HYPERTENSIVE DISORDERS OF PREGNANCY:

OBSERVATIONS ON

CARDIOVASCULAR PATHOPHYSIOLOGY

by

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Abstract

Hypertension affects one in ten pregnancies and its sequelae can affect women and their offspring in later life. The placenta has been the focus of much of the research into the pathogenesis of hypertension in pregnancy. A functioning placenta requires a functioning cardiovascular system, hence investigating whether concepts from hypertension research (echocardiographic structure and function, altered ventricular and arterial elastance and monocyte biology) apply to pregnancy hypertension is of great interest. Evidence for cardiovascular changes in hypertensive disorders of pregnancy is mounting, but the adaptations in subsequent pregnancy are unclear. The aim of this study was to compare cardiovascular (patho)physiology in pregnant women with and without previous hypertension in pregnancy. Monocyte subset heterogeneity was studied to provide mechanistic insight. Prospective changes in study parameters were analysed in each trimester of pregnancy and healthy non-pregnant women were studied as a control group at baseline. I found more prehypertension and increased arterial stiffness in pregnant women with prior hypertension and a corresponding increase in the CD14++CD16–CCR2+ (Mon1) and CD14+CD16++CCR2– (Mon3) monocyte subsets and their aggregates with platelets in these women. Changes in cardiovascular performance and their relationship to monocyte subsets may potentially be mechanisms leading to increased long-term cardiovascular risk.

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Abbreviations

ACE: Angiotensin-converting enzyme

ACOG: American Congress of Obstetricians and Gynecologists

AIx: augmentation index

Aix75: augmentation index standardised to heart rate 75 beats per minute

ANOVA: analysis of variance

AoD: aortic root diameter

BD: Becton Dickinson

BMI: body mass index

BP: blood pressure

BSA: body surface area

BSE: British Society of Echocardiography

CD: cluster of differentiation (also known as classification determinant)

CI: cardiac index

CO: cardiac output

CV: coefficient of variation

DBP: diastolic blood pressure

DIC: disseminated intravascular coagulation

DICOM: digital imaging and communications in medicine

DT: deceleration time

DVP: digital volume pulse analysis

Ea: arterial elastance

EaI: arterial elastanc index

ECCHO: Evaluating Cardiovascular Changes in Hypertension in Obstetrics

ECG: electrocardiogram

EDP: end diastolic pressure

Eed: end diastolic elastance

$E_{Nd(avg)}$: group-averaged normalised left ventricular elastance at onset of ejection

$E_{Nd(est)}$: non-invasive estimated normalised left ventricular elastance at onset of ejection

ESP: end systolic pressure

GH: gestational hypertension

Hb: haemoglobin

Hct: haemoatocrit

HELLP: haemolysis, elevated liver enzymes and low platelets

HR: heart rate

ICAM: intracellular adhesion molecule

ICVS: University of Birmingham Institute of Cardiovascular Sciences

IL: interleukin

IQR: interquartile range

ISSHP: International Society for the Study of Hypertension in Pregnancy

IVRT: isovolumetric relaxation time

LA: left atrium

LMWH: low molecular weight heparin

LPS: lipopolysaccharide

LV: left ventricle/ventricular

LVEF: left ventricular ejection fraction

LVFS: left ventricular fractional shortening

LVOT: left ventricular outflow tract

LVM: left ventricular mass

LVMI: left ventricular mass index

MAP: mean arterial pressure

MCP-1: monocyte chemoattractant protein 1

MFI: median fluorescent intensity

MMP: matrix metalloproteinase

MoM: multiple of the median

Mon1: CD14++CD16-CCR2+ monocytes (monocyte subset 1)

Mon2: CD14++CD16+CCR2+ monocytes (monocyte subset 2)

Mon3: CD14+CD16++CCR2- monocytes (monocyte subset 3)

MPA: monocyte-platelet aggregate

MRI: magnetic resonance imaging

NHS: National Health Service

NICE: National Institute for Health and Care Excellence

NKκB: nuclear factor kappa B

NTP: normotensive pregnant control

OI: operator index

PAI-1: plasminogen activator inhibitor-1

PBS: phosphate buffered saline

PCI: percutaneous coronary intervention

PCR: protein:creatinine ratio

PET: preeclampsia

PLAX: parasternal long axis view

RCOG: Royal College of Obstetricians and Gynaecologists

SBP: systolic blood pressure

SD: standard deviation

SGA: small for gestational age

SOMANZ: Society of Obstetric Medicine of Australia and New Zealand

SOP: standard operating procedure

STEMI: ST elevation myocardial infarction

SV: stroke volume

SVI: stroke volume index

SVR: systemic vascular resistance

t_{Nd}: ratio of left ventricular pre-ejection time to total systolic time period

TDI: tissue Doppler imaging

TLR: toll-like receptor

TNF: tumour necrosis factor

TTE: transthoracic echocardiography

TVR: total vascular resistance

TVRI: total vascular resistance index

UK: United Kingdom

USA: United States of America

VAC: ventricular-arterial coupling

VTI: velocity time integral

WHO: World Health Organization

Preface

I begin my thesis by introducing the topic cardiac function in pregnancy, the interface of cardiovascular and obstetric medicine. The hypertensive disorders of pregnancy, gestational hypertension (GH) and preeclampsia (PET), are introduced in **Chapter 1** as my research interest. The pathogenesis and clinical aspects of hypertension in pregnancy are explored. In **Chapter 2**, I provide a systematic review the literature relating to cardiac structure and function in gestational hypertensive disease, with a focus on echocardiography. To further set the scene for my laboratory work I then review the literature pertaining to monocytes and their subsets in women's health. **Chapter 3** outlines the objectives for my experimental work and the hypotheses to be tested. In **Chapter 4**, I describe the methodology for Evaluating Cardiovascular Changes in Hypertension in Obstetrics (ECCHO), my prospective study. The results from ECCHO are found in the following three chapters. The echocardiographic observations are contained in **Chapter 5**. Within **Chapter 6** are my experimental data relating to monocytes and their subsets. In **Chapter 7**, I integrate my findings and relate them to pregnancy outcomes, followed by **Chapter 8** to conclude and propose future work.

CHAPTER 1:
INTRODUCTION

1.1 Pregnancy and the heart

1.1.1 Physiological adaptations of the cardiovascular system in pregnancy

The maternal cardiovascular system must adapt to the pregnant state as the gravid uterus, placenta and developing fetus place a new demand on the circulation. Maternal haemodynamics adjust as early as the fifth week of pregnancy[2] and cardiac structure and function must adjust in turn. The hyperdynamic circulation tests the cardiac functional reserve.[3] Failure of physiological cardiovascular adaptation to pregnancy is associated with adverse pregnancy outcomes. The study and early recognition of maladaptation could lead to the development of novel screening techniques.[4]

An increase in heart rate by 10-30%[5-10] is the one of the first responses to the pregnant state.[11] Blood pressure is a function of the cardiac output (CO) and systemic vascular resistance (SVR). SVR decreases in pregnancy.[5-8, 11-13] Cardiac output (CO) is determined by heart rate and stroke volume. CO increases in pregnancy.[5-8, 12] This is primarily due to an increase in stroke volume rather than in heart rate.[14, 15] CO has been shown to rise in the first and second trimesters then either plateau[8, 16], increase[13, 17] or decrease[9, 10, 15, 18] towards term. The different findings in the third trimester may be due to variation between individual patients or to different timing of the measurements between early third trimester and close to term. In uncomplicated pregnancy there should be no significant change in systolic blood pressure (SBP). The diastolic blood pressure (DBP), and hence the mean arterial pressure (MAP) decreases during the first trimester, then plateaus in the second trimester before rising in the final weeks of pregnancy.[17, 19] Physiological changes in the renal, haematological and respiratory systems also affect haemodynamics.[20]

The myocardium hypertrophies during pregnancy in order to compensate for the increased load on the left ventricle (LV).[21] The increased load has two elements, the preload (the physiological blood volume expansion in pregnancy) and afterload (increased peripheral resistance). The intrinsic myocardial contractility may increase.[6, 11] Left ventricular mass (LVM) increases during pregnancy[7, 8, 12, 17] by 10 – 52%.[4, 8, 13, 21, 22] This reversible increase in LVM is an adaptation to maintain the necessary cardiac output, whilst aiming to reduce wall stress and oxygen demand.[5] LV hypertrophy means that the cardiac myocytes increase in size. This is usually concentric hypertrophy whereby the LV wall thickness increases without an increase in cavity size.[17, 19] In parallel with this fibroblasts also hypertrophy so cardiac structure changes (remodelling) and the myocardium becomes less compliant.[7] The LV becomes more globular or spherical in shape as pregnancy progresses,[5] as it becomes dilated by increased blood volume.[15] Some studies report no change in LV dimension during pregnancy.[7, 12]

Loading conditions, contractility and heart rate all contribute to left ventricular function.[5] Some investigators report that ejection fraction stays the same throughout pregnancy[5, 7, 14] whilst others report a decrease in the third trimester.[8, 12] A left ventricle subjected to increased loading conditions for some time will become stiffer and diastolic function will be impaired. Diastolic dysfunction means there is resistance to filling of the LV. When diastolic function is normal, the LV should fill to its expected volume at the end of diastole without the pressure in the chambers becoming abnormally high.[19] As the LV becomes less compliant, due to realignment of collagen,[21] the pressure relative to its volume is increased. This means it takes longer for the to fill with blood and longer to relax. Myocardial contractility and

relaxation (both energy-dependent processes) have been shown to be altered at term in normal pregnancy.[3]

The consequences of a shorter diastole are reduced coronary artery perfusion and less filling time.[4] Reduced blood supply is damaging to the heart. Myocardial perfusion in diastole is also reduced by the physiological increase in heart rate. Diastolic dysfunction's preceding systolic dysfunction in cardiac disease is well recognised outside of pregnancy[23-27] but only more recent studies in pregnancy have shown this.[4, 7]

Arterial compliance, which refers to how the pulse wave is propagated throughout the arterial system, increases by about 30% in pregnant women.[19, 22, 28] Arterial compliance is defined as volume change per unit change in blood pressure . This elastic property is necessary to offset the effect of increased blood volume (which causes a similar rise in venous return) without a rise in mean arterial pressure. Large arteries with preserved elastic properties are able to stretch during systole, thus retaining some of the blood volume to be returned to the circulation during diastole. This reduces cardiac cycle-dependent fluctuations in blood pressure, reduces cardiac afterload and improves coronary perfusion pressure during diastole. Increased vascular and LV stiffening on the other hand may lead to an alteration in ventriculo–arterial coupling, which can be assessed reproducibly by echocardiographic measurement of arterial and cardiac elastance.[29-32] Despite the growing evidence of abnormal arterial stiffness and diastolic dysfunction in pregnancy, the mechanisms of these changes and mutual relationship between arterial and cardiac abnormalities are not clear.[33] Abnormal cardiovascular interactions (coupling) are likely to take place, but more data are required to support this hypothesis.

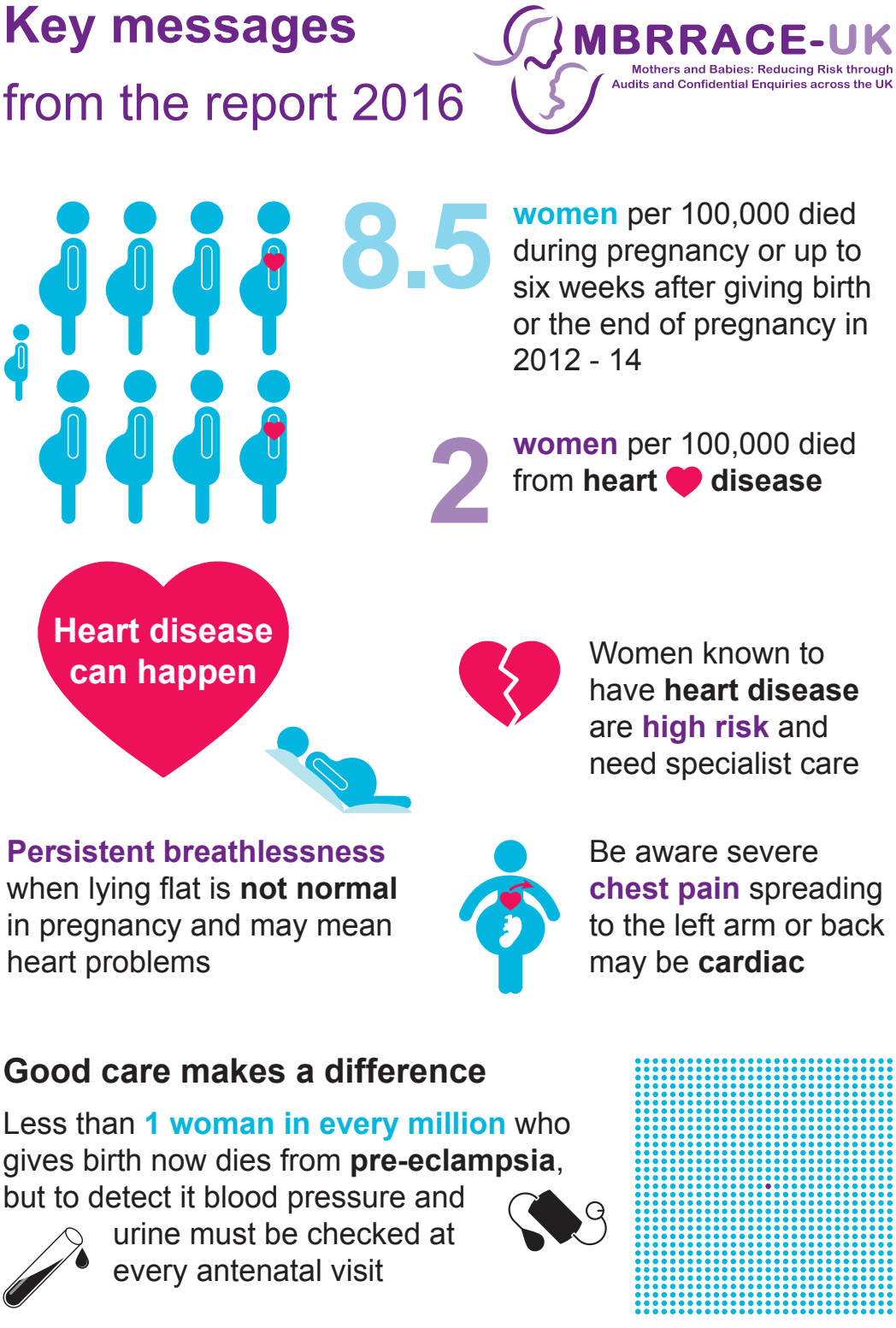
1.1.2 Cardiac disease in pregnancy

Cardiac disease is the leading cause of death in pregnancy and the puerperium in the United Kingdom.[34-36] This has been case since the turn of the millennium and the rate of deaths due to cardiovascular complications continues to rise.[34] The overall maternal mortality rate from cardiac disease, reported in the Confidential Enquiries into Maternal Deaths for the period 2012–2014 was 2.18 per 100,000 maternities[37] and from 2014–2016 was 2.39 per 100,000 maternities.[38] Hypertension in pregnancy causes one third of severe maternal morbidity.[1] Up to 15% of maternal deaths in the developing world are due to pregnancy hypertension and a similar proportion of direct of obstetric deaths in the UK and USA are attributable to complications of hypertension.[39] Investigations into maternal and neonatal morbidity and mortality due to hypertensive disorders in pregnancy suggest that improvements in the care provided is required to achieve better outcomes.[40]

1.1.3 Echocardiography in pregnancy

Methodology and technology for measuring cardiac structure and function has improved greatly over the last century since cardiac output was first measured in 1915.[17] Grollman demonstrated increased cardiac output in pregnancy by measuring concentrations of exogenous gases in the blood.[41] Hamilton later used right heart catheterisation, a technique developed by Cournand and colleagues,[42] to determine cardiac output based on the Fick principle.[9, 43] Until about 1970 other invasive methods including indicator dye dilution and thermodilution techniques were used.[44, 45] Invasive hemodynamic monitoring is the most reliable[6] but may not reflect the resting state (due to effects of anxiety and autonomic stimulation) and outside the context of management of critical illness it is not used as a

Figure 1: Infographic focusing on cardiovascular disease from MBRRACE-UK[37]



research tool as the risks to a pregnant woman are unacceptable in modern practice. Electrical impedance cardiography and M-mode echocardiography emerged in the 1970s as safe and acceptable non-invasive techniques which can provide serial measures in pregnancy.[8, 11, 16, 46] Doppler ultrasound imaging technology has been used since 1985 to assess volumetric flow.[47, 48] Doppler echocardiography is operator-dependent, requiring training in technical skills to provide reliable and reproducible measurements.[46] Temporal variability of Doppler echocardiography is small,[49] and it is a validated modality in pregnancy[17] making it suitable for longitudinal studies.

Tissue Doppler Imaging (TDI) can detect the low velocity, high intensity echoes of myocardial deformation so can demonstrate subtle changes in myocardial performance.[5] TDI is also relatively independent of preload[50] making it a better measure of LV diastolic function in pregnancy.[3, 21] Three dimensional imaging, strain measurements and speckle tracking technology provide further information about cardiac geometry.[51]

Pulsed wave Doppler echocardiography is the most common modality used to assess LV diastolic function.[21] Blood flow from the left atrium (LA) across the mitral valve to the LV in diastole has a measurable E-wave (early filling) and A-wave (late filling), illustrated later in **Figure 4**. These indicate the pressure gradients between the two left-sided chambers in early diastole and after atrial contraction in late diastole. Increased preload increases the maximum velocity of both E- and A-waves. Increased afterload decreases the maximum velocity of the E-waves and increases the maximum velocity of the A-wave.[4] Several studies have shown that in normal pregnancy the E/A ratio decreases towards term, which corresponds to an increase in the A-wave maximum velocity.[4, 7, 17, 21, 52] Atrial contraction has been shown to be

more important to ventricular filling as pregnancy progresses,[21] which is different to the situation in healthy, non-pregnant young women where LV filling is almost completed in early diastole before the atrial 'kick'.[7] The atrial contribution to LV filling also becomes more pronounced with increasing maternal age.[10] Enhanced LV end-diastolic function in healthy pregnancy is an important adaptation to handle the increased preload.[21]

The velocity of lengthening of the left ventricular myocardial fibres can be measured in early diastole (E') and after atrial contraction in late diastole (A') by TDI. The ratio E'/A' measured at the mitral annulus has been shown to decrease in the third trimester when the atrial contraction becomes more important to maintain ventricular filling.[10, 21, 53] As a measure of diastolic function, E' has the advantage of being relatively load-independent compared with the transmitral E-wave which depends on preload.[10] The ratio E/E' reflects left atrial pressure,[54] which has shown to remain normal in healthy pregnancy.[7, 10]

1.2 Hypertensive disorders of pregnancy

1.2.1 Definition

Hypertensive disorders of pregnancy are an important cause of morbidity and mortality, impacting on both maternal and fetal wellbeing. There are two hypertensive disorders specific to pregnancy, namely gestational hypertension (GH) and preeclampsia (PET). The diagnosis and classification of hypertensive disorders in pregnancy depend on the gestation at which elevated blood pressure is identified (both occur in the second half of pregnancy), the presence or absence of significant proteinuria (traditionally the hallmark of PET), and whether the blood pressure normalises in the postnatal period (if it does not then the diagnosis

is chronic hypertension).

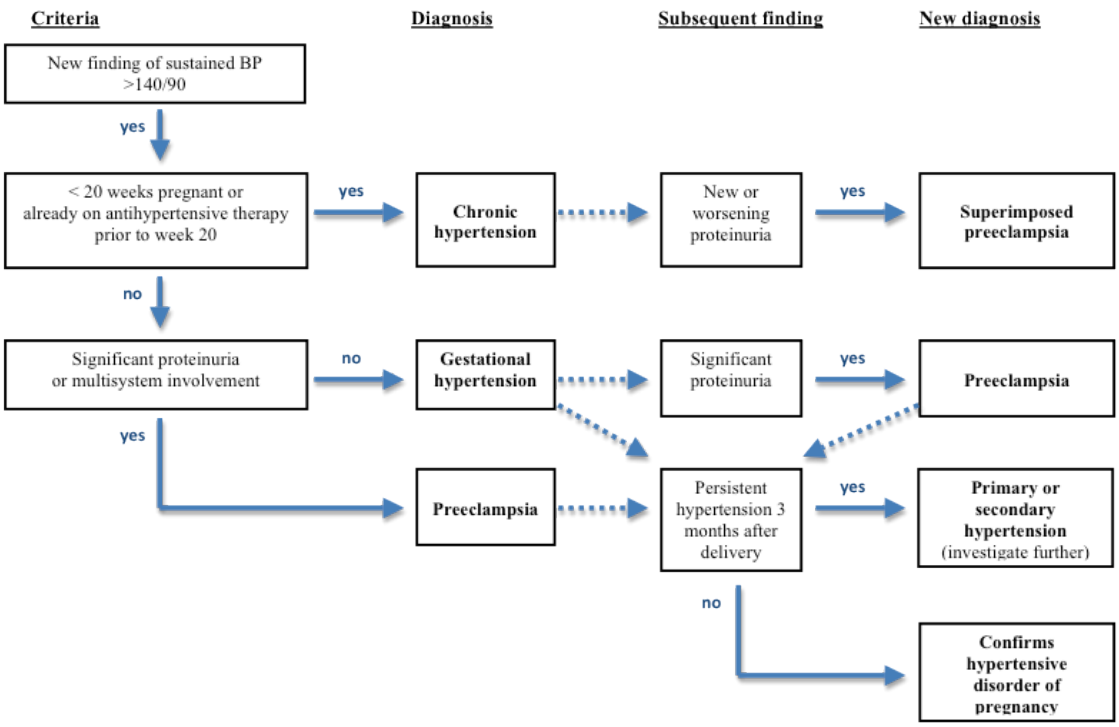
The definition of hypertension in pregnancy requires either a systolic blood pressure of at least 140mmHg or a diastolic blood pressure of at least 90mmHg, with a second confirmatory reading separated in time usually by 4 hours.[55] Blood pressure should be measured with a device validated in pregnancy.[56] Proteinuria has traditionally been the second criterion required for the diagnosis of preeclampsia. Proteinuria is defined as at least 300mg protein in a 24 hour urine collection or a urine protein/creatinine ratio of at least 30mg/mmol.[1] A urine dipstick showing "1+" proteinuria or more can be used as a guide to prompt laboratory confirmation.

In the most recent guidelines from the American Congress of Obstetricians and Gynecologists, the Society of Obstetric Medicine of Australia and New Zealand and the Society of Obstetricians and Gynaecologists of Canada there is not an absolute requirement for proteinuria, with various alternative maternal and fetal sequelae of the disease listed in the diagnostic criteria for PET.[57-60] This wider definition of preeclampsia is in accordance with the recommendations of the International Society for the Study of Hypertension in Pregnancy (ISSHP)[61, 62] and the concept of preeclampsia as a syndrome comprising hypertension and end organ dysfunction, with renal, hepatic, haematological, neurological or placental manifestations. The National Institute for Health and Clinical Excellence (NICE) have not updated their guideline since 2011, therefore proteinuria is still a mandatory criterion for a diagnosis of PET in the UK.[1]

Women with chronic hypertension may become pregnant and the condition may be

discovered for the first time in pregnancy. Secondary hypertension can present in pregnancy, meaning that the raised blood pressure is a consequence of an underlying disease, for example endocrine or renal problems. Although most definitions require the onset of hypertension to be in the second half of pregnancy in order for it to be considered pregnancy-induced, severe PET has been known to present before 20 weeks of gestation when associated with other pregnancy complications such as molar or multiple pregnancy. PET can affect women with no known previous hypertension or can be superimposed on chronic hypertension. A basic algorithm to differentiate between the hypertensive disorders of pregnancy is shown in **Figure 2**, which is based mostly on the NICE Clinical Guideline with the concept of 'superimposed' PET drawn from North American and ISSHP guidance. [1, 57, 59, 61]

Figure 2: Diagnosis of hypertensive disorders in pregnancy



A flow chart for contemporary diagnosis of the hypertensive disorders of pregnancy based on international guidelines.

1.2.2 Epidemiology

Hypertension in pregnancy is common, with around 10% of women diagnosed at some point in pregnancy.[39] The incidence of preeclampsia is 2%.[63] In a systematic review of controlled studies, the following maternal factors present at the start of pregnancy were associated with increased risk of preeclampsia: antiphospholipid antibodies; history of preeclampsia; diabetes; multiple pregnancy; family history; nulliparity; increased BMI; maternal age >40; renal disease; chronic hypertension; ≥ 10 year birth interval; increased blood pressure.[64]

There is much interest in finding a genetic basis for preeclampsia, with the plasminogen activator inhibitor-1 (PAI-1) gene polymorphism and the soluble fms-like tyrosine kinase-1 (sFlt-1) gene showing consistent associations.[65, 66]. Several genes, such as certain lipoprotein lipase alleles and SERPINE1 variants, have been linked to preeclampsia, cardiovascular disease and adverse lipid profiles, demonstrating the complex interplay between genes and environment contributing to the pathogenesis of hypertension in pregnancy. A recent 'review of reviews' highlighted the importance of metabolic syndrome, with obesity and polycystic ovarian syndrome conferring increased risk of hypertension in pregnancy, as well as increased cardiovascular risk in later life.[65] Diabetes mellitus, being overweight before pregnancy and gaining excessive weight during pregnancy and high levels of triglycerides are associated with development of preeclampsia.[66-68]

Ethnic variation is reported in various pregnancy-related complications, but it is important to bear in mind the difference between ethnicity (which has a cultural basis) and racial groups (more related to geography and genetics).[69] The relationship between ethnicity and health outcomes is complex and caution is required when interpreting any associations. Although

hypertension in pregnancy has been shown to vary depending on ethnicity and geographical region, this may reflect other influences such as lifestyle and access to healthcare.[70, 71] Nevertheless it is acknowledged that women from African ancestral groups are at increased risk of hypertension in pregnancy, with nulliparous black women in the United States twice as likely to develop preeclampsia compared to nulliparous white women.[72]

1.2.3 Pathophysiology

The traditional theory to explain the rise in blood pressure in PET is the disordered invasion of the placenta and its vasculature into the uterus.[73] There is a consequent reduction in blood flow to the fetus, which results in compensatory mechanisms to increase the blood flow to avoid fetal compromise. Late onset preeclampsia is thought to be related to endothelial dysfunction and an exaggerated systemic inflammatory response.[74] More recently the placental origin hypothesis has been challenged and contemporary research is focusing on the role of the maternal cardiovascular system in relation to placental syndromes.[75] If the maternal haemodynamic adaptation to pregnancy is impaired or inadequate, subsequent placental dysfunction may manifest as PET and/or fetal growth restriction (FGR).[76] There may be a genetic predisposition to preeclampsia since it is more common in women whose mother or sister was affected.[39] Maternal medical problems affecting the vasculature or blood clotting are associated with increased risk of preeclampsia, with the commonest conditions being diabetes, systemic lupus erythematosus and antiphospholipid syndrome.

1.2.4 The immune system and preeclampsia

The maternal immune system must adapt in pregnancy in order protect the semi-allogenic fetus from rejection.[77] This immunological tolerance is an essential feature of the

immunology of pregnancy.[78] Cells of the innate immune system have been implicated in the pathophysiology of gestational hypertensive disease.[79] Pathological inflammatory processes, both systemic and localised to the placenta, occur in PET.[80] An imbalance between placental factors and the maternal adaptation to them may result in the syndrome of PET.[81] Molecules involved in cell signaling, regulation and activation at the placental interface continue to attract scientific interest in pregnancy hypertension research.

During physiological implantation, the newly formed uterine decidua and underlying myometrium are invaded by fetal trophoblastic cells. The extravillous cytotrophoblast invades the maternal vasculature, transforming its endothelium and muscle layer leading to decreased resistance and increased placental blood flow.[78] The impaired vascular remodeling of the uterine spiral arteries in PET, associated with altered trophoblast invasion, leads to high-resistance vessels and a reduction in placental perfusion.[80, 82] The altered trophoblast invasion in PET has been attributed to an abnormal maternal immune response in early pregnancy.[78, 79] The fetal/paternal alloantigens to which the maternal immune system responds generate partner specificity, which explains the increased incidence of preeclampsia in first pregnancies, or pregnancies with a new partner, where there is no immune memory and the maternal adaptation may be defective.[81]

Sargent *et al* suggest two stages in the immunological origin of PET.[83] Firstly immune regulation by cytokines and angiogenic factors is impaired as HLA-G, a class 1 major histocompatibility complex molecule, is underexpressed and therefore decidual NK cells are not activated. Secondly a systemic inflammatory response involves leucocytes and endothelial cells. The immune response is thought to start in the placenta, as cellular debris

from syncytiotrophoblast necrosis or apoptosis, due to placental hypoxia and infarction, is released as antigen into the maternal circulation. It has been proposed that these placental microparticles activate maternal monocytes through their toll-like receptors and lead to endothelial activation.[80, 84, 85] Histology from placentas of preeclamptic women is consistent with these principles, revealing shallow trophoblast invasion, thrombosis and changes associated with inflammation and ischaemia/infarction.[80]

The progesterone and cytokines produced by the placenta alter the usual equilibrium between the two subsets of T helper (Th) cells. There should be a shift towards the Th2 immunological state in healthy pregnancy, with the diminished Th1 response creating an immune-tolerant environment.[86] In PET this shift is thought not to occur since preeclamptic women have a cytokine profile characteristic of a Th1 response.[79] Th1 cells trigger cell-mediated immunity and phagocytosis, with elevated levels of pro-inflammatory cytokines such as IL-6 and TNF-α.[78]

1.2.5 Clinical symptoms and signs

Women with mild disease may have no symptoms. In severe disease there may be clinically detectable end organ dysfunction affecting the liver, kidneys, brain and coagulation. These abnormalities lead to symptoms including headache, visual disturbance and epigastric pain. The most serious complications are rare, and include eclampsia (seizure), stroke, the syndrome of haemolysis, elevated liver enzymes and low platelets (HELLP) or disseminated intravascular coagulation (DIC). Fetal risks are associated with prematurity and growth restriction, of which the consequent morbidity may manifest throughout the life of survivors.

1.2.6 Clinical management

The only known cure for PET is to empty the womb of the placenta, thus ending the pregnancy. The timing of this definitive intervention has implications on the short and long term outcomes for the neonate. Management of PET remote from term is more challenging, as the risks to the woman must be balanced with the complications of prematurity resulting from iatrogenic preterm delivery. In severe PET presenting extremely preterm when the fetus is not viable, termination of pregnancy may be indicated. If safe, pregnancy can continue to term and with the default mode of delivery being vaginal birth unless Caesarean section is indicated for another reason. Preterm delivery is required when the maternal or fetal risks of continuing the pregnancy outweigh the benefits of prolonging the gestation for the fetus. Delivery should be expedited when there is severe hypertension which is difficult to control, worsening biochemical or haematological parameters, worsening symptoms, maternal complications such as eclampsia or placental abruption, or evidence of fetal compromise in the form of growth restriction or cardiotocograph abnormality.

Hypertension can usually be controlled with antihypertensive medication. Blood pressure control will usually be dependent on antihypertensives until after delivery. Labetalol and nifedipine are the first line oral medications. Methyldopa is also safe to use. Severe hypertension is an indication for admission to hospital and may require intravenous antihypertensives such as hydralazine. Magnesium sulphate is used for prophylaxis and treatment of eclampsia (seizures in the context of hypertension in pregnancy) and also has the benefit of neuroprotection for the early preterm fetus.[87] Antenatal corticosteroids for fetal lung maturity should be given in case of anticipated preterm delivery.[88]

Routine practice in the UK involves the prescription of low dose aspirin (acetylsalicylic acid, an antiplatelet and cyclooxygenase inhibiting drug) to women considered at high risk of developing PET based on maternal history.[1] NICE recommend 75mg aspirin daily from 12 weeks until delivery for women with one "high risk" factor or more than one "moderate risk" factor, as shown in **Table 1** below.

Table 1: Risk factors for preeclampsia

High risk	**Moderate risk**
Hypertensive disease in previous pregnancy	First pregnancy
Chronic kidney disease	Age 40 or older
Lupus erythematosus	Pregnancy interval of more than 10 years
Antiphospholipid syndrome	Body mass index of 35kg/m^2 or more at first visit
Pre-existing diabetes (Type 1 or Type 2)	Family history of preeclampsia
Chronic hypertension	Multiple pregnancy

From *Hypertension in pregnancy: The management of hypertensive disorders during pregnancy*. NICE Clinical Guideline 107. London: National Institute for Health and Clinical Excellence, 2011. Aspirin recommended if >1 factor from first "high risk" column or ≥2 factors from second "moderate risk" column.

Development of prediction models for preeclampsia is an interest of many research groups worldwide. The Fetal Medicine Foundation has proposed a screening algorithm which

combines maternal characteristics with biophysical (uterine artery pulsatility index and mean arterial pressure) and biochemical (placental growth factor and pregnancy-associated plasma protein A) measurements at 11-13 weeks gestation.[63] This model detects 76% of preterm preeclampsia at a screen positive rate of 10%. When women identified as being at ≥1:100 risk of developing preterm preeclampsia were randomised to prophylaxis with 150mg aspirin or placebo to take from the time of first trimester screening until 36 weeks gestation, the incidence of preterm preeclampsia was reduced in the treatment arm of the trial by over 80%.[89] Calcium supplementation for high risk women with low calcium intake has been recommended by the World Health Organization (WHO) and the ISSHP.[90]

1.2.7 Prognosis

PET will recur in a future pregnancy in one in six women.[1] The risk of recurrence increases according to the severity of the disease, with those who delivered before 28 weeks having more than 50% chance of developing PET again.[1] A woman affected by PET is at higher risk of future cardiovascular morbidity and mortality,[91, 92] This may be due to persistent changes in cardiac structure and function, or to irreversible injury to the cardiovascular system.[50, 93] Women with preterm PET are at significantly increased risk of LV dysfunction and essential hypertension within two years of delivery.[50] Women with PET are more likely to develop hypertension, ischaemic heart disease, stroke or venous thromboembolism later in life as these conditions share aetiological factors with pregnancy hypertension.[92] A recent systematic review and meta-analysis reported that PET confers a 4-fold increased chance of heart failure and a 2-fold increased chance of death due to cardiovascular disease, after adjusting for potential confounders.[94]

1.2.8 Unanswered questions

Current research in the field of hypertension in pregnancy is focused on prediction and prevention of PET, as well as on improving treatment once the disease is clinically apparent. More data are required to aid clinicians with the decision regarding timing of delivery. Cardiovascular imaging is emerging as an important tool in obstetric medicine and may represent a novel strategy to improve the detection of patients at risk of adverse pregnancy outcomes and to guide the management of hypertensive pregnancies.[95-97] Post-pregnancy follow up, where clinicians use learning from a woman's response to pregnancy in order to optimise future health outcomes, is likely to become a key public health strategy. Many of the "gestational" syndromes have a counterpart for which the obstetric complication reveals a risk or tendency, for example gestational diabetes and Type 2 diabetes. Understanding the underlying pathophysiology leading to the late effects of hypertension in pregnancy is an important area of research in order to discover how to improve women's cardiovascular health.

The interaction between the heart and vasculature is an important aspect of cardiovascular performance. Adaptations in the arterial system are important in healthy pregnancy. The concept of arterial stiffness is introduced in the next section.

1.3 Arterial stiffness

1.3.1 Introduction to arterial stiffness

Large arteries should be able to regulate blood flow. Their elastic property allows for a smoother passage of blood through the arterial tree compared to the initial strong pulsation of

ejected blood from the heart.[98] A healthy vessel is compliant (normal volume change per unit change in blood pressure) and distensible (normal compliance relative to initial volume). A vessel should expand and contract to adapt to pressure changes.[99] Arterial stiffness is a measure of the ability of the vessel to adapt to pressure changes appropriately. Arterial stiffness, or elastance, describes the change in pressure (ΔP, stress) relative to a change in volume (ΔV, strain).[100] Elastance is the inverse of compliance, which is ΔV/ΔP. Non-invasive assessment of arterial stiffness can be performed using Pulse Wave Analysis and/or cardiac imaging.

Altered arterial stiffness can be caused by vascular aging, endothelial damage or increased mean arterial pressure. Increased arterial stiffness means there is less capacitance in the central arterial reservoir which causes impaired cardiac performance and a reduction in end organ perfusion.[101] If the aorta and other large arteries lose their elasticity, the pulse wave reaches smaller vessels in distal organs and tissues without having been buffered and this can be harmful and may induce remodelling.[101] Arteries should be more compliant in pregnancy in order to accommodate increased intravascular volume and maintain smooth perfusion to vital organs.[22] A recent systematic review and meta-analysis has demonstrated a significant increase in arterial stiffness indices in women with preeclampsia compared women with gestational hypertension and normotensive pregnant women.[102] Arteries stiffen in hypertension due to hyperplasia and hypertrophy of vascular smooth muscle, leading to deposition of collagen.

1.3.2 Pulse Wave Analysis

Pulse wave analysis employs applanation tonometry to assess central haemodynamics and

arterial stiffness. It estimates the augmentation of systolic blood pressure by pulse wave reflection from the periphery. The principle involves studying the shape of the arterial waveform, providing more information than the two traditional systolic and diastolic, peak and trough, measurements of peripheral blood pressure.[103] The pressure waveform obtained non-invasively has been shown to be well matched to invasive measures.[101] The height of the pulse wave is determined by both the advancing wave and the retrograde flow caused by reflections from resistance in the arterial tree.[103] In a recent consensus statement from the International Working Group on Maternal Hemodynamics, the principle of wave reflections has been likened to when a stone is dropped in a small pond and the waves hitting the pond edge are reflected back and summate with advancing waves.[98] Usually the retrograde blood flow reaches the aorta only during diastole when the aortic valve is closed, so it boosts the central diastolic blood pressure. This is beneficial for coronary artery perfusion. Increased arterial wall stiffness, or sites where the arterial walls are less elastic, cause the reflected wave to return more rapidly. The returning wave therefore meets the advancing wave earlier in systole, thus amplifying the systolic peak rather than the diastolic portion of the waveform. It is this meeting of forward-moving and reflected pressure waves that makes central aortic pressure higher when arteries are less compliant, and results in increased cardiac work and less coronary perfusion in diastole. Key findings from previous studies of Pulse Wave Analysis in pregnancy are summarised in **Table 2**.

