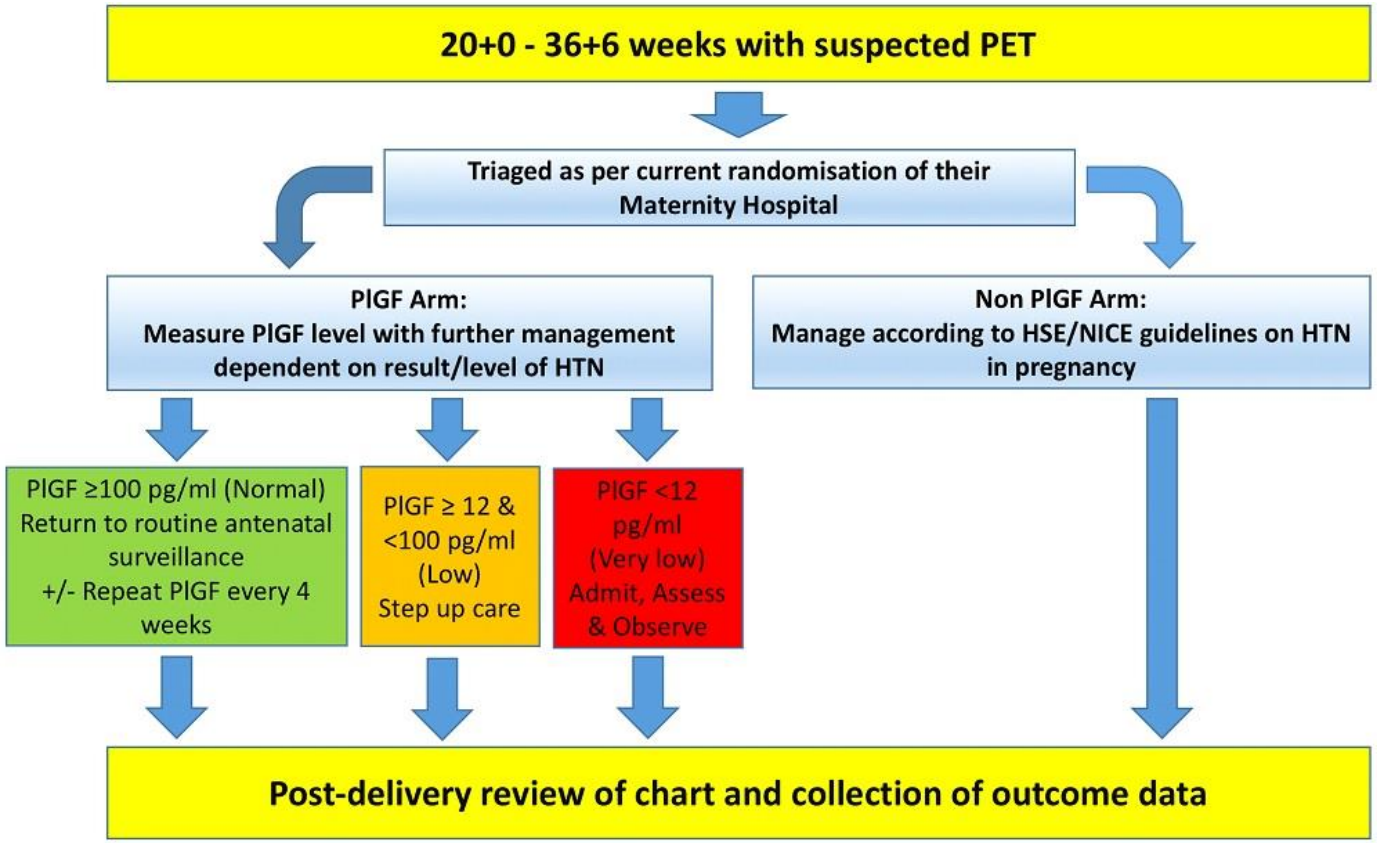
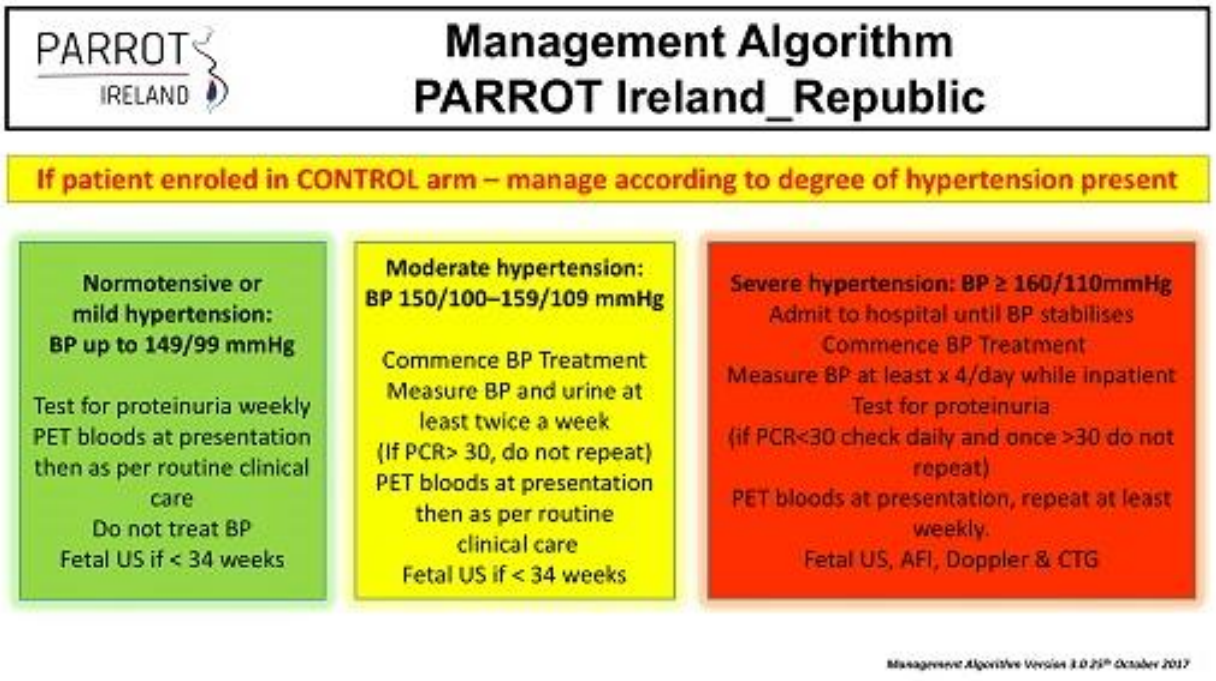


Figure 5. 2: *Trial Schematic for PARROT Ireland.*



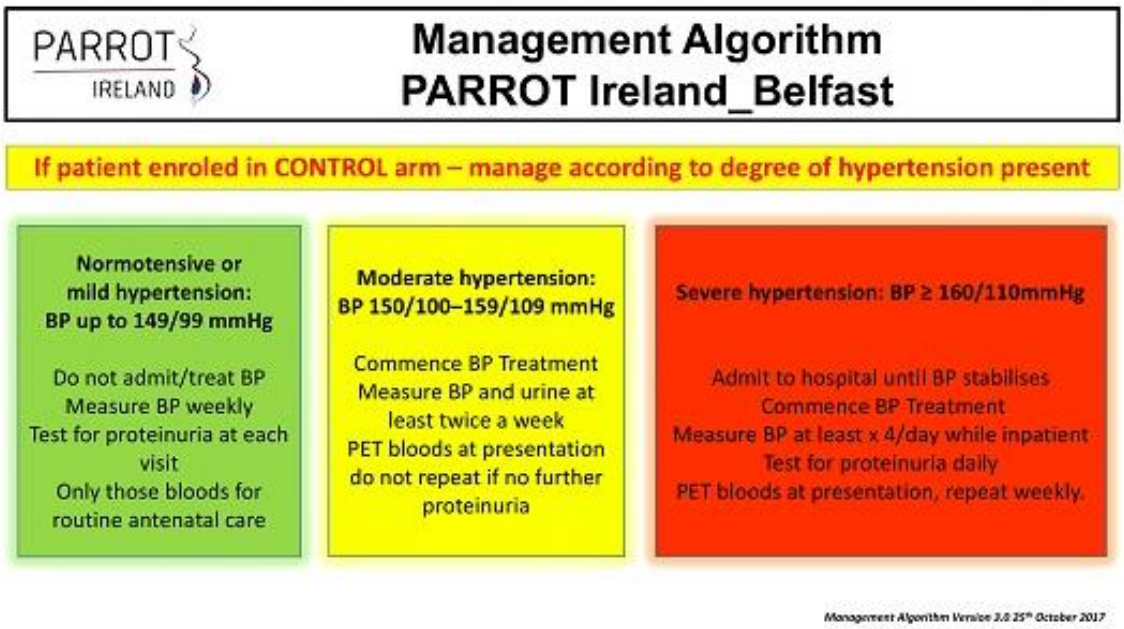


Figure 5. 3a & Figure 5.3b: *Management Algorithm for Control arm based on HSE guidelines for PARROT Ireland.*

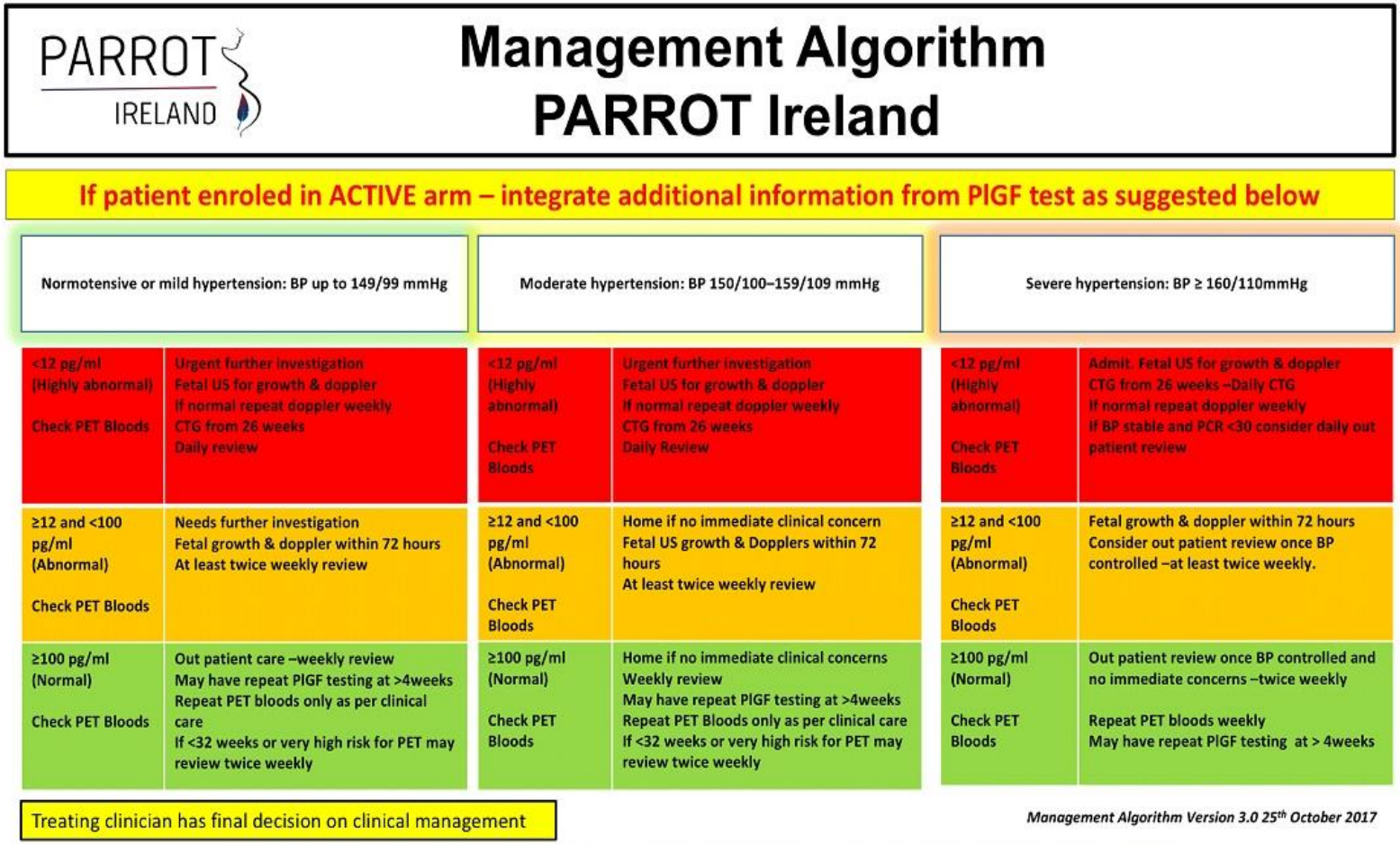


Figure 5. 4: *Suggested Management Algorithm for Intervention for PARROT Ireland.*

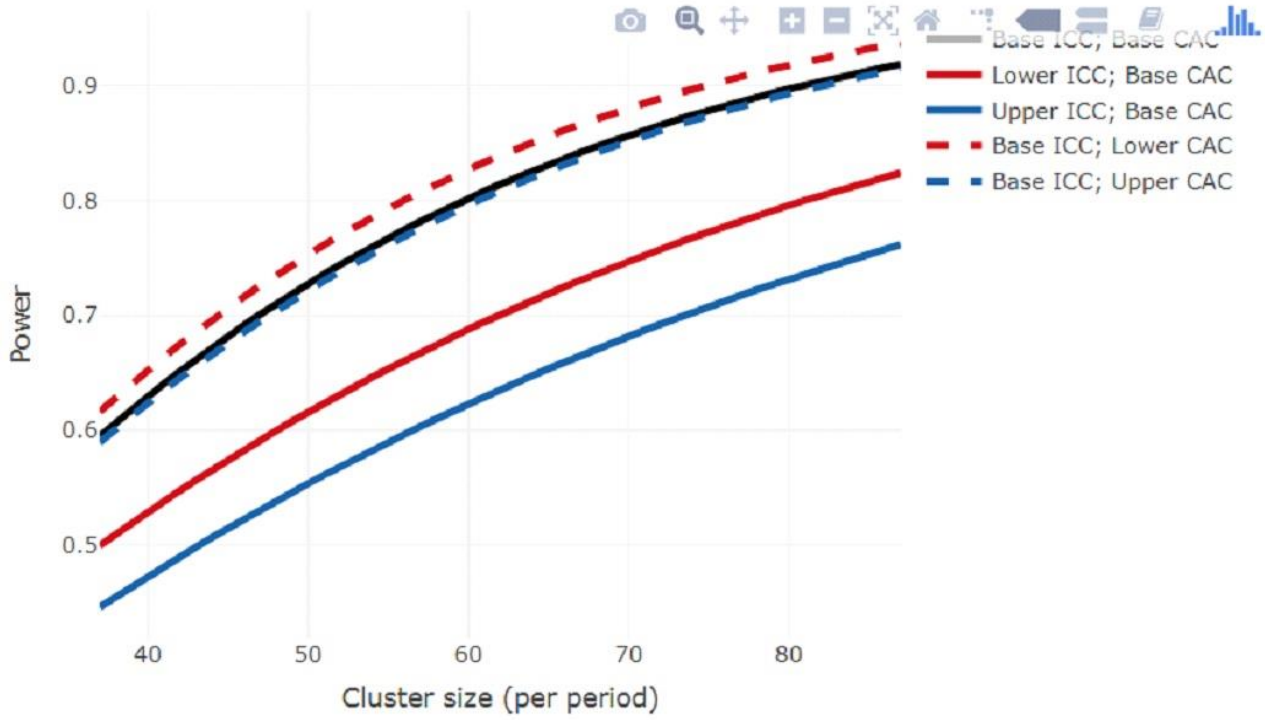


Figure 5. 5: *Power Curve for PARROT Ireland for Maternal Adverse Outcomes*

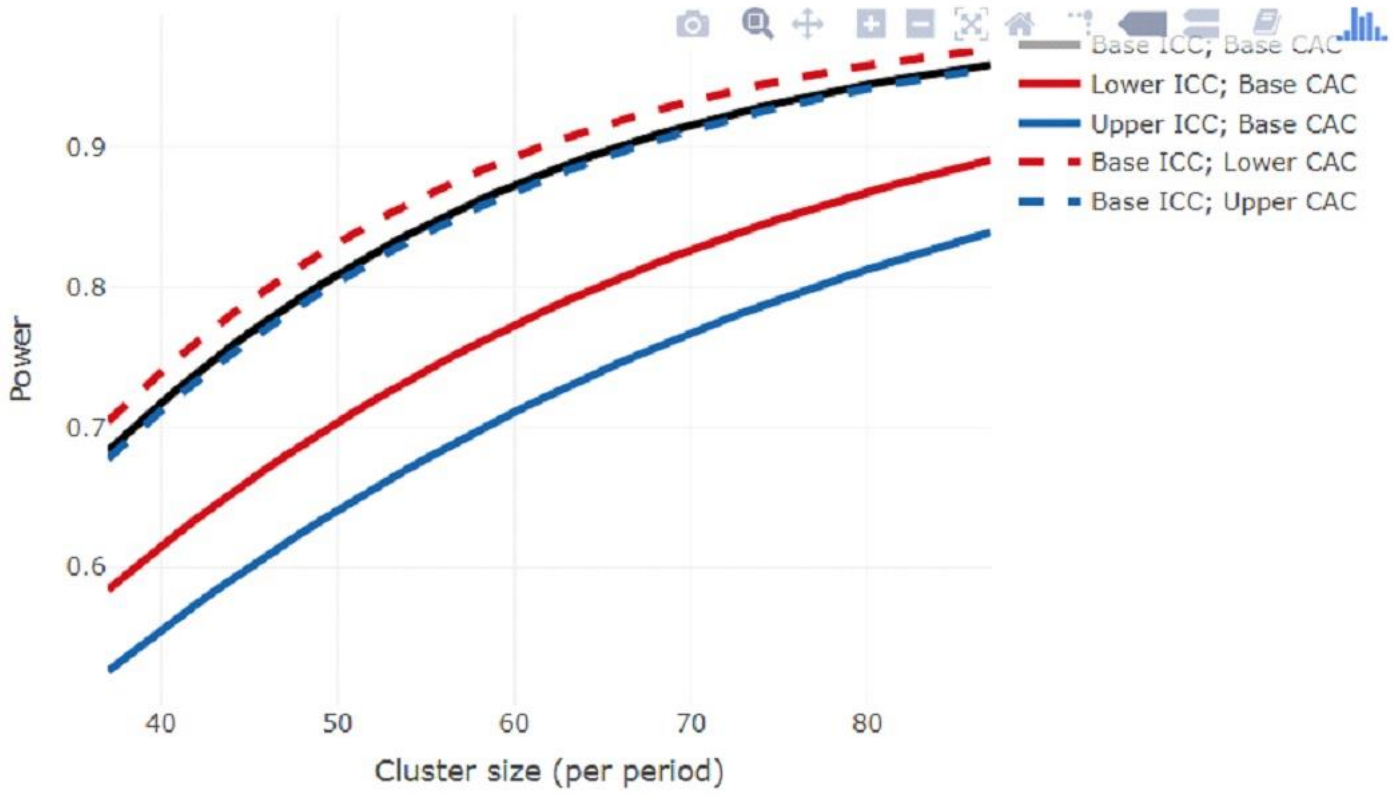


Figure 5. 6: *Power Curve for PARROT Ireland for Neonatal Adverse Outcomes*

5.2 PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: Results of Interim Analysis

5.2.1 Abstract

In order to evaluate the impact of incorporation of PlGF testing into routine clinical care, a national multi-site randomised control trial was conducted. PARROT Ireland recruited from 29th June 2017 to 26th April 2019 in which timeframe over 2000 eligible pregnant women with suspected preterm pre-eclampsia were enrolled. The result of this confidential interim analysis is of no significant reduction in either maternal or neonatal morbidity with the integration of point of care PlGF based testing. These results however, are based on an interim analysis, performed on just the first 1092 participants. Our trial has finished recruitment, outcome data is collected, and shortly we will analyse the clinical outcomes and report the primary endpoints. Should our trial demonstrate a positive impact on maternal morbidity, without a negative impact on neonatal morbidity, we intend to advocate the incorporation of PlGF testing into routine clinical investigations for women presenting with suspected pre-eclampsia before 37 weeks' gestation.

5.2.2 Introduction

Pre-eclampsia is a clinical manifestation of placental dysfunction. Complicating 2-8% of pregnancies, it is associated with significant maternal

and neonatal morbidity and mortality (17). Current diagnosis of pre-eclampsia or placental dysfunction is reliant on objective signs of end stage disease such as; maternal hypertension, significant proteinuria, abnormal biochemical/haematological indices or ultrasound evidence of fetal growth restriction (302-306). A robust diagnostic test for pre-eclampsia/placental dysfunction would prevent unnecessary hospitalisations and investigations for many pregnant women while also enabling earlier identification and focusing of resources on those who require it the most (325). Herein lies the potential of placental growth factor (PlGF); as a diagnostic biomarker for pre-eclampsia/placental dysfunction (110).

As a member of the vascular endothelial growth factor (VEGF) family, PlGF regulates angiogenic events in pathological conditions (95). Circulating levels of PlGF in the maternal plasma increase alongside development of the placenta, peaking at about 32 weeks' gestation then declining until delivery (87). However, in pre-eclampsia due to the endothelial changes that exist, this rise and fall is considerably lower throughout pregnancy, and maternal plasma levels are significantly lower when the condition presents clinically. Observational studies have demonstrated the potential for PlGF in aiding diagnosis of pre-eclampsia in those presenting preterm with signs or symptoms of the disease (88, 89). However, an abnormal PlGF result may prompt earlier intervention by clinicians, resulting in maternal benefit at the expense of neonates, neonates, highlighting the need for adequately powered, ideally randomised controlled trials, to determine the clinical utility and overall cost effectiveness (155).

In 2016 The National Institute for Health and Clinical Excellence, UK (NICE) published guidance on PlGF testing, in addition to clinical assessment, in women presenting with suspected pre-eclampsia from 20-34^{+6} weeks' gestation. It advocated that PlGF testing should not be used to diagnose pre-eclampsia until further research was available, on how an abnormal PlGF result would affect management decisions regarding timing and gestation of delivery and specifically the consequent outcomes associated with this (326).

The objective of this randomised trial was to evaluate the impact of knowledge of PlGF measurement on both maternal and neonatal outcomes. Our hypothesis was that the addition of PlGF measurement to current clinical assessment of women with suspected pre-eclampsia prior to 37 weeks' gestation would reduce associated maternal morbidity, without increasing neonatal morbidity, through improved risk stratification, earlier diagnosis and targeted management of women with the disease.

5.2.3 **Methods**

Chapter 5, section 5.1.3 describes in detail the methodology of the trial and links with paper 5 ".PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial Research Study Protocol" (179).

Study Design

PARROT Ireland was a multi-centre, stepped wedge cluster-controlled trial of PlGF measurement in women presenting with suspected pre-eclampsia and a singleton pregnancy from 20 weeks and prior to 37 weeks' gestation. The seven largest maternity units in the country were involved in this trial; The Coombe Women and Infants University Hospital Dublin, Cork University Maternity Hospital, University Maternity Hospital Limerick, The Royal Jubilee Maternity Hospital Belfast, University College Hospital Galway, The National Maternity Hospital Dublin and The Rotunda Maternity Hospital Dublin. The trial ran for a period of twenty-two months, commencing 29th June 2017 and ceasing 26th April 2019. Detailed inclusion and exclusion criteria are presented in section 5.1.3 (Table 5.1 & 5.2). All units transitioned from control phase to intervention phase over this time period, with a short transition week in between (Figure 5.1). Data collected during this transition period were not included in any analysis of outcomes. Outcome data were collected until the last recruited participant was 12 weeks postnatal and the last neonate was discharged.

Ethical approval and consent

The trial was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with Good Clinical Practice and applicable regulatory requirements. The local ethics committee at each participating site reviewed the trial protocol, including the patient information and informed consent form, and full ethical approval was granted

(Table 5.8). Each eligible woman identified was required to give written informed consent prior to her inclusion in the trial. A GCP trained researcher at the local site obtained this consent.

Table 5. 8: *Ethical approval from the clinical research committee at each participating site*

Cork	Clinical Research Ethics Committee Cork: ECM 3 (h) 08/11/16
Galway	University College Hospital Galway EC: Ref 50/12
Coombe	Coombe Womens & Infants University Hospital EC: Study No 20-2016
Limerick	University Hospital Limerick EC: Ref: 68/16
Rotunda	Rotunda Hospital EC: REC-2016-020
National Maternity	National Maternity Hospital EC: EC 20.2016
Belfast	Health Research Authority (Belfast):16/WM/0484

Randomisation

Once an eligible woman had given written informed consent for inclusion in the study, the site researcher revealed her maternity hospitals randomisation to her. Participants enrolled in the control arm received usual hospital care as

per National Guidelines; Health Service Executive/Institute of Obstetrics and Gynaecology Irish guidelines for those in the Republic or the NICE guidelines for those in Northern Ireland (3, 14). Participants enrolled in the intervention phase had immediate maternal plasma PlGF quantified in addition to routine hospital investigations. The PlGF test was performed by an appropriately trained researcher at each site, using a CE marked validated point of care platform; the automated Triage® Meterpro (Quidel, San Diego, CA). The PlGF result was made immediately available to the participants' clinical team and documented clearly in the participant's medical notes. A suggested further management algorithm was provided to the clinician based on both the degree of hypertension present and the PlGF result (Figure 5.3b).

Outcome Measures

The primary outcome was a composite measure of both maternal morbidity (Table 5.33) and neonatal morbidity (Table 5.4). This co-primary approach was chosen to ensure maternal morbidity is not reduced at the expense of earlier delivery and worse neonatal outcomes.

Data Monitoring

A detailed monitoring plan (MP) was developed for PARROT Ireland by the project manager and myself (Appendix 2). The trial had an assigned study monitor who ensured that the trial was adequately monitored in conjunction with the procedures outlined in the MP. Onsite trial monitoring visits were conducted as per the schedule in the MP throughout the trial. During these

visits the monitor performed a number of tasks to ensure protocol adherence was maintained (Table 5.9).

Table 5. 9: *Data monitoring tasks at each local site visit*

Necessary % required as per MP	Monitoring Task
100%	QC check on the ICFs
100%	Protocol Deviations/Violations
100%	Investigator Site File
100%	PlGF quantification (frequency, result)
5%	Participant Source Data Verification
-	Recruitment targets locally
-	Site Staff, Facilities and Equipment:

To provide protection for study participants an independent data monitoring committee (DMC) was appointed for this trial. Any serious adverse events, such as stillbirth/neonatal death or profound maternal morbidity, in the Intervention arm of the study was reported immediately to the DMC. No major clinical concerns with morbidities occurred. The DMC also received regular updates on the progress of the trial every quarter for the purpose of;

- ensuring the quality of data collection
- ensuring that the intervention was rolled out according to the randomisation plan
- monitoring balance between arms to monitor for potential selection biases and

ensuring PlGF testing is not overwhelmingly better or worse than no PlGF testing with respect to maternal morbidity & neonatal morbidity

Analysis

The trial statisticians conducted this Interim Analysis in accordance with the DMC charter, when over one thousand participants had been delivered and their final clinical outcomes were available.

5.2.4 **Results**

The trial started recruiting on 29th June 2017 and finished on 26th April 2019. In this timeframe, 2313 eligible pregnant women consented to enrollment. In this confidential interim analysis, I present the results of the first 1092 participants (Figure 5.7). At the time of writing this paper, clinical outcome data for the remainder of the participants is ongoing, with the final data analysis planned for early 2020. This confidential interim analysis was presented to the DMC of PARROT Ireland, as per their charter, in May 2019. It is also being presented here for the purposes of my thesis.

There were no significant differences between the control and intervention groups in terms of maternal age, ethnicity, type of antenatal care, pre-existing medical co-morbidities, booking blood pressure or proteinuria at booking (Table 5.10). However, there was some disparity between the two groups in terms of parity and smoking status with significantly more multiparous women (67.71%, n=151 v 59.26%, n=515; p=0.021) and more smokers enrolled in

the intervention arm (14.35%, n=32 v 11.89%, n=103; p=0.004) compared to the control.

Median gestational age at the time of recruitment was 33 weeks, and did not differ between the two arms (Table 5.11). No differences were noted in diagnosis of gestational diabetes, dipstick level of proteinuria prior to study entry, nor presence of fetal growth restriction prior to enrolment between the two groups. In the intervention cohort, significantly more participants were taking aspirin when recruited (23.8%, n=87 v 19.9%, n=173; p<0.0001). There was disparity in location of recruitment, with more recruitment occurring from antenatal clinic (40.36%, n=90 v 23.01%, n=200; p<0.0001) among those in the intervention arm. Both systolic (132.7mmHg, n=220 v 136.1, n=855; p=0.013) and diastolic (82.1mmHg, n=220 v 84.5mmHg, n=855; p=0.014) blood pressures were also lower among those in the intervention arm at time of recruitment.

All participants recruited to the trial were eligible based on the presence of signs or symptoms concerning for evolving pre-eclampsia or placental dysfunction. The main clinical outcome, as well as any additional adverse diagnosis, for all participants are shown (Table 5.12). Gestational age at the time when final outcome diagnosis was reached ranged from 29 to 36 weeks with a median of 33.5 weeks and did not differ significantly between the two arms. There were also no differences in the rates of each clinical outcome between the two groups, with less than 5% of participants having no pathology subsequently identified. The control arm was noted to have significantly higher rates of “no additional diagnosis” compared to the intervention (93.1%, n=809

v 89.24%, n=199; p=0.004) however individual numbers are small and need to be interpreted with caution.

Results were adjusted for cluster and time given the stepped wedge design and then more fully adjusted analysis was performed taking into account; maternal age, BMI, smoking, ethnic origin, gestational age at booking, public versus private obstetric care and maternal co-morbities such as chronic hypertension/renal disease, systemic lupus erythematous (SLE) or antiphospholipid syndrome (APS) and pre-existing diabetes.

The overall incidence of maternal morbidity observed in the trial was 37% (n=411) with no significant difference between the two groups (34.25%, n=75 v 39%, n=336; RR 0.82, 95% CI 0.61-1.12) even when adjusted (RR 0.84, 95% CI 0.62-1.14) (Table 5.13).

The overall incidence of neonatal morbidity present in the trial was 46% (n=501) with those in the intervention arm trending towards less morbidity (41.3%, n=92 v 47.1%, n=409; RR 0.81, 95% CI 0.64-1.02) however this was not statistically significant even when adjusted (RR 0.78, 95% CI 0.60-1.02) (Table 5.14).