Table 2: Summary of findings of studies using pulse wave analysis in pregnancy

Findings	Reference
AIx is significantly lower throughout pregnancy compared to non-pregnant women.	Smith, 2004[104] Macedo, 2008[105] Khalil, 2009[103]
Aortic stiffness falls significantly in the second trimester and rises in the third trimester.	Macedo, 2008[105] Guerin, 2008[106] Khalil, 2009[103]
There is no difference in arterial stiffness between White European and Black African ethnic groups.	Khalil, 2009[103]
Central blood pressures and Aix are higher in hypertensive disorders of pregnancy compared to normotensive pregnant women.	Elvan-Taspinar, 2004[107] Spasojevic, 2005[108] Ronnback, 2005[109] Khalil, 2009[110]
AIx is higher in preeclampsia compared to normal pregnancy and gestational hypertension.	Khalil, 2009[110] Spasojevic, 2005[108]
AIx is significantly higher in early onset preeclampsia compared to late onset preeclampsia.	Khalil, 2009[110]
AIx is raised in preeclampsia and remains elevated postpartum even once blood pressure has normalised.	Robb, 2009[111]
Increased Aix is associated with severity of preeclampsia, reduced time to delivery and need for antihypertensives in women in the third trimester.	Fullerton, 2014[112]

1.3.3 Aortic augmentation

There are two inflection points in the aortic pulse waveform, representing the incident pressure wave generated by the heart and the reflected wave from when that wave meets resistance (see **Figure 3**). The aortic pressure is the difference between the first and second inflection points. The augmentation index (AIx) is the augmentation pressure (AP) expressed as a percentage of the central pulse pressure, which is then standardised to a heart rate of 75 beats per minute (AIx75).

Figure 3: The arterial waveform

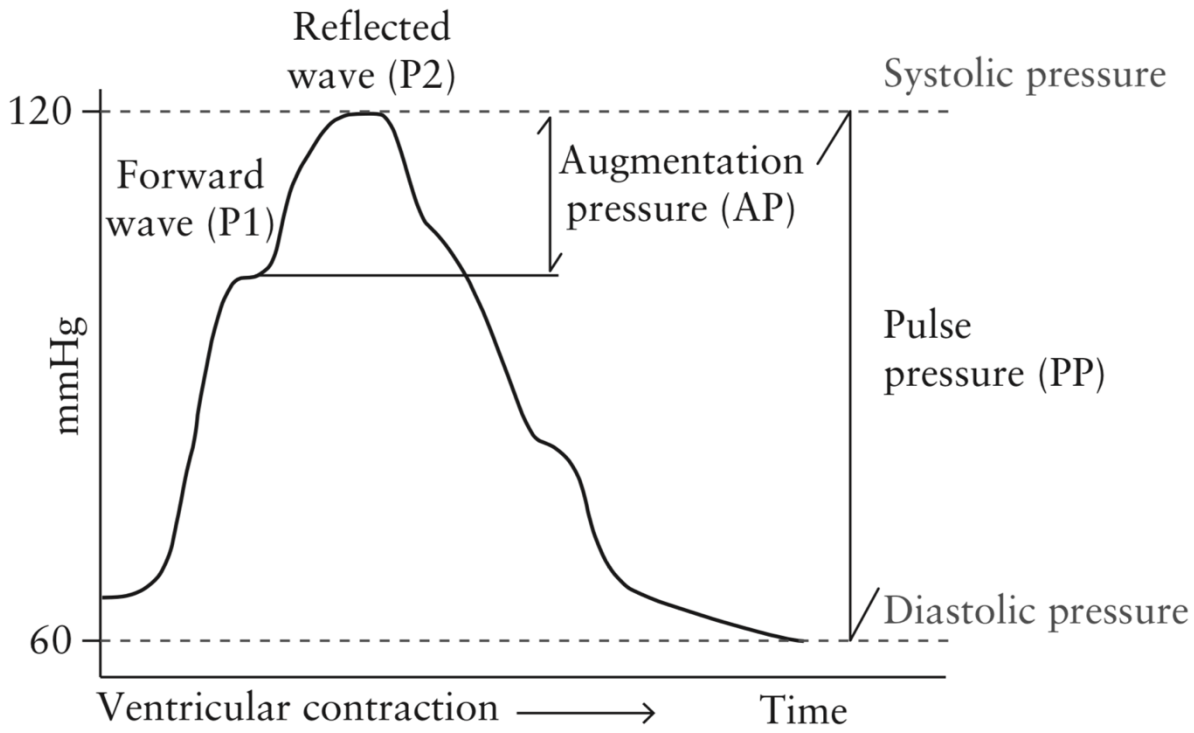


Figure reproduced under license (reference 4555290597134) from the citation Foo FL, Mceniery CM, Lees C and Khalil A. Assessment of arterial function in pregnancy: recommendations of the International Working Group on Maternal Hemodynamics. *Ultrasound in Obstetrics and Gynecology* 2017; 50; 324 – 331.

The augmentation pressure is calculated as the difference in amplitude between the forward wave (P1) and the reflected wave (P2). The augmentation index (Aix) is the ratio of this pressure difference in relation to the pulse pressure (PP).

$$\text{AIx}\ (\%) = \frac{\text{P2} - \text{P1}}{\text{PP}} \times\ 100$$

The adjustment to standardise to a heart rate of 75 beats per minute is done at an inverse rate of 4.8% for each 10 beats per minute increment in heart rate, according to the following formula, which is derived from previous published studies[113]:

$$\text{AIx75}\ (\%) = \text{Aix} + \frac{4.8\ \times\ (\text{HR} - 75)}{10}$$

The augmentation index is a marker of the interaction between the arterial tree and the left ventricle or so called ventricular-arterial coupling which reflects the interaction between the pump (heart) and the load (arterial system). The augmentation index is a more sensitive early marker of arterial stiffness than measurement of pulse wave velocity.[98] There is no significant association between AIx-75 and maternal ethnicity, parity, smoking status or body mass index.[33]

1.3.4 Aortic blood pressure

Pulse wave analysis also estimates the central systolic and pulse pressures. Central blood pressure may be more important than peripheral blood pressure as this is the pressure to which the left ventricle is exposed, for which brachial measures are only a surrogate.[101, 103]

The mathematical transfer function used to derive the aortic waveform from the radial artery waveform is not validated in pregnancy. It has been validated in a wide variety of other physiological and pathological conditions. The properties of the arterial tree between the heart and the radial artery should not change in women of childbearing age. With this in mind the transfer function can be applied in pregnancy.

1.4 Chapter 1 Summary

This opening chapter has introduced maternal cardiac function and the hypertensive disorders of pregnancy as my research themes. At the heart of this thesis is a study of the underlying pathophysiology of hypertension in pregnancy and associated cardiovascular dysfunction. I have explored the physiological adaptation of the heart to the pregnant state, concepts which are important to appreciate in order to understand what happens when this goes wrong. Echocardiography has been introduced as the key modality of interest in my investigations, with further detail to follow in the form of a systematic review in the next section. The concept of ventricular-arterial interaction has been described, and an overview of the Pulse Wave Analysis technique was given. The interface of immunology and cardiology was covered, and this will be explored in more detail in **Chapter 2** when monocytes, their subsets, and their role in women's health are the subject of a literature review.

CHAPTER 2:
LITERATURE REVIEW

2.1 Echocardiographic structure and function in hypertensive disorders of pregnancy: A systematic review

2.1.1 Introduction

Cardiac disease is the leading non-obstetric cause of death in pregnancy and the puerperium.[35] In uncomplicated pregnancy there is no significant change in systolic blood pressure.[17, 19] Diastolic blood pressure and mean arterial pressure decrease during the first trimester, then plateau in the second trimester before rising in the final weeks of pregnancy.[17, 19] Hypertension is seen in 6-8% of pregnancies[1] and the incidence is increasing as the obstetric population becomes older and more obese.[114] Hypertension causes one third of severe maternal morbidity[1] and is the second most common direct cause of maternal mortality worldwide, accounting for approximately 14% of maternal deaths.[115] The hypertensive disorders specific to pregnancy are gestational hypertension (GH) and preeclampsia (PET), as defined previously. Adverse fetal outcomes include preterm birth, growth restriction and stillbirth.[1]

Understanding the structure and function of the heart in pregnancy is vital if we are to recognise abnormalities at an early stage and plan appropriate interventions to avoid adverse outcomes. Echocardiography is a safe, non-invasive technique to assess cardiac structure and function in pregnancy.[8, 11, 16, 46] Although operator-dependent and requiring training to provide reproducible measurements[46], the temporal variability of echocardiography is small.[49] Modern ultrasound technologies can demonstrate subtle changes in cardiac geometry and performance[3, 5, 21, 51], and echocardiography has important potential for longitudinal assessment in view of its non-invasive nature. However, evidence for the role of

echocardiography for serial measurements in pregnancy is lacking, and despite common use in clinical practice, the application of echocardiography to study hypertensive disorders of pregnancy is inconsistent.

My aim was to systematically review the current literature to assess changes in echocardiographic structure and function in women developing GH and PET. I hypothesise that echocardiography would be a useful screening tool to identify: (1) high-risk women in whom cardiovascular changes occur before manifesting clinically as a hypertensive disorder; and (2) women at increased risk following a diagnosis of GH or PET.

2.1.2 Methods

Information sources and search strategy

Studies using echocardiography in pregnancies complicated by GH or PET were eligible for inclusion in the systematic review. The definitions of GH and PET used by each individual study were accepted. **Figure 2** shows a typical algorithm for the classification of hypertensive disorders of pregnancy. My search included MEDLINE, EMBASE, CINAHL and the Cochrane Library from inception to March 2015, as well as relevant reference lists. The MEDLINE search strategy is shown in **Table 3**, which was adapted for the requirements of the other databases. There was no restriction on the type of study design or publication language. Full text articles were obtained after screening the title and/or abstract of eligible studies by two authors (JSC and FT). The review process was conducted according to the

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [117], and prospectively registered with the PROSPERO database (CRD42015015700); (http://www.crd.york.ac.uk/PROSPERO/DisplayPDF.php?ID=CRD42015015700).

Table 3: Search strategy

1. PREGNANCY/ (MeSH)
2. gestation* .mp
3. pregnan* .mp
4. HYPERTENSION, PREGNANCY-INDUCED/ (MeSH)
5. PRE-ECLAMPSIA/ (MeSH)
6. HYPERTENSION/ (MeSH)
7. BLOOD PRESSURE/ (MeSH)
8. HEART/ (MeSH)
9. cardi* .mp
10. "cardiac function" .mp
11. "cardiac structure" .mp
12. "cardiac geometry" .mp
13. diastolic OR systolic .mp
14. function OR dysfunction .mp
15. 13 AND 14
16. ECHOCARDIOGRAPHY/ (MeSH)
17. CARDIAC IMAGING TECHNIQUES/ (MeSH)
18. DIAGNOSTIC IMAGING/ (MeSH)
19. ELASTICITY IMAGING TECHNIQUES/ (MeSH)
20. IMAGING, THREE-DIMENSIONAL/ (MeSH)
21. 1 OR 2 OR 3
22. 4 OR 5 OR 6 OR 7
23. 8 OR 9 OR 10 OR 11 OR 12 OR 15
24. 16 OR 17 OR 18 OR 19 OR 20
25. 21 AND 22 AND 23 AND 24

Key to syntax used:

*	after a word is used as a truncation ("wildcard") to retrieve plurals or different endings, e.g. gestation* would retrieve 'gestational' and 'gestation'
MeSH	Medical Subject Heading
/	at the end of a phrase, searches the phrase as a subject heading
.mp	mapping alias (searches title, abstract, heading words, table of contents and key phrase identifiers)

Eligibility criteria and study selection

The population of interest was pregnant women with GH or PET. My inclusion criteria required an echocardiogram during pregnancy (before and/or after the diagnosis of GH/PET). Pregnant women with normal blood pressure were included as a comparison group. The exclusion criteria were: (1) studies published only in abstract form; (2) duplicate publications or publications using the same dataset (in the latter case only the largest study including the same patients would be included, unless different data were presented in each paper); (3) case reports, editorials, opinion articles, commentaries and letters; (4) animal studies; (5) studies including only multiple pregnancies; (6) studies assessing therapeutic effects; and (7) studies of pregnant women with chronic hypertension, unless a group with GH or PET were also included.

Data collection, outcomes and quality assessment

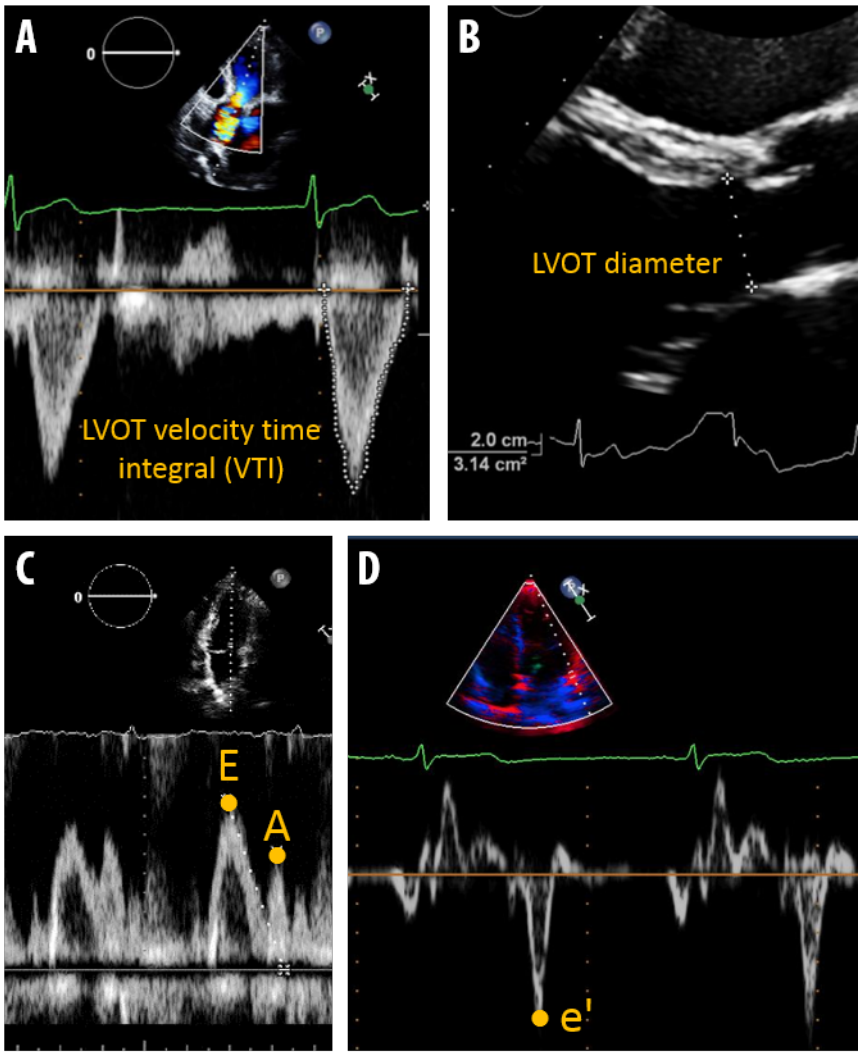
A standardised data extraction form was used. Outcome measures included any echocardiographic assessment of left-ventricular (LV) structure or function (see **Table 4** and **Figure 4**). The Risk of Bias Assessment Tool for Non-Randomised Studies (RoBANS)[118] was used to critique methodological and reporting quality of the included manuscripts, addressing key criteria such as selection bias, exposure measurement, blinding, the completeness of outcome data and selectivity of reporting. Two authors (JSC and FT) completed the data quality assessment independently, and any disagreements were resolved by consensus.

Table 4: Outcome measures using echocardiography

Parameter	Measurement
Stroke volume (SV, ml) – see Figure 2	π x (Left ventricular outflow tract diameter / 2)^2 x velocity time integral; measurements in cm
Cardiac output (CO, L/min)	Stroke volume [mL] x heart rate [beats/min]
Total vascular resistance (TVR, dyne.s/cm^5)	80 x (Mean arterial pressure* / cardiac output)
Left ventricular ejection fraction (LVEF, %)	(End diastolic volume – end systolic volume) / end diastolic volume x 100; measurements in mL
E/A ratio see Figure 2	Mitral valve E wave [early filling] peak velocity / mitral valve A wave [late filling during atrial contraction] peak velocity
E/e' – see Figure 2	Mitral valve E wave peak velocity / e' wave [early diastolic mitral annular] velocity on tissue Doppler imaging; measurements in m/s (can be derived from the septal or lateral mitral valve annulus)
LV geometry	Left ventricular wall thickness, including hypertrophy that is *eccentric* (wall thickness increased in proportion to the increase in chamber size) and *concentric* (increased wall thickness without dilatation).
Left ventricular mass (LVM, g)	0.8 x [1.04((left ventricular end diastolic dimension + posterior wall thickness + interventricular septum thickness)^3 – left ventricular end diastolic diameter^3)] + 0.6; measurements in mm, at end diastole

*Mean arterial pressure (mmHg) is calculated as [Systolic Blood Pressure + (2 x Diastolic Blood Pressure)]/3. Values for stroke volume, cardiac output and left ventricular mass are often indexed to body surface area (m^2) using the Du Bois formula [119]: 0.20247 x height(m)$^{0.725}$ x weight(kg)$^{0.425}$.

Figure 4: Echocardiographic measurements



Assessment of cardiac output (Panels A and B) and left-ventricular diastolic function (Panels C and D) using transthoracic echocardiography. LVOT, left-ventricular outflow tract. See also **Table 3**.

Data synthesis

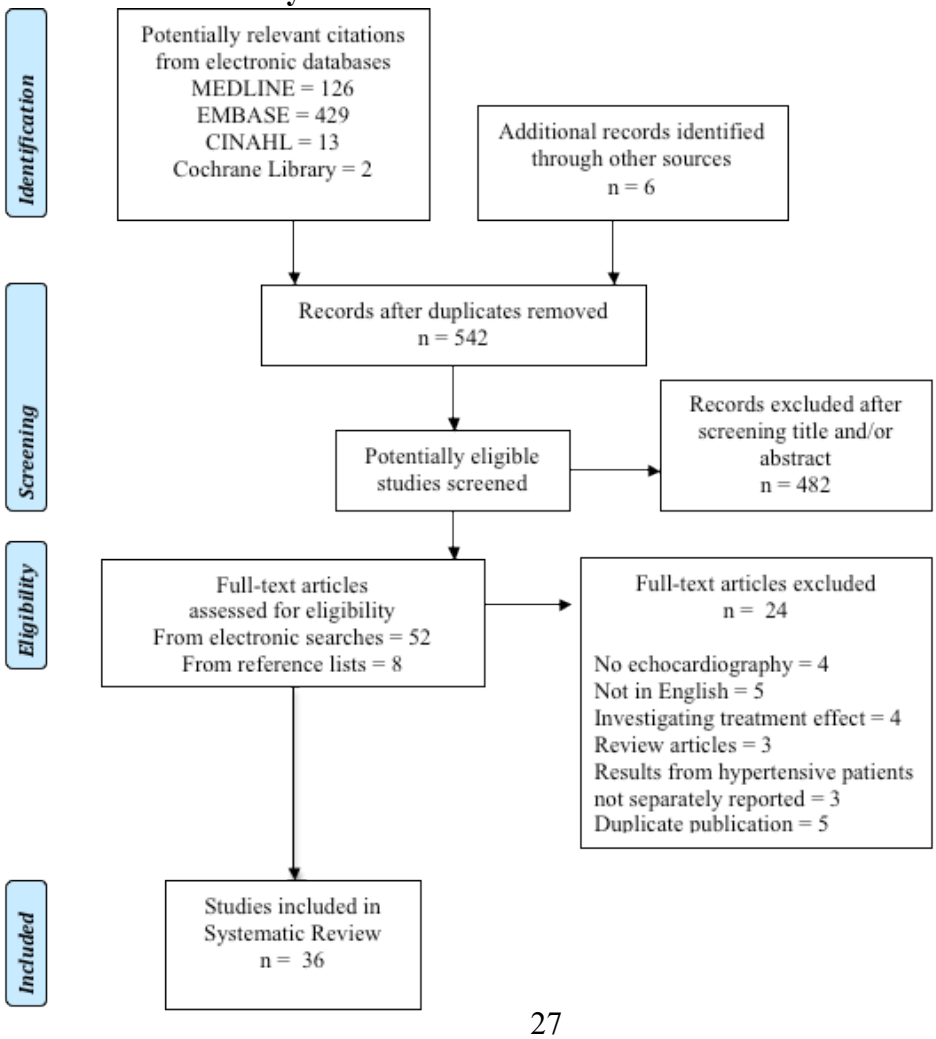
Statistical pooling of data from separate studies was not possible because of marked variation in study design and reported outcome measures, thus precluding meta-analysis. Data were therefore synthesised qualitatively.

2.1.3 Results

Study selection and study characteristics

The search strategy identified 36 studies, including 745 women with GH, 815 women with PET and 7189 normotensive pregnant controls (see **Figure 5**).

Figure 5: Flow chart of systematic review



The characteristics of included studies are shown in **Table 4**. All studies had an observational design, with 25 case-control studies,[120-144] 8 cross-sectional studies [145-152] and 3 longitudinal cohort studies.[153-155] The majority of studies (n=20) were of women with PET.[120, 125, 127, 128, 130, 131, 137-140, 142-145, 147-149, 152, 154] There were nine studies assessing GH only [122, 124, 126, 129, 135, 136, 141, 151, 155] and seven studies evaluating both GH and PET.[121, 123, 129, 132, 146, 150, 153] All investigators recruited women during antenatal visits to hospital. In three of the studies where women were scanned prior to the onset of hypertension, the women had already been identified as a high risk group due to fetal growth restriction,[145] raised uterine artery Doppler [133] or PET in a previous pregnancy.[154]

The majority of studies investigated patients with a single echocardiogram during the third trimester (n=29). Of the three longitudinal studies, one included echocardiography in each trimester[153] and the other two covered two trimesters.[154, 155] Considerable heterogeneity was seen between and within the study populations (see **Table 6**), such that meta-analysis was not deemed appropriate. In six studies, a proportion of the patients were on antihypertensive therapy.[131, 137, 141, 146, 154, 155] In two studies, the PET group included a small number of women with chronic hypertension and superimposed PET.[146, 154]

Other obstetric outcomes, for example birthweight and gestation at delivery, were recorded in 15 of the studies.[127, 130, 132, 137, 138, 143-145, 147, 149-154] Only three authors analyzed the relationship between these pregnancy outcomes and echocardiographic measurements[130, 132, 151] (see **Table 7**).

The risk of bias assessment identified variable study quality (see **Table 8**). Due to the nature

of the studies, the risk of specific biases (particularly blinding) was uncertain due to limited reporting in the individual studies.

Table 5: Characteristics of included studies

Trimester	Author, year	Population (Country)	Inclusion criteria	Exclusion criteria	Controls/comparison	Timing of echocardiography (gestational age in weeks)
Longitudinal cohort studies						
1-3	Bosio, 1999 [153]	Antenatal patients attending hospital (Ireland)	GH or PET	Parity >0; cardiac disease; essential hypertension; chronic illness; long term medication; multiple pregnancy; significant obstetric or medical complication	Nil	5 appointments: 10-14; 20-24; 28-32; 34-36; 37-40
1-2	Sep, 2011[154]	Women with PET in previous pregnancy (Netherlands)	Previous early onset PET	Multiple pregnancy; renal disease; missed > 2 appointments	Nil	Prior to pregnancy and 12, 16, 20 weeks
2-3	Vlahovic-Stipac, 2010 [155]	Antenatal patients attending hospital (Serbia)	GH	Essential hypertension; diabetes; structural heart disease	Normotensive pregnant	24±3 and 36±1
Cross sectional studies						
1	De Paco, 2008 [146]	Antenatal patients attending hospital (UK)	Live singleton pregnancy	Multiple pregnancy; missing outcome data; miscarriage; termination of pregnancy; major anomalies at birth	Normotensive pregnant women split into two groups: SGA (n=532) and uncomplicated (n=3591)	11+0 to 13+6
	Khaw, 2008 [147]	Antenatal patients attending hospital (UK)	PET	Parity >0; medications; unavailable outcomes; fetal loss; maternal disease	Nil	11-14
2	Melchiorre, 2013[149]	Uterine artery Doppler pulsatility index > 95th centile (UK)	Uterine artery pulsatility index >95th centile	Parity >0; essential hypertension; proteinuria prior to 20 weeks gestation; comorbidities; smoking; medication; fetal anomalies; persistent hypertension after 12 weeks post-partum	Women with normal uterine artery pulsatility index and women with raised pulsatility index (term delivery)	20-23
2	Valensise, 2008 [152]	Normotensive pregnant women with bilateral notching of umbilical artery at 20-22 weeks (Italy)	Bilateral umbilical artery notching	Multiple pregnancy; undetermined gestational age; smoking; multiple pregnancy; cardiac disease; pre-existing medical problem; fetal anomalies; persistent hypertension at 1 year follow up	Normotensive pregnant	24

Trimester	Author, year	Population (Country)	Inclusion criteria	Exclusion criteria	Controls/comparison	Timing of echocardiography (gestational age in weeks)
3	Bamfo, 2008 [145]	Pregnant women with fetal growth restriction (UK)	Diagnosis of fetal growth restriction	GH; multiple pregnancy; co-morbidities; medication; fetal anomalies; chromosomal abnormalities; genetic syndromes; infections	Normotensive with fetal growth restriction	28 (24-35)
	Estensen, 2013 [148]	Antenatal patients attending hospital (Norway)	PET	Essential hypertension; diabetes; renal impairment; hyperlipidemia; uncontrolled endocrine or rheumatological disease; cardiovascular disease	Non-pregnant with previous PET	36
	Shahul, 2012 [150]	Antenatal patients attending hospital (USA)	GH or PET	Multiple pregnancy; age < 18 years; gestation < 24 weeks pre-existing cardiovascular disease; pulmonary disease; diabetes; poor image quality; preterm prelabor rupture of membranes	Nil	NTP 38 (35.6-39.6); GH 36.4 (33.4-38.1); PET 36.6 (32.7-37.4)
	Valensise, 2006 [151]	Antenatal patients attending hospital (Italy)	Mild GH	Systolic blood pressure >150; diastolic blood pressure >100; proteinuria; essential hypertension; hemolysis, elevated liver enzymes and low platelets ('HELLP'); antihypertensive therapy; small for gestational age fetus; abnormal fetal Doppler; abnormal liquor volume; undetermined gestational age; smoking; multiple pregnancy; maternal heart disease; maternal chronic medical problems; fetal anomaly	Normotensive pregnant	28-31
Case control studies						
3	Borghi, 2000 [120]	Antenatal patients attending hospital (Italy)	PET	Essential hypertension; secondary hypertension; obesity; diabetes; cardiomyopathy; valvular heart disease; major electrocardiogram abnormality	Normotensive pregnant and non-pregnant	NTP 30.9±4.0; PET 28.4±6.0
	Borghi, 2011 [121]	Antenatal patients attending hospital (Italy)	GH or PET	Possible double or overlapping diagnosis	Chronic hypertension, normotensive pregnant	NTP 30.5±5; GH 31.2±4; PET 30.0±5
	Cho, 2011[122]	Antenatal patients attending hospital (South Korea)	GH	Diabetes; essential hypertension; cardiac disease	Normotensive pregnant	NTP 35.1±3.4; GH 33.3±3.6
	Degani, 1989 [123]	Antenatal patients attending hospital (Israel)	GH or PET	Multiple pregnancy; previous hypertension; previous heart disease; antihypertensive therapy	Normotensive pregnant	third trimester

Trimester	Author, year	Population (Country)	Inclusion criteria	Exclusion criteria	Controls/comparison	Timing of echocardiography (gestational age in weeks)
	Demir, 2003 [124]	Antenatal patients attending hospital (Turkey)	GH	Essential hypertension	Normotensive pregnant	38
	Dennis, 2012 [125]	Antenatal patients attending hospital (Australia)	PET	In labour; smoking; vasoactive medication; critically ill requiring urgent antihypertensive or magnesium sulfate	Normotensive pregnant and non-pregnant	36±4
	Escudero, 1988 [126]	Antenatal patients attending hospital (Argentina)	GH	Parity >0; age under 16; history of heart disease; multiple pregnancy	Non-pregnant	26-42
	Hamad, 2009 [127]	Antenatal patients attending hospital (Sweden)	PET	Parity >0; smoking; assisted conception; multiple pregnancy; clinically unstable; antihypertensive therapy; chronic disease; extreme obesity	Normotensive pregnant	NTP 33±4; PET 35±4
	Ingec, 2005 [128]	Antenatal patients attending hospital (Turkey)	PET	Not stated	Normotensive pregnant	NTP 38±1; PET 37±3
	Kuzniar, 1982 [130]	Antenatal patients attending hospital (Poland)	PET	Multiple pregnancy; uncomplicated pregnancy; cardiorespiratory disease	Normotensive pregnant and pregnant with essential hypertension	30-40
	Kuzniar, 1992 [129]	Antenatal patients attending hospital (Poland)	Mild GH	Previous hypertension; renal disease; persistent hypertension 3 months post-partum; hypertension prior to 3rd trimester; SBP > 160; DBP >110	Normotensive pregnant	32-41
	Lang, 1991 [131]	Antenatal patients attending hospital (USA)	PET	Parity >0; regional wall motion abnormalities	Normotensive pregnant	"early labour" "late third trimester"
	Melchiorre, 2011[132]	Antenatal patients attending hospital (UK)	GH or PET	Multiple pregnancy; co-morbidities; smoking; antihypertensive therapy	Normotensive pregnant	37 (37.5 – 39)
	Melchiorre, 2012[133]	Antenatal patients attending hospital (UK)	PET	Multiple pregnancy; comorbidity; smoking; medication;	Normotensive pregnant (50 term; 54 preterm)	preterm NTP 32 (28.6 – 35.7); preterm PET 35.5 (28.1-35.8)
	Novelli, 2003 [134]	Antenatal patients attending hospital (Italy)	GH	Multiple pregnancy; medications other than vitamins/iron; indeterminate gestational age; smoking; cardiac disease; antihypertensive therapy; pre-existing medical problem	Normotensive pregnant and non-pregnant with essential hypertension	31(3) weeks

Trimester	Author, year	Population (Country)	Inclusion criteria	Exclusion criteria	Controls/comparison	Timing of echocardiography (gestational age in weeks)
	Oren, 1996 [135]	Antenatal patients attending hospital (Israel)	GH	Essential hypertension; diabetes; renal disease; molar pregnancy; hydrops	Normotensive pregnant and patients with gestational diabetes mellitus	NTP 32±3.3; GH 32±2.4
	Sanchez, 1986 [136]	Antenatal patients attending hospital (Argentina)	GH	Complicated pregnancy; cardiorespiratory disease	Normotensive pregnant; non-pregnant; pregnant with essential hypertension	32
	Simmons, 2002 [137]	Antenatal patients attending hospital (Australia)	PET	Medical co-morbidities; essential hypertension; diabetes; multiple pregnancy; vasoactive medication	Normotensive pregnant and non-pregnant	NTP 12±2, 22±1, 35±5; PET 35±4
	Solanki, 2011 [138]	Antenatal patients attending hospital (India)	PET	Multiple pregnancy; unsure of dates; essential hypertension; cardiac disease; moderate or severe anemia; multiple pregnancy; alcohol use; smoking	Normotensive pregnant	> 34 weeks
	Thompson, 1986[139]	Antenatal patients attending hospital (USA)	PET	Essential hypertension; medication	Normotensive pregnant	32-38
	Tyldum, 2010 [140]	Antenatal patients attending hospital (Norway)	PET	Diabetes; essential hypertension; cardiac disease; multiple pregnancy	Normotensive pregnant	27-40 (mean 35)
	Veille, 1984 [141]	Antenatal patients attending hospital (USA)	GH	Essential hypertension; antihypertensive therapy other than magnesium sulfate or diuretics; multiple pregnancy	Normotensive pregnant	38±2
	Yuan, 2006 [142]	Antenatal patients attending hospital (China)	PET	Essential hypertension; renal disease; cardiac disease; diabetes	Normotensive pregnant	mean 39
	Yuan, 2014 [143]	Antenatal patients attending hospital (China)	PET	Parity >0; multiple pregnancy; GH; essential hypertension; risk factors for arterial stiffening (smoking; obstructive sleep apnea; in vitro fertilization; diabetes; hypercholesterolemia)	Normotensive pregnant	35.6±3.4
	Zieleskiewicz, 2014 [144]	Antenatal patients attending hospital (France)	PET	Age under 18; post-partum PET	Normotensive pregnant	NTP 37 (36-39); PET 34 (31-35)

Data are presented as means ± standard deviation or medians (interquartile range).
GH, gestational hypertension; NTP, normotensive pregnant control; PET, preeclampsia

Table 6: Characteristics of patients in included studies

Author, year	Number of women	Number of cases			Age			Parity		
		NTP	GH	PET	NTP	GH	PET	NTP	GH	PET
Bamfo, 2008 [145]	36	19	0	17	26±6	n/a	29±7	38% P0; 21% P1; 5% P2	n/a	94% PO; 6% P2
Borghi, 2000 [120]	85	35	0	40	31±3	n/a	31±5	2±7	n/a	2±1
Borghi, 2011 [121]	112	39	24	33	31±4	29±5	32±5	2±1	2±1	2±1
Bosio, 1999 [153]	378	334	24	20	24 (95% CI 24, 25)	28 (95% CI 26, 30)	24 (95% CI 23, 26)	100% P0	100% P0	100% P0
Cho, 2011 [122]	199	93	106	0	30±4	32±4	n/a	not reported		
De Paco, 2008 [146]	4617	4123	87	83	32 (range 15-47)	32 (range 17-46)	32 (range 18-49)	48% P0	56% P0	64% P0
Degani, 1989 [123]	32	14	18	0*	27±6	25±5	n/a	100% P0	100% P0	n/a
Demir, 2003 [124]	92	56	36	0	26±6	29±9	n/a	not reported		
Dennis, 2012 [125]	100	40	0	40 (6 early; 34 late)	32±4	n/a	31±5	25% P0	n/a	65% P0
Escudero, 1988 [126]	29	10	9	0	27 (SD not given)	24 (SD not given)	n/a	100% P0	100% P0	n/a
Estensen, 2013 [148]	145	65	0	40	32±5	n/a	32±6	58% P0	n/a	67% P0
Hamad, 2009 [127]	65	30	0	35 (8 early; 27 late)	31±4	n/a	31±5	100% P0	n/a	100% P0
Ingec, 2005 [128]	37	17	0	20	29±6	n/a	32±7	not reported		
Khaw, 2008 [147]	534	457	0	27	30 (25 - 33)	n/a	without SGA 31 (22 -33); with SGA 31 (24 – 35)	100% P0	n/a	100% P0
Kuzniar, 1982 [130]	47	19	0	19	26 (range 17 – 31)	n/a	27 (range 15 – 32)	100% P0	n/a	100% P0
Kuzniar, 1992 [129]	72	27	22	23	24±4	24±4	22.5±4.1	100% P0	100% P0	100% P0
Lang, 1991 [131]	20	10	0	10	22±5	n/a	20±4			
Melchiorre, 2011 [132]	120	50	20	50	32 (26-36)	n/a	32.0 (29-37)	100% P0	n/a	100% P0
Melchiorre, 2012 [133]	181	104 (50 term; 54 preterm)	0	77 (27 preterm; 50 term)	32 (28-36)	n/a	30 (27-35)	59% P0	n/a	67% P0

Author, year	Number of women	Number of cases			Age			Parity		
		NTP	GH	PET	NTP	GH	PET	NTP	GH	PET
Melchiorre, 2013 [149]	214	168	0	46 (18 preterm; 28 term)	low risk 32 (26-34); high risk 32 (26-35)	n/a	term 32 (30-37); preterm 30 (24-34)	100% P0	n/a	100% P0
Novelli, 2003 [134]	114	38	36	0	32±6	31±6	n/a	not reported		
Oren, 1996 [135]	30	10	10	0	23±2	23±3	n/a	not reported		
Sanchez, 1986 [136]	69	22	16	0	23 (range 21-24)	26 (range 16-36)	n/a	100% P0	100% P0	n/a
Sep, 2011 [154]	34	24	0	10	33±5	n/a	30±5	100% parous	n/a	100% parous
Shahul, 2012 [150]	39	17	11†	11 (3 severe; 8 mild)	29 (25-33)	35.5 (28-39)	32 (26-34)	0 (0-0)	0 (0-1)	0 (0-2)
Simmons, 2002 [137]	71	44	0	15	29±5	n/a	32±6	not reported		
Solanki, 2011 [138]	40	20	0	20 (12 mild; 8 severe)	25±2	n/a	26±4	not reported		
Thompson, 1986 [139]	35	11	0	10	24 (range 19-29)	n/a	24 (range 16-34)	mean 1 (range 0-5)	mean 0 (range 0-1)	n/a
Tyldum, 2010 [140]	40	20	0	20	27±4	n/a	29±5	65% P0	n/a	65% P0
Valensise, 2006 [151]	309	41	268	17 (in comp. group)	32±3	uncomp. 32±4; comp. 33±4	n/a	27% P0	Uncomp. 29% P0; comp. 44% P0	n/a
Valensise, 2008 [152]	1226	1119	0	107 (75 early; 32 late)	32±5	n/a	early 34±4; late 32±4	100% P0	n/a	100% P0
Veille, 1984 [141]	40	17	23	0*	29±4	25±5	n/a	21% P0	96% P0	n/a
Vlahovic-Stipac, 2010 [155]	47	12	35	0	30±4	30±6	n/a	not reported		
Yuan, 2006 [142]	56	24	0	32	27±3.1	n/a	27±3	not reported		
Yuan, 2014 [143]	63	40	0	23	27±3	n/a	29±6	100% P0	n/a	100% P0
Zieleskiewicz, 2014 [144]	40	20	0	20	30 (26-34)	n/a	31 (26-38)	35% P0	n/a	45% P0

Data are presented as means ± standard deviation or medians (interquartile range).
* Definition of GH could include patients with PET; † GH group includes patients with essential hypertension.
Comp., complicated; uncomp., uncomplicated; GH, gestational hypertension; n/a, not applicable; NTP, normotensive pregnant control; P1, parity 1 etc.; PET, preeclampsia; SGA, small for gestational age fetus.

Table 7: Obstetric outcomes

Author, year	Obstetric outcomes
Bamfo, 2008[145]	Earlier delivery and lower birthweight in PET.
Bosio, 1999[153]	Perinatal mortality in GH = 2/24 (8.3%). Perinatal mortality in PET = 6/20 (30%) Earlier delivery and lower birthweight in PET.
Hamad, 2009[127]	Earlier delivery and lower birthweight in PET.
Kuzniar, 1982[130]*	Positive correlation between cardiac index and fetal birthweight. Inverse relationship between infant birthweight and TVR.
Khaw, 2008 [147]	Lower birthweight in PET.
Melchiorre, 2011[132]*	In the PET group there were the following outcomes: Hemolysis, elevated liver enzymes and low platelets (HELLP syndrome) = 3/50 (6%) Acute kidney injury = 1/50 (2%) Pulmonary edema = 1/50 (2%) Fetal growth restriction = 11/50 (22%)
Melchiorre, 2013[149]	No significant difference in birthweight or gestational age at delivery between groups.
Sep, 2011[154]	Earlier delivery and lower birthweight in PET.
Shahul, 2012[150]	Earlier delivery in PET. No significant difference in birthweight in PET. More women with PET had a history of GH in a previous pregnancy.
Simmons, 2002[137]	Earlier delivery and lower birthweight in PET.
Solanki, 2011[138]	Earlier delivery and lower birthweight in PET.
Valensise, 2006[151]*	In the GH group there were the following outcomes: Perinatal death = 2/268 (0.7%) PET = 17/268 (6.3%) Placental abruption = 3/268 (1.1%) Hemolysis, elevated liver enzymes and low platelets (HELLP syndrome) = 6/268 (2.2%) Fetal growth restriction = 19/268 (7.1%) Neonatal unit admission = 5/268 (1.9%)
Valensise, 2008[152]	Lower birthweight in early PET (<34 weeks). Earlier delivery in early and late PET.
Yuan, 2014[143]	Lower birthweight in PET.
Zieleskiewicz, 2014[144]	In the PET group there were the following outcomes: Perinatal mortality = 3/20 (15%) Hemorrhage = 2/20 (10%) Eclampsia = 2/20 (10%) Pulmonary edema = 4/20 (20%) Placental abruption = 2/20 (10%) Fetal growth restriction = 5/20 (25%) Preterm delivery before 34 weeks = 6/20 (30%)

* analysis includes obstetric outcomes in relation to cardiovascular parameters.
GH, gestational hypertension; PET, preeclampsia; TVR, total vascular resistance.

Table 8: Risk of bias assessment

Author, year	Selection of participants	Confounding variables	Intervention (exposure) measurement	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting
Bamfo, 2008[145]	Low risk	Low risk	Low risk	High risk	Low risk	Unclear risk
Borghi, 2000[120]	Low risk	High risk	Low risk	Low risk	High risk	Unclear risk
Borghi, 2011[121]	Low risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Bosio, 1999[153]	Low risk	High risk	Low risk	Unclear risk	High risk	Unclear risk
Cho, 2011[122]	High risk	High risk	Low risk	Low risk	Low risk	Unclear risk
De Paco, 2008[146]	Low risk	Low risk	Low risk	Unclear risk	High risk	Unclear risk
Degani, 1989[123]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Demir, 2003[124]	Unclear risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Dennis, 2012[125]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Unclear risk
Escudero, 1988[126]	Unclear risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Estensen, 2013[148]	High risk	High risk	Low risk	Unclear risk	High risk	Unclear risk
Hamad, 2009[127]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Ingec, 2005[128]	Unclear risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Khaw, 2008[147]	Low risk	Low risk	Low risk	Unclear risk	High risk	Unclear risk
Kuzniar, 1982[130]	High risk	High risk	Unclear risk	Unclear risk	High risk	Unclear risk
Kuzniar, 1992[129]	Low risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Lang, 1991[131]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Melchiorre, 2011[132]	Low risk	Low risk	Low risk	High risk	Low risk	Unclear risk
Melchiorre, 2012[133]	Low risk	Low risk	Low risk	High risk	Low risk	Unclear risk
Melchiorre, 2013[149]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Unclear risk
Novelli, 2003[134]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Oren, 1996[135]	Unclear risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Sanchez, 1986[136]	Unclear risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Sep, 2011[154]	Low risk	High risk	Unclear risk	Low risk	High risk	Unclear risk
Shahul, 2012[150]	Low risk	Low risk	Low risk	Low risk	Low risk	Unclear risk
Simmons, 2002[137]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Solanki, 2011[138]	Unclear risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Thompson, 1986[139]	Unclear risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Tyldum, 2010[140]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Valensise, 2006[151]	Low risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Valensise, 2008[152]	Low risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Veille, 1984[141]	Unclear risk	High risk	Low risk	Unclear risk	High risk	Unclear risk
Vlahovic-Stipac, 2010[155]	Low risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Yuan, 2006[142]	Unclear risk	High risk	Low risk	Unclear risk	Low risk	Unclear risk
Yuan, 2014[143]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Unclear risk
Zieleskiewicz, 2014[144]	Low risk	High risk	Low risk	High risk	Low risk	Unclear risk

Synthesis of results

Results are summarised below according to hemodynamic parameters and systolic function, diastolic function, and cardiac structure. The details of extracted parameters from all studies, including the earlier screening studies, are presented in **Table 9**. **Table 10a** presents an overview of findings for the main echocardiographic variables investigated in the third trimester studies. **Figure 6** highlights the major echocardiographic changes that may be seen in hypertensive disorders as compared to normal pregnancy. **Figure 7** provides representative images from echocardiograms of women with and without gestational hypertensive disease.

Haemodynamics and systolic function

Total vascular resistance was significantly higher in GH compared with normotensive pregnant controls [123, 134, 135, 141, 153] but lower than in PET.[129, 153] In GH, there were conflicting reports for cardiac output, including an increase[121, 146, 153, 155] or no change compared to normal pregnancy.[123, 134, 135] Myocardial performance index and LV function were unchanged in a longitudinal study of GH with second and third trimester measurements.[155] In GH studies with a third trimester echocardiogram, only one showed a significant reduction in LV ejection fraction [135], whilst three others showed no difference.[122, 135, 155]

In PET, studies covering early trimesters demonstrated that the preclinical phase is characterised by a hyperdynamic circulation with high cardiac output and low peripheral resistance.[146, 147, 149, 153] In the second trimester, women who go on to develop PET have increased total vascular resistance at mid-gestation, with lower cardiac output.[149, 152] Once PET manifests clinically, there is reduced cardiac output and increased resistance,[148, 153] described as a “hemodynamic crossover” in the clinical phase of PET.[153] The increased total

vascular resistance seen in PET[121, 125, 129, 138, 145, 148] is an independent predictor of adverse maternal and fetal outcomes.[151] In the clinical phase, early onset PET (<34 weeks gestation) is characterised by significantly lower cardiac output and higher total vascular resistance compared with late onset PET.[149, 152] Pregnant women who develop recurrent PET have also been shown to have lower cardiac output and higher peripheral resistance than women without recurrent disease.[154]

Stroke volume is lower in PET than in normal pregnancy [129, 130] and in the first trimester this is an independent predictor of subsequent development of PET.[147] Due to the factors discussed, cardiac output in PET has been shown to be both lower [121, 125, 130, 137, 145, 148] and higher [125, 129, 138, 146, 147] compared to normotensive pregnancies. The variation in cardiac output is shown in **Table 9**, which also indicates its derivation, since the use of different calculations (based either on Doppler or volume calculation) is likely to contribute to the disparity for this parameter. There was similar variability with regard to LV ejection fraction, with the majority of studies showing no significant difference compared with normotensive pregnant women[125, 139, 143, 144, 148] and only one showing a decrease.[120] Myocardial performance index was reduced in a study of women with PET and fetal growth restriction in the third trimester.[145] Systolic dysfunction, with marked LV hypertrophy, is significantly more common in preterm PET compared to term PET [133], even before the condition manifests clinically.[149]

Table 9: Summary of extracted numerical data for key parameters

Trimester	Author, year	Results: systolic function	Results: diastolic function	Results: Cardiac structure
Longitudinal cohort studies				
1 -3	Bosio, 1999 [153]	[F] Presented as Box and Whisker plot and then numerically as relative risk ratios		
1 -2	Sep, 2011 [154]	[V] Only pre-pregnancy data given numerically		
2 -3	Vlahovic-Stipac, 2010 [155]	***At baseline (24±3 weeks):*** **CO [V]:** NTP 3.5 (0.8); GH 4.6 (1.3) **TVR:** NTP 2046 (464); GH 1933 (537) **LVEF:** NTP 64 (5); GH 61 (7) ***At follow up (36±1 weeks):*** **CO:** NTP 3.7 (0.7); GH 4.6 (1.1) **TVR:** NTP 1998 (486); GH 1821 (454) **LVEF:** NTP 60 (8); GH 58 (6)	***At baseline (24±3 weeks):*** **E/A:** NTP 1.9 (0.9); GH 1.3 (0.5)* **E/e':** NTP 6.2 (1.1); GH 7.6 (1.9)*	***At baseline (24±3 weeks):*** **LVMI:** NTP 110 (23) GH 127 (33)
Cross sectional studies				
1	De Paco, 2008 [146]	**Mean log MoM CO [F]:** NTP 0.000 (95% CI -0.0025 to 0.0025); PET 0.0261 (95% CI 0.0065 to 0.0457)*; GH 0.0257 (95% CI 0.0079 to 0.0435)*		
	Khaw, 2008 [147]	**CO [F]:** NTP 4.9 (4.3-5.5); PET not SGA 6.2 (5.4-7.1)*; PET with SGA 4.9 (4.1-5.6)* **CI:** NTP 2.9 (2.6-3.3): PET not SGA 3.3 (3.0-4.0)*; PET with SGA 2.8 (2.4-3.0)* **TVR:** NTP 1260 (1110-1460); PET not SGA 1105 (920-1280); PET with SGA 1410 (1300-1530) **SV:** NTP 67 (11.7); PET not SGA 87.9 (15.2); PET with SGA 66.3 (10.9)		
2	Melchiorre, 2013 [149]	**CI [V]:** term PET 3.2 (2.8-3.5); preterm PET 2.6 (2.3-2.8) **TVRI:** term PET 2138 (1995-2469); preterm PET (3090 (2351-3376) **LVEF:** term PET 60 (51-69); preterm PET 64 (48-67);	**E/A ratio:** term PET 1.6 (1.3-2.2); preterm PET 1.4 (0.9-1.6) **Average E/e':** term 6.2 (5.3-6.7); preterm 6.5 (5.3-7)	**LVMI:** term PET 65 (57-71); preterm PET 62 (57-71)

2	Valensise, 2008 [152]	**CO [F]:** NTP 6.61 (1.1); early PET 4.49 (1.09)*; late PET 8.96 (1.83)* **TVR:** NTP 990 (179); early PET 1605 (248)*; late PET 739 (244)* **SV:** NTP 83 (11): early PET 61 (13)*; late PET 102 (19)* Significant difference between early and late PET groups and controls, and between early and late PET.	**E/A:** NTP 1.17 (0.23); early PET 1.68 (0.5)*; late PET 1.09 (0.15)*	**LVMI:** NTP 33 (6); early PET 35 (7)*; late PET 43 (12)* Significant difference between early and late PET groups and controls, and between early and late PET.
3	Bamfo, 2008 [145]	**CO [F]:** NTP 4.79 (0.52); PET 5.52 (1.21)* **CI:** NTP 2.94 (0.30); PET 3.12 (0.71) **TVR:** NTP 1434.05 (255.93); PET 1573.51 (268.87) **SV :** NTP 61.06 (8.80); PET 75.10 (15.88)*	**E/A ratio:** NTP 1.39 (0.23); PET 1.33 (0.42) **E/e' septal:** NTP 6.13 (1.04); PET 8.70 (2.31)* **E/e' lateral:** NTP 4.61 (1.12); PET 6.27 (2.15)*	
	Estensen, 2013 [148]	**CO [F]:** NTP 5.8 (1.1); PET 6.3 (1.2)* **SV:** NTP 75 (13); PET 76 (16)* **LVEF:** NTP 54 (7); PET 56 (6)		**LVMI:** NTP 80 (17); PET 92 (25)*
	Shahul, 2012 [150]	**LVEF [F]:** NTP median 65.0 (64.6-66.5); GH 66.7 (65.4-67.5); PET 67.5 (64.2-70.0)		
	Valensise, 2006 [151]	**CO [F]:** NTP 6.75 (0.96); complicated PET 5 (1); uncomplicated PET 7.4 (1.2)* **TVR:** NTP 949 (150); complicated PET 1754 (425); uncomplicated PET 1138 (183)* **SV:** NTP 77 (10); complicated PET 62 (11); uncomplicated PET 82 (11)*		**LVM:** NTP 148 (20); complicated PET 169 (32); uncomplicated PET 176 (25)* **LVMI:** NTP 40 (6); complicated PET 45 (9); uncomplicated PET 47 (8)*
Case control studies				
3	Borghi, 2000 [120]	**CO [F]:** NTP 7.9 (1.0); PET 6.5 (1.0)* **TVR**: NTP 779 (203); PET 1320 (278) **LVEF**: NTP 67.7 (5.0); PET 65.9 (6.0).	**E/A ratio:** NTP 1.59 (0.3); PET 1.29 (0.3)*	**LVMI:** NTP 101.1 (16.0); PET 110.3 (19.0)*
	Borghi, 2011 [121]	**CO [F]:** NTP 6.6 (2); GH 8.1 (2); PET 5.6 (2) **TVR:** NTP 939 (192); GH 1006 (253); PET 1517 (295)		**LVMI:** NTP 101.1 (16); GH 109.5(21); PET 111.9 (18)
	Cho, 2011 [122]	**LVEF:** NTP 60.7 (7.8) GH 62.3 (9)	**E/A:** NTP 1.27 (0.22); GH 1.00 (0.29)*	**LVMI:** NTP 86.1 (14.5); GH 95.6 (17.3)*
	Degani, 1989 [123]	**CO:** NTP 5.4 (1.1); GH 5.6 (2.3) **CI:** NTP 3.52 (0.7); GH 3.55 (0.7) **TVR**: NTP 1340.1 (211.0); GH 1636.6 (521.3)* **SV:** NTP 68.4 (13); GH 70.5 (22.2) **LVEF:** NTP 72.0 (0.2); GH 75.0 (7.0)		

Demir, 2003 [124]	**LVEF:** NTP 37 (6); GH 72 (3)		**LVMI:** NTP 117 (15); GH 138 (13.8)* 61% of GH patients had abnormal LV geometry compared with 21% NTP*
Dennis, 2012 [125]	**CO ^{F}:** NTP 4.109 (0.595); PET 4.789 (1.419)* **TVR:** NTP 1613 (315); PET 2016 (625)*	**E/A:** NTP 1.45 (0.24); PET 1.29 (0.34) **E/e':** NTP 6.7 (1.3); PET 10.4 (2.4)*	**LVM:** NTP 131 (21); PET 189 (40)*
Escudero, 1988 [126]			**LVM:** non-pregnant 161 (29.6); GH 185 (53.1)*
Hamad, 2009 [127]		**E/A:** NTP 1.54 (0.07); PET 1.29 (0.07)* **E/e' septal;** NTP 7.49 (0.40); PET 10.92 (0.38)* **E/e' lateral:** NTP 5.72 (0.20); PET 8.23 (0.43)*	**LVM:** NTP 180 (8); PET 239 (8)*
Ingec, 2005 [128]			**LVMI**: NTP 82.8 (26); PET 127.6 (42)*
Kuzniar, 1982 [130]	**CO ^{V}:** NTP 7.5 (1.3); PET 5.9 (1.0)* **CI:** NTP 4.3 (0.7); PET 3.3 (0.5)* **TVR:** NTP 877 (197); PET 1727 (408)* **SV:** NTP 101.3 (14); PET 89 (15.9)*		
Kuzniar, 1992 [129]	**CI ^{V}:** NTP 4.23 (0.65); GH 4.72 (0.67); PET 3.62 (0.62)* **TVRI:** NTP 1670 (312); GH 1881 (396); PET 2555 (365)* **SVI:** NTP 57.1 (6.4); GH 58.9 (6.3); PET 51.2 (5.6)*		
Lang, 1991 [131]	**CO ^{F}:** NTP 6.18 (1.91); PET 5.52 (1.55) **TVR:** NTP 1203 (466); PET 1786 (496) **SV:** NTP 72 (17); PET 65 (10)		**LVMI:** NTP 105 (13); PET 108 (19)
Melchiorre, 2011 [132]	**CI ^{V}:** NTP 3.2 (2.2-3.9); GH 2.8 (2.4-3.2); PET 2.9 (2.1-3.8) **TVRI:** NTP 645 (570-840); GH 951 (756-1086); PET 716 (574-1036)	**E/A:** NTP 1.14 (0.88-1.43); PET normal diastolic function (n=30) 1.34 (1.27- 1.55); PET diastolic dysfunction (n=20) 0.81 (0.73-0.95) **Average E/e':** NTP 6.1 (5.2-6.5); PET normal diastolic function 6.5 (6.3-6.9); PET diastolic dysfunction 7.9 (6-8.3)	
Melchiorre, 2012 [133]	**CI ^{V}:** NTP 3.2 (2.7-3.7); preterm PET 2.6 (2.1-3.1)*	**E/A:** NTP 1.5 (1.4-1.8); preterm PET (1.2 (0.7-1.5)* **E/e'** NTP 4.8 (4-6.5); preterm PET 7.7 (7.4-10.7)*	

	Novelli, 2003 [134]	**CO [V]:** NTP 4.9 (0.9); GH 5.3 (1.1) **TVR:** NTP 1422 (221); GH 1621 (358)* **SV:** NTP 68 (10); GH 68 (12)	**E/A:** NTP 1.61 (0.31); GH 1.31 (0.28)*	**LVMI:** NTP 33 (7); GH 50 (8)*
	Oren, 1996 [135]	**CI [V]**: NTP 3.64 (0.64); GH 3.55 (0.89) **TVR:** NTP 14 (2); GH 16.7 (4.0)* **LVEF:** NTP 68.5 (5.6); GH 63 (7.3)*	**E/A:** NTP 1.6 (0.5); GH 1.2 (0.3)*	**LVMI:** NTP 86 (21); GH 99.4 (28)*
	Sanchez, 1986 [136]			**LVM:** NTP 154.65 (31); GH 170.36 (47)
	Simmons, 2002 [137]	**CI [F]:** NTP 4.2 (0.9); PET 4.1 (1.1)* **TVR:** NTP 852 (190); PET 1129 (319)*		**LVMI:** NTP 76 (16); PET 90 (18)*
	Solanki, 2011 [138]	**TVR [F]:** NTP 1204.5 (71.18); PET 1396.85 (156.2)* **SV:** NTP 70.8 (3.22); PET 73.3 (14.19)	**E/A:** NTP 1.35 (0.224); PET 1.497 (0.492)	
	Thompson, 1986 [139]	**LVEF [F]:** NTP 71.6 (7.1); PET 70.8 (6.0)		
	Tyldum, 2010 [140]	**CO [V]:** NTP 5.4 (1.4); PET 5.8 (1.2) **SV:** NTP 68 (23); PET 76 (11)	**E/A:** NTP 1.50 (0.37); PET 1.41 (0.36) **E/e':** NTP 5.7 (1.0); PET 8.6 (1.5)*	**LVM:** NTP 127 (30); PET 161 (28)*
	Veille, 1984 [141]	**CO [V]:** NTP 5.5 (1.8); GH 5.8 (1.4)		**LVM:** NTP 105 (14): GH 117 (20)*
	Yuan, 2006 [142]	**CI [F]:** NTP 4.0 (0.6); PET 3.9 (0.9) **SV:** NTP 71.0 (17.7); PET 79.6 (23.4) **LVEF:** NTP 0.66 (0.08); PET 0.72 (0.07)*	**E/A:** NTP 1.4 (0.2); PET 1.2 (0.2)*	
	Yuan, 2014 [143]	**CI [F]:** 2.99 (0.56); PET 3.07 (0.72) **LVEF:** NTP 0.66 (0.05); PET 0.68 (0.10).	**E/A:** NTP 1.41 (0.31); PET 1.24 (0.36)	**LVMI:** NTP 96.3 (17.8); PET 110.9 (29)*
	Zieleskiewicz, 2014 [144]	**TVRI***: NTP 723 (123); PET 894 (304)	**E/A:** NTP 1.4 (1.2-1.6); PET 1.1 (1.0-1.5) **E/e':** NTP 6.6 (5.8-7.0); PET 7.9 (5.9-8.9)*	

Data are presented as means (standard deviation) or medians (interquartile range).
* statistically significant difference with p-value <0.05; [F] Flow-based hemodynamic calculation (SV calculated from the velocity time integral of the pulsed Doppler waveform multiplied by the left ventricular outflow tract cross sectional area); [V] Volume-based hemodynamic calculation (SV calculated by subtracting the left ventricular end systolic volume from the left ventricular end diastolic volume).

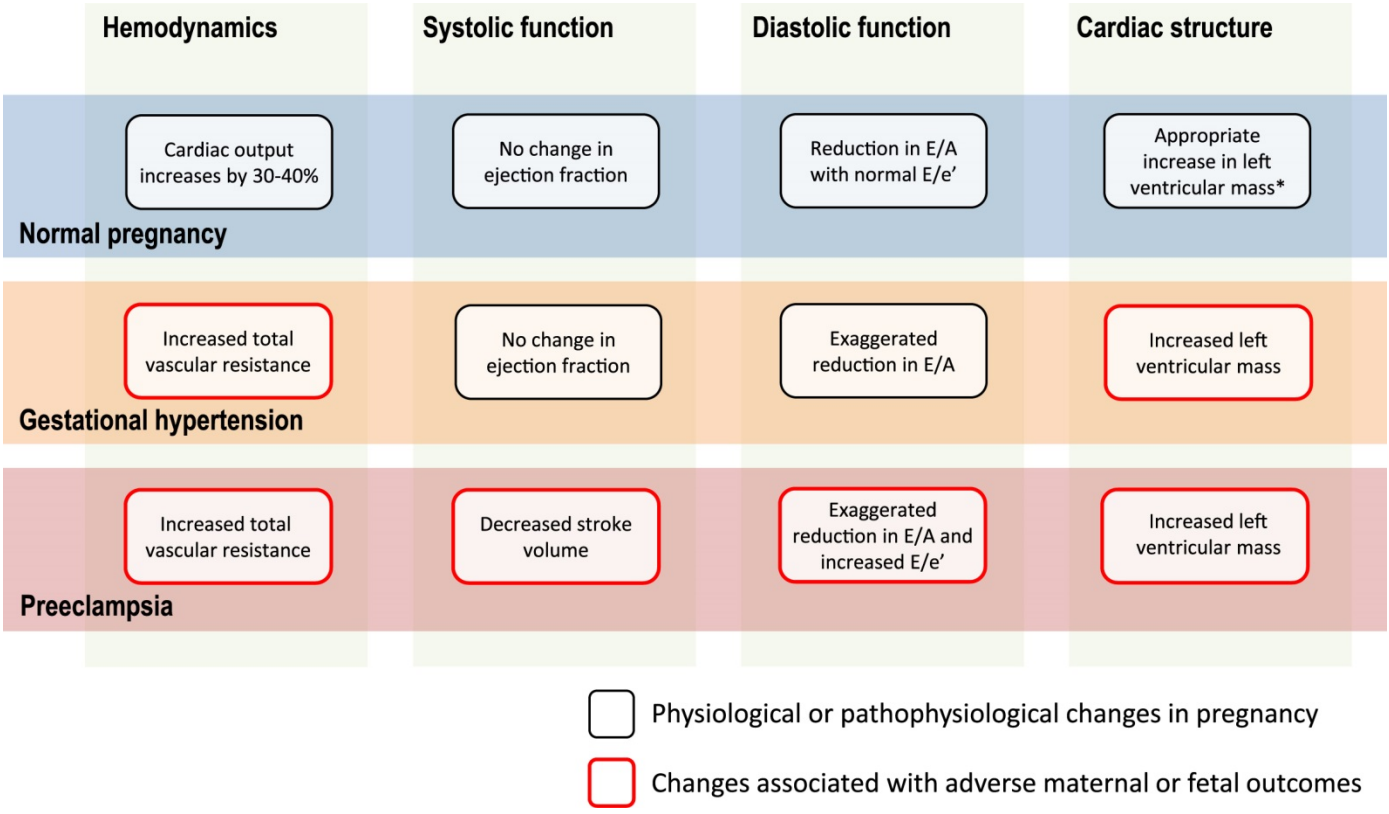
A, late diastole transmitral wave peak velocity (cm/s); CO, cardiac output (L/min); CI, cardiac index (L/min/m^2); E, early diastole transmitral (filling) peak velocity (cm/s); e', peak early diastolic velocity at mitral valve annulus (cm/s); GH, gestational hypertension; LVEF, left ventricular ejection fraction (%); LVM, left ventricular mass (g); LVMI (g/m^2), left ventricular mass index; NTP, normotensive pregnant controls; PET, preeclampsia; SGA, small for gestational age fetus; SV, stroke volume (ml); SVI, stroke volume index (ml/m^2); TVR, total vascular resistance (dynes/ s^1/cm^5); TVRI, total vascular resistance index (dynes s^{-1} cm^{-5}/m^2).

Table 10a: Summary of findings in third trimester studies

Study	Vlahovic-Stipac, 2010 [155] ‡	Bamfo, 2008 [145]	Borghi, 2000 [120]	Borghi, 2011 [121]	Cho, 2011 [122]	Degani, 1989 [123]	Demir, 2003 [124] ‡	Dennis, 2012 [125]	Escudero, 1988 [126]	Estensen, 2013 [148] ‡	Hamad, 2009 [127]	Ingec, 2005 [128]	Kuzniar, 1982 [130]	Kuzniar, 1992 [129] ‡	Lang, 1991 [131] ‡	Melchiorre, 2012 [133]	Novelli, 2003 [134]	Oren, 1996 [135]	Sanchez, 1986 [136] ‡	Shahul, 2012 [150] ‡	Simmons, 2002 [137]	Solanki, 2011 [138] ‡	Thompson, 1986 [139] ‡	Tyldum, 2010 [140]	Valensise, 2006 [151] ‡	Veille, 1984 [141]	Yuan, 2006 [142]	Yuan, 2014 [143]	Zieleskiewicz, 2014 [144]
TVR		**P** =	**P**† =	**P** ↑		**G** ↑							**P** ↑	**P** ↑	**P** =		**G** ↑	**G** ↑			**P** ↑	**P** ↑			**P** ↑				
CO	**G** ↑	**P** ↓	**P**† ↓	**G** ↑ **P** ↑		**G** =		**P** ↑		**P** ↑			**P** ↓		**P** =	**P** ↓	**G** =	**G** =			**P** ↓	**P** ↑		**P** =	**P** ↓		**P** =		
LVEF			**P**† ↓		**G** =	**G** =	**G** =	**P** =		**P** =								**G** ↓		**G** =			**P** =				**P** ↑	**P** =	**P** =
E/A	**G** ↓	**P** =	**P**† ↓		**G** ↓						**P** ↓					**P** ↓	**G** ↓	**G** ↓						**P** =			**P** ↓	**P** =	**P** =
E/e'	**G** ↑	**P** ↑									**P** ↑					**P** ↑								**P** ↑					**P** ↑
LVM	**G** ↑			**P** ↑	**G** ↑		**G** ↑	**P** ↑	**G** ↑	**P** ↑	**P** ↑	**P** ↑			**P** =		**G** ↑	**G** ↑	**G** =		**P** ↑				**P** ↑	**G** ↑		**P** ↑	

* third trimester results from longitudinal study; † all cases early preeclampsia (before 34 weeks gestation); ‡ studies with postnatal follow up.
↑, significant increase; ↓, significant decrease; =, no significant difference compared to controls; CO, cardiac output; G, gestational hypertension; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; P, preeclampsia; TVR, total vascular resistance.

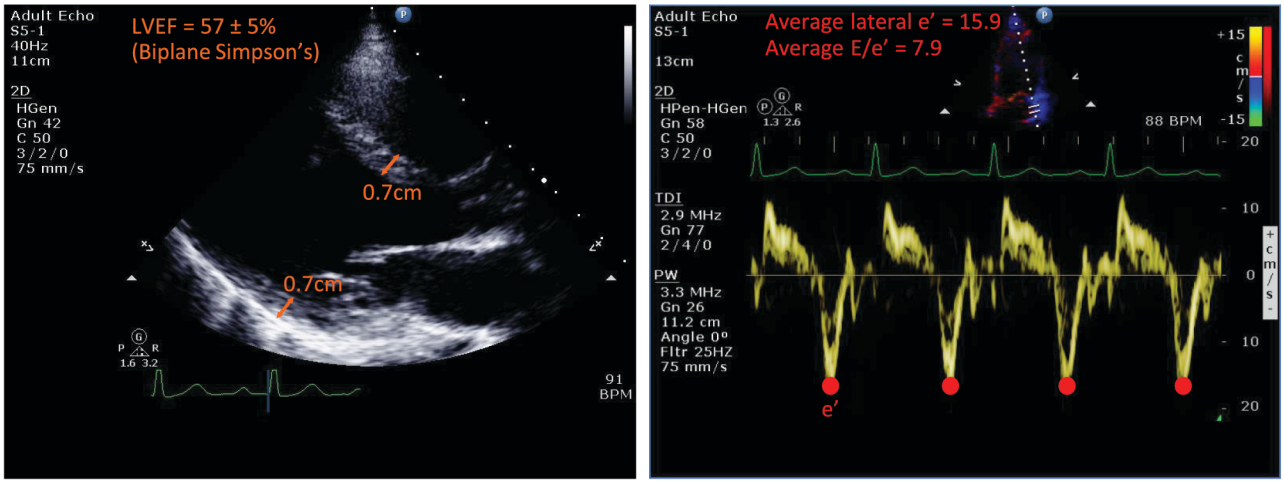
Figure 6: Summary of results



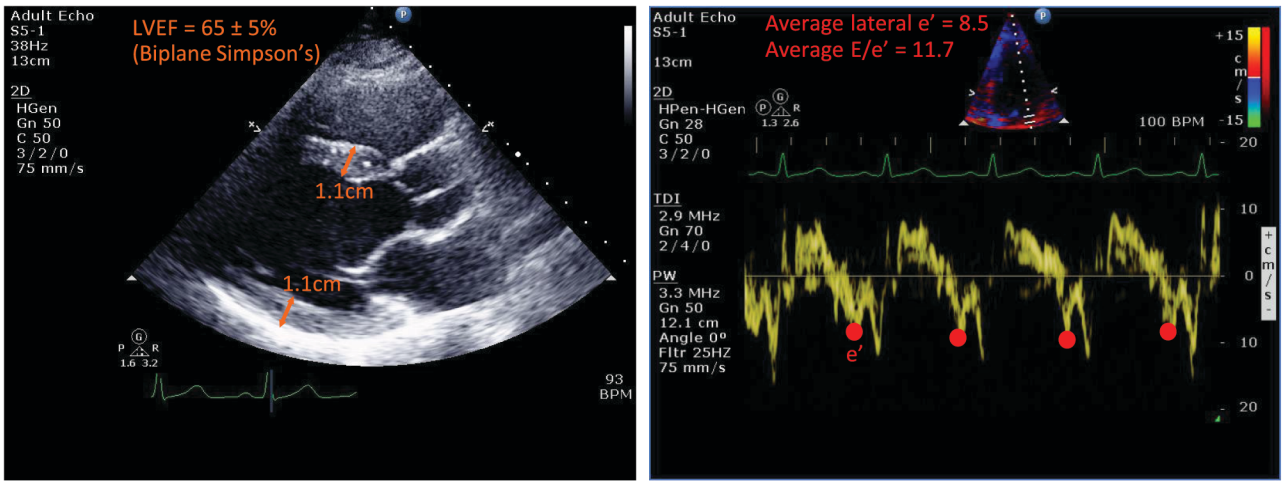
Summary of major findings comparing normotensive pregnancy with gestational hypertension/preeclampsia and association with adverse outcomes. * A progressive and slight increase in left ventricular wall thickness and mass is seen during normal pregnancy that regresses post-partum.[7, 156]

Figure 7: Representative echocardiographic images

Patient 1: Normal pregnancy



Patient 2: Hypertensive disorder of pregnancy



E, early diastole transmitral (filling) peak velocity (cm/s); e', peak early diastolic velocity at mitral valve annulus (cm/s); LVEF, left ventricular ejection fraction (%)

Both echocardiograms were performed at 30 weeks gestation and both patients had normal blood pressure and were not on any medications. Patient 1 is a 23 year-old with one previous uncomplicated pregnancy. Her blood pressure remained normal throughout the current uneventful pregnancy, and she delivered at 40 weeks. Patient 2 is a 25 year-old with one previous pregnancy affected by early onset preeclampsia. The current pregnancy was complicated by gestational hypertension and fetal growth restriction from 34 weeks. She delivered at 35 weeks after spontaneous preterm labour.

Diastolic function

Several studies have shown that in normal pregnancy the E/A ratio decreases towards term.[4, 7, 17, 21, 52] A greater reduction in E/A has been shown in GH compared to pregnancy unaffected by hypertension.[122, 134, 135, 142, 155]

Diastolic function is also impaired in PET,[127, 133, 140] where the usual reduction in E/A is exaggerated.[120, 127, 133, 142] The ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/e'), was significantly higher in women with PET in five studies, suggesting higher LV filling pressures.[125, 127, 140, 144, 149] Interestingly E/e' was shown to be significantly higher in an early-onset PET subgroup compared with a late-onset subgroup.[127] One study used a composite of diastolic indices to diagnose diastolic dysfunction and demonstrated diastolic dysfunction in 40% of pregnancies complicated by PET at term, compared with 14% of controls.[132] In another study, diastolic dysfunction was already present at 20-23 weeks in women who developed preterm PET, but not PET at term.[149] Diastolic dysfunction in PET is more marked in cases associated with fetal growth restriction[145], despite evidence that left atrial mechanical function is similar in PET and normotensive pregnant controls.[128]

Cardiac structure and remodeling

In most studies, LV mass was significantly increased in GH compared to normotensive pregnant controls in the second [135] and third trimester [122, 124, 126, 134, 135, 141], and increased in the second half of pregnancy when measured serially.[155] One study identified ventricular remodeling or hypertrophy in 91% of patients with GH .[134] The concentric hypertrophy seen

in GH[122, 124] is an independent predictor of adverse pregnancy outcomes.[151] Other investigators found no change in LV mass in GH, showing this instead to be a feature of chronic hypertension.[136, 139] LV/left atrial diameters were increased in GH compared to normotensive controls.[125, 142]

LV remodeling is more common in PET compared to normotensive pregnant women in the third trimester,[143] with numerous studies confirming increased LV mass in PET.[120, 122, 125, 127, 128, 132, 137] Hypertrophy in PET tends to be of the concentric type[137], and has been shown in preterm[120, 127, 133] and term PET.[128, 132, 133, 142] In one study, concentric LV remodeling was demonstrated at 20-23 weeks gestation in 33% of women who subsequently developed PET.[149] In women who progressed to PET from GH, 27% had abnormal LV structure and function at the time of echocardiography.[122]

2.1.4 Discussion

I performed a systematic review of all literature pertaining to the use of echocardiography in pregnant women with a hypertensive disorder. My major findings were increased peripheral resistance in GH and PET, diastolic dysfunction in PET and conflicting evidence regarding changes in cardiac output. The echocardiographic changes in cardiac structure and function can be detected before the condition is clinically apparent. Current evidence suggests that alterations in PET are not due to hypertension alone, but rather reflect PET as a multisystem disorder. PET has a greater impact on the heart than GH, and changes are most pronounced in early onset, severe disease.

Currently, echocardiography is not widely used in the clinical management of hypertensive

disorders in pregnancy, or as a screening tool for PET. The application of echocardiography in pregnancy has traditionally been in patients with adult congenital cardiac disease, in acute illness or for research purposes. The management of hypertensive disorders in pregnancy is based on maternal clinical assessment (symptoms, blood pressure and laboratory parameters) and fetal wellbeing. A decision to deliver the baby can be for maternal or fetal reasons. Whereas other reviews have focused on congenital heart disease[157] or described echocardiographic changes in the context of a broad overview of the management of PET[158], ours is the first systematic review of cardiac structure and function in gestational hypertensive disease.