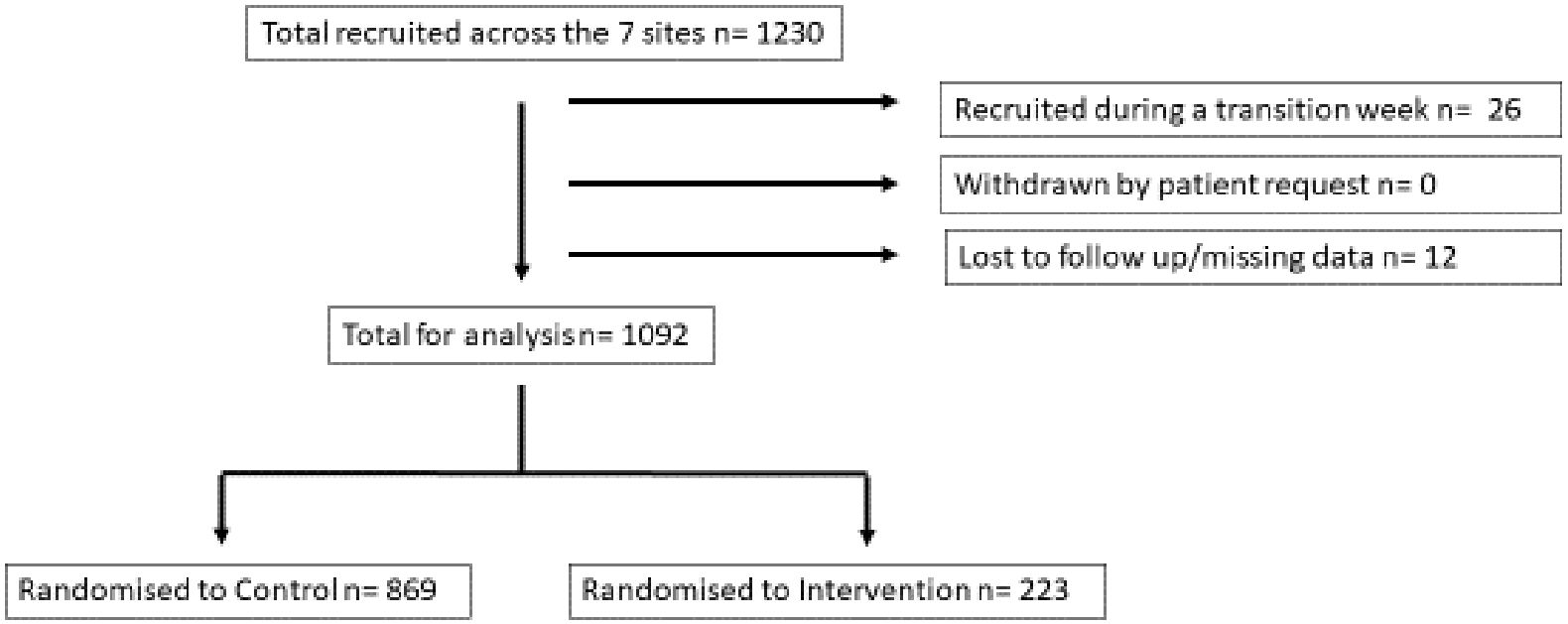


Figure 5. 7: *Diagram of first 1092 participants, June 2017-December 2018*

Table 5. 10: *Baseline characteristics of participants*

	Control, n (%)	**Intervention, n(%)**	**p-Value**
	n=869	**N=223**	
Age, y, mean (SD)	31.9(5.79)	31.3(5.74)	0.178
Ethnicity			0.571
European	787(90.56)	211(94.62)	
African Caribbean	1 (0.12)	0 (0)	
African	23 (2.65)	3(1.35)	
Bangladeshi	2(0.23)	1(0.45)	
Indian	14(1.61)	1(0.45)	
Middle Eastern	5(0.58)	0(0)	
Pakistani	5(0.58)	0(0)	
South East Asian	11(1.27)	2(0.9)	
Other	21(2.42)	5(2.24)	
Parity			0.021

Nulliparous	354(40.74)	72(32.29)	
Multiparous	515(59.26)	151(67.71)	
Previous PET			
Previous Stillbirth			
Medical Co-morbidities			
Chronic Renal Disease	28/869(3.22)	6/223 (2.69)	0.684
Chronic HTN	76/869 (8.75)	23/223(10.31)	0.467
SLE/APS	4/869(0.46)	0/223 (0)	0.310
Pre pregnancy Diabetes	23/869(2.65)	7/223(3.14)	
Obstetric Care			0.156
Public	797 (91.71)	211(94.62)	
Private	71(8.17)	11(4.93)	
Unknown	1(0.12)	1(0.45)	
Gestation at booking (weeks, days), mean (SD)	13.23(3.82), **n(861)**	12.53(3.14)	0.0114
Body mass index, kg/m2, mean (SD)	28.2(7.03), **n(863)**	28.72(6.76), **n(221)**	0.35

Smoking	N=866	N=223	0.004
Current smoking	103(11.89)	32(14.35)	
Quit smoking	34(3.93)	20(8.97)	
Never smoked	729(84.18)	171(76.68)	
Booking BP reading			
Systolic mm Hg, mean (SD)	120.7(15.25), n=860	121.0(12.90), n=223	0.79
Diastolic mm Hg, me mean (SD)	73.5(10.64), n=860	74.3(9.89), n=223	0.28
Proteinuria at booking			0.066
No	763(87.90)	208(93.27)	
Not done	64(7.37)	8(3.59)	
Yes	41(4.72)	7(3.14)	
Proteinuria at booking, Yes			0.562
Trace	17(41.46)	3(42.86)	
+1	11(26.83)	3(42.86)	
+2 or >	13(31.71)	1(14.29)	

Table 5. 11: *Participant characteristics at time of enrollment to the study*

	Control	**Intervention**	**p-Value**
Gestation at enrollment (weeks), median (IQR)	33(30, 35)	33(30, 35)	0.30
Aspirin use in current pregnancy	173/869(19.9)	87/223(23.8)	<0.0001
Gestational Diabetes in current Pregnancy	104/869(11.97)	24/223(10.76)	0.618
Location at enrollment			<0.0001
Antenatal Clinic	200(23.01)	90(40.36)	
Antenatal ward	163(18.76)	37(16.59)	
Labour Ward	1(0.12)	0(0)	
Day Ward	308(35.44)	87(39.01)	
Emergency room	105(12.08)	9(4.04)	
Other	92(10.59)	0(0)	
Highest BP reading recorded in the 48 hours prior to study entry			
Systolic mm Hg, mean (SD)	136.1(18.21), n=855	132.7(17.59), n=220	0.013
Diastolic mm Hg, mean (SD)	84.5(13.0), n=855	82.1 (14.11), n=220	0.014

Highest dipstick level of proteinuria in the last 48 hours prior to study entry			0.438
Trace	122(14.07)	32(14.35)	
1+	93(10.73)	16(7.17)	
=2	66(7.61)	13(5.83)	
None	550(63.44)	153(68.61)	
Not Done	36(4.15)	9(4.04)	
Suspected Fetal growth restriction prior to enrolment			0.144
No	250(50.51)	66(43.71)	
Yes	245(49.49)	85(56.29)	
AC <10th centile	135/245(42.45)	41/85(48.24)	0.002
EFW <10th centile	227/245(92.65)	80/85(94.12)	0.792
Umb Art PI >95th centile	37/245(15.10)	14/85(16.47)	0.338
AREDF	12/244(4.92)	4/85(4.71)	0.237

Table 5. 12: *Clinical outcome diagnosis 12 weeks post-delivery for women in each arm of the study*

	Control	**Intervention**	**p-Value**
Final Diagnosis			0.142
Pre-eclampsia	130(14.96)	29(13)	
Gestational Hypertension	236(27.16)	49(21.97)	
Gestational Proteinuria	16(1.84)	3(1.35)	
Transient Hypertension	112(12.89)	25(11.21)	
Superimposed Pre-eclampsia (background CHT)	6(0.69)	0(0)	
Superimposed Pre-eclampsia (background renal disease)	4(0.46)	1(0.45)	
HELLP	1(0.12)	0(0)	
Suspected SGA only	57(6.56)	21(9.42)	
Chronic Hypertension only	46(5.29)	11(4.93)	
Superimposed pre-eclampsia (background CHT and renal disease)	5(0.58)	0(0)	
Isolated SGA	151(17.38)	51(22.87)	
Chronic Hypertension and SGA	11(1.27)	2(0.90)	
None of these	44(5.06)	10(4.48)	

Other	15(1.73)	12(5.38)	
Gestation at diagnosis (weeks, days), median (IQR)	33.5(29, 36), n=832	33(29, 36), n=218	0.773
Additional Diagnosis			0.004
No additional diagnosis	809(93.10)	199(89.24)	
Eclampsia	2(0.23)	0(0)	
HELLP	4(0.46)	0(0)	
DIC	0(0)	0(0)	
ELLP	4(0.46)	0(0)	
Placental Abruption	7(0.81)	0(0)	
Transient Hypertension	15(1.73)	7(3.14)	
IUGR at delivery	7(0.81)	9(4.04)	
IUGR undetected antenatally	11(1.27)	5(2.24)	
Cholecystitis	0(0)	1(0.45)	
Obstetric Cholestasis	10(1.15)	2(0.90)	

Table 5. 13: *Maternal morbidity composite co-primary outcome*

Maternal Morbidity Composite	**Control**	**Intervention**	**RR (95% CIs)*;**	**RR (95% CI)****
No	526/862(61.0)	144/219(65.75)		
Yes	336/862(39.0)	75/219(34.25)	0.82 (0.61, 1.12); p=0.22	0.84 (0.62,1.14); p=0.26

Table 5. 14: *Neonatal morbidity composite co-primary outcome*

Neonatal Morbidity Composite	**Control**	**Intervention**	**RR (95% CIs)*;**	**RR (95% CI)****
No	460/869(52.9)	131/223(58.7)		
Yes	409/869(47.1)	92/223(41.3)	0.81 (0.64, 1.02); p=0.07	0.78 (0.60,1.02); p=0.07

*Poisson regression models were adjusted for time and hospital.

5.2.5 **Discussion**

We set out to examine if the incorporation of PlGF testing into routine care, improved maternal outcomes without negatively influencing neonatal outcomes. The result of this interim analysis found no significant reduction in either maternal or neonatal morbidity with the integration of point of care PlGF based testing, in addition to usual clinical care, in women with suspected preterm pre-eclampsia and a singleton pregnancy. These results however, are based on an interim analysis, performed on only the first half of participants recruited to the PARROT Ireland trial and should be interpreted with caution. Given the stepped wedge design and the timing of this interim analysis, the proportion of participants represented here is unbalanced; with many more control than intervention cases.

Our interim results contrast to those of the UK PARROT trial which published earlier this year, reporting PlGF testing to be beneficial (188). These findings were based upon an observed reduction in time to diagnose preterm pre-eclampsia (from 4.1 to 1.9 days) as well as a reduction in maternal adverse outcomes in those with revealed PlGF testing.

Similarly to our trial, the UK PARROT trial used a stepped wedge design, recruited women with suspected preterm pre-eclampsia and utilised the Triage® Meterpro for PlGF quantification . The UK trial began a year prior to the Irish trial and was slightly shorter in duration, finishing eighteen months ahead of ours. Subtle differences exist between the two PARROT trials, specifically in terms of the definition of pre-eclampsia utilised, PlGF sampling

in all participants and the primary outcome employed, which must be considered when interpreting and comparing the results (Table 5.15).

The incidence of pre-eclampsia among the UK trial participants was approximately 35%. In comparison, our incidence was much lower at approximately 14%, potentially explained by the use of differing definitions for pre-eclampsia between the two trials. To harmonise clinical diagnosis across all seven maternity units in our trial, we adhered to the 2010 NICE Hypertension in pregnancy guidelines which necessitate the presence of significant proteinuria for the diagnosis of pre-eclampsia (3). In contrast, the UK trial adopted the newer International Society for the Study of Hypertension in Pregnancy (ISSHP) 2014 statement, which advocates that proteinuria is not essential for the diagnoses of pre-eclampsia, if either maternal organ dysfunction or fetal growth restriction are present (15).

Importantly, the primary outcome measures of the two trials differ. The UK trial measured “time from presentation with suspected pre-eclampsia to documented pre-eclampsia in women enrolled in the trial who received a diagnosis of pre-eclampsia by their treating clinicians” as the primary outcome measure, with maternal and neonatal morbidity analysed as secondary outcomes. Given the importance of ensuring clinical benefit and out ruling any clinical harm with the intervention, we opted for a co-primary composite of maternal and neonatal morbidity as the primary outcome measure for PARROT Ireland.

Our trial has limitations that we acknowledge; the first of these being a lack of PlGF testing in the control arm. The UK trial performed venepuncture and

quantified PlGF in all recruited participants but only revealed the PlGF result for those randomised to the intervention (188). This allows for comparison of not just participant demographics and clinical parameters but also of the PlGF results itself, ensuring the two arms of the study are equal in all aspects (327). Given both the financial and technical constraints of PlGF testing, it was not feasible for us to perform PlGF testing on all participants in all maternity units at the commencement of the trial. Biobanking samples locally to facilitate later analyses was also considered but as the infrastructure at most sites was not conducive with the necessary high quality standards required for biobanking, practically this was not a realistic option.

Significantly lower blood pressure was present at the time of enrollment among those in the intervention in our interim analysis. Potentially this is a reflection of the increased interest and engagement of staff locally in the trial once their unit transitioned to the intervention. Unfortunately, this is a challenge when using an unblinded randomisation design, where it is not possible to blind the clinicians at site as knowledge and education is required for adaption of PlGF into clinical practice. A final limitation to consider is potential confounders. Aspirin, multiparity and smoking are known to confer a protective effect against pre-eclampsia and placental dysfunction (328, 329) and a significantly higher incidence of all of these was noted in the intervention group compared to the control of our interim analysis. Potentially, the lack of difference in the primary outcome of our interim results may have been influenced by this imbalance.

Our trial has a number of notable strengths. Thanks to the collaborative work of many maternity units, universities and the health research board mother and baby clinical trial network Ireland (HRB-MBCTNI), we have demonstrated

that a national, multi-site, randomised control trial among the pregnant population is possible in Ireland. Additionally, very few eligible women approached to take part declined, demonstrating the acceptance of clinical research among pregnant women in Ireland. These findings help pave the way for future perinatal research studies, critical for the practice of evidence-based care.

A second strength of the trial is its overall timing, with the publication of the UK trial results occurring just at the very end of our recruitment in April 2019. This enabled equipoise, regarding the potential merits of PlGF testing, to be maintained for the duration of our trial and thus did not influence the participants' decision to enrol nor clinicians' decision to refer their patients. An additional strength of our trial is its design. The cluster randomisation allowed a change in clinical management to occur at a hospital level rather than at an individual patient level, facilitating a pragmatic approach to PlGF use. This approach demonstrated the likely day-to-day practice should PlGF be introduced into clinical practice in the future. In the intervention cohort, a higher incidence of recruitment was observed to occur from antenatal clinic, highlighting the utility of point of care PlGF testing as a rule out test for pre-eclampsia in an outpatient setting. The stepped wedge design also ensured that each hospital had an opportunity to experience the intervention and thus remained engaged with the trial and committed to not adopting any off protocol PlGF testing until the trial ceased.

5.2.6 **Conclusion**

On foot of the PARROT UK publication, the National Health Service (NHS) in the UK has now endorsed PlGF testing to aid diagnosis of preterm pre-eclampsia in maternity units throughout the UK (187). Our trial has finished recruitment, outcome data is collected, and shortly we will analyse the clinical outcomes and report the primary endpoints. Despite some limitations, given the large number of participants that have completed the study we are optimistic we will be able to report adequately powered, high quality data on the effect of PlGF testing. Should our trial demonstrate a positive impact on maternal morbidity, without a negative impact on neonatal morbidity, we would advocate the incorporation of PlGF testing into routine clinical investigations for women presenting with suspected pre-eclampsia before 37 weeks' gestation.

Table 5. 15: *Similarities and Differences in PARROT UK versus PARROT Ireland RCTs*

	UK	**Ireland**
Date of commencement of Trial	June 2016	June 2017
Date of cessation of Trial	October 2017	April 2019
Number of clusters (Maternity Units)	11	7
Duration of trial (months)	17	22
Duration of each block (weeks)	6	12
Gestational age of participants at recruitment	20-36+6 weeks	20-36+6 weeks
Inclusion Similarities	• new-onset or worsening of existing hypertension • dipstick proteinuria • epigastric or right upper-quadrant pain • headache with visual disturbances • fetal growth restriction	• new-onset or worsening of existing hypertension • dipstick proteinuria • epigastric or right upper-quadrant pain • headache with visual disturbances • fetal growth restriction

Inclusion differences	If abnormal maternal blood tests that were suggestive of disease (thrombocytopenia or hepatic or renal dysfunction) participants were still eligible for inclusion	If abnormal maternal blood tests that were suggestive of disease, participants were not included
Number of Participants Enrolled	1035	2313
Guidelines used for diagnosis of Pre-eclampsia	ISSHP 2014	NICE 2010
Primary Outcome	Time to diagnose confirmed pre-eclampsia	Maternal and Neonatal Morbidity

Chapter 6: Research in a Pregnant Population

Paper 7: An exploration of women's experience of taking part in a randomised controlled trial of a diagnostic test during pregnancy; a qualitative study

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6.1 An exploration of women's experience of taking part in a randomised controlled trial of a diagnostic test during pregnancy; a qualitative study

6.1.1 Abstract

Objective: To explore pregnant women's views of participation in a clinical research trial while pregnant

Design: Prospective nested qualitative cross sectional study embedded within a national, multi-site randomised controlled trial of a diagnostic test for pre-eclampsia; Placental Growth Factor. One to one in depth semi-structured interviews were undertaken with 19 women who had recently participated in the trial at a single recruiting site. The interviews were conducted in private, recorded digitally and transcribed verbatim.

Setting: Single tertiary maternity hospital currently recruiting eligible women onto an ongoing randomised controlled trial (NCT 02881073)

Participants: Women who had participated in the PARROT Ireland randomised controlled trial during their recent pregnancy

Methods: Thematic analysis was utilised. Each line of the transcribed interviews was coded into a category by two researchers. The resultant categories were reviewed and those with similarities were pooled allowing the development of themes.

Main Outcome Measures: Women's opinions and experience of participation in a randomised controlled trial of an interventional diagnostic test during their pregnancy

Results: Four major themes were identified: 1) Understanding of pre-eclampsia, 2) Motivators for clinical trial participation 3) Barriers to decision-making and 4) Influence of PARROT Ireland on pregnancy experience

Conclusions: Women are generally interested and positively inclined to participate in research during pregnancy. The potential of risk is an important consideration for eligible pregnant woman. Information and support by both researchers and clinicians are paramount in aiding women's understanding of a research trial.

Keywords: Pre-eclampsia, Pregnancy, Research, Randomised Controlled Trial, Diagnostic Test, Qualitative

6.1.2 Introduction

A randomised controlled trial (RCT) is regarded as the gold standard when testing efficacy of any new drug, intervention or diagnostic test (189, 191). The use of drugs such as thalidomide and diethylstilbestrol in pregnant women has had long lasting repercussions; with women of childbearing age traditionally being excluded from clinical trials owing to safety concerns and fear of litigation (203, 204). Nevertheless, up to 74% of pregnant women take mediation for chronic or acute conditions while the use of prescription medications by pregnant women has risen by more than 60% over the last three decades, with

most of these drugs being used off-label (201, 218). The paucity of evidence available on the use of medications in pregnancy means that some pregnant women may not be receiving optimal treatment, as clinicians are often unsure regarding correct dosage due to the physiological and metabolic changes that occur in pregnancy (208, 330). Further, lack of inclusion of women in clinical trials has resulted in a lack of evidence-based care for pregnant women. Interventions such as cardiotocography and fetal fibronectin testing were integrated into clinical practice prior to robust evidence of their benefit (205). Once use of such clinical interventions are established within a system, withdrawal becomes challenging (206).

In the last number of years concerns have arisen over the ethics of actively excluding pregnant women from clinical trials (207, 331-333). In 1993 the Food and Drug Administration (FDA) lifted its ban on the testing of medicinal products in women and the National Institute for Health (NIH) legally endorsed the inclusion of pregnant women in trials (334, 335). Since the mid 90's there has been a concerted effort to ensure minorities, such as women and children, are represented in research in order to help guide scientific based practice for all societal groups (336-338). With the advent of perinatal research centres, each year more trials specific to pregnant women are developed, funded and conducted globally (339, 340). Literature is sparse in relation to women's willingness to take part in in clinical research while pregnant (341-343). In addition, lack of experience in including pregnant women in trials may lead to poor trial design and hence recruitment difficulties (211, 344, 345). It is well documented that under-recruitment is often an issue in RCTS, with a third not reaching target and over 50% requiring extensions (346). Given that we are

involved in conducting an RCT of an interventional diagnostic test on a pregnant population; we determined we had a unique opportunity to explore pregnant women's views of participation in research, within a clinical trial. Our aim was to explore women's experience on being involved in a clinical trial, specifically a randomised controlled trial, while pregnant.

6.1.3 **Methods**

This qualitative study was nested within a randomised controlled trial of a point of care diagnostic test (NCT 02881073) for preterm pre-eclampsia in Ireland. We first describe the PARROT Ireland RCT and then describe the nested qualitative study.

PARROT Ireland

PARROT Ireland was a multi-site, national study recruiting women in the seven largest maternity units in Ireland, from 29th June 2017 until 26th April 2019. The trial aimed to examine if the addition of point of care Placental Growth Factor (PlGF) testing to routine clinical care improved both maternal and neonatal outcomes for women with a preterm singleton pregnancy, and signs or symptoms of pre-eclampsia or placental dysfunction (179). If an eligible pregnant woman consented to participate, she was randomised to either control (routine care) or intervention (immediate additional PlGF testing) based on the current randomisation of her hospital at the timepoint of her enrolment. As randomisation was unblinded, both the participant and her

clinical care team were aware of her allocation and her PlGF result if she was randomised to the intervention.

Nested Qualitative Study Design

Participants at a single study site were invited to participate in the qualitative study. We employed a qualitative study design, using semi-structured interviews, to explore women's views, experience and beliefs regarding pregnancy research. A semi-structured topic guide which was developed based on existing literature (211-215). Qualitative research has been utilised for many years to provides insight into problems, help develop hypotheses and to gain an understanding of underlying reasons, opinions, and motivations (347). Interviews rather than surveys were employed as they facilitated a relationship of trust to be established between the researcher and participant (348). One to one interviews allowed for an environment where each participant was able to express themselves more openly than perhaps a focus group may have allowed (349).

Recruitment

Purposive sampling of women who had recently completed the PARROT RCT was employed to ensure each arm of the trial was represented. Due to the stepped wedge design of the trial, the interval from recruitment until the interview was longer for those recruited in the control than the intervention. These women had previously consented to be approached about further trial-related research. Each received a patient information leaflet and invitation to

participate by post. Women who agreed to participate attended to the maternity hospital in-person (n=16) or were interviewed by telephone (n=3) if in-person attendance was not feasible. Informed written consent was obtained, and the interview process took approximately 30 minutes. Interviews were conducted over a three month period (September 2018-December 2018) by DHR until data saturation was reached. At the time of interview, the PARROT Ireland trial was ongoing so results were not yet available to participants or researchers. Eighteen of the nineteen interviews were recorded digitally, and transcribed verbatim. The one woman that did not wish to be digitally recorded, gave consent for note taking by the researcher throughout the interview. These notes were used to inform the analysis.

Analysis

Interview transcripts were thematically analysed by DHR and SM (220, 221). In the initial analysis, each line of the transcript was coded into categories. The categories were then reviewed and refined, and themes were developed. These key themes were then presented and agreed upon by the entire research team.

6.1.4 **Results**

Nineteen women were interviewed, ten of whom had been randomised to the control arm and nine who had been randomised to the intervention arm of the trial. Time from completion of study ranged from 6 to 15 months at the time of

the interview. All women interviewed were Caucasian, age ranged from 24 to 42 years and 52% were nulliparous prior to their recent pregnancy. Four themes were identified; 1) Understanding of pre-eclampsia, 2) Motivators for clinical trial participation 3) Barriers to decision-making and 4) Influence of PARROT Ireland on pregnancy experience. Direct quotations from the women, presented in-text, are used to illustrate these themes.

Understanding of pre-eclampsia

Women were recruited to the PARROT Ireland trial on the basis that they were exhibiting signs or symptoms of preterm pre-eclampsia in their pregnancy. Some women were diagnosed with pre-eclampsia. They described the significant impact this had on their pregnancy, emphasising how it took over their lives leaving them with a lack of control and autonomy.

> *"…suddenly I was in hospital and I didn't leave for a month. I had loads of planning to do and my mat leave was happening the next day and just small things that were kind of taken away in a sense, putting the nappies in where I wanted to put them and the vests and washing them and all those little preparation stuff that I was kind of looking forward to" P8*

Most of those interviewed reported only having vaguely heard of the condition prior to their pregnancy with friends or social media being the main source of their knowledge. As most of the participants were nulliparous women at the

beginning of their pregnancies, these women did not feel that pre-eclampsia was of concern to them.

"It didn't really affect me, it wasn't anything to do with me" P3

Most of those interviewed were not aware of risk factors for pre-eclampsia and had misconceptions about who might develop pre-eclampsia. Women commonly stated that they were shocked when the possibility of pre-eclampsia arose later in their pregnancy.

"Like I thought it was something kind of third world people got" P7

"I would have heard of it, but I wouldn't have known much about it" P15

"Before being pregnant I don't think… maybe I had heard the word pre-eclampsia, but I definitely wouldn't have known any specific information" P17

Those who had been pregnant previously, especially those previously investigated for pre-eclampsia, had better knowledge of the risks, symptoms and potential consequences of the condition.

"I definitely thought that you were overweight and unfit and like you'd brought it on yourself kind of thing" P9

"I was definitely more aware of it, because they had mentioned it's a possibility or that it is more probable the second time round. So, I would have been aware of at least the symptoms of it and watching out more in the second pregnancy" P17

When subsequently informed that they required investigation for pre-eclampsia, women were eager for simple, clear, concise information about the condition from a reliable source. Some felt they got the necessary information from their clinical care team. Others searched for the information themselves, usually using online sources, and often felt overwhelmed at the vast amount and varied quality of information available.

> *"You don't know where to look regarding wanting to get correct information because there's so much now online" P8*

Motivators for participation in clinical research

Despite limited previous experience with participation in research, especially medical research, the women had extensive knowledge regarding research. All were convinced about the merit and importance of research, especially in pregnancy, for increasing knowledge of conditions and improving future clinical care.

> *"I just think information is power and the numbers don't lie and if you have information, you can do something" P9*

> *"I felt like whatever we could do it would be a benefit, so I was happy to participate to be honest" P13*

Almost all of those interviewed reported it was a straightforward decision to take part in PARROT Ireland, and that it did not require a lot of time for consideration nor involve discussion with others.

"…as far as I was concerned it was my body and they didn't really have a say" P2

"The study was quite easy-going, you know, it's a blood test or no blood test and then a questionnaire, so that was no real decision on my part in that it was handy enough" P13

The women reported altruism and the potential to help others in the future as a key motivator for participation. Many felt their contribution was essentially "paying it forward" for the knowledge that could be garnered from their current pregnancies in order to benefit those in the future. A second key motivator to participate was a lack of burden associated with participation in the PARROT trial. Even though women reported they would be happy to give extra time or attend for extra appointments if required for research purposes, one of the main factors was how straightforward it was to take part in the PARROT trial.

"other people being involved in research previously surely helped me when I was pregnant" P3

" the fact that it was so simple….you could say yes or no…and then you have a blood test or you didn't and then there was no extra travel or filling out huge surveys or anything like that" P17

The potential of participation facilitating an opportunity of an earlier diagnosis, or identification of a problem, also influenced women's decision to enrol. Women felt that by being part of the study they might know sooner than others if they developed a pregnancy complication. The demeanour of the PARROT researcher was also reported to be an important factor in their decision to take

part. Women discussed the researcher's style of approaching eligible women remarking that the researcher was kind and friendly, made them feel at ease, explained the study in clear simple terms, all without rushing or pressurising women in any way.

> *"she couldn't have been nicer, she really couldn't…had she not been so sensitive and understanding I possibly wouldn't have you know" P5*

> *"It was completely up to me and I didn't feel at all under pressure. I mean I could have just left it. I remember there was a window just next to me so I could have just left it on the window sill and you know, nothing would have ever come of it" P17*

Women reported being positively influenced to take part if they heard about the trial from their treating clinician. Most believed the trial would not have been suggested to them unless it was useful and would be beneficial to them. They also reported being more likely to take part if they recognised the name of one of the Principal Investigators (PI) of the study. The women discussed how these PI's are senior consultants who are well respected clinically and are highly sought after for private obstetric antenatal care locally.

> *"I think that was less pressure in a sense, because you know when the researcher approached me directly, it's kind of my responsibility "do you want to take part?" whereas when it comes from you know your consultant it's an easier step to take then" P5*

Barriers to decision making

The main deterrent to participation in clinical research identified by the group was risk. The group reported being highly reluctant to take part in any research should they perceive it as being potentially harmful to the developing fetus. Taking a medication while pregnant, as part of a research trial, was an example of what women would consider risky. Most would be very reluctant to take part in such a trial and admitted it would require very careful consideration and discussion with their partner if they were approached in the future about such a research study.

> *"I'd be very slow, I'd have to really think about it, purely because it's somebody else's life you're putting on the line, not just your own, it's somebody else's future " P7*

The potential requirement for blood tests from the woman as part of a clinical study was not found to be a deterrent among the group, however if blood sampling were required from the baby following delivery, this would be considered a deterrent. Any test that was invasive, and potentially would cause distress or pain to the baby, that was not required outside of a research setting would not be well received.

> *"You don't want to be the guinea pig and you certainly don't want the baby to be the guinea pig either… you're like well can some people in some other countries sign up first and see what the outcome is. You always want somebody else to stick their toe in the water first" P9*

The clinical situation of the individual woman, at the time point when she is approached to participate in a trial, was flagged by those interviewed as important to consider. Many participants reported that if they had recently received sensitive or distressing information or were currently experiencing serious complications in their pregnancy; they may have been less willing to take part in the trial. The women interviewed mentioned that the language used by researchers when approaching eligible pregnant women is important to consider. Using complicated words and medical jargon could be frightening for some women and many stated it would sway them against participation.

> *"Like I do think it is very dependent on you know the news that you have been given, like if you have been given very sensitive news, you might feel like, why should I be…. the guinea pig to help future cases" P5*

> *"I think any pregnant woman would happily take part, I think the only time that someone might not want to do it is if they are facing a crisis, and they are in a bit of a fog and they can't really think" P2*

> *"I think the only thing that would turn people off is saying its medical research –that can be scary. Maybe, say its more for women's health" P4*

Influence of PARROT on pregnancy experience

When approached for further interview, all those who agreed to take part reported recalling the trial and their agreement to take part. The name of the trial; PARROT, was found to be memorable. Most were uncertain as to why a

trial concerning pregnant women and pre-eclampsia had used an acronym representing a bird. Some understood this was an abbreviation for a longer name. Nobody reported the name to have negative connotations, but some did mention that if it had mentioned a baby in the title it may have been more inviting.

> *"no I remember the name because I have a parrot! So, if it had been another name … but I remember this" P6*

> *"It did cross my mind…"why parrot"? but I didn't think too much about it really. I remembered it straight away so maybe the name was good" P14*

All enrolled participants in PARROT Ireland were asked to complete a five page paper Quality of Life questionnaire at the time point of their enrolment and again prior to their discharge from hospital postnatally. When asked about their thoughts on the questionnaire, most respondents had little memory of it and did not report it as being off-putting or time-consuming. Some commented that they were a welcome distraction while waiting in a busy antenatal clinic and allowed them time to reflect on their current self.

> *"The researcher … she was dropping the follow-up questionnaire, so she could know if I needed to get on to somebody" P13*

> *"One thing I did like about it was it makes you think, it made me think about where I was at in the weeks after having my baby, it gives you time to think what level of anxiety am I now, so that was nice, that was a really good benefit of it" P8*

The majority of participants were aware of the concept of randomisation in a research setting and recognised the importance of assigning participants to groups in order to be able to examine the outcomes when evaluating a new test etc.

> *"I guess controls are important I suppose, to some extent you know, you need a certain amount of people to take part in order to get a certain level of statistics, that you need you know" P10*

> *"While I would still like to be part of the group of people that get the test, for research to develop and everything they need people on the other side of it, so I suppose that I would accept the plan whatever it was" P12*

A distinct difference was expressed between those in the control and those in the intervention regarding the overall experience of the trial on their pregnancy. Those from the control group had a poorer recollection of what the purpose of the trial was and that a blood test was offered to some participants. The women recruited to the control arm felt that the trial did not impact on or influence their pregnancy in any way but were still happy to have taken part.

> *"It was really personable, it actually wasn't like we were just another case number. If anything it brought me on sense of…somethings going right. Like there was never anything bad to come out of the trial for me, like worst case scenario you got nothing, you were just like you were when you started. it was all beneficial in one form or another depending on what way you looked at it" P2*

"I suppose, all I was thinking of was if it will help me or help other people I will. But to be honest information probably went a little over my head, I actually can't tell you now what the Parrot Study is, and that ….through no fault of anybody, but I personally had so much going on P16

Those who were in the intervention arm had a better recollection of the purpose of the trial and overwhelmingly felt being enrolled onto the trial was beneficial to their pregnancy. They felt that knowledge of the PlGF result, whether it was normal or abnormal was useful to the clinicians caring for them and positively influenced their pregnancy. They also felt they received extra care from their clinicians due to their involvement in the trial and having had the extra blood test performed.