Clinicians now recognise that PET should no longer be considered as a single disease process. There is evidence to suggest that preterm hypertension and proteinuria associated with fetal growth restriction is different to hypertension and proteinuria at term when birthweights tend to be normal or increased.[159] The possible difference in the mechanism of disease and how it manifests clinically[74] may be responsible for the conflicting results between studies when early- and late-onset PET are considered as one entity. The contradictory hemodynamic models described can be explained by noting the distinction between early PET [149, 152] and late-onset disease.[151, 153]

Although based on observational data, my review suggests that echocardiography has the potential to improve the management of patients with hypertension during pregnancy and categorise patients with GH or PET into high and low risk groups. Patients with increased vascular resistance and LV mass are more likely to have complications.[151] As a predictor of long term cardiovascular morbidity, diastolic dysfunction in pregnancy is important to

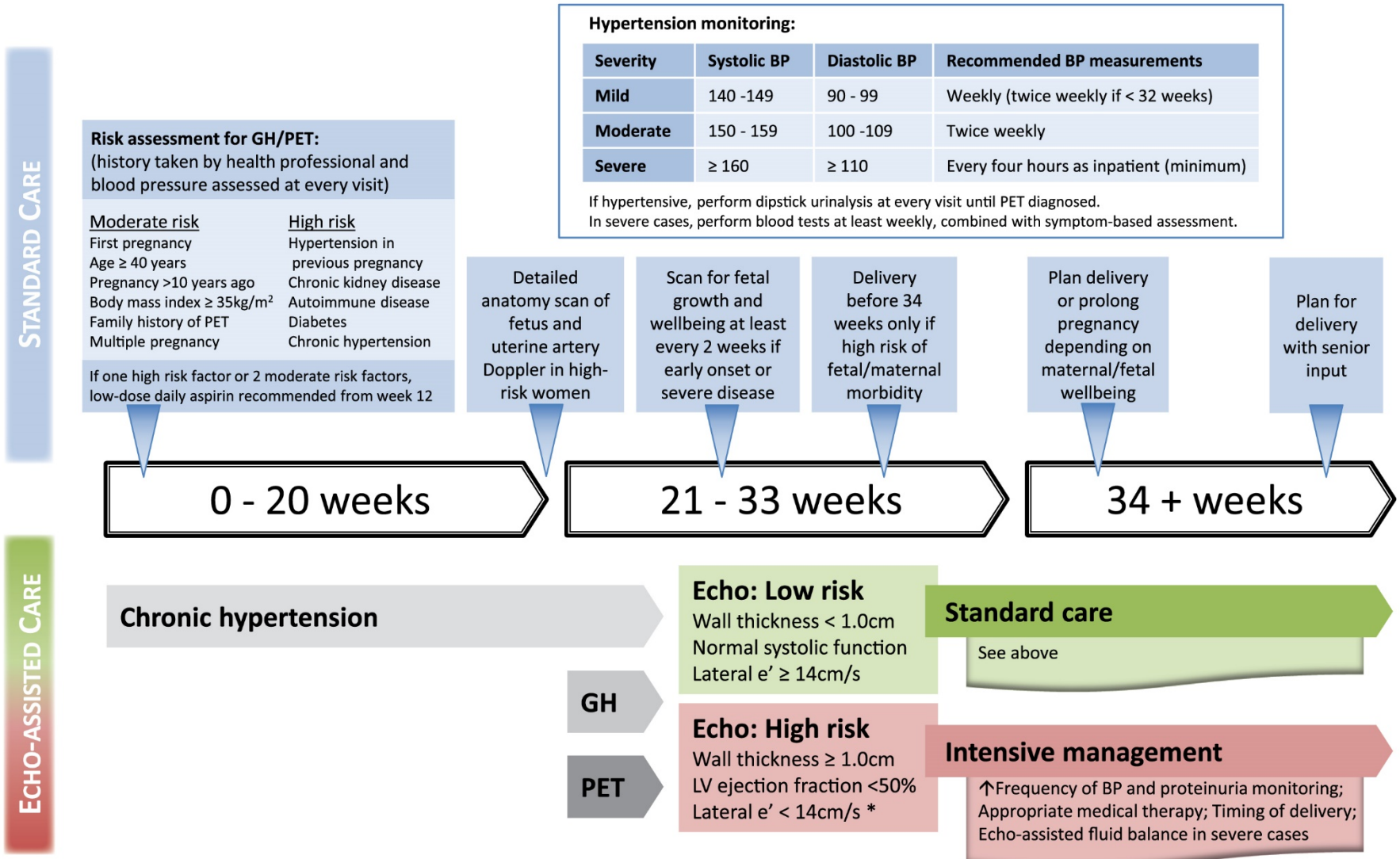
identify,[132] and reduced e' (and therefore elevated E/e') may be a useful and early predictor of PET.[145] Echocardiography can also help to identify the small numbers of women with LV systolic impairment who are more likely to deteriorate during pregnancy or post-partum. With the currently available data, I suggest the most efficient use of echocardiography is after the diagnosis of hypertension, to direct resources to the most vulnerable patients in order to improve maternal (and fetal) outcomes (see **Figure 8**). The optimal timing of echocardiography needs further study. Whereas an early echocardiogram in the first and second trimesters may be helpful for risk stratification, the available data on clinical impact is currently limited. It has also been suggested that echocardiography can stratify hypertensive pregnant women into hemodynamic subgroups, thereby enabling clinicians to tailor their choice of antihypertensive therapy.[147] Hypertension characterised by vasoconstriction responds better to beta-blockade whereas in hypertension with reduced plasma volume, calcium channel blockers are preferable, as they reduce afterload and improve cardiac function.[160] Echocardiography can also have an important role in guiding fluid balance, one of the most challenging aspects in the management of PET. Overzealous fluid administration can lead to pulmonary edema, and conversely if a patient is under-filled, end-organ dysfunction may worsen. In selected centers and patients, myocardial strain imaging has been shown to be more sensitive than LV ejection fraction in detecting differences in LV systolic function in women with and without PET.[134] Strain measurements can potentially provide more information about cardiac function but due to limited data[131, 132, 137, 150], further investigation is required.

In summary, echocardiography can reveal cardiac impairment in GH and PET, which changes antenatal management (medication, frequency of monitoring, timing of delivery) and can

indicate when postnatal follow-up is warranted. More longitudinal studies are required to evaluate the cardiovascular changes in hypertensive disorders throughout pregnancy and to further define the role of echocardiography in the antenatal assessment of women with GH and PET, and in subsequent pregnancies. It would be useful in clinical and research practice to define an ideal dataset for echocardiographic assessment in pregnancy, and agreed outcome measures for studies of cardiac structure and function, so that results are comparable and pooled data can be analyzed quantitatively. Clinicians should follow the American and European consensus guidelines[161] with specific focus on the variables listed in **Figure 8.**

Diastolic dysfunction can be further graded into impaired myocardial relaxation (E/A <0.73, deceleration time [DT] >194ms, isovolumetric relaxation time [IVRT] >83ms), pseudonormal filling (E/A 0.73-2.33, DT 138-194ms, IVRT 51-83ms) and restrictive filling (E/A >2.33, DT <138ms, IVRT <51ms). Left atrial dilatation is also a useful echocardiographic marker. For further details, see Melchiorre *et al.*, 2011 [132], adapted from recommendations by Nagueh *et al.*[162] The criteria listed in the "echo-assisted care" pathway in **Figure 8** have not yet been subjected to statistical assessment or validation, but rather represent my proposed indices and thresholds for triage into low- and high-risk categories based on the findings of my systematic review. Developing collaboration between Cardiologists and Obstetricians has the potential to open up new areas of research and further improve patient care.

Figure 8: Potential value of echocardiography in hypertensive disorders of pregnancy



BP, blood pressure; GH, gestational hypertension; PET, preeclampsia. * GH/PET risk assessment based on UK NICE Excellence guidelines.[1]

Limitations

The main limitation of my assessment was the wide variation in patient groups (age, ethnicity, body habitus, parity, timing of assessment, disease severity) and reported outcome measures. In several studies the participants were grouped based on outcomes other than hypertensive disorder diagnosis. This heterogeneity restricts quantitative synthesis of results and meta-analysis. Many of the included studies involve small numbers of patients, with varying levels of risk for important bias and likely different levels of echocardiographer experience. A substantial amount of data is derived from load-dependent indices, which may be inferior to measurements that take into account the different loading conditions seen in pregnancy. The cross sectional studies capture women at different stages of the disease and offer only a snapshot at a single point in time. At present, there is a paucity of longitudinal data in pregnancy. Only one of the longitudinal studies considered the reproducibility of the echocardiographic measurements, and whilst these results were encouraging (intraobserver variability 2.4% for cardiac output and 2.0 % for total vascular resistance[153]), further data in pregnant patients are clearly needed.

2.1.5 Implications for current research

This systematic review demonstrates that cardiac structure and function using echocardiography are altered in the preclinical and clinical phases of gestational hypertension and preeclampsia. For women with preeclampsia, diastolic dysfunction and increased peripheral vascular resistance correlate with disease severity. Recognition of impairment in cardiac function is important in the contemporary management of gestational hypertension and preeclampsia, in order to improve pregnancy outcomes and long-term cardiovascular health.

2.1.6 Update to systematic review

Some six eligible studies have been published between March 2015 and March 2019.[163-168] **Table 10b** presents an overview of the main findings from these studies, which all included a third trimester echocardiogram for women with preeclampsia and a normotensive pregnant comparison group. Inconsistent findings relating to cardiac output in preeclampsia are again evident.

Table 10b: Update to Table 10a including more recent studies

	Borges, 2018 [163]	Buddeberg, 2018 [164]	Caglar, 2016 [165]	Cong, 2015 [166]	Shahul, 2016 [167]	Yu, 2018 [168]
TVR		↑	↑			
CO		=	=	↓		↓
LVEF			=	↓		↓
E/A	↓	=	↓		↓	
E/e'		↑	=	↑	↑	
LVM	↑	↑	↑	↑	↑	

↑, significant increase; ↓, significant decrease; =, no significant difference compared to controls; CO, cardiac output; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; TVR, total vascular resistance

2.2 Monocyte subsets in pregnancy: A narrative review

2.2.1 Introduction

Cardiac disease is the leading indirect cause of death in pregnancy and the puerperium.[35] Hypertension is seen in 6-8% of pregnancies and causes one third of severe maternal morbidity.[1] Elevated blood pressure in pregnancy, which includes chronic hypertension, gestational (pregnancy-induced) hypertension and preeclampsia, accounts for approximately 14% of maternal deaths[115] and is a risk factor for future cardiovascular disease.[92] Gestational hypertensive disease is also an important cause of fetal morbidity and mortality, mostly due to growth restriction and preterm birth.[1]

The prediction and early detection of preeclampsia require improvement and new strategies for prevention and management are needed. A greater understanding of the underlying mechanisms of disease is required in order to improve outcomes. Hypertension in pregnancy and disorders of the cardiovascular system share many of the same risk factors and underlying pathophysiology. For example, endothelial dysfunction, vascular inflammation and remodelling are involved in preeclampsia and in atherosclerosis.[169, 170] Monocyte subsets are implicated in the pathophysiology of cardiovascular disease and in pregnancy complications. Monocytes may play a role in the pathogenesis of pregnancy hypertension.

In this review I provide an overview of the biology of monocytes and their subsets and then discuss separately their roles in cardiovascular disease and in maternal health. Gestational hypertensive disease is then proposed as an important area for monocyte research in order to investigate the link between pregnancy outcomes and long-term cardiovascular risk.

2.2.2 Search strategy

Any published work on monocyte biology or the roles of monocyte subsets in cardiovascular or women's health was eligible for inclusion in this review. My search included MEDLINE, EMBASE, CINAHL and the Cochrane Library from inception to December 2018, as well as relevant reference lists. The search strategy included the keywords "monocytes", "subsets", "women", "cardiac", "hypertension" and their synonyms. There was no restriction on the type of study design or publication language. Full text articles were obtained after screening the title and/or abstract of relevant studies.

2.2.3 Monocytes

About 3-8% of peripheral blood leucocytes are monocytes.[171] Their phagocytic function gives them a principal role in innate immunity.[172] They are also involved in procoagulant states by their interaction with platelets and play a part in regeneration after tissue injury.[173] Monocytes are the largest pool of progenitor cells, able to differentiate into many phagocytic cells types.[174, 175] They demonstrate the plasticity to differentiate into a wide range of cells including macrophages, dendritic cells, osteoclasts, microglia and Kupffer cells. Differentiation of monocytes in various cellular milieu allows them to perform tissue-specific roles in different parts of the body, for example they may also act as endothelial progenitor cells.[176]

Monocyte trafficking allows them to migrate out of the circulation, where their half-life is around three days[177], into areas of injury where they are involved in resolving inflammation and restoring the tissue, for example in healing after myocardial infarction.[172, 178] Cytokines whose expression is induced by monocytes include TNF-α, the interleukins IL-1 and IL-6,

transforming growth factors, macrophage colony stimulating factor and insulin-like growth factor.[172] Monocyte subsets express receptors involved in angiogenesis, tissue repair and remodelling, which may point to the nuclear factor κB (NFκB) pathway in effecting the reparative role of monocytes.[179]

Monocytes in the peripheral blood can be divided into three types (Mon1, Mon2 and Mon3), based on their expression of cell surface receptors (see **Figure 9**).[175] These receptors are the lipopolysaccharide CD14 and the Fcγ-III receptor, CD16. Updated evidence-based guidance was recently released and endorsed by the European Society of Cardiology.[171]

Figure 9: Nomenclature for human monocyte subsets

Pre-2010	**CLASSICAL MONOCYTES** CD14+CD16- CD14++CD16- CD14highCD16-	**NON-CLASSICAL MONOCYTES** CD14+CD16+ CD14lowCD16+ CD14-CD16+	
2010	**CLASSICAL MONOCYTES** CD14++CD16-	**INTERMEDIATE MONOCYTES** CD14++CD16+	**NON-CLASSICAL MONOCYTES** CD14+CD16++
2016	**Mon1**	**Mon2**	**Mon3**
% of monocytes	**~85%**	**~9%**	**~6%**

The cluster of differentiation molecules or markers (CD) 14 and 16 are the standard cell surface markers used to define monocyte subsets. A '+' designates where the CD expression level is more than 10-fold above the isotype control and '++' where CD expression is increased more than 100-fold.[175] When Mon2 and Mon3 are not separately defined (in older studies) they have been collectively referred to as CD16+ monocytes.[175] Unfortunately these studies cannot provide reliable information on phenotype and roles of either of these very different subsets. Both the phenotypic definition and the terminology for the monocyte subsets should be understood when interpreting the literature, since the variation in nomenclature over the years may cause confusion when older studies are compared with contemporary work.

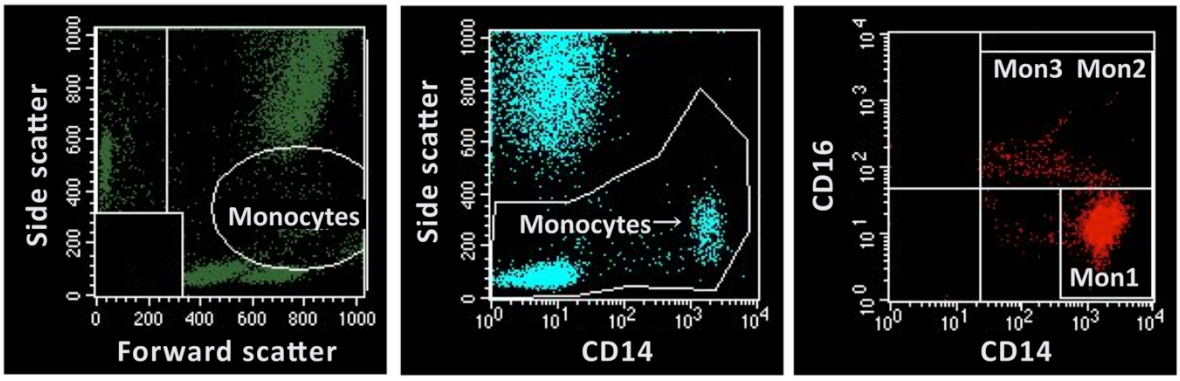
In terms of their developmental origin, previous work has shown that the three monocytes subsets are already in the bone marrow, although at this stage the Mon2 subset is proportionally greater compared to in the blood.[174] Monocyte levels in the blood may not reflect levels in the bone marrow, marginal pool and other tissues. Monocytes within a subset do not have the same role in every disease process, for example Mon2 is associated with improved outcomes in stroke but adverse outcomes in renal failure.[180-182] Further work is required to understand how these cells contribute to pathophysiology within different disease milieu. The three monocyte subsets are described in more detail below and their functional roles are summarised in **Table 11**.

Table 11: Functional roles of the monocyte subsets

Mon1	Mon2	Mon3
Phagocytosis Cytokine production Clearance of cellular debris Release of matrix metalloproteinases Release of reactive oxygen species	Angiogenesis Cytokine production Phagocytosis	Anti-inflammatory cytokine production Tissue healing Immune surveillance by patrolling endothelium

Flow cytometry can differentiate between monocyte subsets based on their light scatter properties and cell surface markers and has superseded morphological and cytochemical analysis.[175] Flow cytometry is relatively observer-independent and allows data to be collected rapidly from a large number of cells.[171] Cells must firstly be clearly defined as monocytes by using antibodies for specific epitopes, their light scatter properties and careful gating strategies to exclude other types of leucocytes. An example of the gating system used in flow cytometry is shown in **Figure 10**. The left panel shows selection of monocytes based on forwards scatter and side scatter. The central panel shows selection of monocytes based CD14 expression and side scatter. In the right panel cells that belong to both left and central panels are selected here to separate monocyte subsets based on their CD14 and CD16 expression.

Figure 10: Example of the gating strategy used in flow cytometry software to distinguish between monocyte subsets based on their granularity and CD14 and CD16 expression



2.2.4 Mon1

These are large cells, which are highly phagocytic. A feature of innate immunity is its not being antigen-specific, therefore monocytes must possess pattern recognition molecules such as Toll-like receptor 4 (TLR4) and CD14. CCR2 is the receptor for monocyte chemo attractant protein-1 (MCP1), which enables Mon1 to be actively recruited to sites of inflammation.[183] Mon1 phagocytose low-density lipoprotein and generate reactive oxygen species.[184] They express CD62L and CD64 and produce increased levels of IL1β, IL6, MCP1, and IKKβ compared to Mon2.[172, 174] This subset expresses genes involved in angiogenesis, wound healing and coagulation.[185]

2.2.5 Mon2

Mon2 expresses genes associated with inflammation and angiogenesis, which the other subsets do not.[186] They also have cell surface receptors linked with angiogenesis and tissue repair.[174, 187] These features, as well as maximal production of the anti-inflammatory IL-10, suggest that this subset of monocytes is important in tissue remodelling.[187, 188] Genetic

sequencing has shown Mon2 to express genes equipping them for antigen processing and presentation, a role that has been demonstrated by functional studies.[186, 189] They show an intermediate phenotype for the cell surface receptors CCR2 and CX3CR1[181, 190] and CCR5 surface-expression is specific to this subset.[181, 190] They have increased expression of the angiogenic receptors CXCR4 and vascular endothelial growth factor (VEGF). They express CD163 (a tissue repair marker), CD115, receptors to intercellular adhesion molecule-1 (ICAM-1), and have the highest surface levels of apolipoprotein B and ferritin.[174, 187] The angiopoietin receptor Tie-2 and major histocompatibility complex class II molecules are also expressed on Mon2.[172] Like Mon1, Mon2 have been shown to produce reactive oxygen species.[186]

2.2.6 Mon3

Mon3 may have a protective function in tissues with a patrolling and reparative role in case of injury. They are smaller and less granular than Mon2 and have a much lower phagocytic activity than Mon1 and Mon2.[171, 174] If there is endothelial dysfunction Mon3 can migrate out of the circulation.[187] CCR2 distinguishes the two distinct populations of CD16+ monocytes, with very few if any of these receptors found on the Mon3 subset, which expresses lower levels of CD14 and high levels of CD16.[174] Mon3 express CX3CR1, cytoskeletal rearrangement genes and CD294[172] and have the highest expression of VCAM-1 receptors and CD204[174] and does not express CD62L.[172] Fractalkine is an adhesion molecule found on activated endothelial cells and is responsible for the recruitment of Mon3.[172]

2.2.7 Monocyte-platelet aggregates

Monocytes combine with platelets to form monocyte-platelet aggregates (MPAs). Platelets

have a role in regulation of circulating cell types and their physical interaction with monocytes is an example of such an interaction that may influence their function.[188, 191] The notion that platelets regulate monocyte activity is supported by the correlation between IκB kinase β levels (a marker of activation of the NFκB pathway) in Mon2 and CD42 expression on MPA2.[179] Formation of MPAs is associated with increased cytokine production by monocytes, with CD16+ monocytes being upregulated[192, 193] and may be associated with induction of monocyte gene activation.[171] Another explanation for monocytes forming aggregates with platelets is as part of phagocytosis of activated platelets. MPAs serve as a marker of monocyte and platelet activation.[188, 194] Platelets are activated when there is denudation of the endothelium, and if they undergo degranulation and adhere to circulating monocytes, then MPAs are formed via an interaction between P-selectin on activated platelets and P-selectin glycoprotein ligand-1 on leucocytes.[171] Monocytes bound to platelets express macrophage adhesion ligand(Mac)-1, activate NFκB and produce IL-1β, IL-6, IL-8 and tissue factor.[173] It is proposed that the cross-talk between monocytes and platelets is the link between thrombosis and inflammation.[193] Aggregation is not permanent as the complexes have been shown to dissociate as they migrate across an endothelial surface.[195]

2.2.8 Monocyte subsets and cardiovascular health

The interface between cardiology and immunology has emerged as an exciting field of research. Leucocytosis is known to be a predictor of cardiovascular events.[196] Evidence has accumulated over the last several years for the distinct subpopulations of monocytes with distinct roles in the pathogenesis of cardiovascular diseases, which are often characterised by tissue injury and inflammatory processes (**Table 12**).

Whilst the primary role of monocytes is protective (neutralisation and elimination of pathogens via cytokine production and phagocytosis) they also have a detrimental role causing injury. When monocytes are high in number or activity, tissue damage may result in adverse outcomes.[171] An example of such host cell destruction is the vascular wall due to its proximity to the circulating monocytes.[197] Hypertension may result in endothelial injury due to shear stress and adherence and infiltration of monocytes in this circumstance contributes to atherosclerosis.

Old platelets are cleared by monocytes by thrombophagocytosis. Monocyte-platelet aggregates might be a precursor to this process leading to foam cell formation which contributes to the formation of atheroma.[194] Monocytes produce tissue factor via the angiotensin II type I receptor. Angiotensin converting enzyme inhibitors therefore reduce plasma tissue factor activity in hypertension.[198] Hypertension is an independent predictor of a low Mon3 count.[199] Monocyte-platelet aggregates in peripheral blood have been shown to correlate directly with blood pressure and are particularly closely related to systolic blood pressure.[200] Elevated blood pressure does not affect monocyte subset mobilisation during an exercise challenge.[201]

Table 12. Subsets of monocytes and their platelet aggregates in cardiovascular disease

Condition	Comparison	Reference
Acute heart failure	Mon1, Mon3 and MPA1 increased compared to stable heart failure, coronary artery disease and healthy controls	Wrigley, 2013[188]
	Mon3 increased compared to healthy controls	
Atherosclerosis	Mon2 associated with increased risk of cardiovascular events	Rogacev, 2012[202]
Chronic kidney disease	Mon2 independently associated with cardiovascular events	Rogacev, 2010[181] Heine, 2008[182]
Coronary artery disease	Mon1 increased and Mon2 decreased in highest risk patients	Czepluch, 2014[203] Hristov, 2010[199]
	CD16+ monocytes associated with increased severity (multiple vessel disease)	Ozaki, 2012[184]
	MPA3 increased compared to healthy controls	Shantsila, 2014[179]
	Diminished CD16+ monocytes on initial presentation with MI, followed by increase. Mon1 peak negative predictor of myocardial salvage.	Tsujioka, 2009[204]
Exercise	CD16+ monocytes decreased in elderly after exercise programme compared to physically inactive	Timmerman, 2008[205]
	CD16+ monocytes transiently increased after a short bout of exercise	Steppich, 2000[206]
Myocardial infarction	Mon1 increased after STEMI and correlates with peak troponin level and LVEF at 6 weeks	Tapp, 2012[207]
	Mon1 negatively correlate with LV function after MI	Tsujioka, 009
	Mon1 and Mon3 involved in myocardial healing after infarction	Dutta, 2015[178]
Obesity	Mon3 count significantly associated with increased BMI	Rogacev, 2009[208]
	CD16+ monocytes increased compared to healthy controls	Krinninger, 2014[209] Rogacev, 2010[208] Zawada, 2012[210]
Sepsis	CD16+ monocytes increased	Fingerle, 1993[211] Skrzeczynska, 2002[212]
Stable heart failure	MPA1 increased compared to healthy controls	Wrigley, 2013[188]
Steroid therapy	Depletion of CD16+ monocytes	Fingerle-Rowson, 2008[213] Heimbeck, 2010[214]
Stroke	Mon2 increased after stroke and higher levels associated with improved outcome	Urra, 2009[180]

BMI, body mass index; LVEF, left ventricular ejection fraction; MI, myocardial infarction; STEMI, ST elevation myocardial infarction

Monocytes are reported to have a role in adverse left ventricular remodelling and in the generation of reactive oxygen species in heart failure.[176, 215] Patients with oedema due to heart failure have higher levels of CD14, monocyte-derived TNF and endotoxin than patients with more mild heart failure, and the levels reduce after clinical improvement. Mon1 and Mon2 remained activated for over three months after an episode of acute heart failure in one study, even if clinically the patient was better.[188] It has been postulated that endotoxins reach the circulation due to increased pressure in the bowel mesentery and increased bacterial translocation.[216] Venous congestion and increased bowel permeability allow the lipopolysaccharide (LPS) in the cell wall of Gram negative bacteria to enter the bloodstream. LPS is a ligand for CD14 and stimulates its cytokine production. Another possible mechanism is increased sympathetic activity reducing blood flow in the splanchnic circulation. The resulting intestinal ischaemia makes the mucosa more permeable. TLR4 is found in organs including the heart, but mostly in peripheral monocytes. TLR4 has been shown to be an important component in the innate immune system and it is required in the monocyte response to LPS, being a co receptor for CD14.[217] The expression of TLR4 on monocytes is increased in heart failure and correlates with disease severity.[218] Monocytes are recruited into areas of remodelling in the myocardium.[217] Increased TLR4 has been shown in association with ventricular remodelling in cardiac failure, demonstrating that monocytes are involved in the adaptive response of the myocardium to heart failure.[219]

2.2.9 Monocyte subsets in Women's Health

Women have a significantly lower total monocyte count in peripheral blood compared to men.[214] The monocyte count is increased after the menopause and decreased with oestrogen replacement therapy.[220] There is emerging evidence that monocytes are activated in obese

women, who have significantly increased Mon2 counts compared to lean controls but overall no significant difference in overall monocyte count.[209] A high fat diet creates a cellular environment akin to inflammation and leads to monocyte emigration from the bone marrow.[221] Chemokine receptor expression on monocytes, in particular CCR2, is increased in obesity and the monocytes therefore show an increased migratory response towards inflammatory stimuli in overweight women.[209]

2.2.10 Monocyte subsets and hypertension in pregnancy

In pregnancy, blood monocytes are increased as part of the innate immune system's adaptation.[222] Mon1 has been shown to comprise a lower percentage of total monocytes in pregnant women, with a greater reduction in women with preeclampsia, attributed to an increase in Mon2.[222] Moreover the Mon2 count has been shown to correlate with the severity of preeclampsia[223] and has been proposed as an inflammatory mediator and predictor of preeclampsia. TLR4 up regulation has been demonstrated in Mon3 and neutrophils in women with preeclampsia.[224]

Preeclampsia has classically been considered a disease of placental aetiology and macrophages play an important role at the fetomaternal interface.[225] Macrophages are involved in immune tolerance and defence, in placentation and in parturition.[225] Melgert and colleagues describe the infiltration of the uterine decidua by monocytes, which become macrophages and dendritic cells involved in spiral artery remodelling and immune tolerance of the "semi-allogenic" fetus.[222] Placental microparticles, which are known to activate monocytes, are increased in preeclampsia.[222] Platelets are activated in preeclampsia and activated platelets lead to an increase in Mon2. These are possible mechanisms for the

increased proportion of CD16+ monocytes have been shown to be increased in preeclampsia[222], and specifically the increased proportion of Mon2.[223] Mon3 has also been shown to be elevated in preeclamptic women.[224]

Monocytes, and their platelet aggregates, are a source of soluble fms-like tyrosine kinase (sFlt-1), which is implicated in endothelial dysfunction.[226, 227] Increased CD14+ macrophages have been shown in the decidua basilis of placentas from preeclamptic women compared to gestational age matched controls, and these cells also stained positive for sFlt-1 suggesting a possible link.[226] Differential cytokine expression has been demonstrated between normotensive and preeclamptic pregnant women, with decreased IL12 and TNFα production in hypertensive pregnancy.[228, 229] Monocytes from preeclamptic women have been shown to be associated with increased production of IL-1β, IL-6, IL-8 and reactive oxygen species.[229-231] Faas *et al* showed that there are factors in the plasma of preeclamptic women, which activate endothelial cells only when cultured with monocytes. These factors may be placental in origin.[232] Work is ongoing to determine the nature of these factors involved in monocyte activation and several candidates have been proposed including microparticles (from the placenta or other cells), fetal DNA and ATP.

CD11b, which binds to intracellular adhesion molecule-1 and causes firm adhesion of leucocytes to endothelium, is upregulated on monocytes in preeclampsia and this is associated with increased metabolic activity in the monocytes as demonstrated by production of reactive oxygen species.[233] CD11b, as well as CD11a, CD11c and CD15 have been shown to be expressed more highly on monocytes in blood drawn from the uterine vein of preeclamptic women at Caesarean section when compared to the monocytes in their peripheral venous

blood.[234] This is consistent with the proposal that monocytes are most likely to be activated by microparticles in the uteroplacental circulation as this is where they have close interaction.[235] The phagocytic function of monocytes has been shown to be reduced in pregnancy, and this function declines further in preeclampsia, which may contribute to reduced elimination of factors causing inflammation and endothelial dysfunction.[236]

Although leucocyte activation is not specific to preeclampsia, there are qualitative differences in the phenotype of monocytes in preeclampsia when compared to pregnancy complications such as preterm labour.[233] A study comparing pregnant women in preterm labour with term pregnancy and non-pregnant controls found increased Mon2 and decreased Mon1 in pregnancy.[237] Mon2 was significantly increased in the preterm labour group leading the authors to suggest that Mon2 is involved in the immunological activation of preterm labour.[237]

2.2.11 Therapeutic intervention

Immunomodulation of the monocyte subsets has generated much interest as a new therapeutic target.[202, 238] Monoclonal antibodies against P-selectin glycoprotein ligand 1 have been shown to reduce MPAs.[239] Modulation of Mon2 function (for example interrupting the platelet chemokine CCR5) has been proposed as a possible mechanism of drug action to prevent cardiovascular events.[181] Current knowledge regarding the effect of cardiovascular therapeutics on monocyte subsets is limited. A difference in Mon2 levels has been shown in heart failure patients on beta blockers.[240] Antiplatelet therapy has been shown to reduce MPAs whereas anticoagulation has little effect.[241, 242] As evidence mounts for the role of monocyte subsets in a variety of diseases, research into agents that alter their biological activity may lead to development of novel therapies. Further research is required to

incorporate new knowledge of monocyte biology, their genomics and proteomics, into drug development.

2.2.12 Implications for current research

Pregnancy is increasingly being recognised both as a stress test for future cardiovascular health and as an important window of opportunity to implement primary and secondary prevention strategies to improve long term outcomes for women.[243] Recent research has encouraged a shift of focus away from the placenta and towards the cardiovascular system as central to the pathogenesis and clinical manifestation of preeclampsia. Maternal cardiac function and acute atherosis are of particular interest.[244, 245] It is time to draw upon knowledge of monocyte biology in relation to cardiovascular disease and bring pregnancy and specifically gestational hypertensive disorders into the equation since monocytes may be key players in women's cardiovascular health in pregnancy and beyond. Further research is also required to relate the altered haemodynamics in healthy and hypertensive pregnancy to the monocyte subsets and to discover whether they are an appropriate biomarker to incorporate into risk prediction models.

CHAPTER 3:
AIMS AND HYPOTHESES

3.1 Objectives

The aim of this work was to investigate how maternal cardiac structure and function is affected by a previous hypertensive pregnancy. Cardiovascular interactions in pregnant women with former GH or PET were compared with those in pregnant and non-pregnant women with no history of hypertension. Techniques including echocardiography, pulse wave analysis and flow cytometry were used to study the properties of the heart and blood vessels, with a focus on their reciprocal performance in pregnancy.

The antenatal booking appointment is a crucial time to assess risk and to plan safe care for an expectant mother and her fetus. Ultrasound imaging is currently used to perform a first trimester risk assessment for the fetus but its role in maternal cardiovascular risk assessment is not yet defined and this work may provide evidence to support increased use of echocardiography in obstetric medicine.

There is clinical value in establishing the echocardiographic changes throughout pregnancy in women with a previous hypertensive disorder, especially if changes manifest while a recurrent hypertensive disorder is at an asymptomatic early stage, as it could lead to development of a screening tool and affect management decisions. Echocardiography could be used in risk stratification for targeted secondary prevention.

It is also novel to correlate echocardiographic changes in pregnancy with changes in monocyte subset heterogeneity. Peripheral blood monocytes have not been related to

vascular changes in pregnancy and this aspect of the study will provide mechanistic insight.

3.2 Hypotheses

I hypothesised that a history of gestational hypertensive disease would be associated with abnormal ventricular-arterial interaction in a subsequent pregnancy, with reduced arterial elastance and altered ventricular function reflecting maladaptation to the pregnant state.

I also hypothesised that there would be altered monocyte subset heterogeneity in pregnant women with previous gestational hypertensive disease, and that levels of proinflammatory monocytes would be inversely proportional to cardiovascular performance.

Finally changes over time may be predictive of development of a hypertensive disorder in the current pregnancy.

CHAPTER 4: METHODOLOGY OF PROSPECTIVE STUDY

4.1 Introduction to chapter

In this chapter I describe the materials and methods for my prospective study, Evaluating Cardiovascular Changes in Hypertension in Obstetrics (ECCHO). I describe how identified and recruited women to participate in my research. The various modalities used to acquire data are explained. To close the chapter, I show my validation and reproducibility data to confirm that I was competent in the experimental procedures and able to record accurate data.

4.2 Study design

4.2.1 Introduction to prospective study

"Evaluating Cardiovascular Changes in Hypertension in Obstetrics" (ECCHO) was a prospective observational study in which women were recruited at the beginning of pregnancy and studied throughout their gestation. Cross-sectional comparison in the first trimester of pregnancy tested the hypothesis that prior hypertension in pregnancy is associated with altered arterial and ventricular function. Pregnant women with prior GH/PET were compared to pregnant women without prior hypertension and to healthy non-pregnant controls. Secondly all measures performed at baseline for the cross-sectional study, were repeated for the pregnant women at around 20 and 32 weeks of gestation (once in each trimester). The longitudinal study of pregnant women tested the hypothesis that women with prior hypertension in pregnancy have altered cardiovascular adaptation to pregnancy, which may be related to increased proinflammatory monocytes and lead to the development of recurrent hypertension.

4.2.2 Study groups

There are were three study groups. Group 1 comprised pregnant women with previous GH or PET. Group 2 comprised pregnant women with no history of hypertension. Group 3 included healthy non-pregnant women as controls. The diagnoses of GH and PET were in accordance with the current definitions according to NICE[1] (see **Figure 2**). The pregnant women were followed throughout pregnancy and the pregnancy outcome recorded. For patients who were hypertensive during the studied pregnancy, post natal follow up was organised in order to ascribe the appropriate diagnosis. Women with persistent hypertension or use of antihypertensives at 3 months post partum would receive a retrospective diagnosis of chronic hypertension whilst those demonstrating normalisation of blood pressure would have a confirmed diagnosis of gestational hypertensive disease.

4.2.3 Medical and obstetric history

A comprehensive medical history was taken from each patient, to assess them against the inclusion and exclusion criteria and to provide the necessary data for the study. Hospital case notes were also interrogated to confirm aspects of the history in order to reduce recall error and bias.

4.2.4 Inclusion criteria

Inclusion criteria are listed in **Table 13**.

Table 13: Inclusion criteria

Group 1 "Previous hypertension"	Group 2 "Previous normotensive"	Group 3 "Non-pregnant controls"
1. Pregnant 2. GH or PET in a previous pregnancy	1. Pregnant 2. No previous hypertensive disorder (May be parous or nulliparous)	1. Not pregnant 2. Not within 1 year post-partum 3. Pre-menopausal (May be parous or nulliparous)

4.2.5 Exclusion criteria

Exclusion criteria are listed in **Table 14**.

4.2.6 Recruitment

Recruitment ran from February 2014 to September 2015. Patients were recruited from women attending the Department of Maternity and Perinatal Medicine at Sandwell and West Birmingham Hospitals NHS Trust (SWBH). The University of Birmingham Institute of Cardiovascular Sciences (ICVS) is on the same campus as the Maternity Unit at Birmingham City Hospital. It has its own dedicated clinical suite for cardiovascular physiology. Professor Lip's group has published all methods and robust Standard Operating Procedures (SOPs) are established (included as **Appendices**). The hospital serves an ethnically diverse population in one of the most deprived inner-city areas of the country.

Table 14: Exclusion criteria

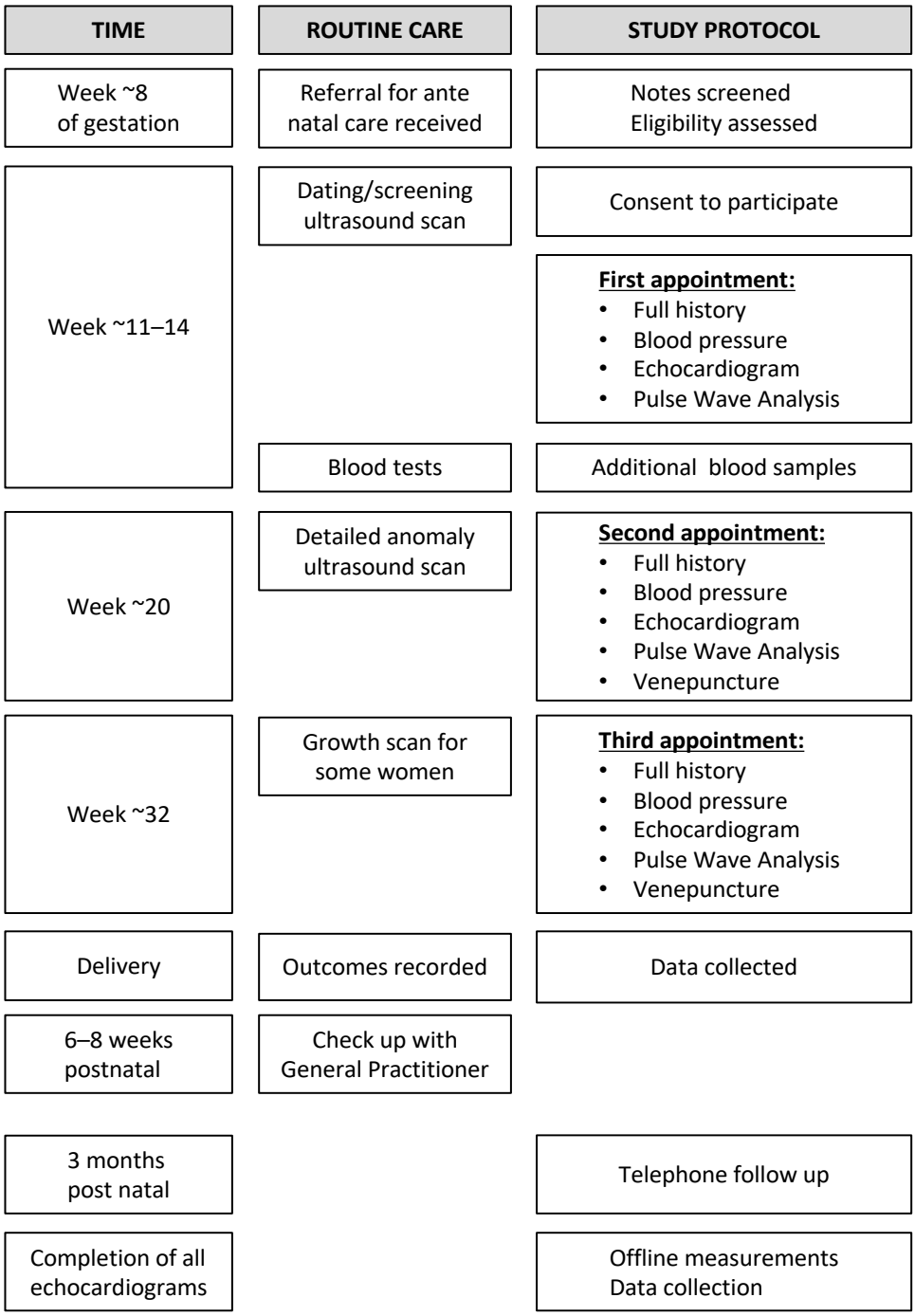
1. Patient refusal
2. Pre-existing cardiac disease (ischaemic heart disease, valvular heart disease, congenital heart defect)
3. Chronic hypertension
4. Significant illness
5. Vasoactive medication
6. Multiple pregnancy
7. Inability to consent (language barrier with no translator available, lacks capacity)
8. Obstetric emergency (haemorrhage, severe symptomatic (pre)eclampsia)
9. In labour
10. Age under 16 years

There are approximately 5800 deliveries in the Maternity Unit each year. Eligible women were identified from referrals for antenatal care and approached during their first hospital visit. Non-pregnant controls were recruited from hospital and university staff.

4.2.7 Timing of investigations

Gestational age was determined by fetal biometry in the first trimester. Pregnant women were studied at baseline (around 12 weeks gestation) and asked to attend two follow up appointments, once in each trimester at 20+ and 30+ weeks (**Figure 11**). Non-pregnant controls were studied only once, following the protocol for the baseline visit for the pregnant women.

Figure 11: Protocol for ECCHO study



4.2.8 Patient management

This was a prospective observational study. As such there were no changes in the routine antenatal care of patients. Clinical management of pregnancy was in accordance with established local protocols, based on national guidelines.

4.3 Echocardiography

4.3.1 Equipment and software

Echocardiography at the baseline visit was performed with a Philips iE33 ultrasound machine (Bothell, WA, USA). A portable echocardiography machine was used for the follow up appointments, namely the Philips CX50 (Bothell, WA, USA). Both machines employed a phased array transducer. The images were converted to Digital Images and Communications in Medicine (DICOM) format. Xcelera software (Philips Medical Systems, Netherlands) was used to analyse the stored images.

4.3.2 Procedure for echocardiogram

After a period of at least 5 minutes rest, the women were examined in a comfortable left lateral position on the couch. Although there was no risk of aortocaval compression in the baseline visit and non-pregnant women, the left lateral tilt was employed throughout the longitudinal study for standardisation. The clinic room was temperature controlled and kept quiet and undisturbed. The women were asked to abstain from caffeine, alcohol and smoking, especially from the night before the appointment. Appointments were routinely made in the morning.

Two investigators performed the echocardiograms throughout the study. Dr Alena Shantsila (AS) performed most of the studies. Dr Richard Brown (RB) deputised during her absence in order to maximise the number of possible appointments. Both of these collaborators are experienced in cardiac imaging and are fully accredited with the British Society of Echocardiography. As post graduate researchers at the ICVS they were also trained in good clinical practice. The images were digitally stored and given unique identification numbers. All echocardiograms were transthoracic (TTE). The examination protocol was in accordance with the latest published guidelines from the international societies.[162, 246, 247]

A three-lead electrocardiogram (ECG) was recorded throughout the cardiac ultrasound examination. The left ventricular outflow tract (LVOT) was visualised in the left parasternal long axis view (PLAX). Since the mitral valve opens in early diastole, end systole is in the frame before this event. End systole is also recognised on the ECG as the onset of the Q wave. The left atrial diameter was also measured in the PLAX view. The LA diameter was measured at the end of the T wave on the ECG. The LVOT velocity time intregral (VTI) was measured in the apical 5 chamber view using pulse wave Doppler to acquire the trace. The mitral valve E and A waves were recorded in the apical 4 chamber view using pulse wave Doppler. Tissue Doppler was used to record the motion of the left ventricular walls at the level of the mitral valve annulus during diastole, in the apical four chamber view to measure the e' and a' waveforms. Isovolumetric relaxation time (IVRT) is recorded from the end of the s' wave until the start of the e' wave. The direct measurements the parameters derived from echocardiography are shown in **Table 15** and **Table 16** respectively.

Table 15: Echocardiography views and direct measurements

Image / View	Measurement
Parasternal long axis	Left ventricular outflow tract diameter (cm) Left atrial dimension (cm) Interventricular septal dimension (cm)* Left ventricular internal diameter (cm)* Left ventricular posterior wall dimension (cm)* *measured at the end of systole and at the end of diastole
Apical 2 chamber	Left ventricular end diastolic area (cm^2) Left ventricular end systolic area (cm^2) Left atrium end systolic area (cm^2) Left atrial major axis (cm)
Apical 4 chamber	Left ventricular end diastolic area (cm^2) Left ventricular end systolic area (cm^2) Left atrium end systolic area (cm^2) Left atrial major axis (cm)
Apical 5 chamber – left ventricular outflow tract ***Continuous wave Doppler***	Left ventricular pre-ejection time (cm/s) Left ventricular ejection time (cm/s) Isovolumetric relaxation time (s) Left ventricular outflow tract velocity time integral
Mitral inflow ***Pulsed wave Doppler***	E wave A wave S wave
Mitral valve annulus (septal and lateral) ***Tissue Doppler***	e’ a’ s’

Table 16: Echocardiography derived measurements

Parameter	Calculation
Heart rate (HR, beats/minute)	Reciprocal of the mean of several consecutive R-R intervals on the ECG, multiplied by 60
Velocity time integral	Mean average of tracings of 4 pulse wave Doppler waveforms for the flow across the aortic valve.
Stroke volume* (SV, ml)	π x (Left ventricular outflow tract diameter / 2)^2 x velocity time integral; measurements in cm
Cardiac output* (CO, L/min)	Stroke volume [L] x heart rate [beats/min]
Total vascular resistance* (TVR, dyne.s/cm^5)	80 x (Mean arterial pressure / cardiac output)
E/A ratio	Mitral valve E wave [early filling] peak velocity / mitral valve A wave [late filling during atrial contraction] peak velocity
E/e'	Mitral valve E wave peak velocity / e' wave [early diastolic mitral annular] velocity on tissue Doppler imaging (TDI); measurements in m/s (The value for e' used is the mean of the septal and lateral e' from TDI)
Left ventricular volume (ml)	Single plane, 4-chamber, method of discs Measured at end-systole and end-diastole, in cm.
Left ventricular mass* (LVM, g)	0.8 x [1.04((left ventricular end diastolic dimension + posterior wall thickness + interventricular septum thickness)^3 – left ventricular end diastolic diameter^3)] + 0.6; measurements in mm, at end diastole This is the Devereux formula.[248]
Relative wall thickness	2 x posterior wall thickness at end diastole / LV end diastolic dimension; measurements in mm

Left ventricular ejection fraction (LVEF, %)	(End diastolic volume – end systolic volume) / end diastolic volume x 100; measurements in mL
Left ventricular fractional shortening (LVFS, %)	100 x (LVIDd-LVIDs) / LVIDd Where LVIDd is the left ventricle internal dimension in diastole and LVIDs is the left ventricle internal dimension in systole.
End systolic pressure (ESP, mmHg)	ESP = systolic blood pressure x 0.9 This can also be calculated from pulse wave analysis.
End diastolic pressure (EDP, mmHg)	EDP = 1.24 x (E/e') + 1.9 e' is the average of the septal and lateral e' values.
Arterial elastance* (Ea)	End systolic pressure / stroke volume
Group-averaged normalised left ventricular elastance at onset of ejection ($E_{Nd(avg)}$)	$0.35695 – 7.2266 \times t_{Nd} + 74.249 \times t_{Nd}^2 – 307.39 \times t_{Nd}^3 + 684.54 \times t_{Nd}^4 – 856.92 \times t_{Nd}^5 + 571.95 \times t_{Nd}^6 – 159.1 \times t_{Nd}^7$ This polynomial function is used according to Chen *et al*[30], where t_{Nd} is the ratio of LV pre-ejection time to total systolic period (pre-ejection time and ejection time).
Non-invasive estimated normalised left ventricular elastance at onset of ejection ($E_{Nd(est)}$)	$0.0275 – 0.165 \times LVEF + 0.3656 \times (DBP/ESP) + 0.515 \times E_{Nd(avg)}$ This is derived from Chen's single beat method to calculate end systolic elastance.[30] LVEF expressed as a decimal.
End-systolic elastance (Ees)	$Ees = (DBP – (E_{Nd(est)} \times ESP) / (SV \times E_{Nd(est)})$ Defined as End systolic pressure / End systolic volume Calculated using Chen's single beat method.[30]
End-diastolic elastance (Eed)	End diastolic pressure / End systolic volume

Ventricular-arterial coupling (VAC)	Ea / Ees

* These measurements are also indexed according to body surface area.

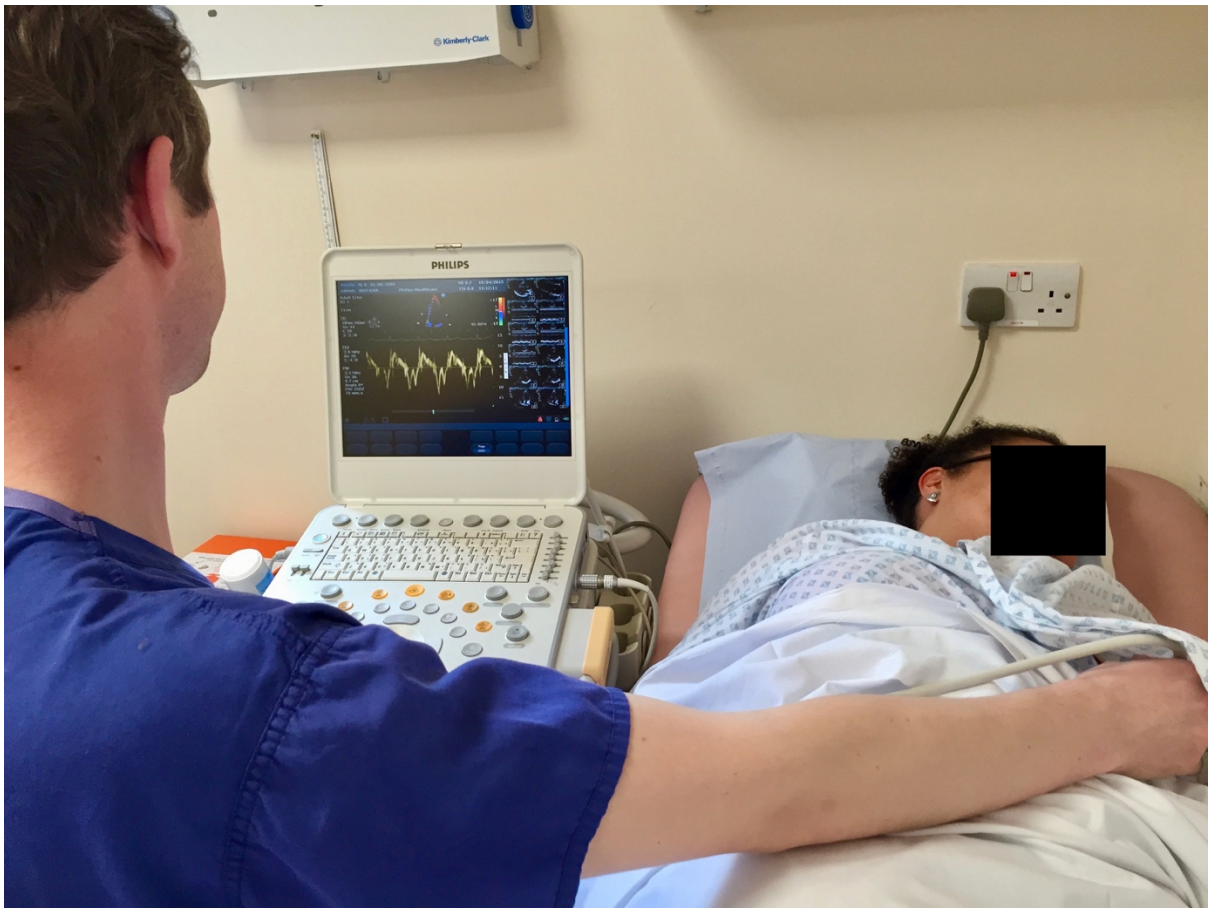
4.3.3 Measurements

The echocardiograms were stored digitally for offline analysis. Measurements were performed by a single observer (JSC). All measurements were performed after completion of the study in a random order, with the investigator blinded to the identity of the patient, their clinical characteristics, including blood pressure, and their pregnancy outcome. Measurements from 2D structural images were recorded once. Measurements based on flow/waveforms were performed on four beats and the mean average was taken.

The LV stroke volume was calculated from the difference between the left ventricular volumes in systole and diastole. The single plane Simpson method was used. The endocardial border of the LV was traced in systole and in diastole. The Xcelera software (Philips Medical Systems, Netherlands) divides the ventricle along its long axis into a series of stacked discs of equal height. The volume is calculated as the sum of these discs.

For quality control ten sets of images from each study group were randomly selected for the assessment of interobserver reliability (see **Table 18**). The images were presented to the second observer (AS) who was blinded to the patient characteristics (including study group) and to the measurements of JSC. Measurements of the parameters of arterial-vascular interactions have been previously validated in the ICVS and were done in accordance with established SOPs.[18]

Figure 12: Photograph of subject having an echocardiogram



4.3.4 Reproducibility data

The reproducibility data in **Table 17** show the results of repeated measures of the same echocardiographic parameters analysed by the same investigator on different days. The co-efficient of variation (CV) measures the dispersion of data points around an expected value (mean) according to the following formula:

$$\text{CV}\ (\%) = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Table 17: Intraobserver variability for echocardiographic measurements

	IVSd Day 1	IVSd Day 2	mean	SD	CV
Controls	0.51	0.58	0.55	0.05	9.08
	0.86	0.61	0.74	0.18	24.05
	0.54	0.43	0.49	0.08	16.04
	0.44	0.49	0.47	0.04	7.60
	0.63	0.68	0.66	0.04	5.40
	0.84	0.81	0.83	0.02	2.57
	0.56	0.76	0.66	0.14	21.43
	0.64	0.97	0.81	0.23	28.99
	0.61	0.56	0.59	0.04	6.04
	0.47	0.52	0.50	0.04	7.14
				Group:	**12.83**
Healthy pregnant	0.56	0.84	0.70	0.20	28.28
	0.66	0.62	0.64	0.03	4.42
	0.59	0.52	0.56	0.05	8.92
	0.54	0.57	0.56	0.02	3.82
	0.85	1.20	1.03	0.25	24.15
	1.00	0.94	0.97	0.04	4.37
	0.56	0.47	0.52	0.06	12.36
	0.47	0.51	0.49	0.03	5.77
	0.94	0.84	0.89	0.07	7.95
	0.91	0.86	0.89	0.04	3.99
				Group:	**10.40**
Previous hypertension	0.59	0.44	0.52	0.11	20.60
	1.10	0.91	1.01	0.13	13.37
	0.91	1.20	1.06	0.21	19.44
	0.74	0.74	0.74	0.00	0.00
	1.10	0.67	0.89	0.30	34.36
	0.65	0.62	0.64	0.02	3.34
	0.69	0.59	0.64	0.07	11.05
	0.91	0.90	0.91	0.01	0.78
	0.69	0.69	0.69	0.00	0.00
	0.50	0.43	0.47	0.05	10.64
				Group:	**11.36**
Combined				**Overall:**	**11.53**

	LVIDd Day 1	LVIDd Day 2	mean	SD	CV
Controls	4.9	4.6	4.75	0.21	4.47
	4.4	5.4	4.90	0.71	14.43
	4.9	5.0	4.95	0.07	1.43
	4.3	3.9	4.10	0.28	6.90
	4.1	4.6	4.35	0.35	8.13
	4.9	4.1	4.50	0.57	12.57
	4.4	4.6	4.50	0.14	3.14
	4.1	3.7	3.90	0.28	7.25
	4.4	4.7	4.55	0.21	4.66
	5.0	5.2	5.10	0.14	2.77
				Group:	**6.58**
Healthy pregnant	4.9	4.7	4.80	0.14	2.95
	4.6	2.9	3.75	1.20	32.06
	5.2	5.2	5.20	0.00	0.00
	5.3	4.3	4.80	0.71	14.73
	4.7	3.8	4.25	0.64	14.97
	5.2	5.2	5.20	0.00	0.00
	4.8	4.9	4.85	0.07	1.46
	3.9	3.9	3.90	0.00	0.00
	4.1	2.7	3.40	0.99	29.12
	4.2	3.1	3.65	0.78	21.31
				Group:	**11.66**
Previous hypertension	4.4	4.8	4.60	0.28	6.15
	3.3	3.8	3.55	0.35	9.96
	4.5	4.5	4.50	0.00	0.00
	5.5	5.8	5.65	0.21	3.75
	3.6	3.8	3.70	0.14	3.82
	4.5	5.3	4.90	0.57	11.54
	4.9	5.3	5.10	0.28	5.55
	5.1	5.4	5.25	0.21	4.04
	4.5	4.7	4.60	0.14	3.07
	4.3	4.2	4.25	0.07	1.66
				Group:	**4.96**
Combined				**Overall:**	**7.73**

	E Day 1	E Day 2	mean	SD	CV
Controls	104.7	115.6	110.12	7.71	7.00
	72.6	77.5	75.02	3.46	4.62
	69.8	73.8	71.80	2.88	4.00
	69.1	71.4	70.27	1.65	2.35
	89.0	94.8	91.90	4.10	4.46
	70.1	75.0	72.58	3.46	4.77
	79.3	88.1	83.70	6.18	7.38
	80.5	86.0	83.27	3.91	4.70
	82.8	89.4	86.08	4.64	5.39
	82.1	89.2	85.65	5.07	5.92
				Group:	**5.06**
Healthy pregnant	65.2	66.2	65.70	0.71	1.08
	96.7	106.5	101.62	6.91	6.80
	84.2	89.0	86.60	3.39	3.92
	70.6	73.3	71.92	1.91	2.65
	73.6	72.8	73.20	0.57	0.77
	120.2	125.5	122.83	3.72	3.03
	60.2	66.0	63.08	4.08	6.46
	95.3	97.3	96.27	1.41	1.47
	89.4	86.6	88.00	1.93	2.20
	77.3	79.2	78.25	1.30	1.66
				Group:	**3.00**
Previous hypertension	84.9	87.2	86.07	1.65	1.92
	81.8	87.3	84.57	3.91	4.63
	101.2	110.2	105.72	6.39	6.04
	88.2	92.6	90.42	3.13	3.47
	53.4	56.8	55.08	2.38	4.32
	95.1	103.8	99.43	6.18	6.21
	110.0	112.5	111.27	1.74	1.57
	76.2	77.6	76.90	1.04	1.35
	68.8	78.6	73.70	6.98	9.47
	81.5	88.4	84.95	4.83	5.69
				Group:	**4.47**
Combined				**Overall:**	**4.18**

	e' Day 1	e' Day 2	mean	SD	CV
Controls	11.4	12.1	11.75	0.49	4.21
	10.2	10.4	10.32	0.16	1.60
	10.6	11.0	10.80	0.33	3.06
	10.8	11.4	11.12	0.45	4.03
	9.0	9.4	9.17	0.28	3.09
	13.0	14.3	13.62	0.92	6.75
	12.8	13.2	12.98	0.26	2.00
	12.4	12.8	12.62	0.31	2.43
	13.8	13.8	13.78	0.02	0.17
	13.4	14.4	13.88	0.68	4.92
				Group:	**3.23**
Healthy pregnant	6.4	6.7	6.52	0.21	3.26
	14.4	14.3	14.38	0.07	0.49
	13.0	13.3	13.15	0.26	1.97
	11.8	12.0	11.90	0.19	1.58
	5.3	5.5	5.42	0.16	3.05
	12.0	8.9	10.47	2.17	20.72
	8.7	8.7	8.70	0.05	0.54
	12.4	12.6	12.52	0.16	1.32
	14.3	15.0	14.62	0.49	3.39
	9.4	9.5	9.45	0.07	0.75
				Group:	**3.71**
Previous hypertension	15.3	16.4	15.88	0.78	4.90
	11.5	11.6	11.58	0.07	0.61
	9.8	11.0	10.38	0.82	7.95
	10.1	9.9	9.98	0.12	1.18
	11.0	11.1	11.05	0.12	1.07
	7.9	8.3	8.10	0.24	2.91
	12.1	12.6	12.37	0.33	2.67
	8.2	8.1	8.12	0.07	0.87
	9.8	10.2	9.98	0.31	3.07
	10.5	11.5	10.98	0.68	6.22
				Group:	**3.14**
Combined				**Overall:**	**3.36**

	E/e' Day 1	E/e' Day 2	mean	SD	CV
Controls	9.2	9.6	9.37	0.26	2.79
	7.1	7.4	7.27	0.22	3.02
	6.6	6.7	6.65	0.06	0.95
	6.4	6.2	6.32	0.11	1.68
	9.9	10.1	10.02	0.14	1.38
	5.4	5.3	5.33	0.11	1.98
	6.2	6.7	6.44	0.35	5.39
	6.5	6.7	6.60	0.15	2.27
	6.0	6.5	6.25	0.33	5.22
	6.1	6.2	6.17	0.06	0.99
				Group:	**2.57**
Healthy pregnant	10.2	9.9	10.09	0.22	2.18
	6.7	7.4	7.07	0.51	7.29
	6.5	6.7	6.58	0.13	1.95
	6.0	6.1	6.04	0.06	1.07
	13.9	13.2	13.52	0.52	3.82
	10.0	14.0	12.03	2.85	23.68
	6.9	7.6	7.25	0.51	7.00
	7.7	7.7	7.69	0.01	0.15
	6.3	5.8	6.03	0.34	5.58
	8.2	8.3	8.28	0.08	0.91
				Group:	**5.36**
Previous hypertension	5.5	5.3	5.42	0.16	2.98
	7.1	7.5	7.30	0.29	4.02
	10.3	10.1	10.19	0.19	1.91
	8.8	9.4	9.06	0.42	4.65
	4.9	5.1	4.98	0.16	3.26
	12.0	12.6	12.27	0.41	3.30
	9.1	8.9	9.00	0.10	1.10
	9.3	9.6	9.48	0.21	2.22
	7.0	7.7	7.38	0.47	6.41
	7.8	7.7	7.74	0.04	0.54
				Group:	**3.04**
Combined				**Overall:**	**3.66**

Table 18: Interobserver variability for echocardiographic measurements

These reproducibility data show the results of repeated measures of the same sample analysed by two researchers (JSC and AS). All CVs are expressed as a percentage.

	IVSd AS	IVSd JC	mean	SD	CV
Controls	0.60	0.58	0.59	0.01	2.40
	0.94	0.61	0.78	0.23	30.11
	0.64	0.43	0.54	0.15	27.76
	0.58	0.49	0.54	0.06	11.90
	0.64	0.68	0.66	0.03	4.29
	0.70	0.81	0.76	0.08	10.30
	0.91	0.76	0.84	0.11	12.70
	0.75	0.97	0.86	0.16	18.09
	0.78	0.56	0.67	0.16	23.22
	0.78	0.52	0.65	0.18	28.28
				Group:	**16.90**
Healthy pregnant	0.85	0.84	0.85	0.01	0.84
	0.67	0.62	0.65	0.04	5.48
	0.58	0.52	0.55	0.04	7.71
	0.64	0.57	0.61	0.05	8.18
	0.78	1.20	0.99	0.30	30.00
	0.82	0.94	0.88	0.08	9.64
	0.78	0.47	0.63	0.22	35.07
	0.63	0.51	0.57	0.08	14.89
	0.70	0.84	0.77	0.10	12.86
	0.97	0.86	0.92	0.08	8.50
				Group:	**13.32**
Previous hypertension	0.86	0.44	0.65	0.30	45.69
	1.10	0.91	1.01	0.13	13.37
	1.10	1.20	1.15	0.07	6.15
	1.00	0.74	0.87	0.18	21.13
	1.00	0.67	0.84	0.23	27.95
	0.73	0.62	0.68	0.08	11.52
	0.74	0.59	0.67	0.11	15.95
	0.98	0.90	0.94	0.06	6.02
	0.91	0.69	0.80	0.16	19.45
	0.60	0.43	0.52	0.12	23.34
				Group:	**19.06**
Combined				**Overall:**	**16.43**

	LVIDd AS	LVIDd JC	mean	SD	CV
Controls	4.8	4.6	4.70	0.14	3.01
	5.0	5.4	5.20	0.28	5.44
	4.9	5.0	4.95	0.07	1.43
	4.3	3.9	4.10	0.28	6.90
	4.1	4.6	4.35	0.35	8.13
	4.9	4.1	4.50	0.57	12.57
	4.4	4.6	4.50	0.14	3.14
	4.2	3.7	3.95	0.35	8.95
	4.4	4.7	4.55	0.21	4.66
	5.0	5.2	5.10	0.14	2.77
				Group:	**5.70**
Healthy pregnant	4.9	4.7	4.80	0.14	2.95
	4.6	2.9	3.75	1.20	32.06
	5.2	5.2	5.20	0.00	0.00
	5.3	4.3	4.80	0.71	14.73
	4.7	3.8	4.25	0.64	14.97
	5.2	5.2	5.20	0.00	0.00
	4.8	4.9	4.85	0.07	1.46
	3.9	3.9	3.90	0.00	0.00
	4.1	2.7	3.40	0.99	29.12
	4.2	3.1	3.65	0.78	21.31
				Group:	**11.66**
Previous hypertension	4.4	4.8	4.60	0.28	6.15
	3.3	3.8	3.55	0.35	9.96
	4.5	4.5	4.50	0.00	0.00
	5.5	5.8	5.65	0.21	3.75
	3.6	3.8	3.70	0.14	3.82
	4.5	5.3	4.90	0.57	11.54
	4.9	5.3	5.10	0.28	5.55
	5.1	5.4	5.25	0.21	4.04
	4.5	4.7	4.60	0.14	3.07
	4.3	4.2	4.25	0.07	1.66
				Group:	**4.96**
Combined				**Overall:**	**7.44**

	E AS	E JC	mean	SD	CV
Controls	104.9	115.6	110.23	7.54	6.84
	78.8	77.5	78.13	0.94	1.21
	72.6	73.8	73.20	0.90	1.22
	73.6	71.4	72.50	1.51	2.08
	97.1	94.8	95.93	1.60	1.67
	74.9	75.0	74.97	0.09	0.13
	87.2	88.1	87.62	0.64	0.73
	85.2	86.0	85.60	0.61	0.72
	88.7	89.4	89.02	0.49	0.56
	88.3	89.2	88.78	0.64	0.72
				Group:	**1.59**
Healthy pregnant	71.6	66.2	68.90	3.82	5.54
	107.6	106.5	107.05	0.78	0.73
	91.4	89.0	90.20	1.70	1.88
	73.4	73.3	73.35	0.12	0.16
	83.6	72.8	78.20	7.64	9.77
	127.5	125.5	126.50	1.46	1.16
	62.5	66.0	64.25	2.43	3.78
	97.6	97.3	97.45	0.26	0.27
	90.1	86.6	88.37	2.45	2.77
	79.9	79.2	79.52	0.49	0.62
				Group:	**2.67**
Previous hypertension	85.6	87.2	86.40	1.18	1.36
	84.1	87.3	85.72	2.29	2.67
	110.0	110.2	110.13	0.14	0.13
	84.9	92.6	88.77	5.47	6.16
	52.3	56.8	54.52	3.18	5.84
	104.2	103.8	104.00	0.28	0.27
	115.4	112.5	113.93	2.03	1.78
	77.6	77.6	77.63	0.00	0.00
	75.9	78.6	77.25	1.96	2.53
	80.3	88.4	84.32	5.73	6.79
				Group:	**2.75**
Combined				**Overall:**	**2.34**

	e' AS	e' JC	mean	SD	CV
Controls	10.8	12.1	11.47	0.90	7.81
	10.1	10.4	10.27	0.24	2.30
	10.7	11.0	10.85	0.26	2.39
	11.5	11.4	11.47	0.05	0.41
	9.5	9.4	9.43	0.09	1.00
	14.1	14.3	14.17	0.14	1.00
	13.0	13.2	13.08	0.12	0.90
	12.7	12.8	12.77	0.09	0.74
	14.0	13.8	13.90	0.14	1.02
	13.9	14.4	14.15	0.31	2.17
				Group:	**1.97**
Healthy pregnant	6.3	6.7	6.48	0.26	4.00
	14.5	14.3	14.43	0.14	0.98
	13.1	13.3	13.22	0.16	1.25
	12.0	12.0	12.00	0.05	0.39
	5.2	5.5	5.35	0.26	4.85
	9.0	8.9	8.97	0.05	0.53
	8.9	8.7	8.80	0.19	2.14
	12.6	12.6	12.63	0.00	0.00
	14.8	15.0	14.87	0.14	0.95
	9.2	9.5	9.35	0.21	2.27
				Group:	**1.74**
Previous hypertension	16.1	16.4	16.25	0.26	1.60
	11.1	11.6	11.37	0.38	3.32
	9.8	11.0	10.40	0.80	7.71
	10.2	9.9	10.07	0.24	2.34
	11.0	11.1	11.08	0.07	0.64
	8.1	8.3	8.20	0.09	1.15
	12.1	12.6	12.33	0.38	3.06
	8.0	8.1	8.05	0.02	0.29
	9.8	10.2	10.00	0.28	2.83
	12.2	11.5	11.82	0.49	4.19
				Group:	**2.71**
Combined				**Overall:**	**2.14**

	E/e' AS	E/e' JC	mean	SD	CV
Controls	9.7	9.6	9.62	0.09	0.97
	7.8	7.4	7.61	0.27	3.50
	6.8	6.7	6.75	0.08	1.17
	6.4	6.2	6.32	0.11	1.67
	10.2	10.1	10.17	0.07	0.67
	5.3	5.3	5.29	0.05	0.87
	6.7	6.7	6.70	0.01	0.17
	6.7	6.7	6.70	0.00	0.02
	6.3	6.5	6.40	0.10	1.57
	6.3	6.2	6.28	0.09	1.45
				Group:	**1.21**
Healthy pregnant	11.4	9.9	10.65	1.01	9.53
	7.4	7.4	7.42	0.02	0.25
	7.0	6.7	6.83	0.21	3.13
	6.1	6.1	6.11	0.03	0.55
	16.2	13.2	14.67	2.14	14.58
	14.2	14.0	14.11	0.09	0.63
	7.0	7.6	7.31	0.43	5.92
	7.7	7.7	7.71	0.02	0.27
	6.1	5.8	5.94	0.22	3.72
	8.7	8.3	8.51	0.25	2.89
				Group:	**4.15**
Previous hypertension	5.3	5.3	5.32	0.01	0.23
	7.6	7.5	7.54	0.05	0.65
	11.2	10.1	10.62	0.80	7.58
	8.3	9.4	8.83	0.75	8.50
	4.7	5.1	4.92	0.26	5.20
	12.8	12.6	12.68	0.18	1.42
	9.6	8.9	9.24	0.45	4.84
	9.7	9.6	9.64	0.03	0.29
	7.7	7.7	7.73	0.02	0.30
	6.6	7.7	7.15	0.78	10.97
				Group:	**4.00**
Combined				**Overall:**	**3.12**

The low CVs suggest that any differences observed in echocardiographic parameters in my study are due to real changes. It is recognised that echocardiography can have around 10% intra- and inter-observer variability for volumes.

4.4 Blood pressure measurement

4.4.1 Method for blood pressure measurement

Blood pressure was measured with a digital blood pressure monitor (Omron Corporation, Tokyo, Japan). This automated, electronic, oscillometric device is validated for use in pregnancy[56] and was calibrated throughout the study. The brachial artery of the non-dominant arm was used for the blood pressure recording. Care was taken to ensure that the arm was free of clothing and that appropriate cuff size was used depending on mid-arm circumference. Blood pressure was recorded whilst the patient was seated comfortably and silently, with the arm supported at the level of the heart. The woman was asked to sit upright and still, with her back well supported, legs uncrossed and feet flat on the floor. This procedure followed the echocardiogram, therefore the patient was rested and the same precautions regarding variables such as caffeine applied. Three readings were taken one minute apart and the mean average was calculated.

4.5 Pulse Wave Analysis

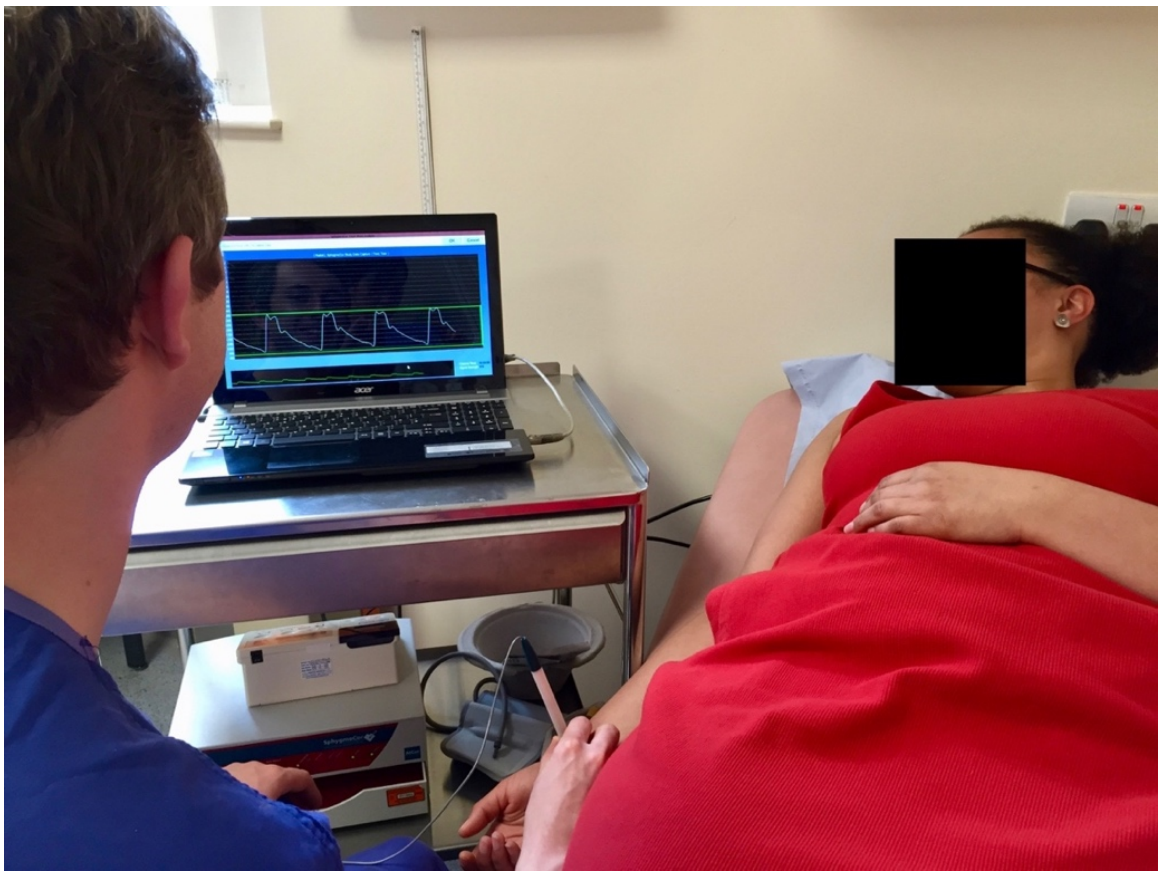
The concept of arterial stiffness was described in the introductory chapter (**section 1.1.3)** along with a description of the Pulse Wave Analysis technique and the augmentation index which it measures.