"I just felt that everyone was giving the best care and all of this research and all of this information was for my baby's good so I thought it was a very positive thing" P18

"I thought it was very good, to be honest. It was hands-on like, you know. I don't remember ever a doctor ringing me like, so….I was happy with that. I think it put a rush on me being monitored, if I'm being honest, it was definitely beneficial" P18

6.1.5 **Discussion**

This qualitative study brings together insight into women's decision-making regarding participation in an interventional clinical trial during pregnancy. We identified that pregnant women are aware of the importance of conducting

research and are interested in taking part, provided participation does not put their unborn baby at any risk. We found there was limited background knowledge of pre-eclampsia among the group and women wanted information on this condition to be clear, concise and provided by a reliable source. In our study, those randomised to the intervention felt participation in the trial directly benefited their pregnancy; with the additional test providing valuable information on placental functioning and the perception of increased care from clinicians. On reviewing the literature, we identified limited numbers of previous studies examining women's experience of participation in a RCT while pregnant (212-215, 350). These RCTS vary in terms of design and methodology, frequently involved administration of a medicinal product or a placebo. Given that our RCT employed a diagnostic test as the intervention, we identified a novel opportunity to gain insight and add new knowledge to this under-examined area.

Participants of our nested study, reported both altruism and the potential of personnel benefit to be key motivators in their enrolment in PARROT Ireland. The prospect of an additional blood test, with its potential of earlier identification and diagnosis of a clinical complication, was an incentive for our study. Others have similarly reported a sense of civic duty, the opportunity to help others and the possibility of an improved outcome for their baby to be driving forces behind participation (213, 214). A Brazilian group reported the main motivator to comprise access to free medicine and an opportunity to engage with healthcare providers (212). This highlights that in countries where health care during pregnancy is not publicly available, participation in clinical research may be the only option those with limited financial means have in

order to access medical care. In such cases, governance of research trials must be closely regulated to ensure this vulnerable group are not exploited.

Respondents in our study reported being more likely to take part in the trial if it was mentioned to them by their treating clinician or a member of the medical team. Endorsement of the trial from medical personnel appears to validate a study for patients. Similarly a lack of interest or support from local clinical staff has been reported as a barrier to participation (211). Accurate knowledge by clinicians of ongoing trials in their unit and the vocalisation of their support is crucial in promoting participation of pregnant women in future trials.

This nested study identified that the main barrier preventing participation of pregnant women in clinical research is the potential of causing harm to the baby. Others have also found pregnant women to be risk adverse, with apprehension and risk-limitation being common barriers prohibiting participation (211, 214). Clincal trials require sponsorship, insurance and undergo rigorous review by national ethical committees prior to their commencement. Ongoing clinical trials are vigilently monitoried by stakeholders, to ensure any trends in adverse events are quickly detected and can be acted upon with possible cessation of the trial if neccessary (331, 340, 351). Changing a pregnant woman's perception of risk is key. Education, through the information and explanation provided by reseachers is paramount. Adequate training of researchers and clinicians, to maximise this skillset, should be prioritised for future studies.

This study revealed that, the decision to take part in the PARROT Ireland trial was made independently by the pregnant woman herself, without any

consultation with her partner, friends or family. Similar findings were reported in the QUOTE study (215), while in contrast the RIPE study (214) reported equal involvement of both the women and her partner in the decision to take part in an RCT. Both RCTs involved taking a medication while pregnant, however in QUOTE women had pre-eclampsia when enrolled, whereas in RIPE healthy pregnant women were recruited. This finding highlights trials that involve taking a medication while pregnant, especially if recruiting healthy pregnant volunteers, likely require a longer time interval from first approach by researchers until signing consent, to facilitate shared decision-making.

Women reported feeling well informed about the PARROT Ireland trial prior to signing the consent and later had a good understanding of the trial when interviewed. Respondents reported that the timing and setting of the researcher's approach was appropriate and the language used was understandable and unambiguous. In contrast, participants of both the MAGPIE (216) and the ORACLE (217) RCTs reported confusion when the trial was initially explained to them. They did not fully understand that randomisation meant they might not receive the intervention and subsequently had limited knowledge and recall about the trials (213, 215). This difference may be attributable to the clinical situation of the women at the time of recruitment. For PARROT Ireland eligible women were approached in a variety of clinical settings; antenatal clinics, wards, assessment units, all while undergoing routine assessments. Both the MAGPIE and ORACLE trials recruited women in Labour Ward/High Dependency Ward, either in preterm labour or close to indicated emergency delivery. Given the complexity of these clinical situations, it is plausible that women may feel over-whelmed and

unable to clearly assimilate information provided about a research study. Designing future trials with recruitment focused in non-emergent situations may provide a solution to this and ensure patient vulnerability is not exploited. An alternative could be to employ the use of a delayed consent process for labour ward based trials (352). This approach has been employed in trials of critically ill patients, is well described and has been found to be acceptable to patients (353).

The women in our study randomised to the control did not report negative experiences. Although they felt the trial had no direct impact on their pregnancy, they were still happy to have taken part. In contrast to our results, others have reported randomisation to the control of an RCT perceived as being disadvantageous (214). A loss of equipoise on the subject under investigation may be one explanation, or equally a familiarity of the intervention among the population. Prior to randomisation in a study, if participants have strong favourable personal opinions on the product being investigated, it may lead to disappointment and disillusionment if they then are randomised to the control. This highlights the need for education of eligible participants by researchers on the purpose and importance of both arms of an RCT. It also highlights the impact background knowledge of the topic under review in the eligible population may have on their willingness to participate and needs to be considered by researchers when planning future studies.

The women we interviewed are likely to be highly motivated and interested in research as they not only took part in the trial during their pregnancy, but also agreed to participate in the qualitative study. Ideally, we would have

interviewed those who declined to take part in the trial also, as this would have better elicited the barriers to research participation in pregnancy. However, as per Good Clinical Practice (GCP) (354) and General Data Protection Regulations (GDPR) (355) we did not retain any information on eligible women who were approached but declined to participate in the trial, thus contacting them for this study was not feasible. Strengths of our study include in-person interviews; facilitating a more personal relationship between the researcher and participant as well as close proximity of interview to time of participation, which greatly aided participants recall. Uniquely, the PARROT Ireland RCT was not blinded, hence even though the trial has not yet published, women subsequently interviewed were aware of their randomisation and, for those in the intervention, knew their PlGF result.

Our findings correlate well with those from a recent systematic review examining the facilitators and barriers to pregnant women's participation in research (350). It reported altruism and the potential to contribute to science to rank highly as motivators to women's participation. The potential for personal benefit, through increased surveillance or earlier detection of medical conditions, was also a commonly reported motivator. Similar to our nested study, the systematic review reported pregnant women to be risk adverse with the potential requirement of taking a medication whilst pregnant a major barrier to participation. Unlike our study, the review reported personnel inconvenience as a barrier to participation amongst pregnant women. This difference may potentially be explained by the design of our trial; with no ongoing assessments or repeat attendance required it was well received by participants. Another difference between the two studies was an underlying

distrust of researchers identified by the authors of the systematic review. This difference may possibly be explained by the demeanour and approach adopted by the research midwife of our study. Her candour and non-pressurising approach was frequently positively commented on by participants.

6.1.6 Conclusion

This study highlights that pregnant women are aware of the importance of research and are generally interested and positively inclined to participate. It identifies that the context, purpose and potential risk of any research are the most important considerations to an eligible pregnant woman. The approach and explanation adopted by both researchers and clinicians is paramount in aiding women's understanding of a research trial. This information may aid the design and conduct of future studies; thereby increasing their acceptability for pregnant women.

Chapter 7: Discussion

7.1 Overview

Worldwide, women who get pregnant are at risk of developing a hypertensive disorder of pregnancy, particularly if pregnant with twins or if conception occurred through use of assisted reproductive therapy (74, 85, 224). Hypertensive disorders arise on a background of placental dysfunction and are a global health issue, as they confer increased risk of both maternal and neonatal morbidity and mortality. Close vigilance of clinical parameters and optimal timing of delivery in women with pre-eclampsia have reduced mortality from this condition in the last numbers of decades in developed countries (21, 27, 356).

Prompt diagnosis of any form of placental dysfunction, including pre-eclampsia, is key to facilitating interventions around management and delivery. The gestation at onset of placental dysfunction as well as clinical symptoms vary between women, necessitating caregivers to be constantly vigilant for concerning signs requiring further investigation. In recent years, the concept of using the angiogenic biomarker placental growth factor to aid diagnosis of placental dysfunction, such as hypertensive disorders, has shown significant promise (89, 183).

There were three main objectives to this thesis. Firstly, to explore the potential of using PlGF in women at increased risk of placental dysfunction such as twin pregnancy. Secondly, to examine the impact of adding PlGF as a diagnostic aid in women with a singleton pregnancy presenting with signs/symptoms of preterm pre-eclampsia and lastly to explore women's experience about being

involved in a clinical trial while pregnant. Five research projects were undertaken to investigate these objectives:

1. The incidence of hypertensive disorders of pregnancy in a twin pregnancy cohort and the maternal and perinatal implications when present (Chapter 2)
2. An overview of placental growth factor (PlGF) knowledge and use to date, both in pregnancy and outside of pregnancy, and identification of areas requiring further research (Chapter 3).
3. Examination of differing immunoassay platforms for PlGF quantification, exploration of the potential for use of PlGF in twin pregnancy and development of a reference range for dichorionic twin pregnancies (Chapter 4)
4. The development, conduct and interim results of a national multi-site randomised controlled trial of PlGF as an adjunctive aid to diagnosis preterm pre-eclampsia in women with a singleton pregnancy and signs/symptoms concerning for placental dysfunction (Chapter 5)
5. The facilitators and barriers to conducting clinical research in a pregnant population through a qualitative study of women's opinions and experience of participation in a randomised controlled trial of an interventional diagnostic test while pregnant (Chapter 6)

For the purposes of this discussion, I have chosen to group similar themed papers together and to present interpretation of each chapter's findings in this way. Given the different aims, content and methods used in the studies, this will allow for easier interpretation of the main findings, strengths and limitations

of my work. It will also allow for greater clarity when discussing the implications my work has for future research and clinical practice.

The papers have been grouped according to three main areas covered by this thesis;

1) Chapters 2 and 4 assess the impact of Hypertensive Disorders of Pregnancy (HDP) in the setting of twin pregnancy (Paper 1) and explore the potential of using PlGF as a potential biomarker of HDP/placental dysfunction in twin pregnancy (Papers 3 & 4).

2) Chapter 3 and 5 give an overview of PlGF knowledge and use to date (Paper 2) and investigate the impact of adding PlGF to routine clinical investigations of women with suspected preterm pre-eclampsia and a singleton pregnancy (Papers 5 & 6).

3) Chapter 6 focuses on the facilitators and barriers to conducting clinical research in a pregnant population (Paper 7).

7.2 Hypertensive Disorders of Pregnancy in twin pregnancy and the potential for using PlGF as a biomarker

Twin pregnancy is well documented to increase the risk of the development of HDP and consequently is associated with an increased incidence of adverse maternal and neonatal outcomes (13, 56, 71, 78, 222). In order to examine the impact of a HDP on a twin pregnancy, I conducted a retrospective study of all

twin pregnancies over a nine-year consecutive period in our own maternity unit in Cork. All women with a twin pregnancy that delivered in the unit from 2009 to 2018 inclusive were included, maternal and neonatal clinical outcomes recorded and compared between those pregnancies that developed HDP and those that did not. From this study I produced a paper on the impact of HDP on maternal and perinatal outcomes in twin pregnancy (Chapter 2). Most research to date on PlGF has been conducted on singleton pregnancies with limited information available on twin pregnancy thus I next conducted a prospective study over the course of a two-year period in the same maternity unit. Women attending with a twin pregnancy underwent plasma PlGF sampling across a variety of gestational time points. This study resulted in the production of two papers (Chapter 4). The first paper compared the results of two different platforms available for PlGF quantification. The second paper gives a detailed description of the course of PlGF in a twin pregnancy, provides a reference range for PlGF in a dichorionic twin pregnancy and compares the PlGF values between uncomplicated twin pregnancies to those complicated by HDP.

7.2.1 Main findings and how they relate to existing research

Chapter 2: Hypertensive Disorders of Pregnancy in twin pregnancy

One out of every five women with a twin pregnancy in our unit developed a HDP, in keeping with the global reported incidence (74, 77, 223, 224). Nulliparity, maternal age over 40 years and conception though use of donor oocytes were noted to be important risk factors amongst our cohort, factors

previously recognised in literature (75, 77, 224). In this paper we demonstrated that the presence of obstetric cholestasis conferred an almost fourfold likelihood of HDP, a more recently recognised risk factor (71, 227). When HDP was present in our cohort, it was more likely to result in iatrogenic late premature delivery. A composite measure of perinatal morbidity did not show any difference between those twin pregnancies complicated by a diagnosis of HDP and those that were not, a similar finding to other research (234). In our cohort, we did note an increased incidence of neonatal hypoglycaemia in the infants born to women with a HDP. Potentially this finding may be related to the administration of antenatal steroids for fetal lung maturation or influenced by the use of beta-blockers to treat maternal hypertension, as both of these have been reported to be associated with neonatal hypoglycaemia (237, 238).

Chapter 4; PlGF in twin pregnancy

The first paper in this chapter indicated that although there is good correlation between the laboratory and point of care immunoassays examined, there exists a significant difference in the range and in the PlGF values obtained between the two assays. This difference in PlGF values may possibly be explained by the use of different antibodies in each assay and their cross reactivity with different PlGF isoforms (104-106, 121, 153, 276). These results highlight that clinical cut-offs developed and validated using one biomarker immunoassay are not transferrable to another immunoassay for the same biomarker. Comparative studies performed in singleton pregnancies have shown similar performance of commercial platforms in their ability to rule out

pre-eclampsia (158). A comparative study of three different automated PlGF immunoassays (Brahms®, Elecsys® and Delfia®) similarly found that PlGF values obtained on automated immunoassay platforms are manufacturer specific, not interchangeable and require separate validation (157).

The second paper in this chapter demonstrated that maternal plasma PlGF in a twin pregnancy follows the same gestational pattern as described in singletons with a steady rise corresponding with development of the placenta, peaking slightly earlier than in singletons at approximately 28 weeks' gestation, and then declining thereafter (126, 159). While research has previously demonstrated maternal plasma PlGF is altered in twin pregnancies at the time of diagnosis of pre-eclampsia (166, 180), this study demonstrated that PlGF levels are significantly lower in twin pregnancies that will later develop pre-eclampsia, independent of gestational age at time of sampling. This finding highlights the potential of using PlGF as a diagnostic aid for evolving placental dysfunction in twin pregnancies.

Through recruitment of a cohort of twin pregnancies, despite the necessary removal of many cases due to subsequent pregnancy complications indicating placental dysfunction, I was successful in developing a reference range for PlGF in dichorionic twin pregnancy, which has not previously been available.

7.2.2 **Major Limitations**

The main limitations in relation to the HDP in twin pregnancy retrospective study (Paper 1) was the lack of certain clinical information in the database. This limited my ability to explore the influence of chorionicity and use of

medications including aspirin on outcomes. It also prevented much exploration of the impact of timing of antenatal steroids and quantity of labetalol on neonatal hypoglycaemia (237, 238). Similarly, the effect of gestational age at time of diagnosis of HDP and exactly what type of HDP was present was not well documented in the cohort. Generally, the earlier the onset of pre-eclampsia the more severe it is and subsequently the poorer the perinatal outcomes (232, 233). Despite the lack of certain data points in this retrospective study, the wealth of information provided through its descriptive collective of nine years of data from a large tertiary maternity unit, with its dedicated twin pregnancy clinic, is immense, highlighted by its successful publication.

In relation to the PlGF twin study, limitations of Paper 4 include the use of a customised birthweight centile not specific to twin pregnancy as well as the exclusion of cases where only both twins were <3rd customised centile. While in relation to Paper 3, and the lack of inclusion of additional automated PlGF immunoassays for comparison of performance as suggested, owing to lack of availability of these locally must be acknowledged (141). Given that biobanking of plasma samples was employed, there is potential for future research of these samples with many different PlGF immunoassays. Serial sampling of all participants would have provided a more robust, informative account of PlGF distribution, however this was a pragmatic study, recruiting women while they attended for antenatal review to facilitate their schedule. This flexibility, with no onus for repeat attendances, increased study acceptability and participation. Despite the large number of participants enrolled, given the high incidence of complications in monochorionic cases,

further analysis of this group was limited. However, we did identify that PlGF was lower in monochorionic twin pregnancy, highlighting the need for further research in this area and specifically the development of a monochorionic specific reference range for PlGF in twin pregnancy.

7.2.3 Implications for health policy, clinical practice, and future research recommendations

As healthcare professionals there is a responsibility to appropriately counsel women in relation to the potential increased risks of HDP and preterm birth when embarking on a pregnancy at an advanced age, especially through use of ART and/or donor oocytes. The Irish ART sector needs regulation , with protocols aimed at minimising the risk of twin pregnancy needing to be introduced nationally (57, 70, 85). In December 2019, plans were announced by the Minister for Health to introduce a publicly funded IVF service in Ireland, once human reproduction legislation is passed (357). Introduction of state funding for fertility treatment in the Republic of Ireland would reduce the numbers of women travelling abroad to seek less expensive fertility treatment which is often poorly regulated and frequently results in twin pregnancy due to the practice of multiple embryo transfer (60, 61). Research has shown that many Irish couples conceiving through use of donor oocytes do not disclose this information to their clinical care providers, due to confidentiality concerns, hindering the ability of care providers to risk stratify them appropriately antenatally (358).

Women with a twin pregnancy are at increased risk of developing a HDP and thus should undergo frequent antenatal review with careful assessment at

each appointment for evolving disease (71, 227). Awareness of HDP among both pregnant women and their healthcare providers needs to be improved. Antenatal education and patient information leaflets could help to increase knowledge among women and their partners in relation to symptoms of concern. Education of health care professionals needs to be encouraged and continuously updated in line with new research developments (230). In addition, the introduction of a dedicated twin antenatal clinic, led by a clinician experienced in the management of twin pregnancy would likely result in harmonisation of care to all twin pregnancies in a maternity unit. This approach would likely reduce adverse outcomes and could be considered by professional organisations (53, 241). Future research on twin pregnancy should explore the relationship between chorionicity and HDP as this is unclear and evidence is conflicting, (224, 226) as well as examine the mechanisms responsible for the predisposition to obstetric cholestasis in twin pregnancy (228).

Further research on PlGF in twin pregnancy will be informed by the final trial results of the PARROT Ireland in which only singleton pregnancies were eligible for inclusion. PlGF has potential for use in twin pregnancy as an adjunct to diagnose evolving placental dysfunction (90, 160, 165, 166, 168). However, plasma levels of circulating PlGF in twin pregnancy differ from singletons and therefore clinical cut-offs developed and validated for use in singletons pregnancies cannot simply be applied to a twin pregnancy cohort. Further research on PlGF in twin pregnancy is required (359, 360). The volume of placental tissue present differs dependent on the underlying chorionicity of a twin pregnancy and chorionicity appears to affect PlGF levels. Having now

developed a reference range for PlGF in dichorionic twin pregnancy, one specific to monochorionic twins also needs to be developed.

Once chorionicity specific references ranges for PlGF are available, an observational study should be conducted to evaluate these PlGF reference ranges and develop and validate clinical cut-offs specific to twin pregnancy for PlGF. Future research work on PlGF should also include comparison of other commercially available platforms such as the DELFIA Xpress PlGF 1-2-3 test, Brahms Kryptor and the Roche Elecsys ratio test, (141). Appropriate cut-offs specific to each immunoassay are required if PlGF quantification is to be introduced as a diagnostic aid in clinical practice (281-283). It is essential that stakeholders, policy makers and clinicians alike are aware that clinical cut-offs developed using one platform are not transferrable to another, when deciding on which immunoassay to introduce locally and what advice to advocate when introducing local and national guidelines (157, 158).

7.3 PlGF use to date and the impact of adding PlGF to routine clinical investigations of women with suspected preterm pre-eclampsia in a singleton pregnancy

In order to understand the structure, function and utility of PlGF, I first conducted a literature review of PlGF research to date (Chapter 3). I identified there was significant potential to improve clinician's ability to detect pre-eclampsia with the addition of PlGF testing to routine care. However, there

was a lack of information regarding the potential impact on both pregnant women and neonates from this additional testing. NICE guidelines on PlGF published in 2016 advocated that while PlGF could be used as a rule out test for pre-eclampsia, further research was warranted before it should be introduced into clinical use to help diagnose pre-eclampsia (141). Recognising this gap in current PlGF knowledge and the need for further research, I led a national multisite randomised controlled trial of PlGF in women with suspected preterm pre-eclampsia in order to adequately examine these outcomes (Chapter 5).

7.3.1 Main findings and how they relate to existing research

Chapter 3: Placental Growth Factor

PlGF, a circulating angiogenic factor, plays an integral role in the development of normal pregnancy along with its soluble receptor sFlt-1 (133, 135, 247, 248). Incorporation of PlGF into screening algorithms in early pregnancy improves prediction of those at increased risk of subsequent development of pre-eclampsia (177). In order to be beneficial, both clinical and health economic benefits of such screening needs to be demonstrated (177, 251). Maternal plasma PlGF concentrations are significantly altered in women prior to the clinical onset of pre-eclampsia and are associated with adverse pregnancy outcomes (87, 88, 128-132, 137, 140, 249, 250, 360, 361). Observational studies demonstrated the potential to improve detection of preterm pre-eclampsia if PlGF was included in routine clinical investigations (88, 89, 147) Smaller cohort studies raised concern however that the addition

of PlGF may lower gestational age at delivery and adversely impact on neonatal outcomes, thus highlighting the need for appropriately powered randomised controlled trials, such as PARROT Ireland, to be conducted (155). The therapeutic potential of PlGF is just beginning to be explored. Experimental animal studies have suggested administration of plasma PlGF in early pregnancy may prevent development of pre-eclampsia while reducing its soluble receptor s-Flt1 by plasma aphesis ameliorates pre-eclampsia symptoms (254, 255).

Chapter 5: PARROT Ireland; Placental growth factor in Assessment of women with suspected pre-eclampsia to reduce maternal morbidity; a Stepped Wedge Cluster Randomised Control Trial

The result of the interim analysis of PARROT Ireland reported no significant reduction in either maternal or neonatal morbidity with integration of point of care PlGF based testing, in addition to usual clinical care, in women with suspected preterm pre-eclampsia. These results should be interpreted with caution as they are based only on an interim analysis, conducted on a larger proportion of control rather than interventional participants, and thus the final trial results may differ.

Our interim results contrast to those of the only published RCT on the use of PlGF as a diagnostic test in women with suspected pre-eclampsia in pregnancy; the UK PARROT trial, which reported PlGF testing to be beneficial (188). The integration of PlGF significantly reduced the time required to diagnosis preterm pre-eclampsia, from 4.1 to 1.9 days in the UK PARROT

cohort. Revealed PlGF testing also resulted in a significant reduction in maternal adverse outcomes, without any detected difference in gestational age at delivery or in adverse perinatal outcomes.

The importance of performing appropriately powered, robust, interventional studies before the integration of a new test is well accepted but not always adhered to in clinical practice. The contrasting results of the PARROT UK trial with the interim analysis of the PARROT Ireland trial demonstrates the necessity of multiple studies on a topic, and not to solely rely on the results of a single study. Of course, the final PARROT Ireland results may demonstrate similar findings to the UK study once complete. Another RCT of PlGF integration (EuroPE Study) is currently ongoing in Spain, aiming to recruit in excess of 2000 participants (362). Unlike the two PARROT trials, where PlGF quantification was performed using the Triage PlGF test, this Spanish study is utilising the Elecsys immunoassay sFlt-1/PlGF ratio test for PlGF assessment. The investigators estimate trial completion in February 2021.

7.3.2 **Major Limitations**

The main limitation of the PARROT Ireland trial has been the deficit in participant recruitment. The aim was to recruit in excess of 4,000 women with suspected preterm pre-eclampsia, however just over half this figure, 2313, were recruited by trial closure. Due to the stepped wedge design utilised, the end date of the trial was fixed and could not be extended in order to increase sample size (195). This under-recruitment may potentially lead to under powering of the final trial results, however it is still a substantial number of participants and will provide valuable knowledge regarding the impact of PlGF

on clinically important outcomes; information which is eagerly awaited by international investigators and health care policy makers (141, 363). A second limitation of the trial was the lack of PlGF testing in the control arm which would have allowed post hoc comparison of the blinded as well as revealed PlGF results, ensuring the two arms of the study were equal in this respect. It would appear however from the interim analysis that the demographics between the two groups are very similar, suggesting the groups are comparable (327).

Some might suggest that the incidence of pre-eclampsia subsequently diagnosed in participants of the trial was low, at approximately 14%. The use of the NICE definition for pre-eclampsia may be contributory, owing to its narrow diagnostic criteria compared to the ISSHP definition employed by the PARROT UK trial. Units in the NHS utilise NICE criteria routinely, hence our Belfast trial site was obliged to use this definition. In order to harmonise all trial sites, a decision was made to adopt the NICE definition of pre-eclampsia at all recruiting sites. When diagnostic outcomes for those recruited to PARROT Ireland were examined, the interim analysis indicated that only 5% of those recruited had a final diagnosis of “none of these”, indicating that in 95% of the sample some form of placental dysfunction was present and that they were appropriately recruited to the trial.

7.3.3 Implications for health policy, clinical practice, and future research recommendations

It was concluded by the PARROT UK trial group in their publication that PlGF testing is beneficial (188) and on foot of this the NHS endorsed PlGF testing

to aid diagnosis of preterm pre-eclampsia in maternity units in the UK (187). The steering committee for PARROT Ireland will examine the final Irish trial results once available. If results demonstrate a favourable outcome, the Institute of Obstetricians & Gynaecologists will likely advocate that PlGF be integrated into clinical care pathways in Ireland. The current HSE guideline on management of hypertension in pregnancy details appropriate referral for investigation and management for symptoms suggestive of evolving pre-eclampsia and stratifies location and frequency of review dependent on the level of hypertension present (1). It would be practical and feasible to add PlGF testing as an adjunct in the national guideline, should the results of the PARROT Ireland trial support this.

An individual participant data (IPD) meta-analysis of participants from both the UK and Irish PARROT trials should next be conducted. This would increase the power of both trials substantially and provide the highest quality evidence in relation to the integration of PlGF testing (364). Given that both trials used the same online electronic clinical report form templates, collecting the same data points, an IPD of the two trials is feasible. An alternative option is to conduct a systematic review of PlGF interventional studies to assess the impact of PlGF testing on maternal and neonatal outcomes. The review could also examine which automated commercial immunoassay platform is best utilised. This information will facilitate the updating of guidelines such as the NICE guidance on PlGF testing and Irish clinical practice guidelines (1, 326).

Having an effective diagnostic test for pre-eclampsia should eliminate protracted hospitalisations of pregnant women and allow limited resources to be better utilised. If indeed PlGF testing is to be integrated into mainstream

clinical care, the financial burden of this additional test needs to be assessed (147). As part of the PARROT Ireland trial, health economic data on cost impact to individual participants, as well as to maternity units, was collected. Planned analysis of these data will enable the cost effectiveness of the implementation of PlGF in Ireland to be determined, which will be a key factor for hospitals in their decision as to whether to finance PlGF testing locally. Acquisition of maternal plasma in pregnant women with suspected pre-eclampsia is an onerous process. Biobanking of participant maternal plasma, upon their initial recruitment, was performed in over 200 participants as part of the PARROT Ireland trial. This resource is priceless in terms of facilitating much quicker evaluation and assessment in future research projects, as the task of sample collection will not be necessary. Many research opportunities for these samples are possible, with potential for PlGF use yet to be explored.

Assessment of different commercially available automated PlGF immunoassay platforms, such as Brahms®, Elecsys® and Delfia®, to compare their efficacy and to validate clinical cut-offs for PlGF and/or sFlt-1 would be both interesting and a clinically useful study (141, 157, 158). A second option worth exploring is the potential of using serial PlGF-based testing in order to reduce adverse perinatal outcomes. The recently launched PARROT-2 randomised controlled trial aims to examine this in women presenting with suspected preterm pre-eclampsia. Recruiting across 19 units in the UK from April 2019 to November 2021, by repeating PlGF testing, every week/fortnight, it will investigate the natural course of PlGF over time and assess if repeat testing is beneficial for neonatal outcomes (365). In PARROT Ireland, repeat maternal plasma PlGF sampling was optional for those women

that initially had a normal (>100pg/ml) PlGF result. Serial samples from consenting participants are biobanked at our research centre in Cork, hence a similar retrospective study of our cohort could be conducted.

The prospect of using PlGF or its receptors as therapeutic agents have begun to be considered with animal studies demonstrating that administration of VEGF early in pregnancy prevented the development of pre-eclampsia (254). Similarly, animal studies inducing a reduction in circulating sFlt-1 levels through plasma apheresis reported an alleviation in pre-eclampsia like symptoms (255). These studies suggest the potential utility of early pro-angiogenic therapies in treating pre-eclampsia in the future.

7.4 Facilitators and barriers to conducting clinical research in a pregnant population

Since the mid 90's there has been a concerted effort to ensure minorities, such as women and children, are represented in research in order to help guide scientific based practice for all societal groups (336-338). Pregnant women are seldomly included in randomised controlled trials and their attitudes and experiences of participation rarely investigated. Gathering feedback of their experience is paramount for future trial design to facilitate participation. In order to examine the factors that influence women in their decision to take part in research during pregnancy, I conducted a qualitative study among women who had participated in the PARROT Ireland randomised control clinical trial during their recent pregnancy in our maternity hospital in Cork. In-person

interviews facilitated a more personal relationship between the researcher and participant, and proximity of interview to the time of participation greatly aided women's overall recall of the trial.

7.4.1 Main findings and how they relate to existing research

Chapter 6: Clinical research in a pregnant population

The main objective of this study was to investigate the barriers and facilitators to pregnant women taking part in research. Overall, the study found that pregnant women are aware of the importance of research and were generally interested and positively inclined to participate. Both altruism and the potential of personnel benefit were key motivators behind women's decision to enrol in PARROT Ireland. Similarly a sense of civic duty, the opportunity to help others and the possibility of an improved outcome for their baby has been reported by other researchers to be driving forces behind participation in research during pregnancy (212-214).

The decision to take part in the PARROT Ireland trial was made independently by the pregnant woman herself, a finding which has been reported by others previously (214, 215). The timing and setting of the researcher's approach and the language she used were reported as key factors in women's decision to participate in PARROT Ireland, a finding not widely reported previously but important for researchers to understand when planning future trials so as to maximise participation.

Women reported feeling well-informed about the PARROT Ireland trial prior to signing the consent and later had a good understanding of the trial when interviewed, in contrast to previous studies which reported limited recall by participants, potentially relating to the researchers approach or to the clinical setting at the time of recruitment. (213, 215-217).

Women randomised to the control group did not report negative experiences and although they felt the trial had no direct impact on their own pregnancy, they were still happy to have taken part as they felt it may have benefited others. In contrast, some researchers have reported randomisation to the control arm in an RCT being perceived as a disadvantage among participants (214).

7.4.2 **Major Limitations**

The interviewed women in this study represent only a subgroup of women, and potentially a biased group, as these women had not only consented to participate in research during their pregnancy, but also agreed to take part in this additional qualitative study. Ideally, to fully evaluate the barriers to research participation in pregnancy I would also have liked to interview those who had declined to take part in the trial. However, as per GCP and GDPR (354, 355) no information on eligible women who were approached but declined to participate in the trial was retained; thus contacting them for this additional part of the study was not feasible.