4.5.1 Equipment and software

Arterial stiffness was measured using the digital volume pulse analysis (DVP) technique. The DVP analysis method is a non-invasive technique of measuring pulse wave reflections to

determine arterial stiffness peripherally. The SphygmoCor device (Atcor Medical, West Ryde, Australia) equipped with a hand-held tonometer like a pencil (Millar Instruments, Houston, Texas, USA) was used to perform pulse wave analysis (see **Figure 13**). There is a micromanometer within the tip of the tonometer to record the pressure within the radial artery.

Figure 13: Photograph of pulse wave analysis



4.5.2 Data acquisition

Pulse wave analysis followed the procedures for echocardiography and blood pressure measurements. The women were rested and had not been exposed to caffeine, cigarettes etc on the day of the study. Subjects were asked not to move or speak during the measurements,

which were performed in a semi-recumbent position with a left lateral tilt to ensure consistency with the echocardiogram methodology. If a cardiac arrhythmia was present on the ECG this was noted. The radial artery was palpated and the point of maximal pulsation identified. The tonometer tip was placed at this point. The radial artery waveforms were recorded over 10 seconds. The aortic pressure waveform is derived from the radial artery waveform by a mathematical transfer function in the Sphygmocor software (Sphygmocor Cardiovascular Management Suite Version 9). Similarly the software was used to derive the aortic pressure and augmentation index.

4.5.3 Reproducibility data

My reproducibility data for Pulse Wave Analysis are shown in **Table 19** and **Table 20**. Good reliability was achieved after a period of supervised training, prior to acquiring measurements for the study. Measurements were obtained from three healthy volunteers on three consecutive days to calculate interassay CVs. For intra-assay CVs, three consecutive measurements were taken on the same day.

Table 19: Inter-assay Coefficient of Variation for pulse wave analysis

Subject	Measurement	Day 1	Day 2	Day 3	Mean	SD	CV
1	AoS	90	95	99	95	5	4.8
	AoD	60	64	73	66	7	10.1
	MAP	72	78	84	78	6	7.7
	HR	67	63	65	65	2	3.1
	AIx	15	10	13	13	3	19.9
	OI	100	100	94	98	4	3.5
2	AoS	113	106	105	108	4	4.0
	AoD	79	73	68	73	6	7.5
	MAP	95	89	85	90	5	5.6
	HR	72	75	73	73	2	2.1
	AIx	25	29	33	29	4	13.8
	OI	99	86	100	95	8	8.2
3	A S	100	100	100	100	0	0
	AoD	74	74	74	74	0	0
	MAP	87	86	86	86	1	0.7
	HR	66	69	66	67	2	2.6
	AIx	19	20	19	19	1	3.0
	OI	93	91	89	91	2	2.2

Measurement	Mean inter-assay CV (%)
AoS (mmHg)	2.9
AoD (mmHg)	5.9
MAP (mmHg)	4.7
HR (beats per minute)	2.6
AIx (%)	12.2
OI (arbitrary)	4.6

Aix, aortic augmentation index (%); AoD, aortic diastolic pressure (central, mmHg); AoS, aortic systolic pressure (central, mmHg); CV, coefficient of variation (%); HR, heart rate (beats per minute); MAP, mean arterial pressure (central, mmHg); OI, operator index (arbitrary); SD, standard deviation

Table 20: Intra-assay coefficient of variation for pulse wave analysis

		Measurement					
Subject	**Parameter**	**1**	**2**	**3**	**Mean**	**SD**	**CV**
1	AoS	90	91	90	90	1	0.6
	AoD	61	61	60	61	1	1.0
	MAP	74	75	72	74	2	2.1
	HR	69	69	67	68	1	1.7
	Aix	14	6	15	12	5	42.3
	OI	96	74	100	90	14	15.6
2	AoS	113	113	112	113	1	0.5
	AoD	79	78	79	79	1	0.7
	MAP	95	95	95	95	0	0
	HR	72	75	75	74	2	2.3
	AIx	25	25	22	24	2	7.2
	OI	99	98	99	99	1	0.6
3	AoS	120	119	119	119	1	0.5
	AoD	89	89	89	89	0	0
	MAP	102	103	103	103	1	0.6
	HR	60	60	64	61	2	3.8
	Aix	36	34	34	35	1	3.3
	OI	97	90	87	91	5	5.6

Parameter	Mean intra-assay CV (%)
AoS (mmHg)	0.5
AoD (mmHg)	0.6
MAP (mmHg)	0.9
HR (beats per minute)	2.6
AIx (%)	17.6
OI (arbitrary)	7.3

Aix, aortic augmentation index (%); AoD, aortic diastolic pressure (central, mmHg); AoS, aortic systolic pressure (central, mmHg); CV, coefficient of variation (%); HR, heart rate (beats per minute); MAP, mean arterial pressure (central, mmHg); OI, operator index (arbitrary); SD, standard deviation

The Sphygmacor device has good repeatability and reproducibility.[249-251]. Healthy young women would be expected to have more compliant vessels, which can result in a negative value for augmentation index and a corresponding increased variability.

4.6 Blood sampling

4.6.1 Procedure for venepuncture

The SWBH SOP for venepuncture is included in the Appendix. Essentially blood was taken from the antecubital vein in the same standardised environment as the rest of the study protocol. Venepuncture was performed last to facilitate prompt processing of the samples. Consent for venepuncture was a separate item on the informed consent form. If a woman was willing to participate in the study but not willing to give blood for research purposes she was not excluded from the study.

4.6.2 Laboratory measurements

The full blood count was performed by the Haematology laboratory at SWBH according to their established SOPs. Similarly the Clinical Biochemistry department at SWBH conducted tests of urea, creatinine and urine PCR.

4.7 Flow cytometry

4.7.1 Equipment and software

I used a FACSCalibur flow cytometer (Becton Dickinson [BD], Oxford, UK). VenturiOne, Verson 3.1 software (Applied Cytometry, Sheffield, UK) was used to process the data.

4.7.2 Procedure for flow cytometry

The methodology has been published by my supervisors previously[174] and the SOPs are well established in our laboratory. Previous researchers in our group have shown that the methods are robust and reliable. The contemporary definitions of the monocyte subsets, recently described in a consensus statement[171], are outlined in **Chapter 2**. A 'Mastermix' of four mouse anti-human monoclonal fluorochrome conjugated antibodies was made in small batches. The antibodies used were anti-CD16-Alexa Fluor 488 (clone DJ130c, Bio-Rad Laboratories, Oxford, UK), anti-CD14-PE (clone MϕP9, BD), anti-CD42a-PerCP (clone Beb1, BD) and anti-CCR2-APC (clone 48607, R&D Systems, Abingdon, UK).

A 12.5μL volume of Mastermix (including anti-CD14-PE 2.5μL; anti-CD16 2.5μL; anti-CD42a-PerCP 5μL and anti-CCR2-APC 2.5μL) was added to a TruCount™ tube (BD, Oxford, UK) containing a precise number of fluorescent count beads. To this tube, 50μL of

EDTA-anticoagulated whole blood was added. The TruCount™ tube was then incubated for 15 minutes. Lysis of the red blood cells was achieved using 450μL FACS Lysing solution (BD) and a further 15 minutes incubation. The sample was diluted with 1500μL of phosphate buffered saline prior to immediate flow cytometric analysis. Appropriate isotype controls were used to define CD14, CD16 and CCR2 and monocyte subsets were defined as CD14++CD16-CCR2+ (Mon1), CD14++CD16+CCR2+ (Mon2) and CD14+CD16++CCR2- (Mon3).[171] Time delay between sample collection and processing was always less than one hour, as delays in processing can compromise results.[252]

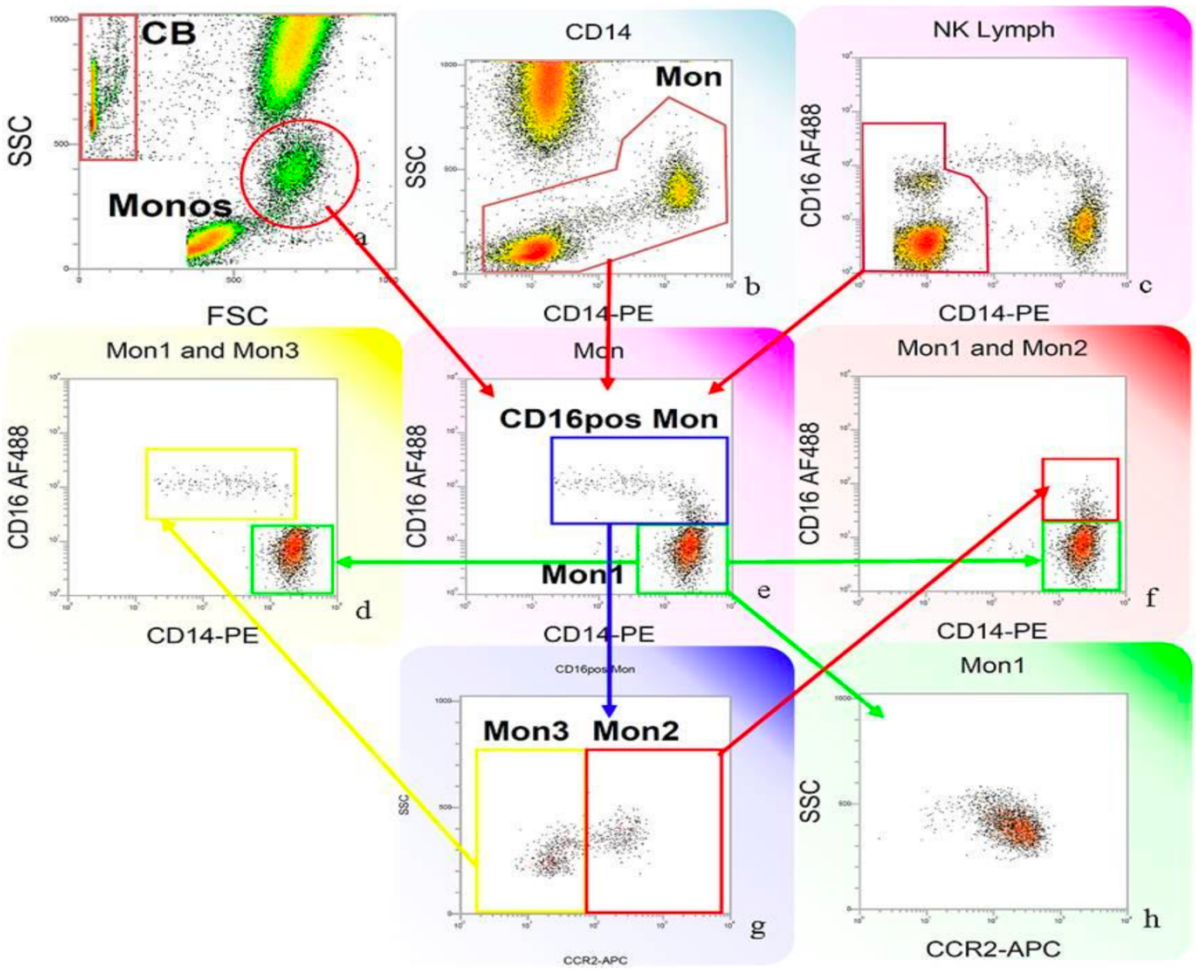
The gating system used in flow cytometry is shown in **Figure 14**. The left panel shows selection of monocytes based on forwards scatter and side scatter. The central panel shows selection of monocytes (and exclusion of granulocytes) based CD14 expression and side scatter. In the right panel cells that belong to both left and central panels are selected here to separate monocyte subsets based on their CD14 and CD16 expression. CD16+ cells must then be separated into Mon2 and Mon3 based on CCR2 expression. This gating technique is

The absolute monocyte count (cells/μL) was calculated from the total number of monocytes proportional to the number of TruCount™ beads. Each subset could be counted individually according to the gating strategy.

Detection of monocyte-platelet aggregates (MPAs) was achieved by their CD42a positivity. CD42a (glycoprotein IX) is a platelet marker and MPA events were positive for monocyte and platelet markers. The MPAs associated with each monocyte subset, and the total number of MPAs, were expressed as a proportion of the number of TruCount™ beads as described

above. Thus, MPAs associated with Mon1 (MPA1) are CD14++CD16-CCR2+CD42a+, MPAs associated with Mon2 (MPA2) are CD14++CD16+CCR2+CD42a+ and those MPAs associated with Mon3 (MPA3) are CD14+CD16++CCR2-CD42a++.

Figure 14: Gating strategy to identify monocyte subsets



Monocytes are identified by their forward and side scatter (a, top left). Granulocytes (neutrophils) are distinguished firstly by their higher degree of side scatter secondly by lack of CD14 expression, and are excluded (b). Panel (c) separates natural killer cells (CD14-) and monocytes (CD14+). Panels (d) (e) and (f) identify the Mon1 subset (in green boxes) as CD14++CD16- cells. CCR2 expression (g) is then used to separate the CD16+ subsets, Mon2 and Mon3. Mon2 are CD14+CD16++CCR2- (in red boxes) and Mon3 are

CD14+CD16++CCR2- (yellow boxes). (Reproduced with permission and thanks to Eduard Shantsila[253]).

4.7.3 Reproducibility data

My reproducibility data for flow cytometry are shown in the following tables. The reproducibility of the method for repeated measures of the same sample (test-retest repeatability) was tested by taking a blood sample from three healthy volunteers. Using the original sample, three further samples were prepared and analysed consecutively to calculate the intra-assay CVs (**Table 21**). Similarly, results of repeated measures of the same sample analysed by two researchers (JSC and RB) were used to calculate the inter-observer CVs (**Table 22**). All CVs are expressed as a percentage.

Table 21: Intra-assay variability for flow cytometry

		Measurement					
Subject	**Parameter**	**1**	**2**	**3**	**Mean**	**SD**	**CV (%)**
1	Mon 1	420	390	391	400	17	4.3
	Mon 2	17	18	13	19	3	12.8
	Mon 3	14	13	12	13	1	9.5
	Overall Mon	451	421	424	432	17	3.8
	MPA 1	46	40	44	44	3	6.9
	MPA 2	2	3	4	3	1	30.9
	MPA 3	2	2	1	2	0	18.6
	Overall MPA	51	45	49	48	3	5.8
2	Mon 1	437	447	427	437	10	2.3
	Mon 2	13	17	52	27	21	77.2
	Mon 3	36	35	34	35	1	2.4
	Overall Mon	486	499	513	500	13	2.7
	MPA 1	37	42	37	39	3	8.3
	MPA 2	3	3	7	4	2	58.2
	MPA 3	4	5	4	4	0	4.4
	Overall MPA	44	50	48	47	3	6.5
3	Mon 1	399	401	397	399	2	0.6
	Mon 2	40	43	38	4	3	6.5
	Mon 3	58	58	56	57	1	1.6
	Overall Mon	497	502	491	497	6	1.1
	MPA 1	31	27	25	28	3	10.1
	MPA 2	3	4	3	3	1	25.7
	MPA 3	5	5	7	6	1	25.1
	Overall MPA	38	37	35	37	2	4.2

CV, coefficient of variation (%); SD, standard deviation

Average intra-assay coefficient for variation for flow cytometry

Measure	**CV (%)**
Mon 1	2.4
Mon 2	32.2
Mon 3	4.5
Total Mon	2.5
MPA 1	8.4
MPA 2	38.3
MPA 3	16.0
Total MPA	5.5

The average intra-assay CV is 2.5% for total monocytes and 5.5% for total MPAs.

Table 22: Inter-observer variability for flow cytometry

Subject		Mon 1	Mon 2	Mon 3	Mon	MPA 1	MPA 2	MPA 3	MPA
1	JC	632	43	44	719	48	4	5	58
	RB	590	70	50	710	49	9	9	67
	Mean	611	57	47	715	49	7	7	62
	SD	30	19	5	7	0	3	3	7
	CV	4.9	32.8	10.1	1.0	0.9	52.0	37.8	10.5
2	JC	311	9	23	343	18	2	2	22
	RB	278	23	18	318	22	7	3	33
	Mean	294	16	20	330	20	4	3	27
	SD	24	10	4	18	3	3	0	7
	CV	8.0	62.3	18.2	5.3	15.1	83.9	13.2	25.4
3	JC	190	9	17	216	13	1	3	17
	RB	180	14	20	214	11	5	4	21
	Mean	185	12	19	215	12	3	3	19
	SD	7	4	2	1	1	3	1	3
	CV	4.0	32.1	12.0	0.7	11.8	99.2	32.1	14.5
4	JC	289	38	61	388	21	5	10	35
	RB	228	44	50	322	15	6	4	25
	Mean	259	41	55	355	18	5	7	30
	SD	43	4	8	47	4	1	4	7
	CV	16.6	10.4	14.4	13.1	22.5	22.1	55.4	22.1
5	JC	331	11	18	359	30	7	3	40
	RB	331	13	18	362	35	5	3	43
	Mean	331	12	18	361	32	6	3	41
	SD	0	1	0	2	3	1	0	3
	CV	0.1	11.8	0.2	0.4	10.7	15.7	5.8	6.6

CV, coefficient of variation (%); SD, standard deviation

Average inter-observer coefficient of variation

Measure	CV (%)
Mon 1	6.7
Mon 2	29.9
Mon 3	11.0
Total Mon	4.1
MPA 1	12.2
MPA 2	54.6
MPA 3	28.9
Total MPA	15.8

The average inter-observer CV is 4.1% for total monocytes and 15.8% for total MPAs. The CVs were consistent with previous work from our group. Diurnal variation and exercise status were not controlled for and as such represent limitations. The Institute for Cardiovascular Sciences have robust SOPs and adherence to principles of good laboratory practice is expected of all researchers. I was assessed as being competent to perform all the necessary assays prior to recruiting women for my research.

4.8 Power calculation

For cross-sectional comparison the power calculation was based on the key elastance parameter, arterial elastance index (EaI). It was hypothesised that Group 1 would have higher EaI compared to Group 2. In previous published work patients with high risk hypertension (which is likely to show similar values to pregnancy hypertension) had EaI of 1.32±0.47 mm

Hg/ml/m^2.[32] It was hypothesised that this parameter will be lower by half of a standard deviation in Group 2 (i.e. 1.09 mm Hg/ml/m^2). Thus, in order to achieve this difference in variance at $1–\beta = 0.8$ and $P<0.05$ (ANOVA F statistic approximately 10), 45 subjects per group were required. However, to provide additional confidence, the recruitment target was 50. This number of participants would also suffice for assessment of longitudinal changes in study parameters. After performing the systematic review in **Chapter 2**, it became clear that there are other outcomes of interest and that a descriptive analysis would provide valuable insight.

The National Institute for Health and Clinical Excellence (NICE) guideline on Hypertension in Pregnancy[1] states that about 10% of pregnancies are complicated by a hypertensive disorder and that this rate is increasing. About 2–3-fold higher rates of hypertensive disorders are expected in patients with hypertension during a previous pregnancy.

4.9 Statistical Analysis

Baseline characteristics were statistically tested for normality of distribution using the Shapiro-Wilk test. Normally distributed data are expressed as mean and standard deviation (SD). Continuous data which are not normally distributed are expressed as median and interquartile range (IQR, described as the 25th and 75th centiles). Categorical data, such as smoking status, are expressed as number and percentage.

Cross-sectional data were subjected to one way analysis of variance (ANOVA) for normally distributed data and Kruskal-Wallis test for non-normally distributed data. Appropriate post hoc testing was performed to account for comparisons between the three groups, using

Tukey's test of pairwise comparisons for normally distributed and the Dunn-Bonferroni method for non-normally distributed data respectively. Categorical data are compared using Fisher's exact test for two groups and the chi-squared test with appropriate degrees of freedom for three groups.

The data acquired in the prospective study was analysed using non-parametric statistical tests for consistency of presentation style. The Kruskal-Wallis test was used to analyse differences between groups and the Mann-Whitney test was applied to comparisons between the two groups of pregnant women.

Longitudinal data were analysed using repeated measures ANOVA, with pairwise comparison of each time point to the baseline time point. Subsequently the Wilcoxon matched-pairs signed rank is applied to within-group comparisons of data from different time points. This analysis required restructuring of the data into a long format with repeated measures from all appointments recorded in a single column. Each appointment represented a time point. These time points were considered as ordinal variables with fixed gaps. The first appointment (baseline) was the reference time point. Before employing the repeated measures ANOVA, data were explored to verify that the test's assumptions were met. Accordingly, the test was only applied to continuous data. The time point of the study appointment was the independent variable ("within subjects factor"), with the same subjects present in each related group with measurements of the same dependent variable (the experimental measurement). If the dependent variable was not normally distributed (Shapiro Wilk $P \geq 0.05$), then the data were log transformed. Box plots were created to ensure that there were no significant outliers which would reduce accuracy. Since the final assumption of sphericity (equality of the

variances of the differences between all levels of time points) is so often not valid, Greenhouse-Geisser procedure is used throughout. The Greenhouse-Geisser correction elicits are more accurate significance value as it takes account of repeated measures ANOVA's being too liberal when sphericity is violated.

Correlations between continuous variables are performed using Pearson correlation analysis. Predictive associations are assessed using regression models. Logistic regression is used for categorical variables and linear regression for continuous data. Prior to correlation and regression analysis, the assumptions that underpin these statistical methods were tested and checked. Non-normally distributed data were log transformed. Pairwise deletion for missing data was done where applicable.

A 2-tailed p-value of <0.05 is considered statistically significant, after appropriate post hoc testing where appropriate. This adjustment reduces the chance of finding an association by chance (type 1 error).

The data were entered into a spreadsheet (Excel, Microsoft) and checked for accuracy. The statistical analysis was performed using Stata® (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

4.10 Ethical considerations

This research was approved by the Research and Development Department of Sandwell and West Birmingham Hospitals NHS Trust (Reference 13CARD65, 27/11/13), following review by the institution's Ethics Committee. Prospective approval for the study was also obtained

from the Local Research Ethics Committee for West Midlands (Reference 13/WM/0472, 07/01/14). All participants gave written informed consent and confirmed ongoing consent at each follow up appointment. The study was conducted in accordance with the Declaration of Helsinki.

4.11 Methods Summary

In this chapter I have provided a comprehensive overview of my study design, experimental methods and analysis plan. I have shown the reproducibility data, which were completed prior to recruitment in order to confirm that my techniques were valid. My coefficients of variation were in line with expectations based on existing literature and previous work within the ICVS.

CHAPTER 5:
MATERNAL CARDIAC FUNCTION IN WOMEN WITH PREVIOUS GESTATIONAL HYPERTENSIVE DISEASE

5.1 Introduction to chapter

This is the first of three chapters containing experimental data. The prospective study at the heart of this thesis, Evaluating Cardiovascular Changes in Hypertension in Obstetrics (ECCHO), was designed to assess the differences in cardiac structure and function in women with a history of gestational hypertensive disease compared to pregnant women without prior hypertension. The demographic and clinical data for all women recruited into the study are outlined at the beginning of this chapter. Thereafter the data from the echocardiographic and vascular studies are presented and discussed, starting with the cross-sectional comparisons at baseline, before continuing to analyse the longitudinal data.

5.2 Aims and hypothesis

The aims and hypotheses of this work are stated in **Chapter 3**. To briefly recap, the hypotheses to be tested are:

1) abnormal ventricular-arterial interaction will be found in women with a history of hypertension in pregnancy.

2) changes in cardiac structure and function over time may be predictive of recurrent gestational hypertensive disease.

5.3 Methods and materials

5.3.1 Study groups

A total of 115 women were enrolled in the study. Group 1 comprised 25 pregnant women with previous hypertension in pregnancy and Group 2 comprised 50 pregnant women without history of hypertension. For cross sectional comparison at baseline there were 40 non-

pregnant controls in Group 3.

5.3.2 Statistical analysis

The statistical analysis plan is described in detail in **section 4.9**. For cross sectional comparison of normally distributed data across the three groups, one-way ANOVA was used, with the post hoc Tukey test applied for pairwise comparisons if a significant difference was detected. For consistency, the Kruskal-Wallis non-parametric test was used to compare the experimental results. Where appropriate, post hoc testing with the Dunn Bonferroni test was applied and differences were considered significant if $P<0.01$ to account for multiple testing. For longitudinal analysis, repeated measures ANOVA was used.

5.4 Results

5.4.1 Patient characteristics

The study groups were well matched for age ($P=0.74$) and ethnicity ($P=0.33$) with a slight majority (50.4%) coming from non-white racial groups **(Table 23)**. There was no difference in maternal medical history apart from gestational hypertensive disease, which by design is unique to Group 1. Where asthma is noted, the cases were mild in severity, with inhaler use required only occasionally and not on the day of the study. The difference in parity between the groups is also a feature of the study design, since women in Group 1 are required to have a previous pregnancy. The number of women taking aspirin was significantly higher in Group 1, since this daily medication is recommended to women previously affected by hypertension in pregnancy for secondary prevention. This is discussed later as a limitation.

Table 23: Demographic and clinical characteristics at baseline

Characteristic	Group 1 **Previous hypertension** (n=25)	Group 2 **Previous normotensive** (n=50)	Group 3 **Non-pregnant** (n=40)	***P***
Age, years	30 (27–33)	29 (25–33)	28 (25–34)	0.74
Parity, *n* (%)				
Nulliparous	0 (0)	22 (44)	28 (70)	<0.001
Parous	25 (100) *†	28 (56)	12 (30)	
Ethnicity, *n* (%)				
White	13 (52)	21 (42)	23 (58)	0.49
South Asian	9 (36)	19 (38)	9 (22)	
Black	1 (4)	7 (14)	5 (13)	
East Asian	0 (0)	1 (2)	2 (5)	
Other/mixed	2 (8)	2 (4)	1 (2)	
White	13 (52)	21 (42)	23 (58)	0.33
Non-white	12 (48)	29 (58)	17 (42)	
Medical history, *n* (%)				
Asthma	3 (12)	2 (4)	3 (8)	0.36
Diabetes	1 (4)	3 (6)	0 (0)	0.30
Medications, *n* (%)				
Aspirin	11 (44) *†	1 (2)	0 (0)	<0001
Hormonal contraception	0 (0)	0 (0) ††	13 (33)	<0.001
Antidepressant	0 (0)	0 (0)	1(3)	0.39
Azathioprine	1 (4)	1 (2)	0 (0)	0.48
Enoxaparin	0 (0)	1 (2)	0 (0)	0.52
Metformin	0 (0)	2 (4)	0 (0)	0.30
Insulin	0 (0)	1 (2)	0 (0)	0.52
Salbutamol	3 (12)	1 (2)	2 (5)	0.19
Thyroxine	0 (0)	3 (6)	0 (0)	0.14
Family history, *n* (%)				
Essential hypertension	11 (44)	13 (26)	15 (38)	0.25
Pregnancy hypertension	3 (12)	4 (8)	5 (13)	0.77
Smoking, *n* (%)				
Current smoker	2 (8)	2 (4)	1 (2)	0.57
Never smoker	17 (68)	43 (86)	38 (95)	0.01

Continuous data are expressed as median (interquartile range). Categorical data are expressed as *n* (%). Post hoc testing: * *P* <0.01 Group 1 vs Group 2; †*P*<0.01 Group 1 vs Group 3; ‡*P*<0.01 Group 2 vs Group 3.

There was no difference in family history of hypertension. There was no significant difference in current smoking status, however previous smoking habits varied, with the pregnant women previously affected by hypertension more likely to have smoked in the past (P=0.03 for Group 1 vs Group 3). Group 1 comprised 9 women with previous gestational hypertension, 9 with previous early onset preeclampsia and 7 with previous late onset preeclampsia. All had normal blood pressure and were free of antihypertensive medication at enrollment, in accordance with the study protocol.

5.4.2 Baseline measurements

The pregnant participants were studied at 13±1 completed weeks of gestation (P=0.42). The non-pregnant women were included for cross sectional comparison at baseline. There was no significant difference in weight (P=0.55) or height (P=0.054), although the non-pregnant women tended to be taller. The groups were matched for BMI (P=0.12) and BSA (P=0.74) **(Table 24)**.

The protocol mandated that blood pressure be normal for all women at the start of the study. Accordingly, systolic blood pressure (SBP) was less than 140mmHg and diastolic blood pressure (DBP) was less than 90mmHg at baseline for all. Women in Group 1 had significantly higher peripheral SBP at baseline compared to Groups 2 and 3 (P<0.01 for both). Peripheral and central DBP and mean arterial pressure (MAP) and central SBP were significantly higher in Group 1 compared to Group 2. Group 2 demonstrated a non-significant reduction in blood pressure compared to Group 3. Heart rate was increased in pregnant women compared to non-pregnant controls (P<0.001).

Table 24: Baseline clinical measurements

Characteristic	Group 1 **Previous hypertension** (n=25)	Group 2 **Previous normotensive** (n=50)	Group 3 **Non-pregnant controls** (n=40)	*P*
Gestation (weeks)	13±1	13±1	n/a	0.42
Height (m)	1.61±0.08	1.64±0.07	1.66±0.08	0.054
Weight (kg)	73 (58–85)	72 (59–81)	67 (58–83)	0.55
BMI (kg/m^2)	27.7 (23.2–31.2)	26.5 (22.3–31.6)	23.8 (21.0–28.8)	0.12
BSA (m^2)	1.78±0.29	1.80±0.22	1.76±0.21	0.74
SBP (mmHg)	115 (113–121) *†	108 (100–114)	112 (101–119)	0.001
DBP (mmHg)	71 (65–75) *	64 (50–69)	66 (60–72)	0.003
MAP (mmHg)	87 (81–91) *	77 (72–82)	81 (74–86)	<0.001
cSBP (mmHg)	102 (95–106) *	91 (85–96)	97 (86–102)	0.001
cDBP (mmHg)	72 (68–77) *	65 (59–70)	67 (61–73)	0.004
cMAP (mmHg)	85 (80–90) *	77 (72–82)	81 (74–86)	0.003
Heart rate (bpm)	79±10 †	74±9 ‡	65±10	<0.001
Hb (g/L)	120±11†	122±11 ‡	132±11	<0.001
WCC (x10^9/L)	9.4 (7.2–10.9) †	8.3 (7.0–9.4) ‡	6.3 (5.3–6.9)	<0.001
Platelets (x10^9/L)	253 (210–291)	247 (202–292)	270 (249–303)	0.20
Creatinine (μmol/L)	51 (49–54) †	53 (50–56) ‡	66 (62–71)	<0.001

BMI, body mass index; bpm, beats per minute; BSA, body surface area; cDBP, central diastolic blood pressure; cMAP, central mean arterial pressure; cSBP, central systolic blood pressure; DBP, diastolic blood pressure; Hb, haemoglobin; MAP, mean arterial pressure; n/a, not applicable; SBP, systolic blood pressure; WCC, white cell count. Data expressed as median (interquartile range) or as mean ± standard deviation.
Post hoc testing:
*p<0.01 Group 1 vs Group 2
†p<0.01 Group 1 vs Group 3
‡p<0.01 Group 2 vs Group

5.4.3 Cross sectional analysis of echocardiographic parameters at baseline

Haemodynamics

Whilst the median stroke volume was the same for both groups of pregnant women at baseline, the increase compared to non-pregnant controls reached statistical significance only for Group 2 (P=0.003), which persisted after adjusting for BSA (see **Table 25**). Cardiac output and cardiac index were significantly increased in pregnant women (P<0.001), with similar values observed regardless of previous hypertension. This higher cardiac output was observed alongside reduced resistance, with total vascular resistance index (TVRI) significantly lower in Group 1 (P=0.001) and Group 2 (P<0.001) compared with Group 3. There was no significant difference in the systolic tissue Doppler average velocity at the septal and lateral mitral valve annuli.

Cardiac structure

The size of the left atrium was unchanged across groups. Pregnant women exhibited a significant increase in left ventricular volume and its index to BSA (P<0.001). Women with previous gestational hypertension had a significantly higher left ventricular mass index compared to non-pregnant women (P=0.006), whilst the increase compared to pregnant women without hypertension history was not significant with the Bonferroni adjustment of alpha to account for multiple comparisons (P=0.03). There was no significant difference in the cross-sectional comparison of left ventricular end diastolic dimension, interventricular septum thickness at end diastole or relative wall thickness. The difference in posterior wall thickness at end diastole was not significantly different when post hoc testing was performed.

Table 25: Echocardiographic parameters at baseline

Parameter	Group 1 **Previous hypertension** (n=25)	Group 2 **Previous normotensive** (n=50)	Group 3 **Non-pregnant controls** (n=40)	***P***
Haemodynamics				
Stroke volume (ml)	68 (59–72)	68 (58–76) ‡	59 (51–69)	0.019
Stroke volume index (ml/m^2)	37.3 (34.0–42.8)	37.6 (33.6–43.6) ‡	32.8 (30.0–37.3)	0.017
Cardiac output (L/min)	5.0 (4.6–5.8)†	4.8 (4.5–5.6) ‡	3.8 (3.1–4.5)	<0.001
Cardiac index (L/min/m^2)	3.0 (2.7–3.2) †	2.8 (2.4–3.3) ‡	2.1 (1.8–2.4)	<0.001
Total vascular resistance (dyne.s/cm^5)	1.4 (1.2–1.5)†	1.2 (1.1–1.5) ‡	1.7 (1.5–2.1)	<0.001
Total vascular resistance index (dyne.s/cm^5/m^2)	0.80 (0.65–0.89) †	0.69 (0.58–0.89) ‡	0.97 (0.83–1.20)	<0.001
Average s' (cm/s)	9.6 (8.5–10.2)	9.5 (8.8–10.1)	9.5 (8.9–10.4)	0.84
Structure				
Left atrium volume (ml)	41 (31–45)	39 (31–46)	42 (30–51)	0.71
Left atrium volume index (ml/m^2)	22 (18–28)	22 (17–26)	23 (18–28)	0.76

Left ventricle volume (ml)	98 (84–127) †	110 (91–121) ‡	91 (80–101)	<0.001
Left ventricle volume index (ml/m^2)	59 (52–64) †	60 (50–71) ‡	51 (44–58)	<0.001
Left ventricular mass (g)	117 (95–152)	119 (97–143)	103 (81–129)	0.10
Left ventricular mass index (g/m^2)	70.9 (57.4–82.5) †	65.4 (56.8–78.7)	61.6 (49.7–69.2)	0.03
Left ventricular end diastolic dimension (cm)	4.5 (4.3–5.0)	4.5 (4.2–4.8)	4.4 (4.3–4.8)	0.93
Posterior wall thickness at end diastole (cm)	0.9 (0.7–1.1)	0.9 (0.8–1.0) ‡	0.8 (0.7–0.9)	0.047
Interventricular septum thickness at end diastole (cm)	0.79 (0.69–0.91)	0.73 (0.66–0.87)	0.72 (0.57–0.82)	0.10
Relative wall thickness	0.38 (0.32–0.49)	0.40 (0.33–0.46)	0.36 (0.29–0.41)	0.11
Diastolic function (mitral inflow)				
Early filling (E) (cm/s)	85 (75–99) †	88 (77–96) ‡	74 (67–85)	<0.001
Atrial filling (A) (cm/s)	62 (51–67) *†	48 (44–62)	49 (40–74)	0.005
E/A ratio	1.40 (1.15–1.64) *†	1.79 (1.55–2.25)	2.03 (1.54–2.32)	<0.001
e' (cm/s) Septal Lateral	 11.2 (9.7–12.5) 14.0 (12.6–16.2)	 11.2 (9.9–12.9) 15.4 (13.5–18.3)	 11.6 (10.2–12.7) 15.7 (14.7–17.7)	 0.90 0.17

E/e' (average from septal and lateral)	6.6 (5.7–8.2) †	6.5 (5.2–7.5) ‡	5.4 (4.6–6.4)	0.001
Derived values including elastance				
Left ventricular ejection fraction	0.63 (0.59–0.67)	0.63 (0.60–0.67)	0.66 (0.62–0.69)	0.066
Left ventricular end diastolic pressure (mmHg)	10.1 (9.0–12.1) †	9.9 (8.4–11.2) ‡	8.6 (7.9–9.9)	0.008
Arterial elastance (Ea)	1.6 (1.4–1.8)	1.4 (1.2–1.7) ‡	1.7 (1.5–1.9)	0.005
Arterial elastance index (EaI)	0.95 (0.79–1.11)	0.79 (0.72–0.95) ‡	0.95 (0.80–1.10)	0.004
End-systolic elastance (Ees)	2.0 (1.8–2.3)	1.9 (1.6–2.2) ‡	2.3 (1.8–2.7)	0.015
End-diastolic elastance (Eed)	0.9 (0.08–0.11)	0.08 (0.07–0.10)	0.08 (0.07–0.10)	0.24
Arterial-ventricular interaction (Ea/Ees)	0.85 (0.78–0.88)	0.81 (0.74–0.90)	0.76 (0.71–0.85)	0.084
Arterial-ventricular interaction index (EaI/Ees)	0.47 (0.42–0.59)	0.44 (0.40–0.53)	0.45 (0.41–0.48)	0.22

Data expressed as median (interquartile range) or as mean ± standard deviation. E, mitral valve early filling on Pulsed-Wave Doppler; e', velocity of early myocardial relaxation measured on tissue Doppler imaging; A, mitral valve late (atrial) filling.
Post hoc testing:
*p<0.01 Group 1 vs Group 2
†p<0.01 Group 1 vs Group 3
‡p<0.01 Group 2 vs Group 3

Diastolic function

Significant differences were observed for mitral inflow velocities. The maximum early mitral valve inflow velocity on Pulsed-Wave Doppler (E wave) was significantly greater in the pregnant women irrespective of former hypertension history (P=0.003 Group 1 vs Group 3; P<0.001 Group 2 vs Group 3). The maximum late diastolic mitral inflow velocity was significantly increased in the women with previous hypertension, compared to both unaffected pregnant women and non-pregnant women (P=0.001 for both comparisons). Similarly the calculated E/A ratio of early to late ventricular filling in diastole, was significantly lower when pregnancy was complicated by previous gestational hypertensive disease (P<0.001). There was no difference in e', the velocity of early myocardial relaxation measured on tissue Doppler imaging, between the three groups. The E/e' ratio of early mitral inflow to the average tissue Doppler early myocardial relaxation at the septal and lateral mitral valve annuli, was significantly increased in both groups of pregnant women compared to the non-pregnant controls (P=0.001 for both).