7.4.3 Implications for health policy, clinical practice, and future research recommendations

In general pregnant women are willing and interested to participate in research, provided it does not confer a risk to their pregnancy (212-215, 350). The attitude, approach and information of their clinicians is a key factor in a woman's decision to participate in research (37). If pregnant women feel well informed, especially if a trusted clinician provides the information, they are much more likely to participate. Accurate knowledge by clinicians of ongoing trials in their unit and the vocalisation of their support is crucial in promoting participation of pregnant women in future trials.

In order to generate evidence based information on the impact of interventions in pregnancy, research is required. Given the stringent pre-initiation examination that clincal trials require (sponsorship, insurance and review by national ethical committees) as well as the ongoing vigilence and monitoring by stakeholders for any trends in adverse events, the safety of any such research is a key priority for all involved, including the pregnant woman herself (331, 340, 351). The context, purpose and potential risk of any research are the most important considerations, thus maximising eligible women's understanding of the research being conducted and allowing shared decision-making, is key to a trial's acceptability. The information and explanation provided by reseachers is crucial. Adequate training of researchers and clinicians, to maximise this skillset, should be prioritised to aid success of future studies.

Designing future trials with recruitment ideally based in non-emergent settings would increase the likelihood of participant recall following the event and reduce the potential for exploitation of potentially vulnerable patients. Alternatively, where the setting of a trial must be emergent, such as a labour ward or emergency theatre, a delayed consent process could be to employed (352). This approach has been used previously in trials of critically ill patients and has been found to be acceptable (353). Using the information generated from my qualitative study on research in pregnancy to design and conduct future pregnancy studies will likely increase the trials acceptability, participation and chance of successful completion.

7.5 Conclusion

The purpose of this thesis was to explore the potential for the use of PlGF in twin pregnancy and evaluate its impact in singletons with suspected preterm pre-eclampsia. The findings from this thesis, though supportive of the current literature in relation to the potential of PlGF, highlight that there is more research required.

The thesis outlines that exact PlGF quantification with the differing commercial immunoassays available differs, and hence clinical cut-offs for PlGF are not transferrable. Further research should ideally utilise multiple immunoassays to facilitate translational work.

PlGF has an important role in healthy placentation in early pregnancy. Aberrations in circulating plasma PlGF as pregnancy progresses are strongly associated with placental dysfunction and adverse pregnancy outcomes. It is clear from the studies presented that there is potential for the use of PlGF in twin pregnancy as a diagnostic aid in conditions of placental dysfunction such as pre-eclampsia. PlGF levels in twin pregnancies differ to those in singletons, as well as differing by chorionicity. Before PlGF can be utilised clinically in twin pregnancy, chorionic specific validated clinical cut-offs need to be established and agreed in clinical guidance.

The research presented in this thesis adds to a growing body of work focusing on utilising PlGF as a diagnostic aid for preterm pre-eclampsia in singleton pregnancies. The potential for use of PlGF in the prediction of other placental dysfunction conditions such as IUGR and stillbirth warrants further investigation but evidence to date is promising.

Pregnancy research is important in order to generate evidence-based information. This thesis highlights that pregnant women are aware of the importance of research and generally satisfied to participate in clinical trials once appropriately informed of the purpose and nature of a study.

The PARROT Ireland trial examined the clinical impact of point of care PlGF based testing in over 2300 women with suspected preterm pre-eclampsia across seven maternity units. While the results of the interim analysis reported in this thesis showed no significant reduction in either maternal or neonatal morbidity with PlGF use, the full trial results are awaited, before making any recommendations in relation to PlGF use..

Combining these Irish trial data with those from the UK PARROT trial, and possibly those of another ongoing RCT, has the potential to provide large scale, high quality results, adequately powered to give a definitive answer in relation to PlGF use in clinical practice. This information is eagerly awaited and will aid the updating of international clinical practice guidelines as well as helping to direct further evidence-based best practice in the diagnosis and management of pre-eclampsia.

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Appendices

Appendix 1: SOP CLIN 003: eCRF Parrot V 2.0

INFANT: Irish Centre for Fetal and Neonatal Translational Research

SOP 003
eCRF
(electronic Case Record Form)

Sponsor: University College Cork (UCC)

SOP Number:	**CLIN 003_PARROT**	**Effective Date:**	**21st Dec 2017**
Version Number & Date:	Version 2.0 21 DEC 2017	**Review Date:**	21st Dec 2019
Author: **Co Author:**	Deirdre Hayes-Ryan Nicolai Murphy	**Title:** **Title:**	Clinical Research Fellow Project Manager
Reviewed By:	Jackie O'Leary	**Title:**	Quality & Regulatory Affairs Manager
Approved By:	Louise Kenny	**Title:**	Principal Investigator

SOP Chronology		
SOP Version Number	**Reason for Change**	**Author**
1.0	Original Document	Blánaid Ní Chuinneagáin

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2.0	Database Amendments	Deirdre Hayes-Ryan

Table of Contents

Tables

Figures

1 Guides

This document (also referred to as a Standard Operating Procedure: SOP) is identified by code CLIN 003_eCRF SOP_PARROT. The code identifies the category of the document; (ADMIN/LAB/CLIN, the number of the document from a list of other PARROT SOPs; 001/002/003, the name of the SOP, the study acronym and the Version of the SOP). The chronological history of this document can be found on the front page.

2 Scope

This SOP applies to any member of the PARROT Ireland study team who will be completing data entry using the eCRF or who is responsible for signing eCRF forms to verify that the data is complete and accurate.

3 Purpose

The purpose of this SOP is to describe the procedure that will be used to enter and complete records for a participant in the PARROT Ireland study on the eCRF and should be used in conjunction with the current version of the MedSciNet_CTF_Users_Manual. MedSciNet are the providers of the eCRF database and all related software and documents.

4 Responsibility

This SOP applies to all study personnel at each recruiting site who will have access to the eCRF, and have been granted investigator or data entry access.

5 Definitions/Abbreviations

Table 1: Definitions

Term/Acronym	Description
ACOG	American College of Obstetrics and Gynaecologists
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body Mass Index
CRF	Clinical Report Form
CS	Caesarean Section
CUMH	Cork University Maternity Hospital
EBL	Estimated Blood Loss
Hb	Haemoglobin
HE	Health Economics
HTN	Hypertension
ITU	Intensive Therapy Unit
LDH	Lactate dehydrogenase
PET	Pre Eclamptic Toxaemia
PlGF	Placental Growth Factor

Term/Acronym	Description
RCOG	Royal College of Obstetrics and Gynaecologists
SGA	Small for Gestational Age
SOP	Standard Operating Procedure
WMA	World Medical Association

6 Materials & Equipment

Table 2: Materials and Equipment

Materials and Equipment
Computer with internet access

7 Procedures

PARROT Ireland Public Website

The PARROT Ireland study has a public website available for participants and clinical staff with information and contact details for any queries; https://parrotireland.medscinet.com/ . This address should be added to favourites in order to easily access throughout the trial.

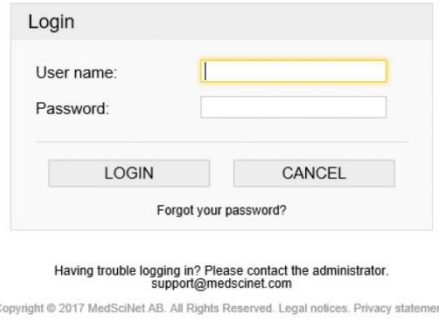
Figure 1: PARROT Ireland Website Homepage

PARROT Ireland Database

The PARROT Ireland eCRF/database where all participant information is stored is accessed through the public PARROT Ireland website via the login icon on the left hand side of the screen on the home page. This side of the secure website is not accessible to the general public. To obtain access to the database researchers should contact the project manager or trial research fellow. A password will be issued to each user, which must be kept confidential and will be required each time the researcher logs onto the eCRF. The database will log all changes made by each individual user.

The researcher will be automatically asked to change his/her password every 3 months.

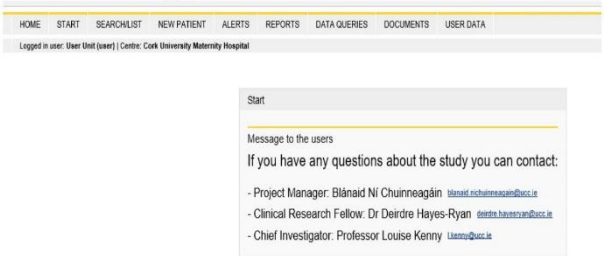
Figure 2: PARROT Ireland Login Page



Start Page

The start page appears following log-in. The text section of the start page is a notice board, headed 'Start Page', where PARROT Ireland information will be posted. It will be used to communicate important messages to all researchers. It will also show current recruitment rates and the number of women who have completed the study. The notice board should be checked daily. For further information on how to navigate the database please see the current version of the MedSciNet_CTF_Users_Manual.

Figure 3: PARROT Ireland Start Page



Documents

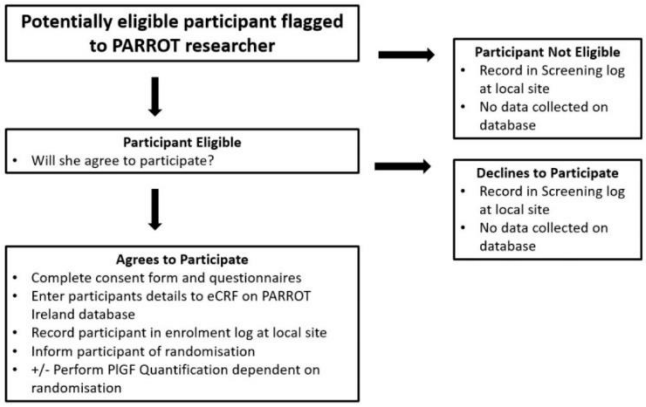
PARROT specific approved documents will be available via the documents tab in the eCRF and in all instances documents that require printing should be printed from this location to ensure the most up to date version is being used.

Data Entry

Once a potentially eligible participant is identified, they will be contacted by the researcher and consent will be obtained (see CLIN_PARROT_001 Informed Consent Process). The researcher will then enter their data in the PARROT Ireland database.

Please see below figure outlining data to be collected from potential participants (both eligible and ineligible).

Figure 4: Diagram of data collection for eligible and ineligible Participants



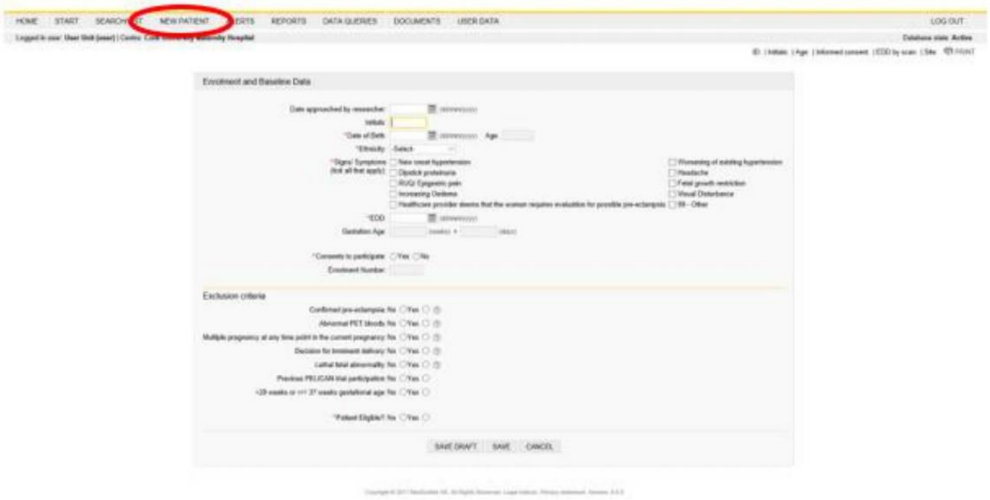
Study Number Assignment

Each participant enrolled in the PARROT Ireland study will have a unique identifying number assigned to them. This number is generated by the database when their enrolment eCRF is completed and saved. It is a combination of letters and numbers. Each site has its own letters while each number is sequential and represents the order of the recruitment, e.g.; CUMH005 is a participant recruited in Cork that was 5th overall to be enrolled in the trial, NMH006 is a participant recruited in the National Maternity Hospital that was 6th overall to be recruited at that site to the trial.

New Participant Entry

To begin entering a new Participant in the database select **'New Patient'**.

Figure 5: New Participant Screen

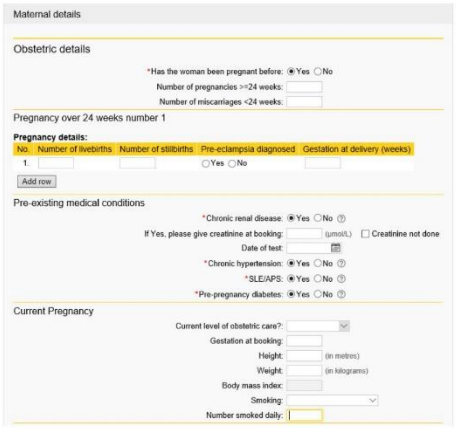


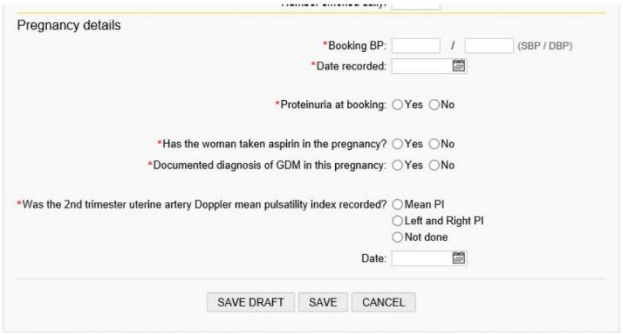
Enrolment and Baseline Data eCRF. The following information will need to be entered:

- Date participant was approached by the researcher
- Demographic data
- Signs/symptoms that make the participant eligible for inclusion
- EDD (Estimated Date of Delivery)
- Participant consent details
- Exclusion criteria and participant eligibility confirmation

Maternal Details eCRF. The following information is required as per screenshots below:

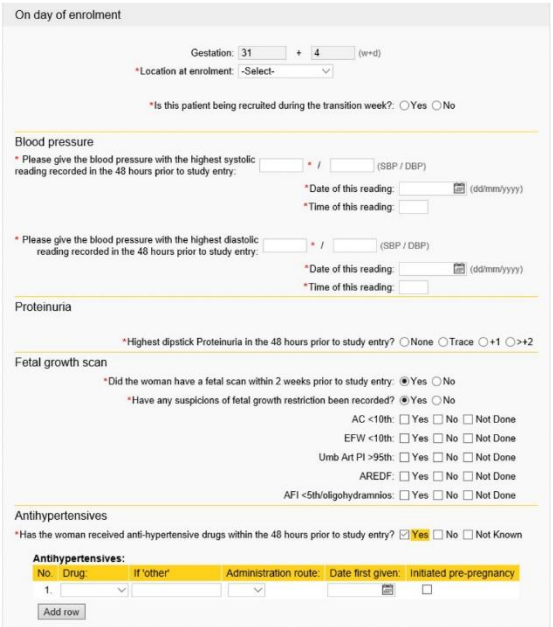
Figure 6: Maternal Details CRF





On day of enrolment eCRF. The following information is required:

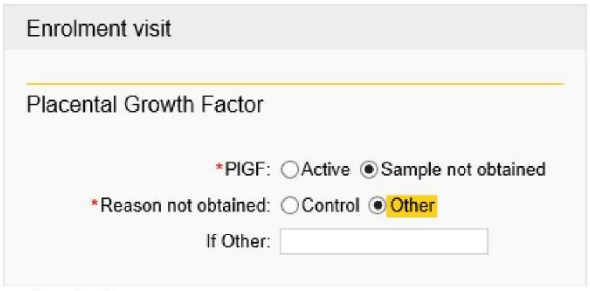
Figure 7: On Day of Enrolment eCRF



Enrolment Visit eCRF. The following information is required:

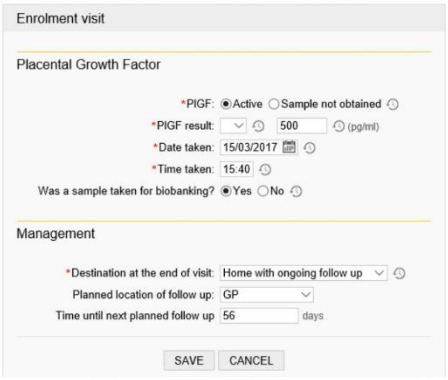
- *Control arm*:
  - Select 'Sample not obtained' and select 'Control' as reason not obtained.

Figure 8: Enrolment Visit - Control



- *Active arm*:
  - Select 'Active' and enter date and time PlGF sample taken.

Figure 9: Enrolment Visit – PlGF sample taken

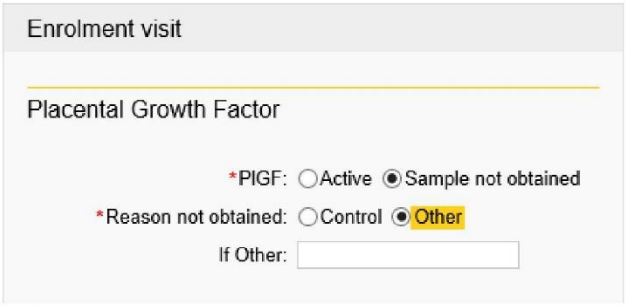


- *Errors*:
  - If there is any issue with obtaining or processing the PlGF sample select 'Sample not obtained' and select 'Other' as reason not obtained.
  - Use the free text box to enter the reason sample not obtained.

Enrolment Visit eCRF. The following information is required:

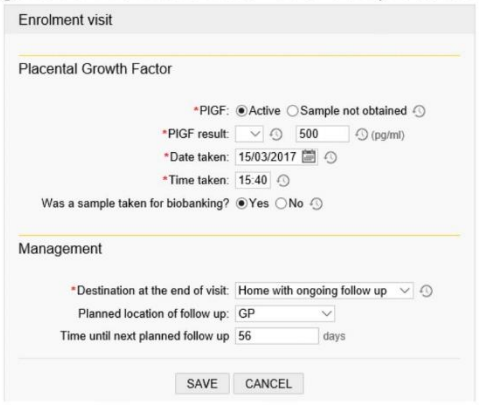
- *Control arm*:
  - Select 'Sample not obtained' and select 'Control' as reason not obtained.

Figure 8: Enrolment Visit - Control



- *Active arm*:
  - Select 'Active' and enter date and time PlGF sample taken.

Figure 9: Enrolment Visit – PlGF sample taken



- *Errors*:
  - If there is any issue with obtaining or processing the PlGF sample select 'Sample not obtained' and select 'Other' as reason not obtained.
  - Use the free text box to enter the reason sample not obtained.

- *Management plan at end of enrolment visit* – Record the documented decision by the clinician reviewing the participant at the end of her review regarding the best location for her immediate future.

Enrolment Visit – Health Economics (HE) eCRF. The following information is required:

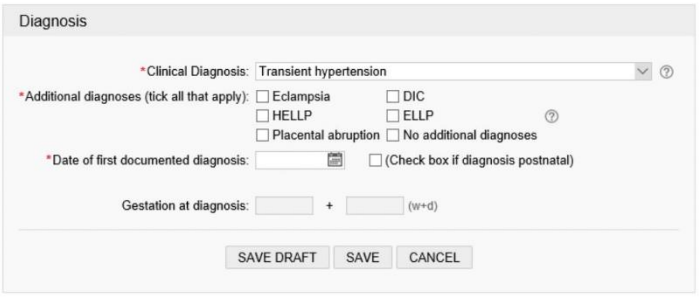
- *EQ-5D Health Questionnaire* – Enter data as completed by participant.
- *SF-36 Health Survey* – Enter data as completed by participant.

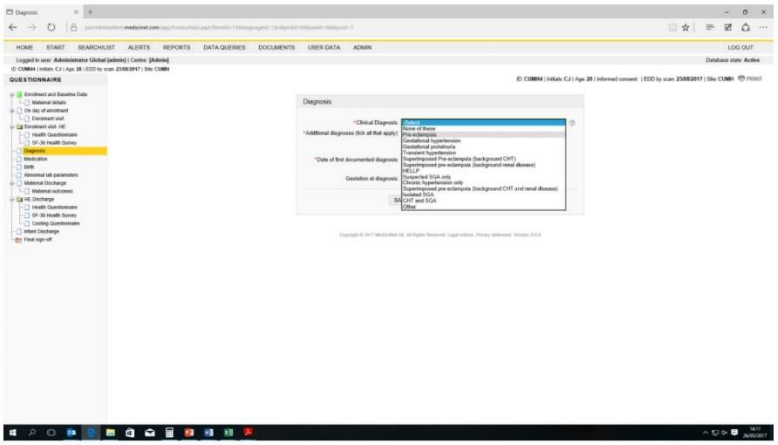
Repeat Attendance Visit – Add new attendance eCRF. If a participant enrolled in the active arm attends for a Repeat Attendance visit (re-admission for suspected pre-eclampsia with PlGF sample taken if >4 weeks since enrolment/last PlGF sample and <37 weeks gestation). The following information is required:

- *Date of assessment*
- *Signs/symptoms that make the participant eligible for inclusion*
- *PlGF result, as per 7.3.7*
- *Management plan, as per 7.3.7*

Diagnosis eCRF. As soon as a participant has delivered and been discharged home and her baby has been discharged from the neonatal unit, their charts and outcome data should be reviewed by the PARROT Ireland researcher at each site. Clinical relevant maternal and neonatal outcomes will be recorded in the database. Note: All maternal outcomes up to 12 weeks post-delivery should be recorded, including if the participant has a readmission postnatally. The following information is required to confirm if the participant had a clinical diagnosis of any conditions outlined in the dropdown menu:

Figure 10: Diagnosis eCRF





If unsure regarding a diagnosis, please refer to the Definitions List, located in the documents section of the trial database, or contact the trial research fellow or project manager to discuss.

Date of First Diagnosis:

- **Small for Gestational Age (SGA)**: suspected/isolated: Date IUGR first detected antenatally
- **Pre Eclamptic Toxaemia (PET):** when fulfils NICE criteria –NB check BP/PCR/24 hour results and do not rely on documentation in the participants chart. Patient may be postnatal when diagnosis occurs.
- **Gestational HTN/Proteinuria**: date of first documented diagnosis
- **Chronic HTN**: Either exists pre pregnancy, occurs before 20 weeks gestation or can only be diagnosed > 6 weeks postnatally if BP remains elevated.

Confirmation _____ eCRF: If any of the above are answered Yes, an additional CRF will open for further information on the diagnosis including;

- Highest recorded blood pressure recordings
- Ultrasound findings
- Medications administered
- Haematological/biochemical findings
- For those with PET confirmation - whether the participant met the American College of Obstetrics and Gynaecologists (ACOG) criteria for a diagnosis of severe Pre-eclampsia.

Medication eCRF. The following information is required:

- *Antihypertensives* – Confirm if participant received any anti-hypertensives from enrolment until delivery, or between delivery and discharge, entering drug, date given, dose and route administered. Additional rows may be added as required.

- *Steroids* – Confirm if participant received any steroids from enrolment until delivery, entering drug, dose and date given.
- *Magnesium* – Confirm Yes/No if magnesium was administered for prophylaxis or treatment of eclampsia in cases of pre-eclampsia.

Birth eCRF. The following information is required:

- *Blood Pressure* - Enter the highest recorded blood pressure, both systolic and diastolic, taken from the participant from time of enrolment onto the trial until her discharge post-delivery, including date.
- *Fetal Ultrasound* – to record if the participant had any formal fetal ultrasound scans *after* her entry to the trial, as per 7.3.6.
- *Delivery* – Enter date the participant delivered (gestational age will automatically be calculated).
  - *Labour onset* – Spontaneous, Induced or Pre Labour Caesarean Section (CS).
    - If induced or pre labour CS enter the most appropriate indication from the drop down list of options.
  - *Mode of delivery* - enter the most appropriate indication from the drop down list of options. **Note:** Pre-labour CS includes all CS whether emergency CS or any CS performed after onset of Labour.
    - For those who have an emergency CS please categorise it from 1-4 as per Royal College of Obstetrics and Gynaecologists (RCOG) good practice guidelines.
  - *Estimated Blood Loss (EBL)* at delivery: Enter the amount of blood loss in millilitres (mls) at time of delivery as recorded in the participants' medical notes.
- Infant Delivery
  - Status at birth: Liveborn –born alive at any gestation >24 weeks / Stillborn – born with no signs of life present >24 weeks of pregnancy / Miscarriage <24 weeks.
  - Sex
  - Birthweight (grams)
  - Customised birthweight - use the following link to calculate a customised infant centile value based on maternal BMI, parity, ethnicity, fetal gender and birthweight. **Note:** if stillbirth occurred, enter gestation on the calculator as gestation when the stillbirth was diagnosed not gestation at delivery.
    - **http://www.gestation.net/cc/5/836998.htm**
  - 5 minute APGAR
- *Cord Blood Gases* - Enter the arterial and venous pHs and base excess taken at the time of delivery (if these were performed). Clarify if there was any evidence of fetal umbilical artery acidosis at birth (arterial cord pH <7.20).

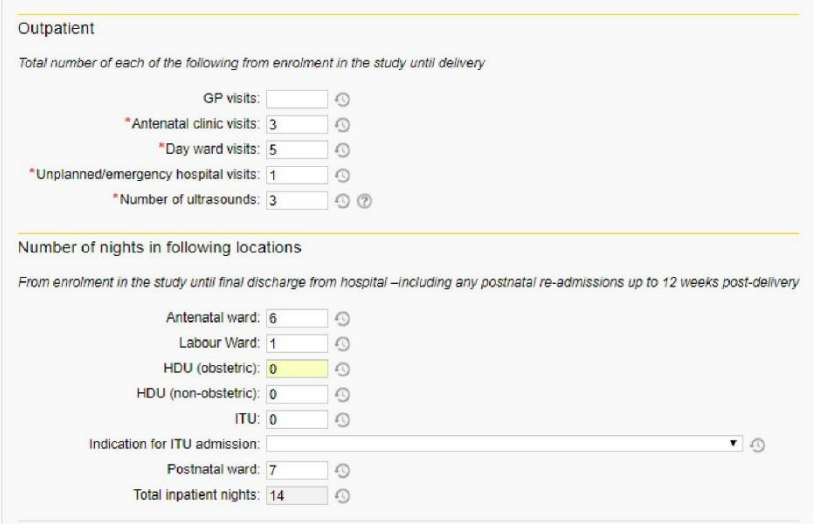
Abnormal Lab Parameters eCRF. The following information is required:

- Enter the most abnormal laboratory values of the following indexes reported for the participant, from the time of her enrolment in the trial until her discharge post-delivery - Haemoglobin (Hb), Platelets, Creatinine, Aspartate Aminotransferase (AST),

Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), Uric Acid, Peripheral blood film.

Maternal Discharge eCRF. The following information is required:

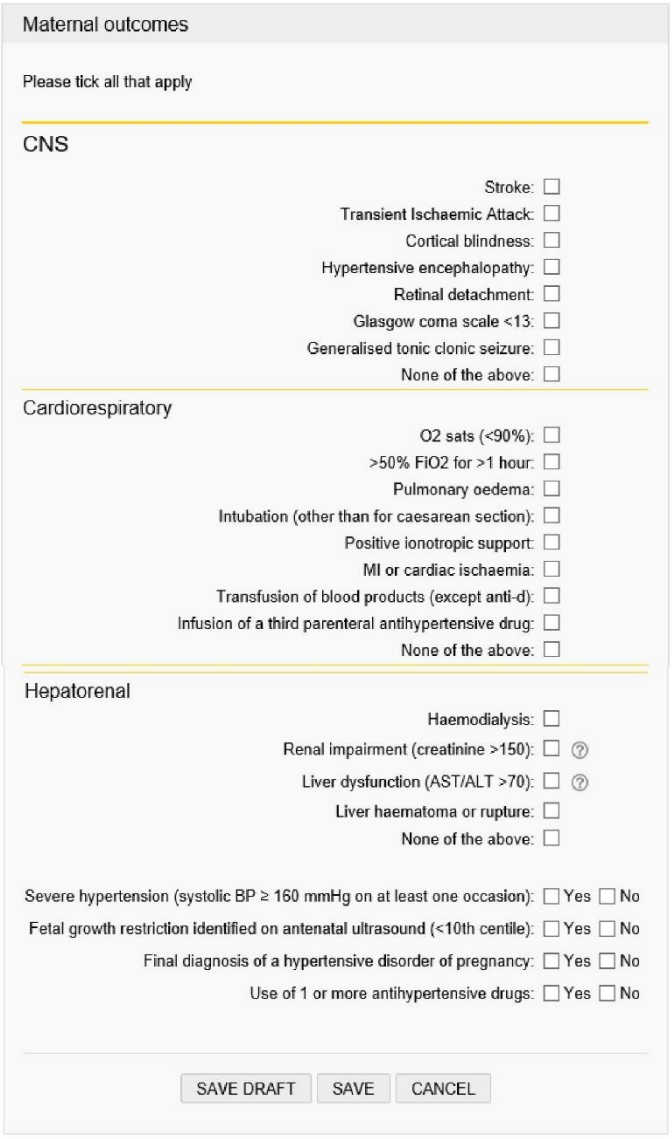
Figure 11: Maternal Discharge eCRF



Number of GP visits may not be available therefore leave blank. Rarely should a patient require Intensive Therapy Unit (ITU) admission but if so please insert reason from dropdown list provided. If the required option is not available, contact trial research fellow/project manager to discuss or select “other” and insert free text.
Note: Number of nights postnatal, should capture **ALL** of postnatal admissions, both initially after delivery and again if a readmission occurs.

Maternal Outcomes eCRF. Record if the participant suffered from any of the following outcomes:

Figure 12: Maternal Outcomes eCRF



If unsure regarding a diagnosis, please refer to the Definitions List, located in the documents section of the trial database, or contact the trial research fellow/project manager to discuss.

Note: MgSo4 should not be considered an antihypertensive agent in this setting

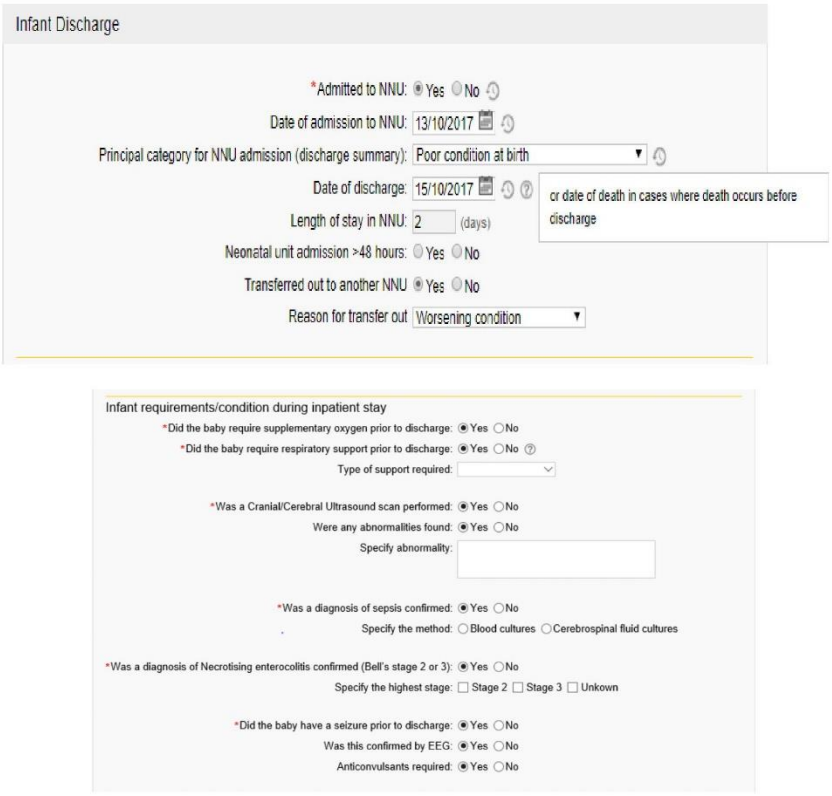
HE Discharge eCRF. The following information is required:

- *EQ-5D Health Questionnaire* – Enter data as completed by participant.
- *SF-36 Health Survey* – Enter data as completed by participant.
- *Costing Questionnaire* – Enter data as completed by participant.