Elastance parameters and other derived values

Left ventricular ejection fraction (LVEF) was similar in all groups (P=0.07). Left ventricular end diastolic pressures were higher in each group of pregnant women compared to non-pregnant controls but not to each other (P=0.008). There was a significant decrease in arterial elastance index (EaI, P <0.001) and left ventricular end-systolic elastance (Ees, P=0.002) in healthy pregnant women in Group 2 compared to non-pregnant controls. Similar values of EaI were seen in Groups 1 and 3 and the reduction in Ees in Group 1 was not statistically significant after post hoc testing. There was no difference in EaI/Ees, the arterial-ventricular interaction index, between the three groups.

Arterial stiffness

Augmentation index, standardised to heart rate 75 beats per minute, was significantly increased in Group 1 with previous hypertension compared to Group 2 (*P*=0.004) and to Group 3 (*P*<0.001, **Table 26**). There was no significant difference in arterial stiffness in pregnancy without hypertension history compared to non-pregnant women.

Table 26: Pulse Wave Analysis at baseline

Parameter	Group 1 **Previous hypertension** (n=25)	Group 2 **Previous normotensive** (n=50)	Group 3 **Non-pregnant controls** (n=40)	***P***
Augmentation index	15 (6–19)	5 (–2–12)	7 (2–16)	0.06
Augmentation index HR75	16 (4–21) * †	4 (–3–14)	2 (–6–11)	0.003
Augmentation index HR75 adjusted for MAP	0.16 (0.05–0.24) * †	0.05 (–0.03–0.17)	0.03 (–0.08–0.13)	0.003

Data expressed as median (interquartile range) or as mean ± standard deviation. HR75 denotes adjustment to standardise for heart rate 75 beats per minute; MAP, mean arterial pressure. Post hoc testing: *p<0.01 Group 1 vs Group 2; †p<0.01 Group 1 vs Group 3; ‡p<0.01 Group 2 vs Group 3

5.4.4 Cross-sectional analysis of clinical and cardiovascular parameters at follow up

Blood pressure was persistently higher in Group 1 compared to Group 2. Systolic, diastolic and mean arterial pressures were significantly increased at 21±1and 32±1 weeks of gestation in women previously affected by gestational hypertension or preeclampsia, compared to healthy pregnant women (**Table 27**). There was no significant difference in heart rate observed between the groups of pregnant women at follow up.

A significantly lower stroke volume was observed in Group 2 in the third trimester, compared to Group 1 at the same timepoint and compared to the values observed early in pregnancy when there had been no difference between the groups. At the follow up appointments, cardiac index was significantly higher in Group 1 compared to Group 2 (P=0.025 for 2nd trimester and P=0.045 for 3rd trimester). There were no other significant differences between the groups when the previously described echocardiographic measurements were subjected to cross sectional comparison at the later gestations (**Table 28**). For augmentation index, the increase in Group 1 compared to Group 2 at baseline, was not observed later in pregnancy, when there was no significant difference between the groups.

Table 27: Clinical measurements at each visit

Characteristic	Group 1 **Previous hypertension**			Group 2 **Previous normotensive**		
	1st visit	**2nd visit**	**3rd visit**	**1st visit**	**2nd visit**	**3rd visit**
Number of women	25	24	20	50	41	34
Gestation (weeks)	13±1	21±1	32±1	13±1	20±1	32±1
SBP (mmHg)	115 (113–121) *	115 (111–127) *	119 (111–125) *	108 (100–114)	106 (97–112)	114 (102–116)
DBP (mmHg)	71 (65–75) *	71 (65–75) *	73 (64–76) *	64 (58–69)	60 (56–66)	65 (60–69)
MAP (mmHg)	87 (81–91) *	88 (79–91) *	87 (83–93) *	77 (72–82)	75 (69–82)	80 (75–86)
cSBP (mmHg)	102 (95–106) *	102 (94–108) *	102 (96–106) *	91 (85–96)	90 (81–96)	94 (87–100)
cDBP (mmHg)	72 (68–77) *	73 (66–77) *	72 (66–79) *	65 (59–70)	62 (57–67)	68 (62–72)
cMAP (mmHg)	85 (80–90) *	88 (79–91) *	85 (82–93) *	77 (72–82)	75 (69–82)	80 (75–86)

Heart rate (bpm)	79±10*	85±14	86±13	74±9	79±12	86±13
Weight (kg)	73 (58–85)	76 (62–94)	80 (68–87)	72 (59–81	74 (62–83)	80 (66–89)
BSA (m^2)	1.78±0.29	1.82±0.28	1.84±0.21	1.80±0.22	1.82±0.23	1.86±0.23
Haemoglobin (g/L)	120±11	115±10	115±10	122±11	116±8	113±10
WCC ($x10^9$/L)	9.4 (7.2–10.9) *	9.9 (7.8–11.3)	9.7 (8.2–11.4)	8.3 (7.0–9.4)	8.6 (7.5–10.0)	9.3 (8.0–10.1)
Platelets ($x10^9$/L)	253 (210–291)	259 (231–287)	263 (218–277)	247 (202–292)	265 (204–291)	248 (197–298)
Creatinine (µmol/L)	51 (49–54)	50 (45–53) *	50 (45–54)	53 (50–56)	53 (50–56)	51 (49–56)

BMI, body mass index; BSA, body surface area; cDBP, central diastolic blood pressure; cSBP, central systolic blood pressure; cMAP, central mean arterial pressure; DBP, peripheral diastolic blood pressure; MAP, peripheral mean arterial pressure; SBP, peripheral systolic blood pressure; WCC, white cell count. Data expressed as median (interquartile range) or as mean ± standard deviation. * $p<0.05$ vs group 2.

Table 28: Cardiovascular parameters at each visit

Parameter	Group 1 Previous hypertension			Group 2 Previous normotensive		
	1st visit	2nd visit	3rd visit	1st visit	2nd visit	3rd visit
Number of women	25	24	20	50	41	34
Gestation (weeks)	13±1	21±1	32±1	13±1	20±1	32±1
Haemodynamics						
Stroke volume (ml)	68 (59–72)	69 (56–75)	65 (53–75)	68 (58–76)	65 (57–72)	56 (49–64)
Stroke volume index (ml/m^2)	37.3 (34.0–42.8)	38.2 (32.0–45.0)	36.4 (28.5–41.5) *	37.6 (33.6–43.6)	35.6 (31.3–40.2)	29.0 (26.7–33.9)
Cardiac output (L/min)	5.0 (4.6–5.8)	5.8 (4.9–6.6) *	5.7 (4.6–6.1)	4.8 (4.5–5.6)	4.9 (4.3–6.0)	5.0 (4.0–6.0)
Cardiac index ($L/min/m^2$)	3.0 (2.7–3.2)	3.2 (2.9–3.7) *	3.0 (2.4–3.6) *	2.8 (2.4–3.3)	2.7 (2.4–3.5)	2.5 (2.2–3.1)
Total vascular resistance ($dyne.s/cm^5$)	1.4 (1.2–1.5)	1.3 (0.96–1.4)	1.2 (1.0–1.6)	1.2 (1.1–1.5)	1.2 (1.0–1.4)	1.3 (1.1–1.7)
Total vascular resistance index ($dyne.s/cm^5/m^2$)	0.80 (0.65–0.89)	0.65 (0.56–0.90)	0.67 (0.54–0.88)	0.69 (0.58–0.89)	0.66 (0.56–0.79)	0.70 (0.59–0.85)

Average s' (cm/s)	9.6 (8.5–10.2)	10.3 (9.1–11.1)	10.0 (8.4–11.3)	9.5 (8.8–10.1)	9.9 (9.1–10.7)	9.6 (8.8–11.1)
Structure						
Left atrium volume (ml)	41 (31–45)	41 (31–49)	34 (29–38)	39 (31–46)	40 (32–44)	39 (33–47)
Left atrium volume index (ml/m^2)	22.1 (18.5–28.0)	23.1 (18.0–25.5)	17.9 (16.3–21.2)	22.2 (17.0–26.3)	20.3 (18.0–26.1)	21.2 (15.7–26.8)
Left ventricle volume (ml)	98 (84–127)	98 (88–113)	103 (94–112)	110 (91–121)	102 (91–114)	112 (98–123)
Left ventricle volume index (ml/m^2)	56.7 (51.6–63.9)	55.3 (50.3–67.0)	63.7 (52.9–68.4)	59.5 (49.9–70.9)	57.5 (50.7–62.3)	63.7 (59.5–66.7)
Left ventricular mass (g)	117 (95–152)	114 (92–142)	132 (96–165)	119 (97–143)	108 (93–142)	130 (108–146)
Left ventricular mass index (g/m^2)	71 (57–82)	63 (53–77)	76 (54–85)	65 (57–79)	64 (54–73)	69 (62–78)
Left ventricular end diastolic dimension (cm)	4.5 (4.3–5.0)	4.5 (4.1–4.8)	4.3 (4.0–4.6)	4.5 (4.2–4.8)	4.5 (4.2–4.8)	4.5 (4.3–4.8)
Posterior wall thickness at end diastole (cm)	0.9 (0.7–1.1)	0.9 (0.7–1.1)	1.0 (0.8–1.2)	0.9 (0.8–1.0)	0.8 (0.7–1.0)	0.9 (0.8–1.1)
Interventricular septum thickness	0.79 (0.69–0.91)	0.80 (0.73–0.90)	0.81 (0.68–1.15)	0.73 (0.66–0.87	0.73 (0.59–0.95)	0.85 (0.73–0.95)

at end diastole (cm)						
Relative wall thickness	0.38 (0.32–0.49)	0.40 (0.32–0.47)	0.46 (0.37–0.57)	0.40 (0.33–0.46)	0.35 (0.30v0.46)	0.42 (0.35–0.49)
Diastolic function (mitral inflow)						
E (cm/s)	84.9 (74.9–99.4)	90.5 (80.7–103.9)	75.7 (71.5–87.8)	87.9 (77.0–95.6)	86.6 (73.8–100.0)	75.3 (64.9–87.4)
A (cm/s)	61.9 (51.0–67.1) *	59.2 (50.1–69.4)	62.7 (54.0–76.2) *	48.5 (44.4–61.5)	52.8 (42.7–62.7)	50.5 (41.7–64.1)
E/A ratio	1.4 (1.1–1.6) *	1.4 (1.3–2.0)	1.3 (1.1–1.4)	1.8 (1.5–1.9)	1.6 (1.4–2.0)	1.5 (1.2–1.8)
Average e' (cm/s)	13.1 (11.6–13.7)	12.9 (11.6–14.4)	10.9 (9.6–12.9)	13.5 (12.1–14.9)	13.4 (11.3–14.8)	12.1 (10.4–13.6)
Average E/e'	6.6 (5.7–8.2)	7.0 (6.2–8.2)	7.4 (6.2–9.2)	6.5 (5.2–7.5)	6.6 (5.4–8.4)	6.3 (5.4–7.3)
Derived values including elastance						
Left ventricular ejection fraction	0.63 (0.59–0.67)	0.61 (0.57–0.63)	0.59 (0.56–0.63) *	0.63 (0.60–0.67)	0.62 (0.58–0.64)	0.60 (0.58–0.65)
Left ventricular end diastolic pressure (mmHg)	10.1 (9.0–12.1)	10.6 (9.6–12.1)	11.0 (9.6–13.2)	9.9 (8.4–11.2)	10.1 (8.6–12.3)	10.4 (8.8–11.9)
Arterial elastance (Ea)	1.6 (1.4–1.8) *	1.5 (1.4–1.9)	1.6 (1.4–2.0)	1.4 (1.2–1.7)	1.5 (1.3–1.8)	1.8 (1.5–2.1)
Arterial elastance index (EaI)	0.95 (0.79–1.11) *	0.92 (0.80–1.07)	0.97 (0.72–1.13)	0.79 (0.72–0.95)	0.86 (0.69–0.97)	0.95 (0.80–1.16)
End-systolic elastance (Ees)	2.0 (1.8–2.3)	2.4 (2.0–2.6)	2.3 (1.9–2.7)	1.9 (1.6–2.2)	2.4 (2.0–2.7)	2.4 (1.9–2.9)

End-diastolic elastance (Eed)	0.09 (0.08–0.11)	0.09 (0.08–0.11)	0.10 (0.08–0.12)	0.08 (0.07–0.10)	0.08 (0.07–0.11)	0.09 (0.08–0.13)
Arterial-ventricular interaction (Ea/Ees)	0.85 (0.78–0.88)	0.72 (0.66–0.81)	0.76 (0.69–0.88)	0.81 (0.74–0.90)	0.69 (0.65–0.73)	0.76 (0.68–0.89)
Pulse Wave Analysis						
Augmentation index	15 (6–19) *	11 (3–18)	12 (3–21)	5 (–2–12)	6 (–2–16)	7 (1–16)
Augmentation index HR75	16 (4–21) *	16 (5–25)	15 (8–23)	4 (–3–14)	8 (2–19)	13 (5–19)
Augmentation index HR75 adjusted for MAP	0.16 (0.05–0.24) *	0.18 (0.05–0.31)	0.17 (0.10–0.27)	0.05 (–0.03–0.17)	0.08 (0.01–0.24)	0.11 (0.04–0.24)

Data expressed as median (interquartile range) or as mean ± standard deviation.
CO, cardiac output; CI, cardiac index; Ea, arterial elastance; EDP, end diastolic pressure; Eed, end diastolic elastance; Ees, end systolic elastance; LA, left ventricle; LAD, left atrial dimension; LVEDd, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; MAP, mean arterial pressure; PWT, posterior wall thickness; RWT, relative wall thickness; SV, stroke volume; SVI, stroke volume index; TVR, total vascular resistance; TVRI, total vascular resistance index. HR75 denotes adjustment to standardise for heart rate 75 beats per minute.
* $P<0.05$ vs group 2.

5.4.5 Longitudinal analysis for Group 1 (previous hypertension in pregnancy)

Heart rate increased as pregnancy progressed (**Table 29**). There was a significant increase in weight throughout the pregnancy. Haemoglobin decreased significantly from the first to second appointment, then plateaued in the third trimester. No significant change in average blood pressure was observed throughout the study period in Group 1.

Several of the echocardiographic measures of cardiac structure and function showed significant changes in the pregnant women with previous hypertension, when studied throughout pregnancy (**Table 30**). Cardiac output increased from baseline to second visit (P=0.01) then remained unchanged into the third trimester. The average s' showed a similar trend (P=0.009). The median left atrial volume index was lower at the third visit (P=0.008). Left ventricular volume and left ventricular mass increased between second and third appointments, but there was no significant longitudinal change.

The maximum velocity of early filling across the mitral valve (E wave) was seen to increase from baseline to second measurement, and then decrease in the third trimester (P=0.02), resulting in a significant reduction in E/A ratio by the last visit (P=0.03). Meanwhile e', the velocity of early myocardial relaxation, reduced significantly during the pregnancy (P=0.01) but E/e' was unchanged. A significant increase in the end-systolic elastance from baseline to follow up measurements (P=0.02) led to a decrease in the arterial-ventricular interaction in the women with previous hypertension (P=0.02). There was no significant longitudinal change in augmentation index in Group 1 during the study.

Table 29: Longitudinal clinical measurements for Group 1 at baseline, second and third trimester

	Baseline at 13±1 weeks (n=25)	2nd visit at 21±1 weeks (n=24)	3rd visit at 32±1 weeks (n=20)	*P*
SBP (mmHg)	115 (113–121)	115 (111–127)	119 (111–125)	0.52
DBP (mmHg)	71 (65–75)	71 (65–75)	73 (64–76)	0.39
MAP (mmHg)	87 (81–91)	88 (79–91)	87 (83–93)	0.61
cSBP (mmHg)	102 (95–106)	102 (94–108)	102 (96–106)	0.85
cDBP (mmHg)	72 (68–77)	73 (66–77)	72 (66–79)	0.45
cMAP (mmHg)	85 (80–90)	88 (79–91)	85 (82–93)	0.28
Heart rate (bpm)	79±10	85±14†	86±13†	0.02
Weight (kg)	73 (58–85)	76 (62-94) †	80 (68–87) †‡	<0.001
Haemoglobin (g/L)	120±11	115±10†	115±10	0.060
WCC ($x10^9$/L)	9.4 (7.2–10.9)	9.9 (7.8–11.3)	9.7 (8.2–11.4)	0.42
Platelets ($x10^9$/L)	253 (210–291)	259 (231-287)	263 (218–277)	0.54
Creatinine (µmol/L)	51 (49–54)	50 (45–53)	50 (45–54)	0.27

BMI, body mass index; BSA, body surface area; cDBP, central diastolic blood pressure; cSBP, central systolic blood pressue; cMAP, central mean arterial pressure; DBP, peripheral diastolic blood pressure; MAP, peripheral mean arterial pressure; SBP, peripheral systolic blood pressure; WCC, white cell count. Data expressed as median (interquartile range) or as mean ± standard deviation. Post hoc testing: † $P<0.05$ vs baseline visit; ‡ $P<0.05$ vs midtrimester 2nd visit.

Table 30: Longitudinal echocardiographic and pulse wave analysis parameters for Group 1 at baseline, second and third trimester

	Baseline at 13±1 weeks (n=25)	**2nd visit at 21±1 weeks (n=24)**	**3rd visit at 32±1 weeks (n=20)**	***P***
Haemodynamics				
Stroke volume (ml)	68 (59–72)	69 (56–75)	65 (53–75)	0.63
Stroke volume index (ml/m^2)	37.3 (34.0–42.8)	38.2 (32.0–45.0)	36.4 (28.5–41.5)	0.43
Cardiac output (L/min)	5.0 (4.6–5.8)	5.8 (4.9–6.6) †	5.7 (4.6–6.1)	0.17
Cardiac index (L/min/m^2)	3.0 (2.7–3.2)	3.2 (2.9–3.7) †	3.0 (2.4–3.6)	0.35
Total vascular resistance (dyne.s/cm^5)	1.4 (1.2–1.5)	1.3 (0.96–1.4)	1.2 (1.0–1.6)	0.30
Total vascular resistance index (dyne.s/cm^5/m^2)	0.80 (0.65–0.89)	0.65 (0.56–0.90)	0.67 (0.54–0.88)	0.12
Average s' (cm/s)	9.6 (8.5–10.2)	10.3 (9.1–11.1) †	10.0 (8.4–11.3)	0.03
Structure				
Left atrium volume (ml)	41 (31–45)	41 (31–49)	34 (29–38) †‡	0.05
Left atrium volume index (ml/m^2)	22.1 (18.5–28.0)	23.1 (18.0–25.5)	17.9 (16.3–21.2) †‡	0.008
Left ventricle volume (ml)	98 (84–127)	98 (88–113)	103 (94–112) ‡	0.13
Left ventricle volume index (ml/m^2)	56.7 (51.6–63.9)	55.3 (50.3–67.0)	63.7 (52.9–68.4)	0.26

Left ventricular mass (g)	117 (95–152)	114 (92–142)	132 (96–165) ‡	0.09
Left ventricular mass index (g/m^2)	71 (57–82)	63 (53–77)	76 (54–85)	0.16
Left ventricular end diastolic dimension (cm)	4.5 (4.3–5.0)	4.5 (4.1–4.8)	4.3 (4.0–4.6)	0.22
Posterior wall thickness at end diastole (cm)	0.9 (0.7–1.1)	0.9 (0.7–1.1)	1.0 (0.8–1.2)	0.13
Interventricular septum thickness at end diastole (cm)	0.79 (0.69–0.91)	0.80 (0.73–0.90)	0.81 (0.68–1.15)	0.09
Relative wall thickness	0.38 (0.32–0.49)	0.40 (0.32–0.47)	0.46 (0.37–0.57)	0.14
Diastolic function (mitral inflow)				
E (cm/s)	84.9 (74.9–99.4)	90.5 (80.7–103.9)	75.7 (71.5–87.8) ‡	0.02
A (cm/s)	61.9 (51.0–67.1)	59.2 (50.1–69.4)	62.7 (54.0–76.2) ‡	0.41
E/A ratio	1.4 (1.1–1.6)	1.4 (1.3–2.0)	1.3 (1.1–1.4) †	0.03
e' (cm/s) Septal Lateral Average	 11.2 (9.7–12.5) 14.0 (12.6–16.2) 13.1 (11.6–13.7)	 10.5 (9.1–12.8) 15.5 (12.5–17.8) 12.9 (11.6–14.4)	 9.7 (7.8–10.9) † ‡ 12.0 (10.2–14.7) ‡ 10.9 (9.6–12.9) † ‡	 0.02 0.02 0.01
E/e'	6.6 (5.7–8.2)	7.0 (6.2–8.2)	7.4 (6.2–9.2)	0.40
Derived values including elastance				
Left ventricular ejection fraction	0.63 (0.59–0.67)	0.61 (0.57–0.63)	0.59 (0.56–0.63)	0.16
Left ventricular end diastolic	10.1 (9.0–12.1)	10.6 (9.6–12.1)	11.0 (9.6–13.2)	0.39

pressure (mmHg)				
Arterial elastance (Ea)	1.6 (1.4–1.8)	1.5 (1.4–1.9)	1.6 (1.4–2.0)	0.75
Arterial elastance index (EaI)	0.95 (0.79–1.11)	0.92 (0.80–1.07)	0.97 (0.72–1.13)	0.71
End-systolic elastance (Ees)	2.0 (1.8–2.3)	2.4 (2.0–2.6) †	2.3 (1.9–2.7)	0.02
End-diastolic elastance (Eed)	0.09 (0.08–0.11)	0.09 (0.08–0.11)	0.10 (0.08–0.12)	0.45
Arterial-ventricular interaction (Ea/Ees)	0.85 (0.78–0.88)	0.72 (0.66–0.81) †	0.76 (0.69–0.88)	0.02
EaI/Ees	0.47 (0.42–0.59)	0.39 (0.36–0.46) †	0.42 (0.38–0.50)	0.005
Arterial stiffness				
Augmentation index	15 (6–19)	11 (3–18)	12 (3–21)	0.58
Augmentation index HR75	16 (4–21)	16 (5–25)	15 (8–23)	0.70
Augmentation index HR75 adjusted for MAP	0.16 (0.05–0.24)	0.18 (0.05–0.31)	0.17 (0.10–0.27)	0.55

Data expressed as median (interquartile range) or as mean ± standard deviation.
CO, cardiac output; CI, cardiac index; Ea, arterial elastance; EDP, end diastolic pressure; Eed, end diastolic elastance; Ees, end systolic elastance; LA, left ventricle; LAD, left atrial dimension; LVEDd, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; MAP, mean arterial pressure; PWT, posterior wall thickness; RWT, relative wall thickness; SV, stroke volume; SVI, stroke volume index; TVR, total vascular resistance; TVRI, total vascular resistance index. HR75 denotes adjustment to standardise for heart rate 75 beats per minute.
Post hoc testing: † $P<0.05$ vs baseline visit; ‡ $P<0.05$ vs midtrimester 2nd visit.

5.4.6 Longitudinal analysis for Group 2 (no prior hypertension in pregnancy)

For the pregnant women with no prior hypertension, there was a significant increase in systolic, diastolic and mean arterial blood pressures throughout the pregnancy. Heart rate increased (P<0.001) and haemoglobin decreased (P<0.001) during the study period. Weight gain was also significant (P<0.001).

A reduction in stroke volume index was observed longitudinally in the women without hypertension history (P<0.001). The volume of the left ventricle increased significantly (P=0.01) with a corresponding increase in left ventricular mass (P=0.02), although this trend did not reach statistical significance once adjusted for body surface area. An increase in interventricular septum thickness at end diastole was observed (P=0.04).

The maximum velocity of early filling across the mitral valve (E) decreased significantly throughout the pregnancies of the women without prior hypertension (P=0.005), whilst the maximum velocity of late filling (A) was unchanged. The ratio of E/A was significantly lower in these women by the third trimester (P=0.01). A significant reduction in average e' was also demonstrated in pregnant controls in longitudinal analysis (P<0.001).

Arterial elastance index (EaI) increased throughout the study period (P<0.001) whilst end systolic elastance (Ees) also increased (P<0.001). The ratio EaI/Ees as a measure of arterial-ventricular interaction showed a decrease from baseline to third trimester assessment (P<0.001).

Augmentation index, adjusted for heart rate and blood pressure, showed a successive increase from baseline through 20±1 and 32±1 weeks of gestation (P<0.001).

Table 31: Longitudinal clinical measurements for Group 2 at baseline, second and third trimester

	Baseline at 13±1 weeks (n=50)	2nd visit at 20±1 weeks (n=41)	3rd visit at 32±1 weeks (n=34)	*P*
SBP (mmHg)	108 (100–114)	106 (97–112)	114 (102–116) †‡	0.02
DBP (mmHg)	64 (58–69)	60 (56–66)	65 (60–79) †‡	<0.001
MAP (mmHg)	77 (72–82)	75 (69–82)	80 (75–86) †‡	<0.001
cSBP (mmHg)	91 (85–96)	90 (81–96)	94 (87–100) †‡	0.008
cDBP (mmHg)	65 (59–70)	62 (57–67)	68 (62–72) †‡	<0.001
cMAP (mmHg)	77 (72–82)	75 (69–82)	80 (75–86) †‡	<0.001
Heart rate (bpm)	74±9	79±12†	86±13†‡	<0.001
Weight (kg)	72 (59–81)	74 (62–83) †	80 (66–89) †‡	<0.001
Haemoglobin (g/L)	122±11	116±8†	113±10†‡	<0.001
WCC ($x10^9$/L)	8.3 (7.0–9.4)	8.6 (7.5–10.0) †	9.3 (8.0–10.1) †	0.02
Platelets ($x10^9$/L)	247 (202–292)	265 (204–291)	248 (197–298)	0.30
Creatinine (µmol/L)	53 (50–56)	53 (50–56)	51 (49–56) †	0.54

BMI, body mass index; BSA, body surface area; cDBP, central diastolic blood pressure; cSBP, central systolic blood pressue; cMAP, central mean arterial pressure; DBP, peripheral diastolic blood pressure; MAP, peripheral mean arterial pressure; rANOVA, repeated measures ANOVA; SBP, peripheral systolic blood pressure; WCC, white cell count. Data expressed as median (interquartile range) or as mean ± standard deviation. Post hoc testing: † $P<0.05$ vs baseline visit; ‡ $P<0.05$ vs midtrimester 2nd visit.

Table 32: Longitudinal echocardiographic and pulse wave analyisis parameters for Group 2 at baseline, second and third trimester

	Baseline at 13±1 weeks (n=50)	**2nd visit at 20±1 weeks (n=41)**	**3rd visit at 32±1 weeks (n=34)**	***P***
Haemodynamics				
Stroke volume (ml)	68 (58–76)	65 (57–72)	56 (49–64) † ‡	<0.001
Stroke volume index (ml/m^2)	37.6 (33.6–43.6)	35.6 (31.3–40.2)	29.0 (26.7–33.9) † ‡	<0.001
Cardiac output (L/min)	4.8 (4.5–5.6)	4.9 (4.3–6.0)	5.0 (4.0–6.0)	0.54
Cardiac index (L/min/m^2)	2.8 (2.4–3.3)	2.7 (2.4–3.5)	2.5 (2.2–3.1)	0.15
Total vascular resistance (dyne.s/cm^5)	1.2 (1.1–1.5)	1.2 (1.0–1.4)	1.3 (1.1–1.7)	0.05
Total vascular resistance index (dyne.s/cm^5/m^2)	0.69 (0.58–0.89)	0.66 (0.56–0.79)	0.70 (0.59–0.85)	0.10
Average s' (cm/s)	9.5 (8.8–10.1)	9.9 (9.1–10.7)	9.6 (8.8–11.1)	0.24
Structure				
Left atrium volume (ml)	39 (31–46)	40 (32–44)	39 (33–47)	0.93
Left atrium volume index (ml/m^2)	22.2 (17.0–26.3)	20.3 (18.0–26.1)	21.2 (15.7–26.8)	0.84
Left ventricle volume (ml)	110 (91–121)	102 (91–114)	112 (98–123) ‡	0.01
Left ventricle volume index (ml/m^2)	59.5 (49.9–70.9)	57.5 (50.7–62.3)	63.7 (59.5–66.7)	0.07

Left ventricular mass (g)	119 (97–143)	108 (93–142)	130 (108–146) ‡	0.02
Left ventricular mass index (g/m^2)	65 (57–79)	64 (54–73)	69 (62–78) ‡	0.14
Left ventricular end diastolic dimension (cm)	4.5 (4.2–4.8)	4.5 (4.2–4.8)	4.5 (4.3–4.8)	0.78
Posterior wall thickness at end diastole (cm)	0.9 (0.8–1.0)	0.8 (0.7–1.0)	0.9 (0.8–1.1)	0.36
Interventricular septum thickness at end diastole (cm)	0.73 (0.66–0.87	0.73 (0.59–0.95)	0.85 (0.73–0.95) †	0.04
Relative wall thickness	0.40 (0.33–0.46)	0.35 (0.30v0.46)	0.42 (0.35–0.49)	0.48
Diastolic function (mitral inflow)				
E (cm/s)	87.9 (77.0–95.6)	86.6 (73.8–100.0)	75.3 (64.9–87.4) † ‡	0.005
A (cm/s)	48.5 (44.4–61.5)	52.8 (42.7–62.7)	50.5 (41.7–64.1)	0.52
E/A ratio	1.8 (1.5-1.9)	1.6 (1.4-2.0)	1.5 (1.2-1.8) † ‡	0.01
e' (cm/s) Septal Lateral Average	 11.2 (9.9–12.9) 15.4 (13.5–18.3) 13.5 (12.1-14.9)	 11.0 (9.7–12.7) 15.7 (13.7–17.0) 13.4 (11.3–14.8)	 10.2 (8.5–11.3) † ‡ 15.2 (12.5–16.3) † 12.1 (10.4–13.6) † ‡	 <0.001 0.03 <0.001
E/e' Average	6.5 (5.2–7.5)	6.6 (5.4–8.4)	6.3 (5.4–7.3)	0.56
Derived values including elastance				
Left ventricular ejection fraction	0.63 (0.60–0.67)	0.62 (0.58–0.64)	0.60 (0.58–0.65)	0.35
Left ventricular end diastolic	9.9 (8.4–11.2)	10.1 (8.6–12.3)	10.4 (8.8–11.9)	0.54

pressure (mmHg)				
Arterial elastance (Ea)	1.4 (1.2–1.7)	1.5 (1.3–1.8)	1.8 (1.5–2.1) † ‡	<0.001
Arterial elastance index (EaI)	0.79 (0.72–0.95)	0.86 (0.69–0.97)	0.95 (0.80–1.16) † ‡	<0.001
End-systolic elastance (Ees)	1.9 (1.6–2.2)	2.4 (2.0–2.7) †	2.4 (1.9–2.9) †	<0.001
End-diastolic elastance (Eed)	0.08 (0.07–0.10)	0.08 (0.07–0.11)	0.09 (0.08–0.13) †	0.11
Arterial-ventricular interaction (Ea/Ees)	0.81 (0.74–0.90)	0.69 (0.65–0.73) †	0.76 (0.68–0.89) †	<0.001
EaI/Ees	0.44 (0.40–0.54)	0.39 (0.34–0.44) †	0.40 (0.36–0.51) †	<0.001
Arterial stiffness				
Augmentation index	5 (-2–12)	6 (-2–16)	7 (1–16)	0.14
Augmentation index HR75	4 (-3–14)	8 (2–19)	13 (5–19)	<0.001
Augmentation index HR75 adjusted for MAP	0.05 (-0.03–0.17)	0.08 (0.01–0.24) †	0.11 (0.04–0.24)† ‡	<0.001

Data expressed as median (interquartile range) or as mean ± standard deviation.
CO, cardiac output; CI, cardiac index; Ea, arterial elastance; EDP, end diastolic pressure; Eed, end diastolic elastance; Ees, end systolic elastance; LA, left ventricle; LAD, left atrial dimension; LVEDd, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; MAP, mean arterial pressure; PWT, posterior wall thickness; RWT, relative wall thickness; SV, stroke volume; SVI, stroke volume index; TVR, total vascular resistance; TVRI, total vascular resistance index. HR75 denotes adjustment to standardise for heart rate 75 beats per minute.
Post hoc testing: † $P<0.05$ vs baseline visit; ‡ $P<0.05$ vs midtrimester 2nd visit.

5.5 Discussion

The peripheral systolic blood pressure in Group 1 at 13±1 weeks of gestation was significantly higher than both other groups after correcting for multiple comparisons (P<0.001 vs Group 2; P=0.007 vs Group 3). Systolic, diastolic and mean arterial pressures remained significantly higher at 21±1 weeks and 32±1 of gestation in those women whose previous pregnancy was affected by hypertension. These findings are similar to those of a two year follow up study of women after preeclamptic pregnancy.[50] My study showed that these changes persist into the subsequent pregnancy. The concept of prehypertension was formally introduced into American guidelines over a decade ago.[254] Patients with a systolic blood pressure of 120–139mmHg or a diastolic blood pressure of 80–90mmHg are classified as prehypertensive. The current European guidelines[255, 256] designate this group 'high normal'. At baseline 5/50 women in Group 2 and 10/25 women in Group 1 would have been classed as prehypertensive according to the Joint National Committee Guideline.[254] The proportion of women with prehypertension in Group 1 is significantly higher (P=0.002).

People with prehypertension have double the odds of developing hypertension compared to those with lower blood pressure.[254] When observed at postpartum follow up over 4–10 years after preeclampsia, prehypertension is associated with asymptomatic heart failure.[257] Over time, prehypertension causes left ventricular hypertrophy and diastolic dysfunction.[258] Previous preeclampsia, especially in association with prehypertension, is independently associated with an increased risk of subclinical cardiac failure.[257] The relationship between blood pressure and risk of cardiovascular disease events is continuous and independent of other risk factors. If prehypertension is identified (rather than simply stating that blood pressure is in the "normal" range), primary prevention strategies, starting with lifestyle

modification, can be implemented.

The increase in stroke volume index was only significant in the group of pregnant women unaffected by previous hypertension. The reduced stroke volume in the healthy pregnant women at 32±1 weeks is a surprise finding, as it was only at the final cross-sectional comparison that this difference was observed. Previous studies have reported a wide range of values for stroke volume in pregnancy, with considerable disagreement regarding the expected physiological, longitudinal changes.[19] Stroke volume is not dependent only on the heart itself as extracardiac factors (preload and afterload) have an important effect. Arterial blood pressure and vascular tone create the afterload against which the ventricles must eject blood. If afterload is increased then the stroke volume will decrease. The interplay between the pressure and volume components means that the reduction in stroke volume with an increased afterload depends on the end diastolic volume, and whether a secondary increase in preload can result in a greater contractile force according to the Frank-Starling principle.

The physiological increase in left ventricular volume and left ventricular mass corresponded with the increase in body surface area and hence did not reach statistical significance after indexation. A large component of the increased body weight throughout pregnancy is the increasing size of the feto-placental unit. Therefore, if the left ventricle increases in size relative to placenta and its demands for blood supply, it makes mathematical sense that indexation would 'cancel out' any significance in the longitudinal comparison of LV structure.

Both groups of pregnant women demonstrated a higher cardiac output, lower peripheral

resistance state at baseline compared to the non-pregnant controls. This hyperdynamic state was reflected by an increase in left ventricular volume index. The reduced peripheral vascular resistance, likely due to the effects of oestrogen, progesterone and relaxin, leads to increased plasma volume in normal pregnancy. This is mediated by the renin-angiotensin-aldosterone system which controls sodium homeostasis.[259] There is a dilution effect leading to a reduction in the haemoglobin level. In pregnancies where there is not a physiological reduction in vascular resistance and where the plasma volume is low, there will be relatively higher haemoglobin concentration unless there is interplay with another factor such as iron deficiency.