The date that these are completed should also be recorded

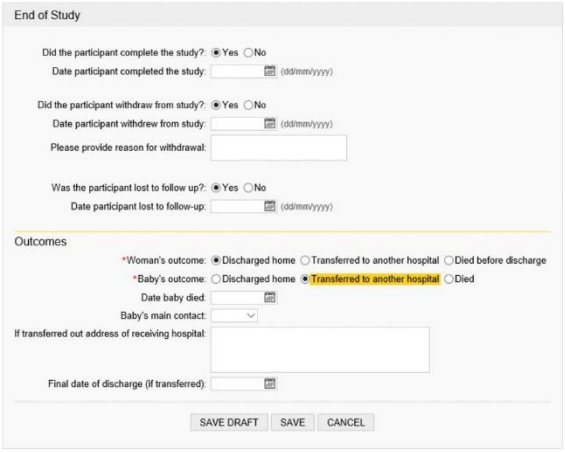
Infant Discharge eCRF. The following information is required:

Figure 13: Infant Discharge eCRF

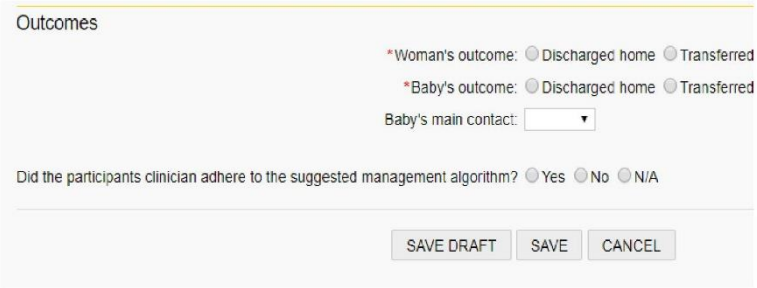


End of Study eCRF. The following information is required:

Figure 14: End of Study eCRF



Please note the date of completion (if the participant completed the study) is the date that the participant has the last outcome recorded therefore if they have outcome data entered up to 12 weeks post natally, then this is the date of completion. If no further outcomes are recorded post birth of the infant, the date of birth of the infant will be the date of completion.



If the participant is enrolled during the control arm of the study, the adherence to the algorithm is N/A. However if the participant is enrolled during the ***intervention arm***, record whether or not the treating clinician adhered to the suggested management algorithm provided.

Protocol Deviation eCRF. The following information is required:

Figure 15: Protocol Deviation eCRF

Source Data

The source data for each item of data collected in the eCRF is defined as the first place that this data is recorded. For the duration of the study, PARROT specific documents (Signed Informed Consent Forms and Health Economic Questionnaires) will be filed in a separate folder and stored by the researcher in a locked drawer separate to the ISF and accessible only to PARROT Ireland Personnel.

Data Storage

At the end of the study, data will be extracted from the database for the analysis. An audit trail exists for all of the data in the database.

Document Storage

All study documents will be maintained by each site in their Investigator Site File (ISF) and by the Trial Management Group in the Trial Master File (TMF).

8 Reference to Other SOPs, Regulations, Guidelines

- PARROT Ireland Trial Protocol (Current Version)
- MedSciNet Clinical Trial Framework USER'S MANUAL Document (Current Version)
- WMA Declaration of Helsinki –Ethical Principles for Medical Research Involving Human Subjects
- RCOG Good Practice No. 11 April 2010
- INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2)
- SOP 001 Informed Consent Process

Appendix 2: Enrolment Log V 2.0

PARROT IRELAND PARTICIPANT ENROLMENT LOG

Study ID Number	Participant Name	Participant Date of Birth	Hospital Number (Mother)	Hospital Number (Baby) when applicable	Date of Consent	Estimated Due Date	Participant Completed (C), Withdrawn (W) or Lost to Follow up (LTFU)	Date Participant Completed (date last piece of data collected), Withdrew or was Lost to Follow up

Appendix 3: LAB 001 Sample Handling V 1.0

INFANT: Irish Centre for Fetal and Neonatal Translational Research

Sample Handling using the Alere Triage® PIGF Meter Pro platform and Biobanking (selected sites only)

Sponsor: University College Cork (UCC)

SOP Number:	**LAB 001_PARROT**	**Effective Date:**	17th AUG 2017
Version Number & Date:	Version 1.0 17th AUG 2017	**Review Date:**	17th AUG 2019
Author:	Caroline Nolan	**Title:**	Research Midwife
Reviewed By:	Deirdre Hayes-Ryan Blánaid Ní Chuinneagáin Jackie O'Leary	**Title:** **Title:** **Title:**	Clinical Research Fellow Project Manager Quality & Regulatory Affairs Manager
Approved By:	Emma Snapes	**Title:**	Laboratory Manager
Approved By:	Louise Kenny	**Title:**	Principal Investigator

SOP Chronology		
SOP Version Number	**Reason for Change**	**Author**
1.0	Original Document	Caroline Nolan

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1 Guides

This document (also referred to as a Standard Operating Procedure: SOP) is identified by code LAB 001_Sample Handling SOP_PARROT_Version 1.0. The code identifies the category of the document; (ADMIN/LAB/CLIN, and the number of the document from a list of other specific PARROT SOPs; 001/002/003). The chronological history of this document can be found on the title page.

2 Scope

This SOP applies to PARROT Ireland personnel involved in processing participant samples using the Alere Triage® PlGF Meter Pro platform. This SOP also applies to personnel from selected PARROT Ireland sites processing the remaining samples for biobanking.

3 Purpose

The purpose of this SOP is to describe the procedure used when installing and calibrating the Alere Triage® PlGF Meter Pro platform for the analysis of biobanked participant samples.

4 Responsibility

The appointed PARROT Ireland personnel in selected sites will be responsible for daily and monthly calibration of the Alere Triage Meter Pro platform, analysis of the participant samples on the Alere Triage Meter Pro platform and biobanking of remaining samples.

5 Definitions & Abbreviations

Table 1: Abbreviations

Term/ Acronym	Description
µL	Microliter
Alere Triage Meter Pro	The Meter
AUX ID	Auxiliary Identification (Initials for the purpose of this SOP)
ID	Identification
IQC	Internal Quality Control
ISBER	International Standards for Biological and Environmental Repositories
LTS	Long Term Storage
NPV	Negative Predictive Value
PlGF	Placental Growth Factor
PPV	Positive Predictive Value
QC	Quality Control
SOP	Standard Operating Procedure- A set of detailed, written instructions to be adhered to, in order to achieve uniformity of the performance of a specific function
ULT	Ultra Low Temperature
WBC	White Blood Cells

Table 2: Definitions

Term/ Acronym	Description
Blood collection tube	Closed tube with an evacuated chamber for venepuncture
Buffy Coat	A blood component. Contains nucleus bearing DNA. Also referred to as the white blood cell layer.
Class II Biosafety Cabinet	Contained units providing personnel and environmental protection and sterile conditions for product when used correctly. The key features include a front access opening with carefully maintained inward airflow, a HEPA-filtered, vertical, unidirectional airflow within the work area and a HEPA-filtered air exhaust.
Cryoboxes	Polypropylene boxes suitable for ULT storage of cryovial used to optimise storage capacity of biobank.
Cryovials	Tubes designed for storage of liquids at ultra-low temperature. Those used in this SOP have a unique 2D code on the base, a visible 250ul ring and are supplied capped and irradiated.
EDTA	An anticoagulant spray of ethylene-diamineteraacetic acid coated inside a lavender topped blood collection tube.
Fibrin clots	Can occur in plasma which has not had sufficient time to clot adequately or it can occur due to incorrect collection method. The presence of clots in the plasma depletes the plasma available for storage. Invisible fibrinogen carried over in aliquots can interfere with some analyses.
Haemolysis	Visible due to the presence of free haemoglobin (>100 mg/L) in plasma as a result of RBC rupture.
Icterus	Bile pigments in the blood can colour resulting blood plasma and serum from dark to bright yellow rather than a straw colour.
PARROT laboratory Log book	A local laboratory based diary to record participant blood processing details in the laboratory.
Lipemia	The presence of excess fats or lipids in blood typically after a heavy meal can cause serum/plasma to appear milky or turbid. The large particles can interfere with instrument methods that are based on light detection or scatter.
Plasma	Liquid fraction produced when whole blood is collected in tubes treated with an anticoagulant. The blood does not clot and the cells are removed by centrifugation.
RBC (Red Blood Cells)	A blood component. Does not contain DNA
SPRECs	Standard Pre-Analytical Codes for use in biobanks as recommended by ISBER
Ultra Low Temperature (ULT) Freezer	A critical component of a biobank used to stably store frozen blood samples at very low temperatures

6 Materials & Equipment

Table 3: Materials and Equipment

Materials and Equipment	Source
Alere Triage® Meter Pro, including Triage meter Power cable, QC device and code chip and Supervisor code chip	Alere (Cat no. 55071)
Centrifuges	Sigma 2-16PK (refrigerated) or equivalent
EDTA blood Collection Tube	Cork University Maternity Hospital
Bulldog clip for grabbing multiple cryovials	Any stationary supplier. Approximately 70mm wide
Gloves (non-latex recommended)	As per institutional standards
Class II Biosafety Cabinet	AGB/VWR Bioquell Microflow ABS1500CLS2-MK2 (no longer commercially available)
Lavender precapped cryovials, 0.65ml for plasma use	W-SER WILMUT (960 per bag), lavender, sterile Biostor Catalogue #W054100LE
Needle/sharps disposal unit	Patron 22L
Pipette 50-250µl	VWR Catalogue 613-5264
Pipette tips Filtered	Axygen TF-350-R-S (VWR Catalogue 732-0646)
Processing cryorack	Empty cryorack, W-ECO WILMUT, no barcode, suitable for holding 0.65ml cryovials Biostor Catalogue #-W000050
PlGF Control 1 and 2 QC Samples, including code chips	Alere
Single channel decapper	Manual decapper single channel for precapped cryovial Biostor Catalogue WS000018
Single cryovial picker	Single picker for precapped cryovial Biostor Catalogue W000040
Test Device box, including 25 Test Devices, 25 transfer pipettes and 1 code chip per box	Alere
Cryorack	Empty cryoracks, W-ECO WILMUT,

	barcoded, suitable for holding 0.65ml cryovials Biostor Catalogue #-W000159

7 Procedures

The Alere Triage® Meter Pro (Figure 1) is used to measure Placental Growth Factor (PlGF) levels in EDTA plasma of women presenting with signs or symptoms of pre-eclampsia. The Alere Triage® Meter Pro is referred to as The Meter throughout this SOP.

Each meter comes with

- AC/DC power convertor.
- Supervisor chip code. This gives the user access to set parameters in the main menu.
- A Quality Control (QC) device box containing the QC device and code chip. The QC device is run each day of sample testing.

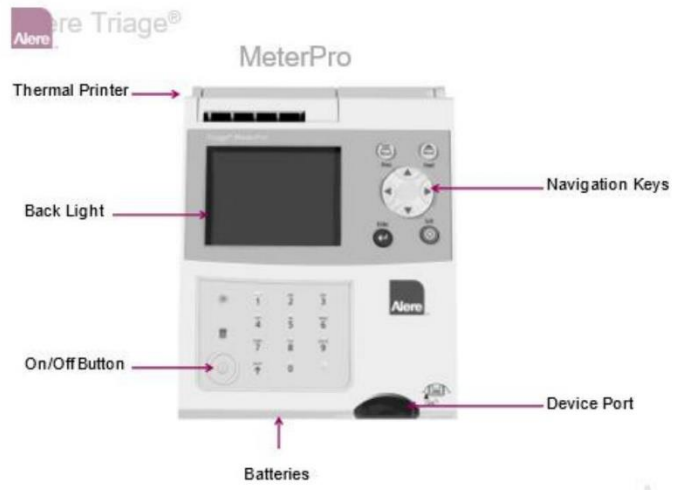


Figure 1: Alere Triage® Meter Pro

7.1 Meter Set Up

- Operate the meter on a level, dry surface away from direct sunlight.

1. Connect the meter to a power source using the power cable provided and press the On/Off button as seen in Figure 1. The meter runs a self-test upon switching on. In self-test mode, the meter scans an internal calibration chip. Each calibration chip scan is used to validate and adjust, if necessary, the meter calibration. Operator calibration is not necessary. When self-test calibration is complete, the meter display screen displays the main menu. The Main Menu has three options: run test, recall results, and install new code chip, as shown in Figure 2.

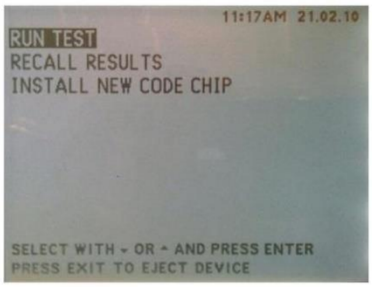


Figure 2: Main Menu

7.1.1 Installing meter code chips

Each new lot of test devices and liquid controls are accompanied by a specific code chip. The meter will prompt the user to install a code chip when required.

1. Install the new individual code chip in the code chip port of the meter (see Figure 3), before a new lot of Test Devices or liquid controls is used.

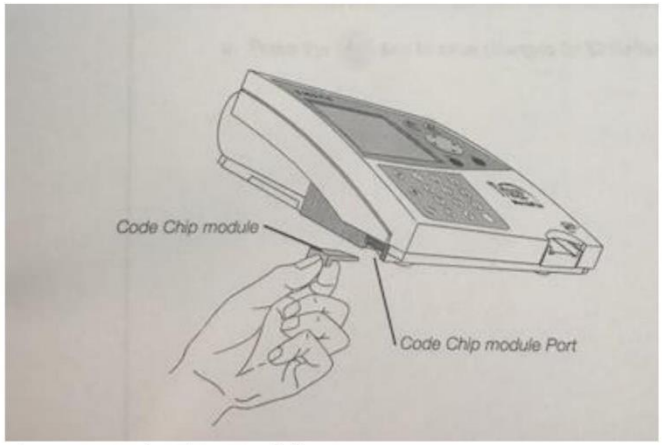


Figure 3: Code Chip Module Port

<u>Code chip installation:</u>

Instructions for installing QC sample chip codes and reagent chip codes for each new lot of Test Devices.

1. Hold only the coloured plastic casing of the code chip when removing it from its clear protective mould. Do not touch the code chip itself.
2. Compare the lot number of the code chip with the corresponding lot number on the source box.
3. From the ***Main Menu*** select ***INSTALL NEW CODE CHIP*** and press ***Enter*** to confirm.
4. Insert the code chip into the port on the lower left hand front corner of the meter (Figure 3).

5. Once installation is confirmed, press ***Enter***.
6. Remove the code chip and store in its original container in a safe place.

Types of code chips:

- QC device code chip - stored in the black opaque QC device box (Figure 4) and labelled with the serial number of the QC device. The QC device code chip is used in conjunction with the QC device.
- QC sample code chip - included in each box of liquid controls. Lot number begins with a **C.**
- Reagent code chips - included in each box of Test Devices. Lot number begins with a **W.**

7.1.2 Installing a new role of paper in the meter

1. Remove the thermal printer cover (Figure 1) by pulling upwards.
2. Remove any paper with sticker or glue on it from around the outside of the new roll of thermal paper roll.
3. Tear or cut a clean straight edge to feed into the printer. Do not cut paper at an angle, as the printer must sense the edge of the paper along the feed path.
4. Remove the spindle from the used roll and place it in the new roll.
5. Place the new roll into the printer with the spindle in the side supports so that the paper feeds from underneath the roll.
6. Pull some of the paper away from the roll and slip it into the guides in front of the feeder. Press the Feed button on the meter if necessary so that blank paper is coming out of the cutter.
7. Sit the roll into the holding space and replace the cover and rip any excess thermal paper off at the cutter.

7.2 Quality Control of the Meter

There are two quality control (QC) stages required for the device:

1) The QC DEVICE – performed on each day of patient testing.
2) QC SAMPLE - performed monthly or prior to use of a new lot of Test Devices using PlGF control 1 (low concentration) and PlGF control 2 (high concentration).

7.2.1 The QC Device

Each meter has its own unique reusable QC device which does not expire. QC devices cannot be used with any other machine.

1. Store the QC device in its black opaque case at room temperature when not in use, (Figure 4) as it is light sensitive.

Figure 4: QC Device Case

Running the QC device:

Run the QC device on each day of patient sample testing.

1. From the ***Main Menu*** (Figure 2), select ***Run Test*** using the arrow keys and press ***Enter***.
2. Select ***QC Device*** if not already selected (Figure 5) and Press ***Enter*** to confirm selection
3. Gently insert the QC device into the meter until QC device catches on the pin internally and an audible click is heard (Figure 6).
4. Press ***Enter*** to start the calibration test as instructed (Figure 6). The meter prompts the operator to install the QC Device Code Chip module for the initial QC Device use. The test takes approximately 4 mins (Figure 7). The meter pulls in the QC device and scans it. The QC device may partially move in and out of the meter several times. Do not be tempted to push the device in further or remove it before the end of the test.

Figure 5: QC Device

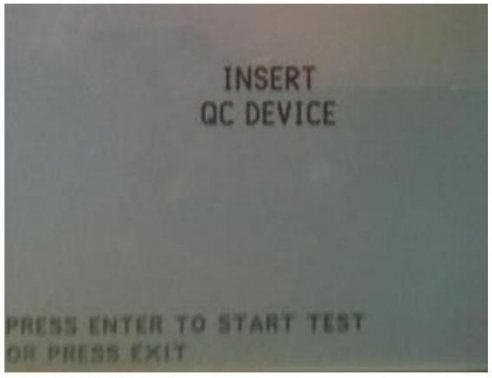


Figure 6: Insert QC Device

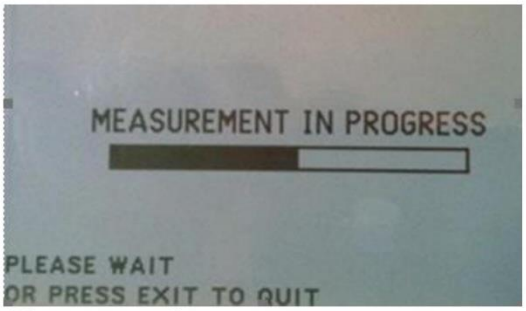


Figure 7: Measurement in progress

5. When the test is complete, the meter beeps, ejects the device and displays a Pass (calibration, laser and alignment as in Figure 8) or Fail result on the meter's screen. Press ***Exit*** to return to main menu.
6. Return the QC Device in its box immediately after ejection to minimise exposure to light.
7. Tear off the printed receipt and file in the QC Device Results Log tab in the local PARROT PlGF folder. **Staple the printed receipt into the log. Take care not to touch the printed ink with fingers or sticky tape as this may cause the ink to fade over time.**

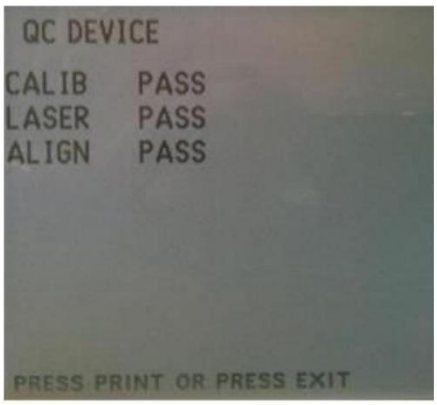


Figure 8: QC Device

8. If the QC Device fails any of the three tests, check for damage to the device and wipe with a clean soft tissue to remove any oils, dust fibres or fingerprints. Do not apply any liquid to the QC device. Repeat the test. If the device fails a second time contact Alere on 0044 7771 642360 for further support and alert the PARROT project manager.

7.2.2 QC Samples

- The QC samples are liquid PlGF controls (PlGF Control 1 & PlGF Control 2 (Figure 9)) and are run to test the accuracy and precision of the meter and Test Devices.
- QC samples should be run once a month, and before using a new lot number of Test Devices.
- Store controls in a -20°C freezer and thaw completely before use. Controls are stable for one hour once removed from the freezer.
- Remove a single control from each control box (PlGF Control 1 and PlGF Control 2) when running a QC sample. Freeze thaw cycles can compromise the quality of the control samples. **Before using a new lot of controls, ensure that the correct code chips have been installed prior to testing** (see section 7.1.1).

Figure 9: PlGF Controls 1 & 2

Preparing the QC sample controls:

Both controls can be prepared before running both, or prepare the second one while the first is running, as is most convienient for the operator.

1. Remove one vial each of PlGF Control 1 and PlGF Control 2 from the freezer (Figure 9) and thaw for 15 mins. Use within 1 hour. Take care not to overly expose the other controls in the boxes to room temperature as freeze thaw cycles may compromise the quality of the controls and therefore the accuracy of the QC sample.
2. A new Test Device is required to run each control. Remove 2 PlGF Test Devices from the fridge and allow to reach room temperature for at least 15 mins while the controls are thawing.
3. Open one Test Device foil pouch and remove the Test Device, label with "Control 1" (see Figure 10) and set on a level surface.
4. Hold the liquid PlGF control 1 vial with the tip upright and flick to settle all the liquid at the bottom.
5. To open - twist the top and snap it off.
6. Carefully dispense the entire contents of the vial into the sample port at the bottom of the device. This inoculation should occur immediately after opening the foil pouch, as Test Devices are moisture sensitive and will absorb moisture from the air that will compromise the test. Inoculation must be performed as soon as possible but no later than 10 minutes after opening the foil pouch.
7. Write the time of inoculation on the Test Device.
8. **Ensure the devices are run through the meter within 20 minutes after inoculation.**

Figure 10: Test Device with control 1

9. Check that the control has absorbed onto the channel in the centre of the Test Device before inserting into the prepared meter.
10. Then repeat steps from 7.2.2.3 to 7.2.2.7 the above with control 2 and a new Test Device, using 'Control 2' to label the Test Device.

<u>To run the QC sample controls:</u>

1. From the Main Menu, select ***Run Test***; and press ***Enter*** (Figure 11).

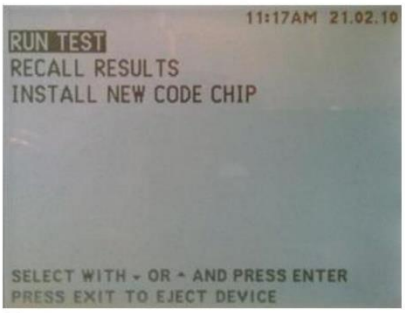


Figure 11: Run Test

2. Select ***QC Sample*** and press ***Enter*** (Figure 12).

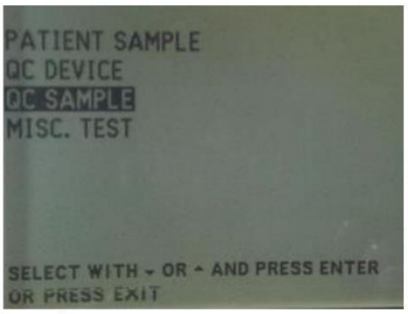


Figure 12: QC Sample

3. Enter the QC 4 digit lot number of the appropriate control (Figure 13) found on the label on the side of the vial containing the control - **do not include the preceeding letters**. Press ***Enter*** to confirm the number. If the following message ***No QC Sample Data in Memory*** appears, press ***Enter*** to continue and install corresponding QC Code Chip module.

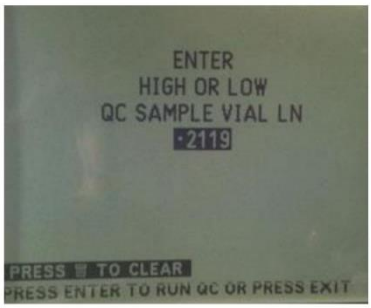


Figure 13: QC Lot Number

4. Holding the Test Device by its edges, gently insert the Test Device face up, following the arrow, gently pushing until it catches on the internal pin and a click is heard. Press ***Enter*** to start the test (Figure 6).
5. Once the test is complete, the meter will beep and partially eject the device for the operator to remove. Check that the reading is within the correct range (see Alere Triage Meter user manual for information on quality control ranges) which is indicated by dark writing on light background on the screen.
6. Tear off the printed receipt and file in the QC Sample Results Log tab in the local PARROT PlGF folder. **Staple the printed receipt into the log. Take care not to touch the printed ink with fingers or sticky tape as this causes the ink to fade.**
7. If the reading is not in the correct range (indicated by lightwriting on dark highlight) repeat the test using a new Test Device and a new control vial. Ensure the control vial and Test Device are at room temperature before repeating. If the results are still not acceptable, please call Alere on 0044 7771 642360 for further support and inform the PARROT project manager.

   Once both PlGF controls have provided satisfactory readings within the acceptable range, the meter is ready to use.

7.3 Participant Sample Testing

7.3.1 Before running a sample

- If opening a new lot of Test Devices, install the accompanying Test Device chip code (see section 7.1.1 for instructions).
- As this is a point of care test a participant's blood sample should be analysed as soon as possible after venepuncture and MUST be analysed within 3 hours of sampling.

Delay in analysis beyond 3 hours post venepuncture or exposure to extremes of temperature prior to analysis may affect the result

- Confirm blood sample is in an EDTA blood collection tube.
- Always use EDTA plasma sample for this test.

7.3.2 Before You Begin

1. Set the centrifuge to 1400 x *g* for 10 minutes.
2. Remove the Test Device from the fridge and leave it for approx. 15 minutes to allow Test Device come to room temperature.
3. Perform the QC device calibration prior to testing if required, see section 7.2.1.
4. Perform QC sample calibration prior to testing if required, see 7.2.2.
5. Devices must be read within 20 minutes of inoculation.

7.3.3 Sample Preparation

1. Collect 1 EDTA blood collection tube of blood and gently invert tube 8-10 times; avoid vigorous shaking.
2. Centrifuge this sample at 1400 x *g* for 10 minutes, with an appropriate balance as advised in the centrifuge instruction manual (Figure 14).

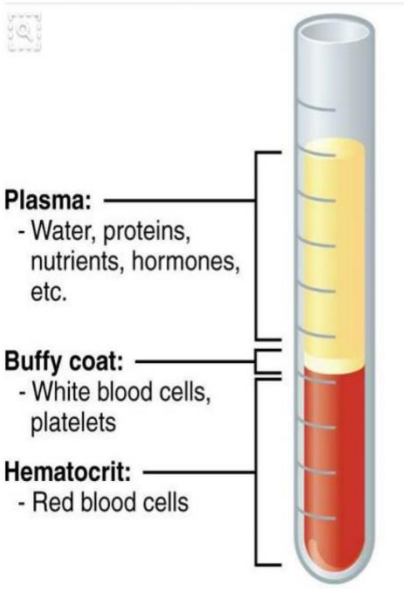


Figure 14: Centrifuged Whole Blood as collected in EDTA Blood Collection Tube

3. Open the foil pouch and remove the Test Device from the foil just before inoculation. It is important to minimise the time that the Test Device will be exposed

to air as it will absorb moisture and not work correctly. **Ideally, inoculate the Test Device within one minute, but no longer than 10 minutes.**

4. **Ensure the Test Device is kept flat on the bench for the duration of the process.**
5. Aspirate 250µl of EDTA plasma using the transfer pipette (Figure 15) supplied with the kit by squeezing the top of the dual bulb (X). Please note any sites involved in biobanking samples should use a pipette to aspirate the required 250 µl so as not to contaminate the remaining plasma.

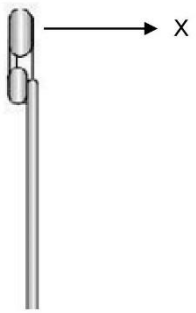
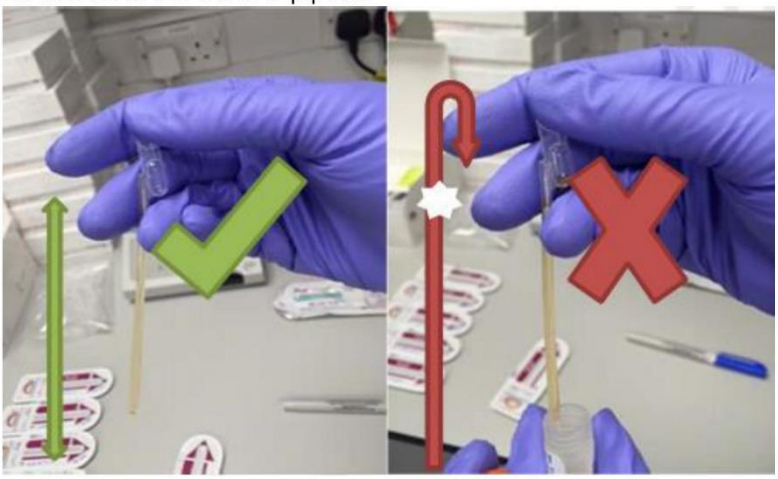


Figure 15: Transfer pipette for PlGF testing

6. Insert the tip into the plasma and release the top bulb to draw up the sample.
7. The stalk of the transfer pipette should be filled up to, but not into the bulb as shown in the images below. **The stalk must be full to allow application of the minimal volume necessary for the test.** Any liquid that falls over into the bulb will stay there and not be transferred to the device. Ensure the full volume is transferred into the sample port on the device and is not lost into the bulb (see Figure 16). This will require a steady thumb to maintain pressure on the bulb.

Figure 16: Correct use of transfer pipette



8. Expel the EDTA plasma into the sample port of a newly opened and labelled (with Pat ID) PLGF Test Device (see Figure 17). Expel sample by squeezing the top bulb fully. **Use a new pipette for each test performed, even if using the same sample.**

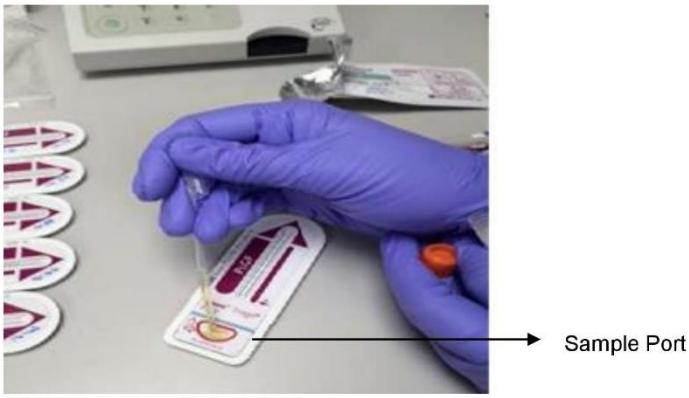


Figure 17: Inoculation of Test Device

9. Once the sample has soaked to the end, it is ready to run. Where possible start entering the information in the meter while the plasma sample is absorbing.
10. Once absorbed, hold the Test Device by its edges, gently insert the Test Device face up, following the arrow, gently pushing until it catches on the internal pin and a click is heard. Press ***Enter*** to start the test (Figure 6).

7.3.4 Running a Patient Test

Follow the on-screen instructions as detailed in the images below:

1. From ***Main Menu***, select ***Run Test*** (Figure 2) and press ***Enter***.
2. Select ***Patient Sample*** (Figure 18) and press ***Enter***.

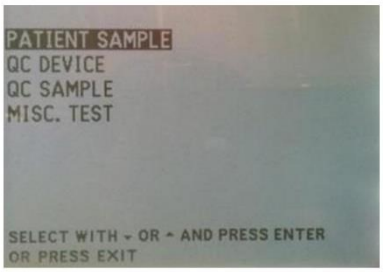


Figure 18: Patient Sample

3. Enter PARROT Participant ID, using the shift key to toggle between letters and numbers, and press ***Enter*** (Figure 19).

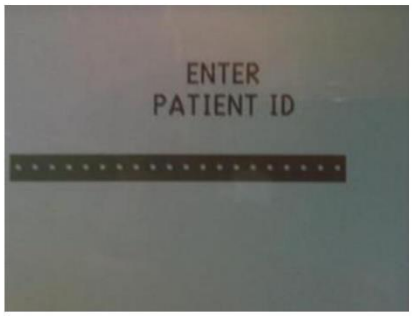


Figure 19: Enter Patient ID

4. Check the information has been correctly entered and press ***Enter*** to confirm, or edit as required.
5. Check and confirm/correct as appropriate (Figure 20).
   Confirm – that the patient ID is correct, OR
   Correct – and re-enter the patient ID again if required.

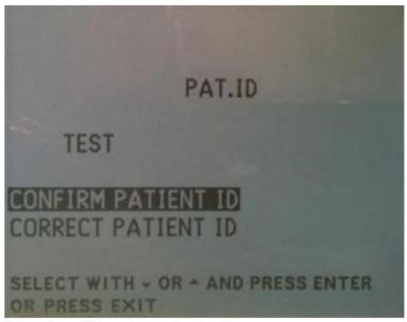


Figure 20: Confirm Patient ID

6. Label the PLGF device with the participant ID using a marker (Figure 21).

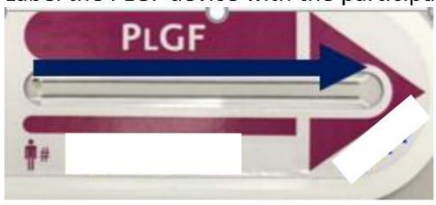


Figure 21: Test Device marked with patient ID

7. Place the Test Device in the port at the front by gently pushing until you hear the click, and press ***Enter*** to start (Figure 22 and 23).

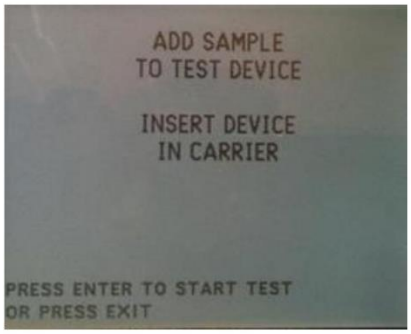


Figure 22: Add sample prompt

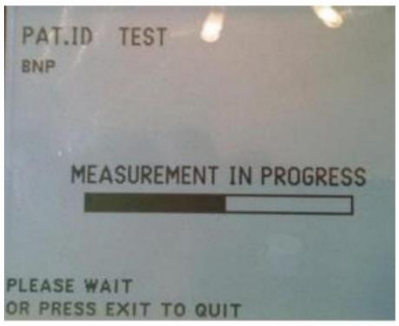


Figure 23: Measurement in progress

8. When the meter has completed analysis, there will be a beep and the result will be displayed on screen and printed.
9. Remove the used device and dispose in clinical waste.

7.3.5 Reading the Result

If a result is abnormal, on the screen it will appear as light writing on a dark highlight.

1. Note the result and tear off the printed result receipt.
2. File receipt in the Sample Log tab in the local PARROT PlGF folder. **Staple the printed receipt into the log. Take care not to touch the printed ink with fingers or sticky tape as this may cause the ink to fade in time.**
3. Log the PlGF result in the Parrot enrolment sticker in the patient's hospital notes.

7.3.6 Recalling Results

The meter stores up to 750 patient results and 250 QC results.

1. To recall results, select ***Recall Results*** from the ***Main Menu***, press ***Enter*** and select the option required. Figure 24 lists the options for viewing/ printing results. For

more information, please refer to the Alere Triage® Meter Pro User Manual in the User Manual tab in the local PARROT PlGF folder.

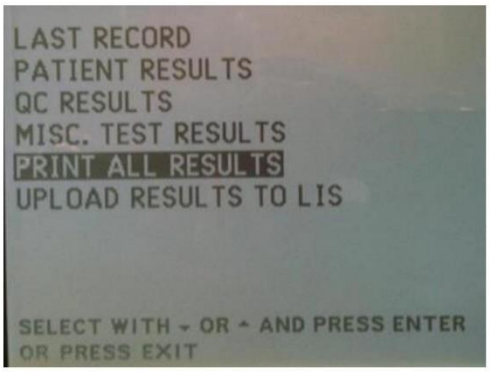


Figure 24: Recall Results

7.4 Biobanking for PARROT Ireland (Cork Site Only)

- Following extraction of the EDTA plasma for the purpose of PlGF testing in the meter selected PARROT sites biobank remaining EDTA plasma.
- Ideally the remaining EDTA plasma is prepared for Long Term Storage (LTS) in the biobank while the meter is running the test.
- EDTA are dispensed as 250µL sample aliquots and stored in cryovials enclosed in 96-well cryoboxes.
- Biobanked sample aliquot information and location is recorded locally in the PARROT Sample Database spreadsheet electronically and a hard copy is kept in the PARROT laboratory notebook. Access to this Sample Database spreadsheet is restricted only to personnel who require it for sample tracking and control.
- The objective is to store aliquots in the Ultra-Low Temperature (ULT) freezer within 3 hours of the venepuncture.

7.4.1 Cryovials

- Each cryovial has a 2 dimensional code laser-etched on its base. This encodes a unique alphanumerical code also printed on the side of the cryovial in a legible format.
- Pre-chill capped empty cryovials prior to use.
- Coded cryovials and pipette tips must be sterile. Do not touch or cover over the cryovial and/or the cap interiors.
- Once filled and re-capped, the cryovial codes can be scanned individually using a handheld data scanner or in multiples using a flatbed scanner and the codes uploaded to the PARROT Sample Database spreadsheet.

7.4.2 Cryoboxes

- Cryoboxes used to organise cryovials have an 8 x 12 format (accommodating 96 cryovials in each box). Each cryobox has a unique linear barcode along its side.
- All cryoboxes are labelled with study name, box number, rack number, shelf number and freezer number using a cryosticker.
- A processing cryobox is used to minimise the amount of time a cryobox is out of the freezer.

7.4.3 Sample Preparation for LTS in the biobank.

1. In the biosafety cabinet with sterile gloves, uncap the chilled coded cryovials in the 96 cryobox using a decapper. Lay the decapper and caps down as a single unit on the cabinet surface, ensuring the caps remain sterile. When pipetting, keep EDTA blood collection tube, cryovials and cryovial caps behind the safety cabinet air curtain to prevent contamination.
2. Using a sterile pipette tip, uptake and dispense 250 µl of EDTA plasma from the EDTA blood collection tube into each open cryovial, keeping the blood tubes and cryovials cold. Carefully refit the caps to the open cryovials. Use caution not to uptake any WBC with the EDTA plasma samples.
3. Continue to fill 250µL of EDTA plasma into cryovials until there is no remaining EDTA plasma. Minimise the time the cryovials remain uncapped once filled with the aliquot volume to prevent evaporation.
4. The cryovials are externally marked with an approximate indication of 250 µl. Visually check for under-filled or unfilled cryovials. The EDTA plasma from under-filled vials can be distributed between the already filled vials.

7.4.4 Transfer to LTS

1. Take the processing cryoboxes and any overflow cryobox on ice to the -80°C freezer designated for PARROT storage.
2. Before opening a freezer door, check both the freezer display temperature on the front of the freezer and the external temperature probe read out to verify it is safe to open the freezer. If either one is approaching -78°C or warmer, do not open the freezer. Prior to opening the freezer know the exact location of the current shelf and rack in use for sample storage.
3. The freezer door should be open no longer than 30 seconds. Open only the doors necessary for access to the storage location. Work quickly as the freezer temperature rises rapidly.
4. Without removing it from its rack, unlock the last cryobox, remove the lid and use a vial picker or cryovial grab clip to move the cryovials from the processing cryobox directly into the last cryobox already within the freezer.
5. Replace and lock all cryobox lids.
6. Place the locked new partially filled cryobox (Figure 27) in the next available racking location in the freezer.
7. Close freezer doors securely.

8. Check freezer door temperature and freezer monitoring system to ensure freezer temperature is within acceptable limits. Refer to your local SOP Freezer Management. The processing cryobox is now empty ready for aliquots resulting from the next recruit.
9. The freezer inventory system is filled in a sequential manner with new cryoboxes being placed in the next available location as nominated by the laboratory and biobank team.

Never place a processing cryobox in a freezer.

7.4.5 Local Recording of Data Generated

When processing is complete, enter the following data into the PARROT Sample Database spreadsheet and also in the PARROT laboratory notebook.

- Study Participant/Patient ID
- Date and time of venepuncture
- Operators initials
- Sample type
- Number of sample aliquots obtained
- Gestation (week and days)
- PlGF result
- Aliquot barcode
- Positions in cryobox (e.g. A1 - .....)
- Cryobox barcode
- Cryobox number (1-6)
- Rack (1-36)
- Shelf (1-4)
- Freezer
- Time sample placed in LTS
- Relevant quality attributes Standard Preanalytical Code (SPREC) V2.0 (ISBER Guidelines), see appendix (Section 9)
- Comments

This data should be double-checked by the PARROT project manager or designee on a monthly basis.

7.5 Sample Quality Control

Blood samples that are haemolysed, icteric or lipemic can invalidate certain downstream analyses.

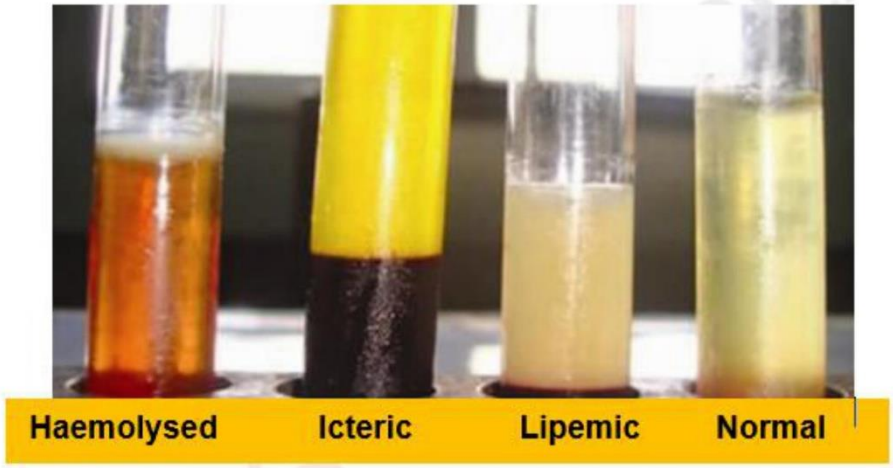


Figure 25: Sample Quality Control

7.5.1 Haemolysis

- Haemolysed EDTA plasma varies in colour from faint pink to bright red, rather than the normal straw colour.
- Note the degree of any haemolysis of samples in the PARROT Sample Database spreadsheet and in the PARROT laboratory notebook.

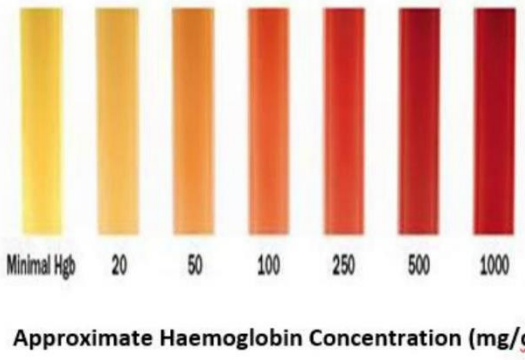


Figure 26: Haemolysis chart for EDTA plasma

7.5.2 Fibrin Clots

- If fibrin clots are present in EDTA plasma post centrifugation, do not squeeze the clot to release EDTA plasma as this can result in inadvertent release of fibrin threads into the surrounding EDTA plasma. Uptake of fibrin threads can cause clotting in the analytical phase potentially leading to erroneous test results.
- Note the occurrence of fibrin clots in the PARROT Sample Database spreadsheet and in the PARROT laboratory notebook.

7.5.3 Icterus

- Icteric EDTA plasma varies in color from dark to bright yellow, rather than the normal straw color (Figure 26).
- While visible detection of icterus is variable and can be unreliable, icterus may affect certain determinations and therefore needs to be recorded in the PARROT Sample Database spreadsheet and in the PARROT laboratory notebook.

7.6 Ultra-low Temperature Freezer Storage Overview

Long term ultra-low temperature storage is crucial to the integrity of the specimens collected for the PARROT Ireland biobank. Following processing, specimens in cryovials are stored in -80˚C upright Panasonic/Sanyo freezers. The laboratory/ biorepository manager will assign freezer space for the long term sample storage of PARROT samples.

It is critical that PARROT biobanked EDTA plasma samples do not rise above -70ºC for more than 50 minutes during long term storage including retrieval and sample movement.

Never store PARROT biobanked EDTA plasma samples in -20ºC freezers as this environment is not sufficiently cold enough to halt degradative effects of enzymes and few biomolecules are preserved well at −20 °C.

7.6.1 Freezer Set Up and Inventory System

Freezer shelves are numbered 1-4 from the top to the bottom of the freezer.

Pack the assigned freezer space with inventory units, storage boxes or other items that will prevent mass loss of cold air upon opening of internal door(s).

- Shelves 1 and 3 hold towers 10 racks high (top left hand corner is rack 1 and bottom right hand corner is 60).
- Shelves 2 and 4 hold towers 9 racks high (top left hand corner is rack 1 and bottom right hand corner is 54).

Each rack has capacity for 6 cryoboxes. Each cryobox accommodates an 8 x 12 array i.e. 96 Nirco 0.65ml W-SER WILMUT freestanding cryovials used for sample aliquots. As the boxes are filled with PARROT Ireland samples, they are sequentially placed on the pull out racks. Boxes are numbered from the back of the freezer to the front, i.e. box number 6 is to the front of the freezer.

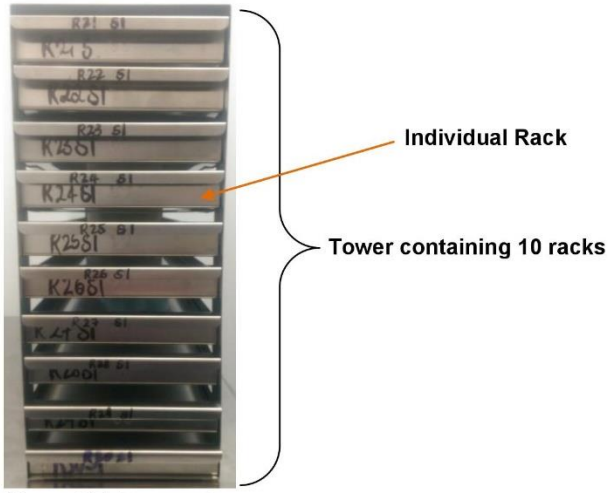


Figure 25: Freezer tower

8 References

- Alere Triage® MeterPro Training Presentation
- Alere Triage Meter Pro User Manual
- Standard Preanalytical Code (SPREC) v2.0 from International Society for biological and environmental repositories (ISBER)

9 Appendix

Appendix A:

SPREC 2.0, applied to fluid samples. Codes in bold come from the Laboratory Data Management System (LDMS).

Type of sample		
Plasma, single spun		**PL1**
Type of primary container		
EDTA and gel		EDG
Other		ZZZ
Pre-centrifugation (delay between collection and processing)		
RT*	<2 h	A
2 to 10 °C	<2 h	B
RT	2-4 h	C
2 to 10 °C	2-4 h	D
Centrifugation		
RT 10 to 15 min	<3000 g no braking	A
RT 10 to 15 min	<3000 g with braking	B
2 to 10 °C 10 to 15 min	<3000 g no braking	C
2 to 10 °C 10 to 15 min	<3000 g with braking	D
Post-centrifugation delay		
<1 h 2 to 10 °C		A
<1 h RT		B
1 to 2 h 2 to 10 °C		C
1 to 2 h RT		D
2 to 8 h 2 to 10 °C		E
Long-term storage		
PP tube 0.5- to 2-mL**	(-85) to (-60) ° C	A

*RT, room temperature: 18 to 28 °C

**PP, polypropylene

***LN, liquid nitrogen, referring to either vapor- or liquid-phase (this information being documented in the biobank's SOPs)

Volumes refer to container size

10 References

- Alere Triage® MeterPro Training Presentation
- Alere Triage Meter Pro User Manual

Appendix 4: Recruitment Guide V 1.0

PARROT IRELAND

PARROT Ireland Suggested Recruitment Guide for Researchers

Hello my name is……I am a Midwife/researcher working for ….. on the PARROT Ireland trial which is currently being run in your Maternity Hospital.

I understand you are being investigated for signs of pre-eclampsia so would it be ok with you if I had a quick chat to you about our research?.....Thank you!

Pre-eclampsia is a complication of pregnancy that causes high blood pressure and protein in the urine, but many other organs in the body can be effected and in a small number of cases it can develop into a very serious disease for both mom and baby.

Previous studies have shown that the level of a protein in your blood called Placental growth factor (PlGF) is very good at telling the difference between women who have a high chance of getting pre-eclampsia and serious complications from it in the near future and those that do not.

Presently this protein is not checked as part of regular clinical care in Ireland for women with suspected pre-eclampsia. Our research is looking at what the effect of doing this extra test would be. We think it should allow women who are at low risk to be recognised earlier and managed outside of hospital and those who are at high risk to be identified sooner so that care to them can be increased and complications for them and their babies reduced.

Half of the women in PARROT will get this extra test while the other half will get regular care. We will follow all women up after they have delivered and see if there is a difference in the outcomes to moms and babies by doing this test.

[Following confirmation of participant's eligibility] As you are eligible for the study is this something you would like to participate in?

Thank you (",)

Please read the consent form carefully and I am happy to answer any questions you might have.

Don't forget to Sm;)e

Appendix 5: Definitions List V 2.2

ADDITIONAL DEFINITIONS FOR PARROT IRELAND

ENROLMENT AND BASELINE DATA

Inclusion Criteria

- **Between 20+0 and 36+6 weeks of gestation (inclusive)**
- **Singleton pregnancy**
- **Aged 18 years or over**
- **Able to give fully informed consent**

And presenting with suspected pre-eclampsia with **at least one** of the following signs/symptoms:

- **Hypertension:** ≥140mmHg systolic or ≥90mmHg diastolic
- **Dipstick proteinuria:** Trace, 1+, 2+ or 3+
- **Headache:** new onset and not attributable to any other cause
- **Visual disturbances:** new onset and not attributable to any other cause
- **Epigastric/right upper quadrant pain**: new onset & not attributable to any other cause
- **Increasing oedema:** not attributable to any other cause
- **Suspected/Confirmed fetal growth restriction:** EFW <10thCentile or AC <10th Centile
- **Healthcare provider deems that the woman requires evaluation for possible pre-eclampsia**

Exclusion Criteria

- **Participant declines inclusion**
- **Confirmed pre-eclampsia**: Sustained hypertension with systolic BP ≥ 140 or diastolic BP ≥ 90 (on at least two occasions at least 4hrs apart) **with** significant quantified proteinuria (>300mg protein on 24hr collection or urine protein creatinine ratio >30mg/mmol or ≥ 3+ on dipstick urinalysis)
- **≥37 weeks' gestation**
- **Abnormal PET bloods:** New onset reduced number of platelets or deranged liver function/renal function tests (urea, creatinine, AST or ALT), identified during routine care prior to enrolment and not attributable to anything other than PET. Elevated urate/uric acid in isolation is not an exclusion criteria.
- **Multiple pregnancy at any time point in the current pregnancy:** Confirmed by a departmental ultrasound
- **Decision for imminent delivery already made:** Delivery of patient is already agreed upon within the next 24 hours
- **Lethal fetal abnormality present**
- **Previous participation in PELICAN trial in a prior pregnancy**: This will only be applicable to participants who attended CUMH for a previous pregnancy from 2011-2012 and were investigated for suspected pre-eclampsia

Maternal Details

Pre-existing Medical Conditions

Pre-existing Chronic Renal disease

Defined as abnormalities of kidney structure or function present for more than 3 months, with implications for health. Includes all people with markers of kidney damage and those with a

glomerular filtration rate (GFR) of less than 60 ml/min/1.73m" on at least 2 occasions separated by a period of at least 90 days (with or without markers of kidney damage)
If present:

- What is the Chronic renal disease secondary to? E.g; Diabetes, High Blood Pressure, PCKD
- what stage of CKD (Chronic Kidney Disease) is present? Should be documented in medical notes from the Renal Physician in charge of patients care

Stage	GFR	Description
1	90+	Normal Kidney function but urine findings or structural abnormalities or genetic trait point to a kidney disease
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease
3A	45-59	Moderately reduced kidney function
3B	30-44	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	<15 or on dialysis	Very severe, or end stage kidney failure (sometimes call established renal failure)

Chronic Hypertension
Chronic Hypertension is hypertension that is present at the booking visit or before 20 weeks or if the woman is already taking antihypertensive medication when referred to maternity services. It can primary or secondary in aetiology.
If present:

- What is the Chronic Hypertension secondary to? E.g; Renal Disease, Diabetes

Systemic Lupus Erythematosus (SLE) or Antiphospholipid syndrome (APS)
Confirmed by a Physician
Pre-pregnancy Diabetes
Diabetes of any type pre pregnancy requiring treatment with insulin or medication.

Current Pregnancy Details

Was the 2nd trimester uterine artery Doppler mean pulsatility index recorded:
This is a measurement of the blood flow through the uterine artery taken during an ultrasound scan –be careful not to confuse it with umbilical artery doppler. It is sometimes performed early in the second trimester in women who are thought to be at higher risk of placental dysfunction/growth restriction. If performed it will be reported on the formal ultrasound scan report of the participant. Most participants in the trial will not have had this performed. If it is performed it may be taken from either the left or right uterine artery of from both.

ON DAY OF ENROLMENT

Is participant being recruited during a transition week:
A transition week is the first week of both the control arm at all sites (aka first week trial is running) and also the first week each site transitions over to the active arm (dates will vary dependent on randomisation). Any data collected during these weeks will not be used in the final analyses.

Did the participant have a formal Fetal Ultrasound within 2 weeks prior to study entry: Only include ultrasounds performed in a dedicated pregnancy ultrasound department by a person certified in fetal ultrasound where a report in generated for the maternal chart. Do not include bedside ultrasounds performed in clinic/emergency rooms by doctors.

DIAGNOSIS

Pre-eclampsia: Sustained hypertension with systolic BP ≥ 140 or diastolic BP ≥ 90 (on at least two occasions at least 4hrs apart) **with** significant quantified proteinuria (>300mg protein on 24hr collection or urine protein creatinine ratio >30mg/mmol or ≥ 3+ on dipstick urinalysis)
Gestational Hypertension: Sustained hypertension with systolic BP ≥ 140 or diastolic BP ≥ 90 (on at least two occasions at least 4hrs apart) that was newly diagnosed during pregnancy and resolved post delivery (Also known as PIH; Pregnancy induced Hypertension. Resolves by 6 weeks postnatal)
Gestational Proteinuria: Significant quantified proteinuria (>300mg protein on 24hr collection or urine protein creatinine ratio >30mg/mmol or ≥ 3+ on dipstick urinalysis) that was newly diagnosed in pregnancy
Transient Hypertension: Isolated elevated blood pressure readings, such as with pain, that did not meet criteria to be diagnosed as gestational hypertension
Superimposed pre-eclampsia: Pre-eclampsia diagnosed on a participant with a pre-pregnancy diagnosis of renal disease or chronic hypertension or both.
HELLP: Severe form of pre-eclampsia associated with haemolysis, elevated liver enzymes and a low platelet count
Eclampsia: Eclampsia is the occurrence of convulsions/seizures in association with the signs and symptoms of pre-eclampsia
Disseminated Intravascular Coagulation/DIC: A syndrome characterised by systemic activation of pathways leading to and regulating coagulation, which can result in the generation of fibrin clots that may cause organ failure, with consumption of platelets and coagulation factors that may result in clinical bleeding
ELLP: Severe form of pre-eclampsia associated with elevated liver enzymes and a low platelet count
Placental Abruption: Antepartum haemorrhage with a clinical diagnosis of placental abruption, or retroplacental clot found at delivery, or both.
Suspected SGA only: Growth Restriction identified in antenatal fetal ultrasound but not confirmed at delivery. Customised EFW at delivery >10th centile
Chronic Hypertension: Chronic Hypertension is hypertension that is present at the booking visit or before 20 weeks or if the woman is already taking antihypertensive medication when referred to maternity services. It can primary or secondary in aetiology.
Isolated Small for Gestational Age: Growth Restriction identified on antenatal fetal ultrasound ***and*** confirmed at delivery. Customised EFW at delivery <10th centile
Chronic HTN and SGA: Combination of the above

The presence of one or more of the following indicates a diagnosis of severe PET as per ACOG guidelines:

- Systolic blood pressure of 160 mm Hg or higher, or diastolic blood pressure of 110 mm Hg or higher on two occasions at least 4 hours apart while the patient is on bed rest (unless antihypertensive therapy is initiated before this time)
- Thrombocytopenia (platelet count less than 100,000/microliter)
- Impaired liver function as indicated by abnormally elevated blood concentrations of liver enzymes (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses, or both
- Progressive renal insufficiency (serum creatinine concentration greater than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease)
- Pulmonary edema
- New-onset cerebral or visual disturbances

Note: One mg/dL of creatinine is 88.4 µmol/L. The typical human reference ranges for serum creatinine are **0.5 to 1.0 mg/dL** (about 45–90 µmol/L) for women and **0.7 to 1.2 mg/dL** (60–110 µmol/L) for men.

1.1mg/dL = 97 µmol/L

BIRTH

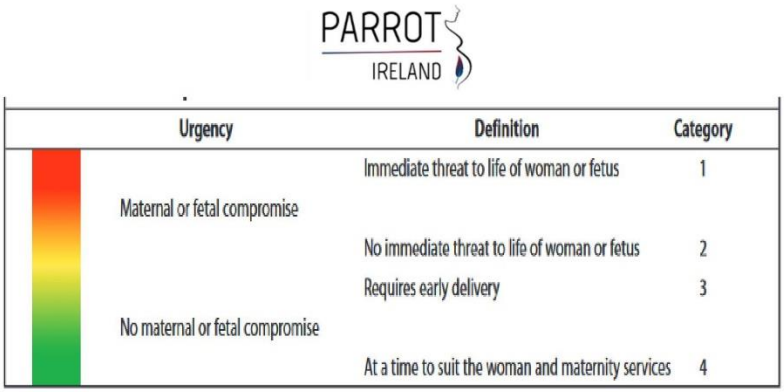
Delivery

Labour onset:

- Spontaneous
- Induced – if any medications were given to the participant in order to induce labour
- Pre labour caesarean section – delivery by Caesarean section before participant was in established labour

Mode of delivery

- Spontaneous vaginal
- Assisted vaginal using ventouse –includes KIWI, Metal Cup and Silastic Cup
- Assisted vaginal using forceps
- Vaginal breech spontaneous –delivery unassisted by a midwife/obstetrician
- Vaginal breech assisted - delivery assisted by a midwife/obstetrician
- Pre-labour caesarean section: any caesarean section performed before participant was in established labour, Includes all Elective Caesarean Sections
- Emergency caesarean section: All Caesarean deliveries performed after participant was in established labour
    - **For those who have an emergency section categorise the urgency of it from 1-4 as per RCOG good practice guidelines:**



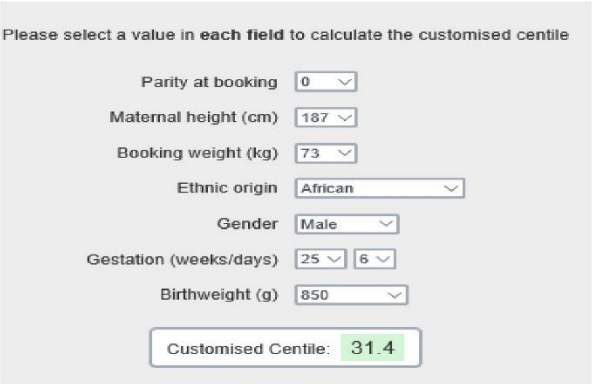
Urgency	Definition	Category
Maternal or fetal compromise	Immediate threat to life of woman or fetus	1
	No immediate threat to life of woman or fetus	2
No maternal or fetal compromise	Requires early delivery	3
	At a time to suit the woman and maternity services	4

Infant Delivery

Status at birth
Liveborn –born alive at any gestation >24 weeks
Stillborn –born with no signs of life present >24 weeks of pregnancy
Miscarriage <24 weeks –pregnancy ended prior to 24 completed weeks of pregnancy

Customised Birthweight
Use the following link to calculate a customised infant centile value based on maternal BMI, parity, ethnicity and fetal gender and birthweight. See example below
http://www.gestation.net/cc/5/836998.htm

Grow Centile Calculator



5 minute Apgar
The Apgar score is a clinical assessment of every baby made at 1 and 5 minutes post-delivery by the birth attendant (midwife/paediatrician). It can range from 0 to a maximum of 10. Record if there was documentation of an Apgar score of ≤ 7 at 5 minutes of life.

Cord Blood Gases
Enter the arterial and venous pHs and base excess taken at the time of delivery (if these were performed). Clarify of there was any evidence of fetal umbilical artery acidosis at birth (Arterial Cord pH <7.20).

MATERNAL DISCHARGE

This should not be completed until 12 weeks post-delivery

Enter the cumulative amount of visits the participant had from the time of her enrolment in the trial until her discharge post-delivery to each of the following locations:

Outpatient

GP: General Practitioner
ANC: Antenatal Clinic of her Maternity Hospital or equivalent (may be domino clinic or community midwife appointment depending on her pregnancy care plan)
Day ward: Day Unit of her Maternity Hospital
ER/emergency unplanned: Any unplanned/emergency/out of hours' presentations to her maternity hospital
Ultrasounds: The number of formal ultrasounds in the ultrasound department from inclusion in the study until delivery

Inpatient

Antenatal ward: Include all overnight stays that occurred antenatally
HDU: High Dependency Unit of her Maternity Hospital
ITU: Intensive Care Unit of her Maternity Hospital
Labour ward
Postnatal ward: Include all overnight stays that occurred post delivery

MATERNAL OUTCOMES

CNS: Central Nervous System Abnormalities

Stroke: Interruption in the blood supply to the brain resulting in clinical disability, confirmed by imaging showing a cerebral haemorrhage/infarct
Transient Ischaemic Attack: A temporary interruption in blood supply to the brain resulting in clinical symptoms that resolves within 24 hours
Cortical blindness: total or partial loss of vision caused by damage to the brain's occipital cortex, confirmed by imaging.
Hypertensive encephalopathy: A syndrome consisting of a sudden elevation of arterial blood pressure usually, usually preceded by severe headache and followed by convulsions, coma or a variety of transitory cerebral phenomena.
Retinal detachment: Separation of the retina confirmed by an Ophthalmologist
Glasgow coma scale <13: Objective neurological scale of consciousness
Generalised tonic clonic seizure: A seizure/convulsion where the whole brain is affected resulting in a loss of consciousness, stiffening of muscles and jerking movements

Cardiorespiratory System Abnormalities
O2 Sats (<90%): Oxygen saturation levels recorded at less than 90% for any period of time
>50% FiO2 for >1 hour: Fraction of inspired oxygen (FiO_2) at greater than 50% (or ≥0.5) for more than 60 minutes
Note that room air includes 21% oxygen, which is equivalent to a FiO2 of 0.21. For any person being provided with additional oxygen (nasal cannula/facemask/ventilator) each additional litre/min of

oxygen adds about 4 percentage points for the first 3 litres and only 3 percentage points for every litre thereafter to their FiO2. (eg; a patient with a nasal cannula with 4L/min of oxygen flow would have an FIO2 of 21% + (3 x 4%)+(1 x 3%) =36%).

Pulmonary oedema: a collection of fluid in the lungs resulting in breathing difficulty.

Intubation: Placement of an endotracheal tube to maintain airway patency (other than for Caesarean Section)

Positive ionotropic support: group of drugs that stimulate and increase the force of contraction of heart muscle, generally given for conditions associated with a low cardiac output (CO)

Myocardial Infarction (MI) or cardiac ischaemia: Interruption of blood flow to the heart muscle resulting in damage to the heart tissue

Transfusion of blood products (except anti-d): administration of any blood products to the participant at any time point. This includes:

- Whole Blood
- RBC (Red Blood Cells)
- Platelets
- Plasma
- Cryoprecipitate
- Fibrinogen

Infusion of third parenteral antihypertensive: Intravenous administration of an agent for lowering blood pressure, subsequent to the administration of 2 previous intravenous agents. NOTE: Use of MgSO4 is NOT considered an antihypertensive agent in this setting.

Hepatorenal

Haemodialysis – due to new onset renal failure

Acute renal insufficiency (creatinine >150mmol/L) - new onset in current pregnancy

Liver dysfunction (AST/ALT >70IU/L) – new onset in current pregnancy

Liver haematoma or rupture

Additional Maternal Outcomes

Severe Hypertension: systolic BP ≥ 160 mmHg on at least one occasion at any time point following enrolment onto the trial

Fetal growth restriction identified on antenatal ultrasound: (<10th centile)

Final diagnosis of a hypertensive disorder of pregnancy: gestational hypertension, chronic hypertension, pre-eclampsia or superimposed pre-eclampsia diagnosed during current pregnancy

Use of 1 or more antihypertensive drugs: Use of any drugs for lowering blood pressure at any time point

Note: BP measurements are worst ones EVER recorded, whether antenatal, postnatal, or on readmission

INFANT DISCHARGE

Most neonates will not require admission to the neonatal unit (NNU) following delivery. However, if they are admitted they will have a separate neonatal chart. Following their discharge from the NNU the following details should be taken from their chart and entered to the database to assess neonatal outcomes:

Neurological Assessment

- If the baby was diagnosed with encephalopathy while in the NNU, specify the highest stage: This information should be available from the clinical notes
    - Stage 1 - mild
    - Stage 2 - moderate
    - Stage 3 - severe

Stages of Encephalopathy

Stage 1 Mild	• Duration < 24 hours with hyperalertness • Uninhibited Moro and stretch reflexes • Sympathetic effects • Normal electroencephalogram
Stage 2 Moderate	• Obtundation • Hypotonia • Decreased spontaneous movements with or without seizures.
Stage 3 Severe	• Stupor • Flaccidity • Seizures • Suppressed brain stem and autonomic functions • The EEG may be isopotential or have infrequent periodic discharges.

Hypoglycaemia: Blood glucose level <3mmol/L

Gastrointestinal Assessment

If the baby was diagnosed with Necrotising Enterocolitis while in the NNU, specify the highest stage using Bell's criteria. This information should be available in the clinical notes. If unclear, enter unknown.

Modified Bells Staging Criteria for Necrotising Enterocolitis

STAGE	SYSTEMIC SIGNS	INTESTINAL SIGNS	RADIOLOGIC SIGNS	TREATMENT
I. Suspected				
A	Temperature instability, apnoea, bradycardia	Elevated pregavage residuals, mild abdominal distension, occult blood in stool	Normal or mild ileus	NPO, antibiotics x 3 days
B	Same as IA	Same as IA, plus gross blood in stool	Same as IA	Same as IA
II. Definite				
A: Mildly ill	Same as IA	Same as I, plus absent bowel sounds, abdominal tenderness	Ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
B: Moderately ill	Same as I, plus mild metabolic acidosis, mild thrombocytopenia	Same as I, plus absent bowel sounds, definite abdominal tenderness, abdominal cellulitis, right lower quadrant mass	Same as IIA, plus portal vein gas, with or without ascites	NPO, antibiotics x 14 days
III Advanced				
A: Severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia, respiratory acidosis, metabolic acidosis, disseminated intravascular coagulation, neutropenia	Same as I and II, plus signs of generalised peritonitis, marked tenderness and distension of abdomen.	Same as IIB, plus definite ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
B: Severely ill: bowel perforated	Same as IIIA	Same as IIIA	Same as IIB, plus pneumoperitoneum	Same as IIA, plus surgery

END OF STUDY

STAGE	SYSTEMIC SIGNS	INTESTINAL SIGNS	RADIOLOGIC SIGNS	TREATMENT
I. Suspected				
A	Temperature instability, apnoea, bradycardia	Elevated pregavage residuals, mild abdominal distension, occult blood in stool	Normal or mild ileus	NPO, antibiotics x 3 days
B	Same as IA	Same as IA, plus gross blood in stool	Same as IA	Same as IA
II. Definite				
A: Mildly ill	Same as IA	Same as I, plus absent bowel sounds, abdominal tenderness	Ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
B: Moderately ill	Same as I, plus mild metabolic acidosis, mild thrombocytopenia	Same as I, plus absent bowel sounds, definite abdominal tenderness, abdominal cellulitis, right lower quadrant mass	Same as IIA, plus portal vein gas, with or without ascites	NPO, antibiotics x 14 days
III Advanced				
A: Severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia, respiratory acidosis, metabolic acidosis, disseminated intravascular coagulation, neutropenia	Same as I and II, plus signs of generalised peritonitis, marked tenderness and distension of abdomen.	Same as IIB, plus definite ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
B: Severely ill: bowel perforated	Same as IIIA	Same as IIIA	Same as IIB, plus pneumoperitoneum	Same as IIA, plus surgery

END OF STUDY

Appendix 6: Documentation of enrollment for Participants Charts V 1.0

This patient is enroled on the PARROT Ireland RCT.

Informed Consent Form Version _____ (version number/date) signed and dated by the patient and PARROT Ireland researcher on _____ (date).

Copy of consent given to patient and filed in chart.

PlGF result ____pg/ml which is normal/abnormal/highly abnormal.

Result conveyed to _____ (attending clinician) at _____(time) on _____(date) and directed to suggested management algorithm as per protocol.

Patient is eligible for a repeat PlGF test from _____ (date).

For any further information please contact _____(researcher) at _____ (telephone/bleep number).

Documentation of Enrolment for Participants Chart_ V1.0_25 MAY 17

Appendix 7: SOP 001: Informed Consent Process V 1.0

INFANT: Irish Centre for Fetal and Neonatal Translational Research

SOP 001
Informed Consent Process

Sponsor: University College Cork (UCC)

SOP Number:	**CLIN_PARROT_001**	**Effective Date:**	**22 June 2017**
Version Number & Date:	Version 1.0 22 June 2017	**Review Date:**	22 June 2019
Author:	Blánaid Ní Chuinneagáin	**Title:**	Project Manager
Co Author:	Deirdre Hayes-Ryan	**Title:**	Clinical Research Fellow
Reviewed By:	Jackie O'Leary	**Title:**	Quality & Regulatory Affairs Manager
Approved By:	Louise Kenny	**Title:**	Principal Investigator

SOP Chronology		
SOP Version Number	**Reason for Change**	**Author**
1.0	Original Document	Blánaid Ní Chuinneagáin

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1 Guides

This document (also referred to as a Standard Operating Procedure: SOP) is identified by code CLIN PARROT__001. The code identifies the category of the document; (ADMIN/LAB/CLIN, the acronym of the study and the number of the document from a list of other PARROT SOPs; 001/002/003). The chronological history of this document can be found on the front page.

2 Scope

This SOP documents the procedure for obtaining and documenting Informed Consent.

3 Purpose

The purpose of this SOP is to describe the Informed Consent Process in full.

4 Responsibility

The responsibility that informed consent is obtained and maintained as source data lies with each site project manager/researcher. As this is a non-regulated study it is not mandatory that an Investigator (Principal or Sub) obtains informed consent therefore this task may be performed by the site researchers. Overall responsibility for obtaining and maintaining informed consent lies with the Principal Investigator at each participating site. The monitor will carry out a 100% check on informed consent at all of the participating sites throughout the study.

5 Definitions/Abbreviations

Table 1: Definitions/Abbreviations

Term/Acronym	Description
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
Informed Consent	A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.
ICF	Informed Consent Form
PIL	Patient Information Leaflet
SOP	Standard Operating Procedure
TMG	Trial Management Group

6 Materials & Equipment

N/A

7 Procedure

7.1 Participant Information Leaflets (PIL) and Informed Consent Forms (ICF) will have been approved in each of the participating sites by the relevant research Ethics Committees.

7.2 The ICF and PIL are version controlled documents and are stored on the eCRF website once released by the TMG. Each individual obtaining consent must always ensure they are using the correct version of the PIL/ICF.

7.3 If new information becomes available during the course of the study it may be necessary for the PIL/ICF to be updated. If this occurs, once the updated PIL/ICF has been approved in each of the participating sites by the relevant research Ethics Committees consent will need to be obtained again from all participants who are still enrolled on the study.

7.4 Researchers involved in the recruitment of participants involved in PARROT Ireland at each participating site will complete mandatory training in Good Clinical Practice

(GCP) and will have PARROT specific training carried out prior to commencement of recruitment.

7.5 The participant will be approached and the Patient Information Leaflet and study in general will be explained to them. Ample time and opportunity will be given to discuss any queries/questions regarding the study with the researcher. Participants will be informed that they can withdraw from the investigation at any time and that this decision will not influence their care. Participants will then be asked if they wish to participate and if so, will be invited to sign and personally date the Informed Consent Form (ICF).

7.6 The ICF will then be checked for completion by the researcher who obtained the consent, countersigned and dated.

7.7 If the participant is unable to read, query or sign the PIL and the ICF, then a translator or independent witness, as appropriate, will read, explain and/or facilitate the asking and answering of questions. This person will also countersign and date the ICF indicating their role in the consenting process.

7.8 Signed ICFs are source documentation and the original will be kept at each participating site in a locked secure location, a copy should be retained in the subject's medical notes and a copy of both the PIL and signed ICF must be given to the participant to keep.

7.9 An entry must also be made by the person obtaining consent in the patient's medical notes detailing the participant's entry into the PARROT Ireland study and stating that the PIL/ICF (including version number) was discussed with the participant, that they signed the ICF and received a copy to take home.

8 Reference to Other SOPs, Regulations, Guidelines

- INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2)

Appendix 8: SOP 002: Personnel Training V 1.0

INFANT: Irish Centre for Fetal and Neonatal Translational Research

SOP 002
Personnel Training Process

Sponsor: University College Cork (UCC)

SOP Number:	**CLIN_PARROT_002**	**Effective Date:**	**22 June 2017**
Version Number & Date:	Version 1.0 22 June 2017	**Review Date:**	22 June 2019
Author: **Co Author:**	Blánaid Ní Chuinneagáin Deirdre Hayes-Ryan	**Title:** **Title:**	Project Manager Clinical Research Fellow
Reviewed By:	Jackie O'Leary	**Title:**	Quality & Regulatory Affairs Manager
Approved By:	Louise Kenny	**Title:**	Principal Investigator

SOP Chronology		
SOP Version Number	**Reason for Change**	**Author**
1.0	Original Document	Blánaid Ní Chuinneagáin

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1 Guides

This document (also referred to as a Standard Operating Procedure: SOP) is identified by code CLIN_PARROT_002. The code identifies the category of the document; (ADMIN/LAB/CLIN, the acronym of the study and the number of the document from a list of other PARROT SOPs; 001/002/003). The chronological history of this document can be found on the front page.

2 Scope

This SOP is applicable to all training carried out during the PARROT Ireland Study. This includes all internal training carried out at each participating site and also all external training attended by PARROT Ireland personnel.

3 Purpose

The purpose of this SOP is to describe the training required, undertaken and completed by personnel involved in the PARROT Ireland Study in order to conduct the study according to the approved protocol and all relevant guidelines and legislation.

4 Responsibility

The Principal Investigator (PI) at each participating site is responsible for ensuring all members of his/her PARROT Ireland team receive training appropriate to their needs and responsibilities within the PARROT Ireland study. The filing, maintenance and retention of the training records also falls under the PIs remit and these will be checked on an ongoing basis by the monitor during the course of the study. The clinical project manager holds responsibility for ensuring that the Trial Management Group (TMG) and all other project personnel (if relevant) are adequately trained in all relevant policies, procedures, ISO standards and directives.

5 Definitions/Abbreviations

Table 1: Definitions/Abbreviations

Term/Acronym	Description
CV	Curricula Vitae
eCRF	electronic Case Record Form
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
ISF	Investigator Site File
PI	Principal Investigator
SOP	Standard Operating Procedure
TMF	Trial Master File
TMG	Trial Management Group

6 Materials & Equipment

N/A

7 Procedures

7.1 All personnel are trained on relevant policies and procedures and will be trained by trainers who are themselves trained.

7.2 All personnel who are responsible for carrying out procedures specifically related to the conduct of the study as documented in the protocol and study specific SOPs, once basic training has been performed and documented, should read and familiarise themselves with all the relevant documentation prior to commencement.

7.3 All personnel are trained in the protocol and the completion of the eCRF (where applicable) and this training should be reflected in their respective training records.

7.4 All personnel listed on the Site Delegation Log must have documented GCP training.

7.5 A training record for each member of the PARROT Ireland team listed on the Site Delegation Log at each participating site is filed in the Investigator Site File (ISF) along with his/her signed and dated CV. Training records for the Trial Management

Group and all other PARROT personnel will be filed in the Trial Master File (TMF) with signed and dated CVs. All CVs must be updated every two years at a minimum.

7.6 A copy of the PIs CV at each participating site must be provided to the project manager for filing in the TMF.

7.7 During site team meetings, if appropriate, training or refresher sessions relevant to the site staff will be performed and documented. This type of training will be communally documented for all personnel present in the form of a memo and signed and dated by the trainer. These memos will be filed in the respective ISFs.

7.8 Training will also take place at each site prior to study commencement in the form of a teleconference, where the clinical research fellow/designee will train or refresh PARROT personnel in all relevant PARROT specific responsibilities and procedures. This training will be documented in a training memo and sent to the sites for filing in the ISF.

7.9 If for any reason any personnel cannot attend a training session, there will be another training session scheduled if appropriate and warranted. There is an option for "Self-Assessment" whereby training is by self-education and on a "Read and Understood" basis. In this instance the trainee will sign off their own training but this type of training is only indicated where there is a minor change to an existing document, procedure or skill that may be considered self-explanatory.

7.10 To establish the need for re-training/re-certification and to identify any gaps in training the training records are reviewed at a minimum on a yearly basis by the PI/designee at each site and/or the study monitor. If gaps are identified then additional training/re-training is added as required including any training relating to legislative updates and where relevant, GCP refresher training.

7.11 In the case of new staff being employed during the course of the study they will be trained in relevant study related documents and procedures pertinent to their job description and will be supervised while performing these tasks for a period of time until they are proficient and feel confident to assume responsibility for their role as outlined in the Site Delegation Log. This training will be documented in their training record.

7.12 Certificates are filed of all relevant courses/classes attended by all project personnel, both for internal or external training. Project personnel Curricula Vitae (CV) should ideally be updated if additional courses have been attended or additional training has been completed.

7.13 Training records for the PARROT Ireland study team are the responsibility of the PI at each participating site. The clinical project manager is responsible for the training records of the Trial Management Group.

8 Reference to Other SOPs, Regulations, Guidelines

- INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2)

Appendix 9: PlGF Devices Training V 1.0

PARROT IRELAND	**PlGF SAMPLE TESTING RESULTS LOG**

Site:	PI:
Participant ID:	

VENOUS EDTA PLASMA TESTING on TRIAGE PlGF	
Date of Measurement:	
Time of Inoculation: (24-hour clock) _____ Sample re-tested? ☐ Yes ☐ No Reason/Comment: PlGf Result: _____ pg/mL	
Comments:	
Test operator:	Date:

STAPLE Triage Meter Print Out(s)

Appendix 10: Pre-Screening Log V 2.0

PARROT IRELAND PARTICIPANT PRE-SCREENING LOG

Participant Initials	Date of Pre-Screening	Reason for not recruiting (See Categories below)	Comment

CATEGORIES:		
A: Participant did not meet inclusion criteria, specify as below -	**B:** Participant met Exclusion criteria, specify as below -	
A1: Participant did not meet criterion of "Pregnant women between 20+0 and 36+6 weeks of gestation (inclusive)" **A2:** Participant did not meet criterion of "Singleton pregnancy" **A3:** Participant did not meet criterion of "Aged 18 years or over" **A4:** Participant did not meet criterion of "Able to give informed consent" **A5:** Participant did not meet criterion of "Presenting with suspected pre-eclampsia with one or more signs/symptoms".	**B1:** Participant met the criterion of "Confirmed pre-eclampsia at point of enrolment: **B2:** Participant met the criterion of "Abnormal PET bloods (new onset reduced number of platelets or deranged liver function/renal function tests, identified during routine care prior to enrollment and not attributable to anything other than PET)." **B3:** Participant met the criterion of "Multiple pregnancy at any time point in the current pregnancy" **B4:** Participant met the criterion of "Decision for imminent delivery already made" **B5:** Participant met the criterion of "Lethal fetal abnormality present" **B6:** Participant met the criterion of "Previous participation in PELICAN trial in a prior pregnancy"	
C: Participant declined participation	**D:** Participant could not be contacted following identification	**E:** Other (Specify in comments column above)

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Appendix 11: QC Device Results Log

PARROT IRELAND	**QC DEVICE RESULT LOG** **To be performed each day of patient testing**

Site Name:	PI Name:

Date	QC Simulator	Passed	Failed	Initials	Printout
	Calibration Laser Alignment	____ ____ ____	____ ____ ____		STAPLE Print Out
	Calibration Laser Alignment	____ ____ ____	____ ____ ____		STAPLE Print Out
	Calibration Laser Alignment	____ ____ ____	____ ____ ____		STAPLE Print Out

* If the QC Simulator Device fails a second attempt, please do NOT use the meter and contact Alere and the PARROT Project Manager.

Appendix 12: QC Sample Results Log

PARROT IRELAND	**QC SAMPLE (PLGF CONTROLS) RESULT LOG** **To be performed once a month and when changing to a new lot number of test devices.**

Site Name:	**PI Name:**

Date	**Low Control (L1) Triage Results**	**Lot #**	**High Control (L2) Triage Results**	**Lot #**	**Initials**	**Comments**

LOW CONTROL **HIGH CONTROL**

STAPLE Print Out	STAPLE Print Out

	LOT #	ACCEPTABLE RANGE
LOW CONTROL (L1) RANGE		
HIGH CONTROL (L2) RANGE		

PARROT Ireland QC Sample Result Log Version 1.0 24 Aug 2017 Page __ of __

Appendix 13: Trial Monitoring Plan

PARROT Ireland: Placental growth factor in **A**ssessment of women with suspected pre-eclampsia to **R**educe maternal morbidity: a Stepped Wedge Cluster **R**andomised Control **T**rial

MONITORING PLAN

Sponsor: University College Cork (UCC)

Version Number & Date:		Version Draft 1.0 19 MAY 2017	
Author:	Blánaid Ní Chuinneagáin	**Title:**	Project Manager
Co Author:	Deirdre Hayes-Ryan	**Title:**	Clinical Research Fellow
Reviewed By:	Jackie O'Leary	**Title:**	Quality & Regulatory Manager
Approved By:	Louise Kenny	**Title:**	Principal Investigator

Monitoring Plan Chronology		
Version Number	**Reason for Change**	**Author**
1.0	Original Document	Blánaid Ní Chuinneagáin

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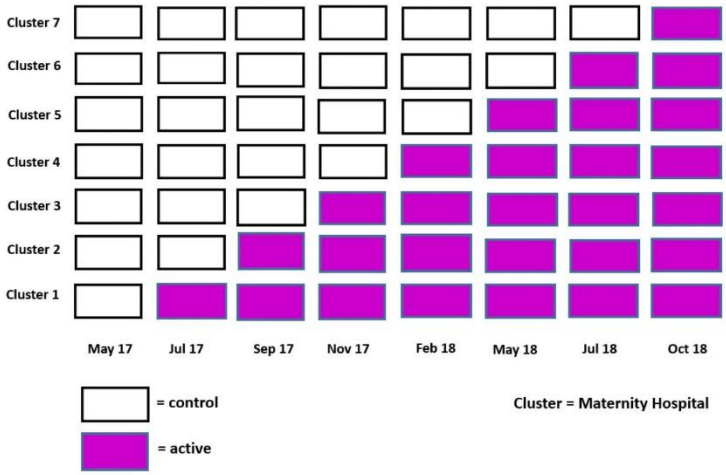
1 INTRODUCTION

The PARROT Ireland trial is a Stepped Wedge Cluster Randomised Control Trial being run in 7 centres across Ireland. The aim of this trial is to establish the effectiveness of plasma PlGF measurement in reducing maternal morbidity (with assessment of perinatal safety in parallel) of women presenting with suspected pre-eclampsia prior to 37 weeks' gestation. The primary outcomes which will be assessed in this trial will be maternal morbidity and neonatal morbidity. As this is a Stepped Wedge design all sites must begin the trial on the same day (See Figure 1 below).

Figure 1: PARROT Ireland Stepped Wedge Design



The PARROT Ireland trial will be monitored to ensure that the trial is conducted in accordance with the protocol/amendments, ICH-GCP E6 (R2), trial specific SOPs and the applicable local and national regulations, to ensure that the rights, safety and well-being of human subjects are protected and that the data integrity and validity of the trial results is protected.

An important part of a monitoring visit is comparing the entries in the case report forms with the original source documents (e.g. laboratory results, patient hospital notes). This procedure is known as Source Data Verification (SDV).
Monitoring will be in addition to any auditing of the trial that may be carried out.

2 PURPOSE

The purpose of this document is to describe the preparation for and the procedures that will be followed to monitor the PARROT Ireland trial.

Infant / PARROT IRELAND	Monitoring Plan	Final Version 1.0 19 MAY 2017

3 SCOPE

This Monitoring Plan (MP) will cover the monitoring procedure to be followed before, during and after the trial by the monitor throughout the conduct of the clinical trial.

4 RESPONSIBILITY

The assigned monitor is responsible for ensuring that the trial is adequately monitored in conjunction with the procedures outlined in this document. The monitor is the first line of contact for trial site staff and investigators regarding monitoring/quality issues. The second line of contact is the project manager, who should also be contacted in the absence of the monitor.

5 DEFINITIONS AND ABBREVIATIONS

Table 1: Abbreviations

Term/Acronym	Description
CI	Chief Investigator
COV	Close Out Visit
eCRF	electronic Case Record Form
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ISF	Investigator Site File
MP	Monitoring Plan
MV	Monitoring Visit
MVR	Monitoring Visit Report
PI	Principal Investigator
PIL	Participant Information Leaflet
PM	Project Manager
SDV	Source Data Verification
SMV	Site Monitoring Visit
SOP	Standard Operating Procedure
TMF	Trial Master File
TMG	Trial Management Group

6 MATERIALS & EQUIPMENT

N/A

7 PROCEDURES

7.1 SITE INITIATION AND TRAINING

The trial will not be initiated until ethical approval has been received at each site, all contracts have been executed and the sponsor greenlight has been given.
Training was completed at each participating site in early 2017 and topics covered included the following:

- Trial Protocol
- Participant Information Leaflet (PIL)/Informed Consent Form (ICF)
- Health Economics Questionnaire
- Centile Calculator
- Trial Website
- Trial database and data collected on the eCRF

Training will be documented either in individual training records or training memos for each training session during all site visits and these will be filed in the relevant Investigator Site Files (ISF).

A teleconference will be organised with site personnel 3-5 working days prior to the expected trial start date and will cover protocol refresher, trial specific SOPs and eCRF data entry training.

If new personnel join the trial team after the trial has commenced they will be trained in all trial related procedures prior to carrying out any trial related activities.

7.1.1 eCRF

The eCRF is in electronic form for the purpose of entering subject data. The eCRF will be accessed via the internet (www.parrotireland.medscinet.com) and data should be entered in real time in as far as is possible for each baby as per the eCRF SOP.

All eCRF pages will be checked for missing or invalid field completion prior to being saved and frozen. Any further data query resolution will be as per eCRF SOP.

7.2 SITE MONITORING VISIT (SMV)

7.2.1 Site Monitoring Schedule

On-site monitoring visits will be performed at each of the 7 sites included in the trial. The first visit should take place shortly after enrolment of the first 10-20 participants in order to ensure that trial procedures are correctly understood and followed at the site. The subsequent visits should be planned according to the inclusion rate at each

centre. The monitoring frequency is dependent on site recruitment targets and is as follows:

Table 2: Monitoring Visit Schedule CUMH, Coombe, NMH and Rotunda

Target Recruitment	712 Participants
Monitoring Visit (MV)	**Timing of MV**
1st MV	Following Enrolment of 10-20 Participants
2nd MV	Following Enrolment of 140 Participants +/-30 (110 – 170)
3rd MV	Following Enrolment of 260 Participants +/-30 (230 – 290)
4th MV	Following Enrolment of 380 Participants +/-30 (350 – 410)
5th MV	Following Enrolment of 500 Participants +/-30 (470 – 530)
6th MV	Following Enrolment of 620 Participants +/-30 (590 – 650)
7th MV	Following Enrolment of 740 Participants +/-30 (710 – 770)

The above monitoring schedule is based on a monitoring visit every 120 participants +/-30. A minimum of 6 MVs will be conducted at the above mentioned sites.

Table 3: Monitoring Visit Schedule Limerick and Belfast

Target Recruitment	426 Participants
Monitoring Visit (MV)	**Timing of MV**
1st MV	Following Enrolment of 10-20 Participants
2nd MV	Following Enrolment of 105 Participants +/-30 (75 – 135)
3rd MV	Following Enrolment of 190 Participants +/-30 (160 – 220)
4th MV	Following Enrolment of 275 Participants +/-30 (245 – 305)
5th MV	Following Enrolment of 360 Participants +/-30 (330 – 390)
6th MV	Following Enrolment of 445 Participants +/-30 (415 – 475)

The above monitoring schedule is based on a monitoring visit every 85 participants +/-30. A minimum of 5 MVs will be conducted at the above mentioned sites.

Table 4: Monitoring Visit Schedule Galway

Target Recruitment	300 Participants
Monitoring Visit (MV)	**Timing of MV**
1st MV	Following Enrolment of 10-20 Participants
2nd MV	Following Enrolment of 80 Participants +/-30 (50 – 110)
3rd MV	Following Enrolment of 140 Participants +/-30 (110 – 170)
4th MV	Following Enrolment of 200 Participants +/-30 (170 – 230)
5th MV	Following Enrolment of 260 Participants +/-30 (230 – 290)
6th MV	Following Enrolment of 320 Participants +/-30 (290 – 350)

The above monitoring schedule is based on a monitoring visit every 60 participants +/- 30. A minimum of 5 MVs will be conducted at the above mentioned sites.

For cause monitoring: If the monitor identifies issues at the site during one of the above visits and feels that additional monitoring is required, a for cause monitoring visit may be requested by the project manager or monitor to the CI or Sponsor and this should be promptly arranged in conjunction with the site.

7.2.2 Before the Monitoring Visit

The monitor should schedule the MV at a time that is convenient for the site trial team, giving the site at least 1 weeks' notice of the visit. The monitor will liaise with the site prior to the visit to confirm the following:

- Date of MV and expected arrival time
- Expected duration of the MV
- Source documents required (i.e. selected participants for review)
- Additional study documents required (e.g. ISF)
- Study personnel required to attend

During the visit the monitor will review previous monitoring visit reports (MVRs) (if applicable), focusing on any outstanding action items and the eCRF.

7.2.3 During the Monitoring Visit

Each monitoring visit should start with a brief meeting between the monitor and a member of the trial team to review the purpose and objectives of the visit.
The monitor will then review the following during the visit to ensure protocol and any applicable regulations adherence:

- <u>Informed Consent:</u>
  The monitor should ensure that informed consent is being obtained based on ICH GCP E6 (R2) and the trial specific procedure (SOP 001).
  The monitor will carry out 100% QC check on the ICFs for all participants enrolled to the trial at each site.

  If participants are required to re-consent to a new ICF during the study the monitor will 100% QC check the updated ICFs at each site.

- <u>Protocol Adherence:</u>
  The monitor will review through source documentation and site questioning the site adherence to the trial protocol. For examples, items such as PlGF testing will be reviewed to ensure it is not less than every 4 weeks. If

participants are failing to complete trial activities and questionnaires the monitor should identify and discuss ways to encourage better adherence with site personnel.

- Protocol Deviations/Violations:
  All protocol deviations/violations are captured on the eCRF and this will be checked during every monitoring visit.

  A protocol deviation is defined as a deviation to the protocol that has no impact on participant safety.
  A protocol violation is defined as a deviation to the protocol that could impact participant safety.

- Source Data Verification:
  During the MV, data will be verified by reviewing source notes at the site against the eCRF. Full SDV will be performed on 5% of participants at each site (rounded to the nearest participant). The participants should be randomly selected from participants recruited since the last MV. Each selected participant should be followed through until all data has been collected for the trial.

  The following should be reviewed when carrying out full SDV:
  1) Existence of the participant: Review the participant file for consistency between information.
  2) Eligibility of the participant: Review source documentation to ensure eligibility for the trial. Inclusion and exclusion criteria should be checked. If any eligibility issues highlighted site should clarify and report as deviation/violation if required
  3) Reporting of trial outcomes: Check to see that all reported outcomes have been correctly documented for participants. If not, site should be prompted, re-trained (as required) and deviation completed if necessary.
  4) Data entry: Check data in eCRF is complete, true, consistent with source notes, and the transcription of worksheet data into database is accurate. All discrepancies should be noted for site resolution or clarification.

  PlGF result should be checked for all participants on the intervention arm at each site.

- eCRF Queries:
  The monitor will identify and query any inconsistencies between the eCRF and the relevant source data. Any outstanding queries should be resolved as soon as possible by the relevant personnel at the site.

- Recruitment:
  At every MV the monitor will review recruitment goals and strategies with the site team to ensure they are on track to meet their recruitment targets as per Tables 2-4. The monitor will identify any recruitment issues or barriers the site is coming across and discuss with the PI and the Trial Management Group (TMG) to identify if a solution can be found.

- Investigator Site File:
  The monitor will review the ISF during the visit to ensure the following documents are filed and current:
  - Current approved trial protocol
  - Current approved PIL/ICF form with site specific logos
  - Local Ethics Committee approval
  - Training records for all trial personnel listed on the delegation log
  - Enrolment Logs
  - Completed ICFs
  - Completed Biobanking Consent Forms (applicable sites only)
  - Completed Health Economic Questionnaires

- Site Staff, Facilities and Equipment:
  If training is required, either on an updated protocol, re-training or training of new staff, the monitor may conduct this during the MV. Alternatively, a trained member of staff may conduct the relevant training. Training may also be conducted via teleconference by the monitor or a member of the TMG.
  The monitor will review the sites facilities and equipment, especially Alere controls and devices to ensure the site has sufficient supplies to conduct the trial.

At the end of the visit, the monitor should meet again with the relevant site staff members, including the PI (if available), review what was accomplished and any actions items that are required.

7.2.4 After the Monitoring Visit

The monitor will generate an MVR which will be sent to the PM or designee at the site for resolution of any outstanding findings. The report should be finalized within 10 working days of the visit and a copy will be sent to the Sponsor and PI for review prior to sending to the site. Once the MVR is finalized the monitor will send it to the site

detailing what was monitored, any issues identified and any actions to be completed including their projected resolution dates. Any outstanding actions should be answered by the site personnel and a copy of the report sent back to the monitor for filing in the TMF. A copy of the answered report should also be filed in the ISF at site.

7.3 REMOTE DATA MONITORING

In between MVs the monitor will review data entered into the database on a monthly basis for the following:

- Missing data trends
- Data completeness
- Protocol outcomes review
- Protocol deviations/violations

If the monitor finds any issues, then the site will be contacted within 5 working days of the review to resolve these issues.

7.4 CLOSE OUT VISIT (COV)

7.4.1 Before the COV

The monitor will liaise with the site to schedule the COV once all participants have completed the trial and at a time that is convenient to the trial team. A confirmatory e-mail will be sent to detail the following:

- Date of COV and expected arrival time
- Expected duration of the COV
- Any study documents that may be required
- Study Personnel required to attend

7.4.2 During the COV

During the COV the monitor will review the outstanding eCRFs, in line with the % SDV agreed, generate queries to be resolved and all discrepancies will be noted in the report. The ISF will be checked to ensure it is complete and all relevant documentation has been filed. Confirmation of the location where the ISF will be archived will also be sought. All outstanding ICFs not checked during previous visits will be 100% SDV'd.

The Monitor will organise for any study equipment loaned to the site for the trial to be returned, if applicable.

The Monitor will meet with the PI and discuss his/her responsibilities following the trial closure.

7.4.3 After the COV

Once the COV has been completed the monitor will generate a COV Report which will be sent to the PM or designee. The report should be finalized within 10 working days of the visit and a timeline for any outstanding actions to be resolved will be agreed with the relevant site personnel.

A copy of the COV Report will be sent to the Sponsor.

Copies of all monitoring visit reports together with the answered reports will be filed in the respective ISFs and TMF.

7.5 ARCHIVING

Once data lock has taken place and all outstanding actions are completed at the sites, the ISF can be archived.
Each site will be responsible for archiving their data as per local hospital requirements.

The eCRF will be archived electronically on servers located in MedSciNet.

8 APPENDICES

8.1 Appendix 1: PARROT Ireland Site List

Site	Principal Investigator
Belfast	Dr Alyson Hunter
Coombe	Prof Deirdre Murphy
CUMH	Prof Louise Kenny
Galway	Prof John Morrisson / Prof Declan Devane
Limerick	Prof Amanda Cotter
NMH (Holles St)	Prof Fionnaula McAuliffe
Rotunda	Prof Fionnaula Breathnach

Appendix 14: Trial Steering Committee Charter

PARROT Ireland Trial Steering Committee Charter

Protocol Title: PARROT Ireland: Placental growth factor in **A**ssessment of women with suspected pre-eclampsia to **R**educe maternal morbidity: a Stepped Wedge Cluster **R**andomised Control **T**rial

ClinicalTrials.gov Identifier (if applicable): NCT 02881073

Chief Investigator: Dr Keelin O' Donoghue

Sponsor: University College Cork (UCC)

PARROT Ireland: Placental growth factor in **A**ssessment of women with suspected pre-eclampsia to **R**educe maternal morbidity: a Stepped Wedge Cluster **R**andomised Control **T**rial

Clinical Trial.gov Ref: NCT 02881073

Sponsor: University College Cork

Prepared by:

Print Name: Nicolai Murphy Title: PROJECT MANAGER

Signature: _____ Date: _____

Authorised by:

Print Name: Dr. Keelin O' Donoghue Title: CHIEF INVESTIGATOR

Signature: _____ Date: _____

This document is prepared by the Sponsor and the TSC to describe the establishment and operations of the TSC for the PARROT Ireland Study

CLINICAL TRIAL.GOV REF: NCT 02881073

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Contents

1. Introduction

PARROT Ireland is a randomised, control trial that is funded by the Health Research Board Ireland (HRB) and Sponsored by University College Cork (UCC) PARROT Ireland will investigate the use of Placental Growth Factor (PlGF) in the assessment of women with suspected pre-eclampsia. PARROT is scheduled to begin in Q2 2017.

The full title of the trial is Placental growth factor in Assessment of women with suspected pre-eclampsia to Reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial (PARROT). This trial is a multi-centre trial conducted in Ireland. All sites will obtain approval from their local Ethics Committee (EC) and governance bodies (if applicable) prior to the start of the trial.

The role of the Trial Steering Committee (TSC) is to provide overall supervision on behalf of the Trial Sponsor and Trial Funder and to ensure that the PARROT Ireland trial is conducted according to the guidelines for Good Clinical Practice (GCP) and all relevant regulations and local policies. In order to assist with the trial, a Data Monitoring Committee (DMC) will also be appointed.

The background to this trial, its objectives, assessments, interventions etc., are described in the trial protocol.

The purpose of this document is to define the roles and responsibilities of the TSC for the PARROT Ireland trial including the nature and constitution of the TSC, the methods of providing information to and from the TSC and the frequency and format of meetings.

2. Roles and responsibilities

2.1. Objectives

The aim of the TSC is to oversee the conduct of the PARROT Ireland study and to safeguard the interests of the study participants and investigators; to assess the safety data, in conjunction with DMC, to monitor the overall conduct of the study; and to protect its validity and credibility.

2.2. Charter preparation

The TSC will be working jointly with members of the Trial Management Group (TMG) to prepare and finalise the TSC charter.

2.3. Roles and responsibilities of the TSC

The role and responsibilities of the TSC are:

- to provide overall supervision of the progress of the trial
- to ensure that the trial is being conducted in accordance with the principles of ICH-GCP, Directive 2001/20/EC and the relevant local and national regulations,
- To ensure that the rights, safety and wellbeing of the participants are the most important considerations and should prevail over the interests of science and society,
- to provide advice to the investigators on all aspects of the trial,
- to provide advice to the TMG on all aspects of the trial,
- to agree proposals for substantial protocol amendments,
- to review adherence to the protocol by study staff and participants and
- to make decisions about continuation or termination of the trial, after consideration of recommendations from the DMC.

The above responsibilities are carried out in conjunction with the DMC.

All members will be provided with relevant trial documents prior to their participation in the TSC.

2.4. Recommendations

A fundamental responsibility of every TSC is to consider the recommendations made by the DMC, with respect to the continuation of the study.

The DMC can recommend the following courses of action to the TSC:

- Recommendations concerning the termination of the study.
- Recommendation of study continuation with major or minor protocol modifications (*e.g.* changes to the recruitment procedures, inclusion criteria, endpoints, data collection, *etc.*).
- Recommendation of temporary suspension of enrolment and/or study intervention until some uncertainty is resolved.
- Recommendation that the study continue without modification.

2.5. Trial termination

All minutes from the TSC meetings should be included in the study Trial Master File (TMF) at the end of the study.

3. TSC membership

3.1. Committee composition

The PARROT Ireland TSC is a multidisciplinary group comprising of the following members who jointly have responsibility for the overall supervision of the clinical research project.

- Chief Investigator (Chair)
- Members of the TMG (e.g. study coordinator/trial manager, study statistician, data manager etc)
- Independent clinician(s) or Scientist(s) with relevant experience
- At least one Principal Investigator
- Representative of the Funder
- Representative of the Sponsor
- Independent lay person

The responsibility for calling and organising TSC meetings lies with the Chief Investigator in association with the Trial Management Group (TMG) The Chief Investigator is responsible for facilitating the meetings and summarising discussions with help from the Project Manager as required. If the votes are equal, the Chief Investigator will have the deciding vote.

The TSC membership is for the duration of the trial. If any members leave the TSC, the TMG should provide replacements promptly for appointment by the Chair.

3.2. Qualifications

3.2.1. Lay person

A layperson will be invited to join the TSC.

3.2.2. Scientific, medical and pharmaceutical related persons

The Sponsor has selected the TSC members based on the following minimum qualifications.

- A TSC member must be an expert in clinical trials and/or the related medical field.
- A TSC member must agree with and follow terms/conditions specified in this charter.
- Each TSC member will provide his/her curriculum vitae signed and dated to the TMG.

The TSC may, with agreement by the Sponsor, ask for external consultation or expertise.

3.3. Competing interests

Members should not serve on TSCs of similar, concurrently active trials as this could compromise the independence of the trial and possibly the confidentiality of the results of the individual trials. Any competing interests, both real and potential, should be disclosed. Although members may well be able to act objectively despite such connections, complete disclosure enhances credibility.

3.4. Membership duration

TSC membership will be scheduled for the entire duration of the study.

If the membership of a TSC member is terminated before the completion of defined membership duration, either voluntarily or due to the study teams decision, the member must still follow the terms in the signed confidential disclosure agreement with the Sponsor.

3.5. Replacement

- If any TSC member should resign, the committee member must discuss the action with the Chief Investigator as well as with the other TSC members
- In the situation where a member is no longer able to participate in the TSC, the remaining members of the TSC can suggest a replacement
- The Sponsor will retain the power to choose and appoint the new member.

3.6. Trial Insurance

The Sponsor ensures that the trial is insured according to the current regulations.

3.7. Termination

The Sponsor maintains the right to dismiss a TSC member and TSC members maintain the right to resign; thirty days notice should be given *in writing* in either case where possible.

4. TSC Organisation

4.1. TSC meetings

4.1.1. General organisation

As mentioned above there will be a TMG which will perform the management duties on a day-to-day basis and the TSC which will meet ('meeting' may be via teleconference or skype) on a quarterly basis, but they will also be available for advice when needed.

Other study team members and ad-hoc experts may participate in some open session discussions at the request of the TSC.

The Project Manager will distribute a draft of the minutes to the other TSC committee members for review within 7 calendar days of the meeting.

The following information will be included in the minutes:

- Meeting date
- Outline of session
- Meeting Attendees
- Discussions and action points
- Next expected date of TSC meeting

Comments on the draft minutes by TSC members will be submitted to the Project Manager within 7 calendar days of receipt of the draft minutes. The Project Manager will query members about their comments, and if needed, incorporate changes and prepare the final minutes for distribution and approval by the committee.

The Project Manager will maintain all documentation detailing DMC recommendations, and TSC meeting minutes. All minutes from the meetings should be included in the study Trial Master File (TMF) at the end of the study.

4.2. Role of the TSC Chair

- Provide experienced opinion if conflicts arise between the needs of the research team, the Funder, the Sponsor and/or any other agencies
- Leading the TSC to provide regular, impartial oversight of the trial, especially to identify and pre-empt problems
- Ensuring that changes to the protocol are debated and endorsed by other members of the TSC
- Establish clear reporting lines – to the Funder, Sponsor etc
- Become familiar with the role of the DMC

4.3. Scheduled meetings

4.3.1. Objective

Following the initial meeting, the scheduled meetings will have the following main objectives:

- Review recommendations from the DMC.
- Review reports on the trial provided by the TMG.
- Discuss the previous minutes, and follow up on any action points.

4.3.2. Frequency

The TSC with the full board invited will aim to meet on a quarterly basis but at a minimum once per year. At least 60% of the Committee members must be present for voting on recommendations of DMC with respect to the continuation of the study. Further meetings may be scheduled if and when necessary.

Every effort will be made to ensure that all TSC members can attend the meetings. The TMG should try and find a date that enables this. The CI must try to attend all meetings, especially if major actions are expected.

If the TSC is considering major actions the TSC Chair should communicate with absent members, as soon after the meeting as possible to determine whether they all agree. If there is disagreement amongst absent members a further meeting should be arranged with the full TSC.

4.4. Final Meeting

The final TSC meeting will be arranged when target recruitment is completed, all data collected and cleaned and the database is locked. This final meeting will be held to discuss final/completed data and interpretation, and publication timelines. If the study is terminated prematurely, no final study meeting is required.

4.5. Unscheduled meetings

Unscheduled meetings may be requested by the Sponsor, TMG or TSC based on unexpected findings and / or other unexpected reasons. The unscheduled meetings should be handled in the same way as scheduled meetings in terms of minutes and format.

In the case of a disagreement between the DMC and the TSC, an extraordinary meeting between the Sponsor and chairpersons/Chief Investigator of both Committees will be called and this group is expected to settle the disagreement to everybody's satisfaction

5. Reporting

Prior to a TSC meeting a report will be prepared by the TMG with input from relevant team members and circulated to TSC members at least a week before the meeting.

An outline of the contents of the TSC report is given below:

- Patient recruitment by month/site/total
- Eligibility violations
- Protocol violations by investigators or participants

- Study Outcome reporting
- Major protocol amendments
- Any matters affecting the trial

6. Appendices

6.1. Appendix 1: TSC Membership Contact List Version 1.0

Name	Role	E-mail Address
Dr. Keelin O' Donoghue	Chief Investigator	K.odonoghue@ucc.ie
Prof. Fionnuala Breathnach	Principal Investigator	fbreathnach@rcsi.ie
Prof. Fionnuala McAuliffe	Principal Investigator	Fionnuala.mcauliffe@ucd.ie
Prof. Amanda Cotter	Principal Investigator	Amanda.cotter@ul.ie
Prof. Deirdre Murphy	Principal Investigator	MurphyD4@tcd.ie
Dr. Alyson Hunter	Principal Investigator	Alyson.hunter@belfasttrust.hcsni.net
Prof. Declan Devane	Principal Investigator	Declan.devane@nuigalway.ie
Prof. JJ Morrisson	Principal Investigator	John.morrisson@nuigalway.ie
Prof. Gene Dempsey	Neonatologist	g.demsey@ucc.ie
Ms. Nicolai Murphy	Project Manager	Nicolai.murphy@ucc.ie
Dr. Deirdre Hayes-Ryan	Clinical Research Fellow	Deirdre.hayesryan@ucc.ie
Dr. Brendan McElroy	Health Economist	b.mcelroy@ucc.ie
Dr. Aileen Murphy	Health Economist	Aileen.murphy@ucc.ie
Dr. Ali Khashan	Statistician	a.khashan@ucc.ie
Dr. Karla Hemming	Statistician	k.hemming@bham.co.uk
Dr. Ruben Keane	Sponsor (Back up Sponsor representative)	Ruben.keane@ucc.ie
Dr. Muiris Dowling	Sponsor (Primary Sponsor representative)	Maurice.dowling@ucc.ie
Dr. Elizabeth Tully	Network Manager	elizabethtully@rcsi.ie
Ms. Emma Snapes	Laboratory	e.snapes@ucc.ie
To Be Confirmed	Lay Person	

Appendix 15: Data Monitoring Committee Charter

Data Monitoring Committee Charter

PARROT Ireland: Placental growth factor in Assessment of women with suspected pre-eclampsia to Reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial

Trial registration

Clinical Trials NCT02881073 (26th August 2016)

Signature Page Data Monitoring Committee

_____ _____

Name, Title Date
Chairperson, DMC

_____ _____

Name, Title Date
Member, DMC

_____ _____

Name, Title Date
Member, DMC

_____ _____

Name, Title Date
Member, DMC

_____ _____

Name, Title Date
Sponsor

Table of contents

1 Introduction

1.1 Outline and scope of the Data Monitoring Committee Charter

The purpose of this document is to describe the roles and responsibilities of the Data Monitoring Committee (DMC) for the PARROT Ireland trial including the nature and constitution of the DMC, the methods of providing information to and from the DMC, the frequency and format of meetings, the statistical issues and the relationships with the study team.

1.2 Trial design

Multi centre, stepped wedge cluster randomised control trial (SWC-RCT), of women presenting with suspected pre-eclampsia from 20 weeks to 36+6 weeks gestation inclusive (Figure 1). Each maternity hospital will act as a cluster and each cluster will transition from control to intervention at pre-defined specified time points.

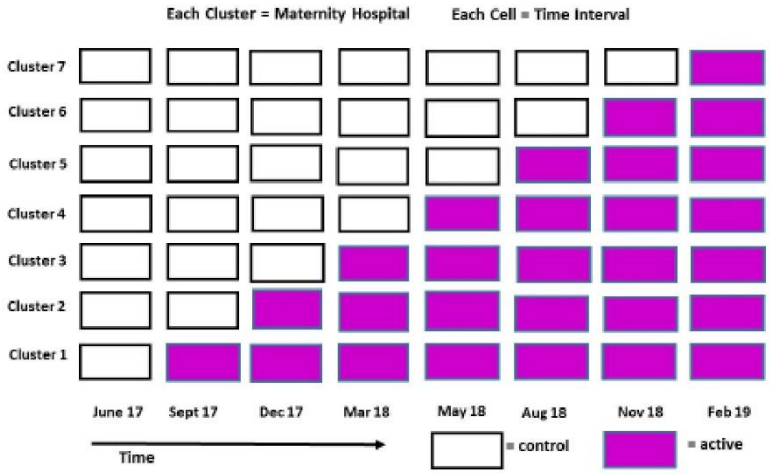

Figure 1; Stepped Wedge Cluster Design

1.3 Study Objectives

The primary aims are to establish the effectiveness of plasma PlGF measurement in reducing maternal morbidity in women presenting with suspected pre-eclampsia prior to 37 weeks' gestation while ensuring its use does not result in an increase in neonatal morbidity.

The long term aim is to demonstrate that knowledge of PlGF measurement enables appropriate stratification of the antenatal management of women presenting with suspected pre-eclampsia, such that those at highest risk receive greater surveillance with a decrease in maternal and neonatal

adverse outcomes, and those at lower risk can be managed without unnecessary admission and other interventions.

1.4 Sample size

Four thousand women with suspected preterm pre-eclampsia will be enrolled onto this trial.

1.5 Trial design

Participants enrolled in the control arm receive usual hospital care as per National guidelines; these are Health Service Executive/Institute of Obstetrics and Gynaecology Irish guidelines for clusters in the Republic or the NICE guidelines for the cluster in Northern Ireland. Participants enrolled in the intervention arm have their plasma PlGF quantified at enrolment in addition to routine hospital investigations.

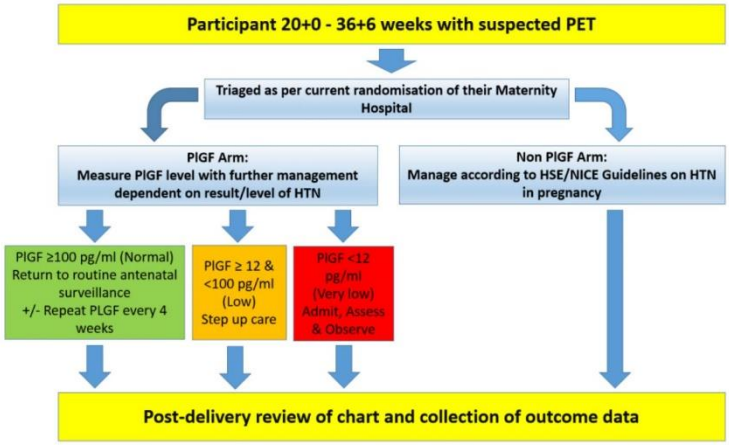

Figure 2; Trial Schema

2 Roles and Responsibilities

2.1 Objectives

The aim of the DMC is to safeguard the interests of the study participants, the investigators and the Sponsor. To monitor the overall conduct of the study and to protect its validity and credibility.

2.2 Charter preparation

The DMC will work jointly with members of the study team to prepare and finalise the DMC charter.

2.3 Roles and responsibilities of the DMC

The roles and responsibilities of the DMC are:

- Assessing data quality, including completeness (thereby encouraging collection of high quality data).
- Monitoring recruitment figures and withdrawals.
- Monitoring compliance with the protocol by participants and investigators.
- Monitoring evidence for treatment differences in the main efficacy measures.
- Making recommendations to the sponsor.
- Monitoring compliance with previous DMC recommendations if applicable.
- Considering the ethical implications of any recommendations made by the DMC.
- Advising on and/or endorsing any major protocol modifications suggested by investigators or Sponsor (*eg.* changes to the inclusion criteria, changes to sample size, endpoints, data collection, *etc.*).
- Suggesting additional data analyses.

2.4 Recommendations

A fundamental responsibility of every DMC is to make recommendations to the Sponsor and the Trial Steering Committee (TSC) concerning the continuation of the study. The DMC will make the recommendation based on a review of the interim data and the expert report.

The DMC may provide the following recommendations to the Sponsor and the TSC:

- Recommendation concerning the termination of the study.
- Recommendation of temporary suspension of enrolment and/or study intervention until some uncertainty is resolved.
- Recommendation that the study continue with modification.
- Recommendation that the study continue without modification.

The DMC should make its recommendations based on the following requirements:

- Recommendations for the termination of the study (see section 5.3 Decision rules for early stopping).
- Recommendations for modifications, other than termination, should be accompanied by the minimum amount of data required for the Sponsor to make a reasoned decision about the recommendation, and the rationale for such recommendations should be as clear and precise as possible.

- The DMC should clearly express its recommendations to the Sponsor and to the TSC. To this end, both a written recommendation and oral communication should be provided, with opportunity for questions and discussion.
- The DMC should document its recommendations and their rationale in a letter that can be reviewed by the Sponsor, Ethics Committees or other interested parties as appropriate.

2.5 Trial Termination

All minutes and letters/documents of recommendations from the DMC meetings should be included in the Trial Master File (TMF).

3 DMC Membership

3.1 Committee Composition

The membership will consist of 4 individuals including at least one clinician and at least one statistician (to provide independent statistical expertise). Members have been chosen because they are experienced in clinical trials and/or the disease area.

The quorum of the DMC is:

- Chairperson:
- Statistician:
- 2 other members:
  - (Obstetrician)
  - (Midwife)

The Chairperson is expected to facilitate and summarise discussions.

The Sponsor's representatives may participate in open DMC discussions only.

The Chief Investigator may also participate in open DMC discussions only.

Other study team members and ad-hoc experts may participate in some open session discussions at the request of the DMC.

3.2 Qualifications

The DMC members have been selected based on the following minimum qualifications.

- A DMC member must be an expert in clinical trials and/or the related medical field.
- A DMC member must have sufficient experience in serving as an expert for interim data monitoring.
- A DMC member must agree with and follow the terms/conditions specified in this charter.
- A DMC member must have signed a confidential disclosure agreement (see appendix 6.1).
- Each DMC member will provide his/her curriculum vitae to the Sponsor.

The DMC may, with an agreement from the Sponsor, ask for external consultation or expertise.

3.3 Competing Interests

The DMC members should be independent of the trial (i.e. they should not be involved with the trial in any way or have any involvement that could impact on the trial). Members should not serve on DMCs of similar, concurrently active trials as this could compromise the independence of the trial and possibly the confidentiality of the results of the individual trials. Any competing interests, both real and potential, should be disclosed. Although members may well be able to act objectively despite such connections, complete disclosure enhances credibility.

A short competing interest form should be completed by the DMC members and returned to the Sponsor (See Appendix: 6.3).

DMC members should not use interim results to inform trading in pharmaceutical shares, and careful consideration should be given to trading in the stock of companies with competing products.

3.4 Membership duration

- DMC membership will be scheduled for the entire duration of the trial
- If the membership of a DMC member is terminated before the completion of defined membership duration, either voluntarily or due to the Sponsor's decision, the member must still follow the terms in the signed confidential disclosure agreement with the Sponsor.

3.5 Replacement

- If any DMC member should resign, the committee member as well as the Chairperson must discuss the action with the sponsor as well as with the other DMC members

- In the situation where a member is no longer able to participate in the DMC , the remaining members of the DMC can suggest a replacement to the sponsor
- The sponsor will retain the power to choose and appoint the new member.

3.6 Termination

The Sponsor maintains the right to dismiss a DMC member and DMC members maintain the right to resign; - thirty days' notice should be given in either case where possible.

3.7 Clinical Trial Insurance

Insurance for the trial is in place and provided by the Sponsor University College Cork. The insurer is NWL Syndicate 1218 at Lloyds of London (newline).

4 DMC Organisation

4.1 Initial Communication

The initial communication between the TSC and the DMC occurred after the confidential disclosure agreement was signed by the DMC members. The initial communication included

- Introduction of the study protocol, amendments, patient notes and eCRF
- Preparation of the DMC Charter
- Completion of the selection of DMC members, if necessary
- Planning for the first and subsequent DMC meetings

At the time of the establishment of the DMC, the following documents must be available:
Study Protocol, electronic Case Report Form (eCRF).

All potential DMC members will have received the protocol before agreeing to join the committee. If a potential DMC member has major reservations about the trial they should report these to the Sponsor and may decide not to accept the invitation to join. DMC members should be independent and constructively critical of the ongoing trial, but also supportive of the aims and methods of the trial.

The TSC and study statistician will work closely with the DMC statistician to define the requested data summaries, listings, tables and graphs for inclusion in the draft Data Summary Report (DSR) for the DMC meeting.

4.2 DMC Meetings

4.2.1 Definitions

- **Closed session**: Portions of the DMC meetings attended only by DMC members. This gives the DMC freedom to discuss all aspects of the trial, without representatives from the sponsor being able to influence discussions.
- **Open session**: Portions of the DMC meetings attended by the members of the DMC, the clinical trial coordinator, and representatives of the sponsor, as relevant. Information in the DSR should not be made available for open sessions since it will contain interim efficacy data.

4.2.2 General Format

The general format of DMC meetings will be:

- **Open session**: The Sponsor may be invited to make brief presentations and be available for questions. The objective of the open session will be to present to the DMC, information about the study conduct (site status, enrolment rates, *etc.*), demographic data, baseline data from enrolled patients and safety data. Further presentation of analysed data may be done by the DMC statistician during the closed session.
- **Closed session**: Issues concerning the protocol and the DSR will be discussed.
- **Open session**: If necessary, any matters arising from the closed session will be discussed.
- **Closed session**: If necessary, any matters arising from the second open session will be discussed.

If additional information is still necessary at the close of a DMC meeting before a recommendation can be made, a final open and/or closed session may be organised within the shortest delay possible. This meeting may take place via a teleconference.

4.2.3 General Organisation

The TSC will send the draft Data Summary report (DSR)) to the DMC committee members prior to the DMC meeting along with confirmation of the meeting time and venue. The cut-off date for data entry will be 4 weeks prior to the meeting.

The first meeting will be face-to-face. It is recommended that all subsequent meetings should be face-to-face also if possible, with teleconference as a second option.

A nominated member of the DMC will document the discussions during each meeting (open and closed sessions). This documentation will be validated by the DMC members prior to being filed as **closed minutes** along with the DSR.
At the close of each meeting, recommendations proposed by the DMC will be voted on. In all votes, a unanimous decision is preferred, but a minimum of two votes may be accepted at the discretion of

the Chairperson (at least the Chairperson and one other member of the board must attend a DMC meeting in order to make recommendations to the Sponsor). In case of voting to close the study for safety reasons, the chairperson of the DMC must be present.

Following each meeting, duly voted and passed DMC recommendations will be transmitted in writing by a nominated member of the DMC to the Chief Investigator and Sponsor within 7 working days of the meeting, or they may be contacted by phone immediately if considered urgent.

A nominated member of the the DMC will distribute a draft version of the **open minutes** to the other DMC committee members for review within 7 working days of the meeting.

The following information will be included in the open minutes:

- Meeting dates, meeting session
- Outline of session
- Meeting Attendees: both open and closed sessions if applicable
- Recommendations
- Points to highlight, requests for further information, requests for changes, other comments
- Next expected date of DMC meeting
- Signature of the Chairperson on behalf of the DMC.

Comments on the draft version of the open minutes by DMC members will be submitted to the nominated DMC member within 7 working days of receipt of the minutes, who will then query the DMC members about their comments, and if needed, incorporate changes and prepare the final minutes for distribution and approval by the committee. Members unable to attend will be contacted by the Chairperson prior to the filing of the report for their opinion.

The approved open minutes will be submitted to the Chief Investigator and Sponsor within 14 working days of the meeting.

A nominated member of the DMC will maintain all meeting recommendations, DSR and minutes of open and closed sessions. These records will be transferred to the Sponsor upon completion of the trial. All minutes from the meetings will be filed in the study Trial Master File (TMF).

4.3 Initial DMC meeting

The aim of the first meeting will be to discuss the workings of the DMC and the information necessary for review at each meeting (to be included in the DSR).

Open session:

The meeting will commence with a brief presentation of the project by members of the TSC, following which the participants will:

- Review the process for transmission of data and analysis results
- Clarify the objectives of the DMC
- Review the draft DMC charter and finalise the charter including:
  - Scheduling of meetings
  - Format of the interim reports to the DMC
  - Timing of the delivery of the interim reports to the DMC members prior to the meeting
  - Definition of a "quorum" of DMC members, including representation of essential scientific and other disciplines
  - Handling of meeting minutes

Closed session:
The DMC members will:

- Review the protocol, data collection instruments and other important trial documents
- Discuss the DSR
- Decide on the recommendations to the Sponsor
- Discuss the open minutes

4.4 Scheduled meetings

4.4.1 Objective

Following the initial meeting, the scheduled meetings will have the following main objectives:

- Review and evaluate the DSR
- Decide on the recommendations to the Sponsor if applicable
- Discuss the open minutes

4.4.2 Frequency

The frequency of the scheduled meetings will be based on the speed of patient recruitment to the study.

- The first meeting took place on 27th Feb 2018
- The next meeting will occur when 1000 postnatal outcome results are available for review by the committee; this is anticipated to be in early 2019

- The DMC have been be provided with quarterly reports via email from the Trial Management Group (TMG). These include an update on overall numbers of; recruited participants, withdrawals, deviations and violations. All Data Summary Reports to date are filed in the Trial Master File
- Any serious events or clinical concerns raised during the course of the trial will be notified to the DMC as they arise.

4.5 Unscheduled meetings

Unscheduled meetings may be requested by the Sponsor or DMC based on unexpected findings, regulatory requests and/or other unexpected reasons. The unscheduled meetings should be handled in the same way as scheduled meetings in terms of minutes and format.

The DMC members should be given at least 2 weeks, and if possible a month, to read and comment on any draft publications that report outcome measures and/or details of the DMC. This may be done simultaneously to other groups reviewing the draft manuscript.

5 Interim data evaluation

5.1 Data source and statistical deliverables

In preparation for the DMC meeting, the TMG will provide the DMC members with a blinded statistical analysis of the primary outcomes. This data will be based on a "snapshot" of the clinical database with a data entry cut-off date typically four weeks prior to the respective DMC meetings.

5.2 Data Summary Report (DSR)

The DMC members will receive the quarterly DSR directly from the TMG. The report will include tables and listings related to the following areas:

A. Ensure the quality of data collection

B. Ensure that the intervention is being rolled out according to the randomisation plan

C. Monitor balance between arms to monitor for potential selection biases

D. Ensure PlGF testing is not overwhelmingly better or worse than no PlGF testing with respect to maternal morbidity with neonatal morbidity

5.3 Decision rules for early stopping

The stopping rules are as follows:

- Proof beyond reasonable doubt that for all, or for some, types of participant, one particular intervention is definitely indicated or definitely contra-indicated in terms of a net difference of

5.3 Decision rules for early stopping

The stopping rules are as follows:

- Proof beyond reasonable doubt that for all, or for some, types of participant, one particular intervention is definitely indicated or definitely contra-indicated in terms of a net difference of a major endpoint.
- There will be no stopping of the trial for futility as the study will be underpowered to detect small effects.

6 Appendices

6.1 Confidentiality Disclosure Statement

Confidentiality Statement for members of the PARROT Ireland Data Monitoring Committee.

Full Name:

Title:

Company:

Position:

To encourage open communication, each member of the ***PARROT Ireland Trial*** *(*Placental growth factor in Assessment of women with suspected pre-eclampsia to Reduce maternal morbidity: a Stepped Wedge Cluster Randomised Control Trial) agrees that any information, notably notes, documents, memoranda, correspondence, emails, work results or other materials provided by the parties to each other either directly or indirectly, in writing or orally, is a private communication from the individual making the contribution and is presented with the restriction that such information is not for public use. The members agree not to transmit, distribute, publish or disseminate any information that (or the transmission, distribution, publication or dissemination of which) infringes any intellectual proprietary rights of the responsible authors.

I agree with the above statement:

Name: _____

Signed: _____ Date: _____

DRAFT

6.2 Competing Interests Form

Agreement and Competing Interests Form for members of the PARROT Ireland Data Monitoring Committee.

Please complete the following document and return to: Nicolai Murphy, Project Manager (nicolai.murphy@ucc.ie)

☐ I have read and understood the DMC Charter Version 1.0 dated XX XXX 2018

☐ I agree to join the DMC for this trial as an independent member

☐ I agree to treat all sensitive trial data and discussions confidential

The avoidance of any perception that members of the DMC may be biased in some fashion is important for the credibility of the decisions made by the DMC and for the integrity of the trial. Possible competing interest should be disclosed via the trials office. In many cases simple disclosure up front should be sufficient. Otherwise, the (potential) DMC member should remove the conflict or stop participating in the DMC. Table 1 lists potential competing interests:

Table 1: Potential competing interests for independent members

1. Stock ownership in any commercial companies involved.
2. Stock transaction in any commercial company involved (if previously holding stock)
3. Consulting arrangements with the Sponsor
4. Frequent speaking engagements on behalf of the intervention
5. Career tied up in a product or technique assessed by the trial
6. Hand-on participation in the trial
7. Involvement in the running of the trial
8. Emotional involvement in the trial
9. Intellectual conflict e.g. strong prior belief in the trial's experimental arm
10. Involvement in regulatory issues relevant to the trial procedures
11. Investment (financial or intellectual) in competing products
12. Involvement in the publication

☐ **NO,** I have no competing interests to declare

☐ **YES,** I have competing interests to declare (please detail below)

_____

_____

Name: _____

Signature:_____ Date: _____

DRAFT