The increase in left ventricular mass compared to non-pregnant women was only significant for the women previously affected by hypertension. This increase in left ventricular mass in Group 1 occurred in conjunction with increased cardiac output at the midtrimester visit, which remained elevated from baseline at the next assessment. In my systematic review (see **section 2.1**) and in a review of cardiac function in normal pregnancy by Melchiorre *et al*[19], cardiac output was a parameter with considerable variation in reported measures and trends. This is likely to be due to different patient characteristics, timing of assessment and methodological variation in relation to measurement of stroke volume. It is also possible that the legacy of a hypertensive disorder in pregnancy is different according to its association with fetal growth restriction. A recent study demonstrated that preeclampsia is associated with increased cardiac output, but when fetal growth restriction coexists, cardiac output is reduced and peripheral vascular resistance is increased.[260] In studies such as this and my own, it remains to be shown whether the cardiovascular profile is altered due to maladaptation in the index pregnancy, or whether there was pre-existing pathology in the cardiovascular system

unmasked by pregnancy. The women with prior hypertension had an increased cardiac index in later pregnancy compared to women without previous gestational hypertensive disease, demonstrating that irrespective of blood pressure these women have an exaggerated increase in their cardiac output in pregnancy. Left ventricular ejection fraction was unchanged, neither by pregnancy nor by prior hypertension, in line with expectations of this asymptomatic cohort.

A recent meta-analysis showed that LVM and RWT both increase in normal pregnancies, which demonstrates concentric rather than eccentric hypertrophy.[261] Eccentric hypertrophy is seen in healthy athletes in response to training. Physiological remodelling is seen in healthy pregnancy, as a woman develops a heart akin to a sportswoman. This type of hypertrophy was previously thought to be a sign of pathology in hypertensive pregnancies.[262] When there is increased volume load, the end diastolic pressure increases. The cardiac myocytes increase so the wall thickness increases. This compensates for the increase in pressure.
A greater increase in LVM and RWT was demonstrated in hypertensive pregnancies. This shows that in some cases the pregnant heart reaches its limit of physiological adaptation (where the remodeling would be expected to be eccentric) and the hypertrophy becomes the kind more associated with pathology. This maladaptation to chronic volume overload even in healthy pregnancy has recently been reported in an echocardiographic study.[263] It has been shown that some 40% of women with preeclampsia have persistently abnormal cardiac structure and function up to one year postpartum, with diastolic dysfunction amounting to subclinical heart failure.[50]

The increased early diastolic mitral inflow (E wave) in pregnancy is typical of healthy, fit

individuals. As the ventricular wall recoils there is negative pressure in the ventricle so blood is sucked down the pressure gradient from the atrium across the mitral valve. This results in a higher E wave. There is relatively little atrial filling as most of the volume has already flowed out of the atrium, leaving a smaller atrial contribution. This is the pattern seen in healthy pregnancy and in the non-pregnant controls. Since there is an increased volume load in pregnancy, the already stretched heart is less compliant. This explains the reduction in E/A seen in normal pregnancy. In the women affected by previous hypertension, late diastolic filling caused by atrial contraction was greater, leading to a significantly lower E/A ratio in this group. The larger A wave usually reflects compensation for reduced early filling, after its being impaired by a stiffer ventricle. In the case of Group 1 at baseline, the results suggest a trend towards a 'pseudonormal' filling pattern which is a marker of diastolic dysfunction. As the left atrial pressure rises as a marker of progressive diastolic dysfunction, an increase in the E wave is caused not by reduced pressure in the ventricle, but rather by increased pressure from the atrium driving blood through the mitral valve in early diastole and in this pattern both E and A are increased as seen in my study group. Women with prior hypertension had a lower E/A at the final follow up compared to baseline. The maximum early mitral inflow velocity peaked in the midtrimester follow up for women in Group 1, whilst for women in Group 2 there was a stepwise decrease observed during the pregnancy. Both groups had similar values at the final follow up. The longitudinal trend in normal pregnancy is consistent with existing literature.[19] The E and A waves are affected by the loading conditions of pregnancy.[132, 264]. Pregnancy affects volume haemostasis with an approximately 1600ml increase in the intravascular compartment (1300ml extra plasma volume and 30ml extra red blood cell volume).[265] I did not assess volume status, which would have provided further insight into the contribution of volume expansion to the load dependency of the

measurements.

The fact that there is no significant difference in e' between groups shows that myocardial relaxation/recoil is unchanged. The velocity of early myocardial relaxation measured with Tissue Doppler Imaging (e') reduced during the pregnancy in both groups and this change was significant. This reflects a degree of diastolic dysfunction in both groups, and if this were to persist after pregnancy this would confer increased cardiovascular risk.[266] Although E/e' was increased in pregnancy, the average for each group was in the normal range and this difference largely reflects the difference in E already described.

In the healthy pregnant women arterial elastance index was decreased significantly at baseline, but this adaptation was not seen in women with prior hypertension. Arterial elastance relates to the ability of the aorta to receive blood. In healthy pregnancy there is increased compliance in the arterial system. In my study the women with prior hypertension did not demonstrate this physiological adaptation. Both peripheral and aortic arterial stiffness has been demonstrated in preeclampsia.[267] Increased arterial stiffness has been shown to be present postpartum.[111, 268] To my knowledge this is the first demonstration of arterial stiffness in a normotensive pregnancy following gestational hypertensive disease. Increased arterial stiffness was demonstrated in the women with prior hypertension at baseline, compared to their pregnant counterparts without hypertension history and with non-pregnant controls. This is consistent with other studies showing increased arterial stiffness after pregnancy hypertension.[143, 148] This adverse effect leads to increased risk of future hypertension, coronary artery disease and heart failure.[269] Although the difference was statistically significant only at baseline, the augmentation index remained increased in women with

previous hypertension throughout pregnancy.

Ventriculo-arterial coupling (VAC) is a marker of cardiovascular performance and has not been widely reported in studies of maternal cardiac function during and after pregnancy hypertension to date. Increased arterial stiffness and impaired systolic function reduces the efficiency of the myocardium which is reflected in decreased VAC. This may be discovered prior to LV dysfunction being detectable. The cross talk between the vascular and ventricular behaviour is important because if normal it means that blood can be ejected from the heart to the periphery without an abnormal pressure change. Normal Ea/Ees signifies mechanical and energetic efficiency. Both study groups demonstrated increased end-systolic elastance during follow up, with evidence of reduced arterial-ventricular interaction when assessed longitudinally. One would expect the left atrial volume to increase in healthy pregnancy due to an increase in preload,[19] however in women with prior hypertension there was a reduction in left atrial volume in the third trimester alongside a plateau in cardiac output. This may reflect a reduced plasma volume in these women, or be a feature of the increased elastance preventing a physiological expansion within this chamber.

I observed an increase in s' in Group 1 during follow up. This measure of LV contraction is a surrogate marker of systolic function.[270] In their echocardiographic studies Buddeberg and colleagues demonstrated a reduction in s' in preeclamptic women at term, and no difference in obese women.[164, 271] This may demonstrate that women affected by previous hypertension in pregnancy are able to optimise their systolic function and that subendocardial fibre function is preserved. Increased posterior wall thickness in Group 1 is an adaptation thought to maintain myocardial perfusion, thereby facilitating oxygenation of the tissue and reducing

wall stress.[19]

5.6 Strengths and limitations

My systematic review demonstrated that most studies of maternal cardiac function have a cross sectional design, with investigations performed on a single occasion in pregnancy. Longitudinal designs, of which my study is an example, are less common. Where serial investigations are performed on the same subjects throughout pregnancy, it is said that the women act as their own control and changes over the time course of pregnancy can be observed.[17] This study has the advantage of using a non-pregnant control group with which to compare in the cross sectional analysis at baseline. Longitudinal studies in pregnancy present challenges to the researcher. Study appointments must be arranged in tight timescales, since the advancing gestation does not slow down to facilitate research. There are many factors which might prevent a pregnant woman from attending an appointment (work commitments, childcare, fatigue), and non-attendance is more likely when there is an expectation for her to attend more than once. Although every effort was made to maximise the convenience for the patient, for example by scheduling research appointments to coincide other commitments in the hospital, we did not have funding to offer other incentives. Therefore, in common with other longitudinal studies, complete data sets were not obtained for every patient.

A full dataset was collected for 19/25 patients in Group 1 and 33/50 patients in Group 2. In common with other longitudinal studies in pregnancy, some women did not return for follow up. The distribution of attendance across all three appointments is shown in **Table 36**. There were no women who did not attend due to finding the echocardiogram uncomfortable or unacceptable to them. No woman withdrew consent for her data to be used in the analysis.

Reasons for non-attendance included inability to travel to the hospital, not answering phone or responding to message in time, or transfer of care to another geographical area. Patients who did not have a complete dataset are unlikely to demonstrate different (patho)physiology to those who completed all follow up appointments.

Table 35: Completeness of echocardiographic follow up data

Appointments	Group 1 **Previous hypertension**	Group 2 **Previous normotensive**
Baseline only	0	8
1st and 2nd appointments (missed 3rd)	5	8
1st and 3rd appointments (missed 2nd)	1	1
Complete dataset (3 appointments)	19	33

In a small minority of cases the echo images were of insufficient quality to evaluate all of the parameters specified. There were no missing data from the parasternal long axis view. Indicative numbers of missing data points at the baseline visit are 2 ejection fractions (it was not possible even to approximate the "eyeball" ejection fraction) and from the third trimester appointment 3 of the ejection fractions were approximated as the measurements were not possible, despite adjusting the machine settings to reduce noise and to delineate the endocardium as clearly as possible.

It is common for gestational hypertensive disease to coincide with other maternal medical conditions, such as diabetes or obesity. These conditions could be viewed as confounders which contaminate the cohort. It can also be argued that the presence of comorbidities reflects the overall clinical condition and the challenges faced in antenatal practice. To

remove women from the study on the basis of pre-existing or gestational diabetes, would have resulted in a smaller sample size. Similarly, during the development of the study protocol, it was decided not to exclude extremes of body weight. Obesity is prevalent in the obstetric population. To have excluded obese women would have been detrimental to the sample size and reduced the generalisability of the results. Indexation of key echocardiographic measurements controlled for variation in body habitus.

There is some controversy in the literature regarding use of cardiac index in pregnancy. Indexing in pregnant women may be misleading because the change in the body contour is not consistent with the standard way in which body surface area is calculated.[272] Furthermore it is debatable whether pre-pregnancy or baseline body weight should be used in the calculations or whether the actual weight at the time of assessment should be incorporated.[272] Scaling according to maternal size is a crude correction, since it is not possible to correct for the metabolic demands of pregnancy. It is clear from **Table 9** in the systematic review in **Chapter 2**, that researchers show variation in practice in terms of adjusting for maternal height and weight. Some correct for maternal stature using height only[273], whilst others advocate indexing according to body surface area.[158] Some investigators index all the cardiac dimensions to body surface area[154] whilst others make no quantitative adjustment for maternal anthropometry.[153] I have used indexing for specific pre-specified derived measurements, and in each case have displayed the raw data alongside the adjusted data. It would be useful to have a consensus amongst the international research community as the current variation in reporting limits comparisons between studies and precludes meta-analysis of the data without recourse to individual participant data.

Some researchers in the field of maternal cardiac function have adjusted tissue Doppler velocity indices to left ventricular long or short axis lengths.[132, 149] In common with the majority of studies of pregnant women, I did not incorporate this adjustment into my analysis. The principle of accounting for the size of the heart is routine practice in paediatrics, and its importance in adult cardiology has been recognised.[274, 275] Dewey and colleagues reviewed the evidence for ratiometric and allometric scaling[276], demonstrating how crucial it is to consider these principles in experimental planning. The confounding effect of body size in cardiovascular medicine and in particular in pregnancy is important, and it would be useful to establish a consensus statement on how indices should be reported. For research purposes, such a consensus would facilitate comparisons between studies and quantitative synthesis of data would be less complex. Arguably this concept is even more important in clinical practice where diagnostic criteria and treatment decisions rely on accurate numerical thresholds, which may be altered depending on scaling.

Adjustment of augmentation index to heart rate of 75 beats per minute is not universally accepted. This method is recommended by the manufacturers of SphygmoCor®, and has been used in previous studies in pregnancy.[103, 112, 277] The correction assumes that the relationship between heart rate and arterial stiffness is uniform across populations. It has been suggested that in longitudinal studies it may be more appropriate to use the unadjusted AIx and use heart rate as a covariate, as it may help to explain the change in arterial stiffness.[278] It has also been suggested that AIx be corrected not only for heart rate but also for blood pressure, therefore I also present a MAP-adjusted Aix75.[98] There has been recent criticism for the use of negative AIx values in analyses of wave reflection as they may distort the results. Negative values are associated with a waveform where peak pressure coincides

with peak flow and a forward travelling decompression wave causes a late systolic shoulder after this peak. The corresponding fall in pressure and flow causes a reduction in peak pressure which is detected by the tonometer and results in a negative AIx.[279] If the backwards wave superimposes on the forward moving wave after the systolic peak, then the systolic blood pressure is unaffected by the backwards waves so the augmentation index will be zero or negative

Researchers affiliated with the Fetal Medicine Foundation have published many studies relating to prediction of adverse pregnancy outcomes. This institution advocates the use of multiples of the (normal) median (MoMs), adjusted for maternal characteristics. The MoM values are established from extensive population studies. This approach has recently been applied to maternal cardiovascular parameters near term in order to investigate whether haemodynamic data improve the sensitivity of a Bayes' theorem-based screening model for late PET.[280] The rationale for use of MoMs is to recognise that a parameter may be altered by a maternal factor, such as age, and therefore adjustment is required to avoid overestimation of an association with the outcome of interest.[281] These values are not transferable to echocardiographic studies since it has been shown that the NICOM device used in the screening study obtains significantly different values to echocardiographic measures, therefore might be considered device-specific.[280, 282] MoMs for echocardiographic measurements in pregnancy have not been established, and since echocardiography is said to represent the Gold Standard in non-invasive assessment of maternal haemodynamics[97], these will be important to ascertain in future large studies.

Aortic root diameter (AoD) does not change as much as the left ventricular outflow tract

diameter in pregnancy, which alters the geometric assumptions involved in calculations involving the latter. When calculating stroke volume some investigators use the AoD multiplied by the AoD VTI in the apical 3 Chamber view. Others use LVOT and its corresponding VTI. I chose the latter as it is the most commonly reported in similar research and forms part of the standard echocardiography protocol.

The pregnant heart is more spherical compared to the more ellipsoid non-pregnant heart. There is no geometrical assumption in the Simpson method of discs to calculate the left ventricular volume. This method was employed to calculate left ventricular ejection fraction, which is the proportion of blood ejected relative to the intraventricular volume. Simpson's method represents the longitudinal contractile function only and does not account for the radial contractile function. Unfortunately, although ventricular volumes were measured in two planes, the software was unable to calculate the biplane Simpson method and therefore a single plane was used.

A strength of this research is that both interobserver and intraobserver reliability is recorded in the methodology. I have demonstrated that as the primary observer, my measurements are consistent with a second observer for a random sample of patients. Ideally, all measurements would be performed by two observers, but demonstrating good interobserver reliability is the next best, pragmatic, option as the human resource to perform all measurements in duplicate was not available. I was able to show that my measurements are reliable by reporting intraobserver coefficients of variation. Lack of reporting of such validation was a weakness of many of the studies in my systematic review.

The echocardiographic image acquisition by experienced cardiologists is another strength of the study. Whilst writing the protocol, consideration was given to my training in echocardiography. It was decided that the echocardiograms should be performed by expert cardiac sonographers, certified by the British Society of Echocardiography. The rationale was twofold, firstly to maximise the quality of the images and therefore the quality of the data, and secondly to ensure that as the investigator I would be truly blinded to all demographic and clinical details relating to the patient during offline analysis. Whilst this is a strength of the study in terms of its generating high quality data, it might also be considered a limitation as scheduling the appointments at times convenient for two researchers and the patient was, on occasion, challenging and a more complete dataset might have been possible if I had performed the scans myself. The experience gained during this fellowship, combined with my training in obstetric ultrasound, should enable me to develop echocardiography skills for future research.

Speckle tracking technology is more independent of the loading conditions of pregnancy and cardiac motion, therefore it is useful to study alterations in cardiac function. It can also be used to assess complex torsional heart movement in order to detect preclinical impairment of LV function.[283] Speckle tracking imaging technology has become more prominent in the lifetime of this study and its not being employed in my methodology results in a less comprehensive evaluation of systolic function, lacking information relating to strain and rotation/torsion data.

Arterial elastance as a measure of arterial load is important as it recognises the pulsatile nature of the pressure and flow in the arterial system. In hypertension, vascular resistance

alone is an inadequate measure as it does not take the oscillatory nature of the pulse pressure into account. The ventricular-arterial interaction should be part of the expected dataset in studies of maternal haemodynamics, and its inclusion in this study should be considered a strength.

Non-invasive devices like the Sphygmacor® have been criticised since the brachial blood pressures to which they are calibrated may underestimate the true (were it to be measured invasively) blood pressures.[284] Central systolic and diastolic pressures may therefore be falsely low. My methodology used the radial arterial pulse for pulse wave analysis, assuming radial systolic and diastolic blood pressures to be equal to the brachial recordings. Unknown, and therefore unaccounted for, amplification or diminution of the wave form between the brachial and radial arteries would make the estimation of central pressure inaccurate. This potential flaw should be considered a limitation.

The Sphygmacor® software reports an 'Operator Index' which is a measure of waveform reproducibility and signal strength. The operator index is a composite quality control parameter. If the average pulse height is unacceptably low or there is too much variation in the pulse height the system will report a low operator index. Prior to commencement of the study I honed my technique to ensure that my operator index was consistently high. High operator indices are achieved when both the operator and the patient have stable and steady forearm position and the tonometer is held close to the base to allow application of constant pressure to the waveform location. Since the operator index is reported in real time, the measurement should be repeated until an Operator Index of at least 75 was achieved, but ideally operator index should be above 80 for best results. There were 26 cases out of 231

pulse wave experiments with operator index of 74 and below. The operator index was above 75 in 89% of cases. Since operator index was not considered in the original methodology all values were included in my analysis. There were 3 studies where a waveform could not be detected and therefore these data points are missing.

According to recommendations of Kaplan and Bennett the ethnicity of subjects in this study is that reported by the women themselves[285], but self-reporting may not be reliable.[69] I have described the study group as ethnically diverse, but it must be borne in mind that all participants were booked for antenatal care at a single institution in a densely populated part of Birmingham. The geographical area from which the patients come is relatively small. I do not have data on the diversity within the different ethnic groups, for example socio-economic factors and diet. The British Journal of Obstetrics and Gynaecology published a commentary by Steer[69], who described the development of accepted practice in reporting on race and ethnic origin, drawing attention to the differences between ethnicity (which has a cultural basis) and racial groups (more related to geography and genetics). The relationship between ethnicity and health outcomes is complex. The ECCHO Study was not powered to make comparisons between different ethnic groups for any of the parameters under investigation. I was not able to study any candidate genes to assess any genetic associations.

The non-pregnant control group included women on the contraceptive pill and I did not obtain information regarding menstrual cycle which can affect vascular function.[286] This should be considered as a limitation and borne in mind when developing future research protocols.

Women with persistently abnormal ventricular structure and function after preeclampsia are

more likely to develop essential hypertension.[50] When women with early onset preeclampsia were studied up to 2 years postpartum, the majority had asymptomatic cardiac failure and 40% developed hypertension.[50] Since women with hypertension at booking were excluded from my study, it is unlikely to capture the full burden of cardiac impairment after hypertensive pregnancy. If there were larger numbers, women with former preeclampsia, former gestational hypertension and women with chronic hypertension could be studied in subgroups to evaluate the differences between these conditions.

5.7 Conclusions

I have described cardiovascular system differences in pregnancy, depending on history of gestational hypertension or preeclampsia. I have shown increased prevalence of prehypertension, and increased arterial stiffness in pregnant women previously affected by gestational hypertensive disease. An increased atrial component to ventricular filling reflects altered diastolic function after hypertensive pregnancy.

CHAPTER 6:
MONOCYTE SUBSETS IN WOMEN WITH PREVIOUS GESTATIONAL HYPERTENSIVE DISEASE

6.1 Introduction to chapter

Monocyte biology, with particular reference to women's cardiovascular health and pregnancy, was discussed in **Chapter 2**. In this chapter I assess monocyte heterogeneity in women in the ECCHO Study and suggest an association between these leucocytes and cardiovascular risk in pregnancy.

6.2 Aims and hypothesis

This part of the study was designed to provide mechanistic insight into the echocardiographic changes described in **Chapter 5**. My objective was to assess changes in monocyte subsets in pregnancy, including those pregnancies with the additional risk factor of prior gestational hypertension or preeclampsia. Functionally distinct monocyte subsets play distinct roles in the pathogenesis of cardiovascular disease, but their implications in hypertension in pregnancy are unclear. I hypothesised that there would be significant differences between pregnant women and controls, and differences in pregnant women according to history of hypertension in pregnancy.

6.3 Methods and materials

6.3.1 Pregnant women

59 pregnant women, comprising 17 with hypertension in a previous pregnancy and 42 with no history of hypertension, were recruited in the ante natal clinic at City Hospital, Birmingham, as described in **section 4.2** according to strict, predefined inclusion and exclusion criteria. For this substudy an additional exclusion criterion was the presence of inflammatory bowel

disease, which affected one woman in each group of pregnant women.

6.3.2 Control group

27 healthy non-pregnant controls were recruited as described in **section 4.2.2**.

6.3.3 Experimental measurements

The experimental design is described in detail in **section 4.2**. Blood sampled from a peripheral vein was promptly processed and analysed by flow cytometry. The BD FACSCalibur flow cytometer (Becton Dickinson, Oxford, UK) would yield the absolute monocyte count, as well as counts of the three monocyte subsets. According to the most recent international consensus statement, the monocyte subsets were defined as CD14++CD16-CCR2+ (Mon1), CD14++CD16+CCR2+ (Mon2) and CD14+CD16++CCR2- (Mon3).[171] Data were also recorded for monocytes' interaction with platelets (monocyte-platelet aggregates, MPAs) and their expression of cell surface markers.

6.3.4 Statistical analysis

The statistical analysis plan is described in **section 4.9**. Where demographic and clinical characteristics were normally distributed, ANOVA was used to assess differences between the three study groups. For the monocyte data, the Mann-Whitney test was applied to comparisons between the two groups of pregnant women. The Kruskal-Wallis test was used to analyse differences between all three groups. Post hoc testing (Dunn-Bonferroni procedure) was performed to account for multiple comparisons.

6.4 Results

6.4.1 Patient characteristics

There were 17 pregnant women in Group 1 (previous hypertension), 42 pregnant women in Group 2 (no previous hypertension) and 27 non-pregnant women in Group 3 (no hypertension). Although the monocyte substudy involved fewer women, the groups remained matched for age, ethnicity and smoking status (P>0.05 for all, **Table 36**). All women in Group 1 were parous, since previous hypertensive pregnancy was an inclusion criterion. Differences in aspirin consumption (higher in Group 1, P<0.001) and hormonal contraception (only in the non-pregnant controls, P<0.001) were present, as discussed in **section 1.4.1**. The pregnant women were studied at 13±1 weeks of gestation (P=0.65). There was a trend towards higher BMI for women in Group 1 (P=0.07). As discussed previously, blood pressures at baseline were higher in Group 1. Heart rate was higher and haemoglobin lower in the pregnant women (P<0.001). White cell count was significantly higher in pregnancy, and higher still in pregnant women with prior hypertension (P<0.001).

Table 36: Demographic and clinical characteristics in monocyte substudy

Characteristic	Group 1 **Previous hypertension** (n=17)	Group 2 **Previous normotensive** (n=42)	Group 3 **Non-pregnant controls** (n=27)	***P***
Age, years	30 (27–33)	30 (25–33)	27 (25–34)	0.68
Parity, *n* (%) Nulliparous Parous	 0 (0) 17 (100) *†	 19 (45) 23 (55)‡	 22 (81) 5 (19)	 <0.001
Ethnicity, *n* (%) White South Asian Black East Asian Other/mixed	 6 (35) 8 (47) 1 (6) 0 (0) 2 (12)	 16 (38) 18 (43) 6 (14) 1 (2) 1 (2)	 18 (67) 2 (7) 5 (19) 1 (4) 1 (4)	 0.28
Other medical history, *n* (%) Asthma Diabetes	 2 (12) 1 (6)	 1 (2) 3 (7)	 3 (8) 0 (0)	 0.34 0.38
Medications, *n* (%) Aspirin Hormonal contraception	 6 (35) 0 (0)	 1 (2) 0 (0) †	 0 (0) 10 (37)	 <0.001 <0.001
Smoking, *n* (%) Smoker Non-smoker	 2 (12) 15 (88)	 2 (5) 40 (95)	 1 (4) 26 (96)	 0.50
BMI (kg/m^2)	27.9 (26.0–30.5)	26.4 (22.3–29.5)	23.7 (20.6–28.6)	0.07
BSA (m^2)	1.79±0.22	1.77±0.20	1.77±0.23	0.91
Peripheral systolic blood pressure (mmHg)	117 (113–121) *	108 (100–113)	113 (101–122)	0.006
Peripheral diastolic blood pressure (mmHg)	72 (66–75) *	64 (58–67)	66 (59–71)	0.007
Peripheral mean arterial pressure	87 (82–92) *	77 (72–81)	81 (73–86)	0.001

(mmHg)				
Central systolic blood pressure (mmHg)	101 (99–105) *	92 (86–96)	98 (89–102)	0.001
Central diastolic blood pressure (mmHg)	72 (68–77) *	65 (59–69)	67 (59–72)	0.004
Central mean arterial pressure (mmHg)	86 (80–92) *	77 (72–81)	81 (73–86)	0.01
Heart rate (beats per minute)	82±10 *†	74±9†	65±11	<0.001
Haemoglobin (g/L)	118±12†	120±9†	132±11	<0.001
White cell count ($x10^9$/L)	10.6 (9.3–11.6) *†	8.3 (7.1–9.2) †	6.3 (5.3–7.1)	<0.001
Platelets ($x10^9$/L)	259 (226–306)	241 (199–289)	270 (249–303)	0.15
Creatinine (μmol/L)	51 (49–54) †	53 (50–55) †	66 (64–71)	<0.001

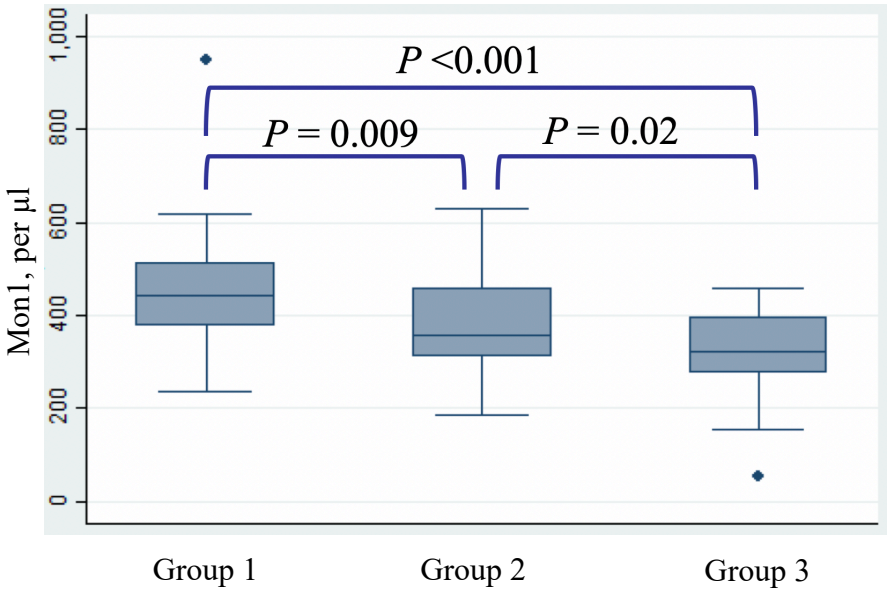
Continuous data are expressed as median (interquartile range). Categorical data are expressed as *n* (%). BMI, body mass index; BSA, body surface area; n/a, not applicable. Post hoc testing: *p<0.01 vs group without previous gestational hypertension; †p<0.01 vs non-pregnant controls; ‡p=0.01 vs non-pregnant controls. Cross sectional analysis of echocardiographic parameters at baseline

6.4.2 Cross sectional analysis of monocytes at baseline

Mon1 count was significantly higher in women with previous hypertension in pregnancy compared to non-pregnant controls (*P*<0.001, **Figure 14** and **Table 37**). The Mon1 count in pregnant women without previous hypertension was not significantly higher than in non-pregnant controls (*P*=0.06) and was lower than Mon1 count in the group with prior hypertension, but this comparison did not reach significance after post hoc testing (*P*=0.02). Mon3 was increased in both groups of pregnant women (*P*=0.002 Group 1 vs Group3; *P*=0.007 Group 2 vs Group 3). The increase in total monocytes in Group 1 was highly significant compared to Group 3 (*P*<0.001) and trended towards significance compared to

Group 2 (*P*=0.053). Mon1-platelet aggregates (MPA1) were significantly increased in the previous hypertension group (*P*=0.006) compared to the non-pregnant control group. Mon3-platelet aggregates (MPA3) were increased in both groups of pregnant women (*P*≤0.005 for both). For total monocyte platelet aggregate count, the only significant difference observed on cross sectional comparison at baseline was a higher count in Group 1 compared to Group 3 (*P*=0.004).

Figure 15: Mon1 count for each study group



The expression of cell surface markers on the monocyte subsets and their aggregates with platelets was also analysed (**Table 38**), with the relative expression demonstrated by median fluorescence intensity (MFI) according to convention. This comparison showed that the C-C chemokine receptor type 2 (CCR2) was expressed less on Mon1 (*P*=0.002), Mon 2 (*P*=0.037) and MPA1 (*P*<0.001) of the pregnant women compared to the non-pregnant women. The relative expression of CD14, CD16 and CD42a was unchanged in cross sectional comparison at baseline.

Table 37: Monocytes and their subsets at baseline

	Group 1 **Previous hypertension** (n=17)	Group 2 **Previous normotensive** (n=42)	Group 3 **Non-pregnant controls** (n=27)	***P***
Monocyte subsets				
Total monocytes, per µl	545 (455–592) †	425 (374–514)	378 (293–463)	<0.001
Mon1, per µl	441 (376–512) †	357 (309–457)	323 (277–397)	<0.001
Mon2, per µl	15 (9–49)	19 (10–41)	22 (10–41)	0.99
Mon3, per µl	51 (38–62) †	38 (29–58) ‡	26 (20–40)	0.002
Monocyte-platelet aggregates				
MPA, per µl	51 (43–69) †	47 (34–66)	41 (26–46)	0.01
MPA1, per µl	40 (33–62) †	35 (26–47)	32 (23–38)	0.02
MPA2, per µl	2 (1–6)	3 (1–6)	4 (2–5)	0.99
MPA3, per µl	6 (4–8) †	5 (4–9) ‡	3 (2–5)	0.002

MPA, monocyte-platelet aggregates; Data expressed as median (interquartile range).
Post hoc testing:
*p<0.01 Group 1 vs Group 2
†p<0.01 Group 1 vs Group 3
‡p<0.01 Group 2 vs Group 3

Table 38: Cell surface expression of monocyte markers

	Group 1 **Previous hypertension** (n=17)	Group 2 **Previous normotensive** (n=42)	Group 3 **Non-pregnant controls** (n=27)	***P***
CCR2 (Mon1), MFI	113 (88–140) †	126 (98–147) ‡	167 (117–203)	0.002
CCR2 (Mon2), MFI	96 (86–112)	91 (78–121) ‡	121 (92–151)	0.037
CCR2 (Mon3), MFI	17 (16–19)	16 (15–18)	18 (16–19)	0.14
CD14 (Mon1), MFI	1586 (1233–1712)	1535 (1408–1729)	1651 (1359–1766)	0.68
CD14 (Mon2), MFI	1199 (887–1612)	1396 (1146–1684)	1566 (1189–1769)	0.23
CD14 (Mon3), MFI	200 (165–217)	183 (136–228)	198 (153–235)	0.91
CD16 (Mon2), MFI	91 (84–120)	91 (77–111)	90 (81–95)	0.60
CD16 (Mon3), MFI	185 (166–237)	215 (172–272)	236 (144–292)	0.39
CCR2 (MPA1), MFI	111 (85–126) †	111 (85–137) ‡	148 (111–193)	<0.001
CCR2 (MPA2), MFI	123 (96–142)	107 (95–142)	133 (102–159)	0.44
CCR2 (MPA3), MFI	17 (14–17)	17 (15–19)	17 (16–19)	0.17
CD42a (MPA1), MFI	46 (43–48)	43 (40–48)	42 (39–49)	0.42
CD42a (MPA2), MFI	57 (48–100)	56 (48–71)	51 (47–60)	0.19
CD42a (MPA3), MFI	46 (44–51)	48 (42–55)	49 (43–58)	0.95

MFI, mean fluorescent intensity. Data expressed as median (interquartile range).
Post hoc testing:
*p<0.01 Group 1 vs Group 2
†p<0.01 Group 1 vs Group 3
‡p<0.01 Group 2 vs Group 3

Table 39: Longitudinal flow cytometry data for Group 1 at baseline, second and third trimester

	Baseline at 13±1 weeks (n=17)	**2nd visit at 21±1 weeks (n=18)**	**3rd visit at 32±1 weeks (n=12)**	***P***
Monocytes (per μl)				
Total monocytes	545 (455–592)	556 (418–673)	513 (430–694)	0.71
Mon1	441 (376–512)	488 (369–557)	452 (379–599)	0.76
Mon2	15 (9–49)	25 (14–43)	33 (10–47)	0.75
Mon3	51 (38–62)	43 (32–58)	55 (23–80)	0.95
MPAs (per μl)				
MPA	51 (43–69)	51 (40–64)	43 (28–52)	0.26
MPA1	40 (33–62)	42 (33–51)	32 (22–43)	0.18
MPA2	2 (1–6)	3 (2–5)	2 (2–4)	0.51
MPA3	6 (4–8)	6 (4–7)	5 (3–7)	0.57

Data expressed as median (interquartile range). MPA, monocyte-platelet aggregates; MFI, mean fluorescent intensity.
Post hoc testing:
† $P<0.05$ vs baseline visit; ‡ $P<0.05$ vs midtrimester 2nd visit.

Table 40: Longitudinal flow cytometry data for Group 2 at baseline, second and third trimester

	Baseline at 13±1 weeks (n=42)	**2nd visit at 20±1 weeks (n=28)**	**3rd visit at 32±1 weeks (n=20)**	***P***
Monocytes (per µl)				
Total monocytes	425 (374–514)	491 (407–581) †	554 (473–673) †	0.02
Mon1	357 (309–457)	421 (344–483) †	465 (423–555) †	0.02
Mon2	19 (10–41)	19 (7–36)	41 (19–69) †	0.04
Mon3	38 (29–58)	39 (31–68)	36 (30–50)	0.52
MPAs (per µl)				
MPA	47 (34–66)	48 (39–61)	50 (25–66)	0.30
MPA1	35 (26–47)	40 (29–48)	41 (18–53)	0.34
MPA2	3 (1–6)	2 (2–6)	4 (2–7)	0.89
MPA3	5 (4–9)	6 (4–9)	5 (3–7) †	0.04

Data expressed as median (interquartile range). MPA, monocyte-platelet aggregates; MFI, mean fluorescent intensity.
Post hoc testing:
† $P<0.05$ vs baseline visit; ‡ $P<0.05$ vs midtrimester 2nd visit.

6.5 Discussion

The associations I have found may be involved in the aetiology of hypertension in pregnancy and its conferring increased cardiovascular risk after affected pregnancy. Whilst it is scientifically plausible that the associations could imply causation, they could also represent coincidental changes, or changes associated with compensatory mechanisms for other disease-related changes. The overall increase in monocyte count in pregnancy is consistent with previous studies[222] demonstrating an immunological response to the gravid state.

The increase in Mon1 counts that I have shown in pregnant women with previous hypertension could be interpreted in several ways. The increased number of these circulating monocytes which release cytokines could be involved in the pathogenesis of prehypertension and hypertension in pregnancy. Endothelial damage/dysfunction and resulting inflammatory response may be responsible for the increased monocyte counts. The increase in monocyte platelet aggregates, especially those associated with Mon1, in women with prior hypertension reflects monocyte activation in these women. Previous work has shown Mon 2 to be increased, and make up a greater proportion of the monocyte count in women with preeclampsia, with counts correlating with disease severity.[222] My results show Mon2 to be unchanged at baseline or during pregnancy in women with prior gestational hypertensive disease. The increase in Mon3 in both groups of pregnant women could point to a pattern of increased immune surveillance.

Monocyte migration can be controlled by the modulation of their cell surface receptors. Monocyte chemo attractant protein-1 (MCP1) binds to CCR2 on Mon1 to promote its active recruitment to sites of inflammation.[183] Bosco *et al* propose that downregulation of CCR2

leads to trapping of monocytes to retain them at pathological sites. A reduction in CCR2 expression has been shown in hypoxic states.[287] Increased expression of CCR2 reflects increased activation of monocytes. CCR2 being less expressed on both subsets on which it is present (Mon1 and Mon2) in pregnant women compared to non-pregnant controls may mean it is attenuated in pregnancy as part of the immunological adaptation to the pregnant state. Reduced CCR2 expression may be detrimental if it results in less clearance of cellular debris and reduced beneficial phagocytosis and cytokine release. In contrast, CCR2 expression has been shown to be increased on monocytes of obese women, with enhanced migration towards inflammatory stimuli.[209]

Monocytes interact with lipopolysaccharide via CD14, which is the molecule on the surface of monocytes involved in their activation.[216] This interaction stimulates the production of cytokines. It has been suggested that altered CD14 expression on monocyte subsets may play a role in the altered immunological state in heart failure.[288] No difference in CD14 expression was shown between the groups in this study so further evidence for the role of this cell surface marker in cardiovascular pathophysiology cannot be provided.

Monocyte count is perhaps less important than monocyte function. The functional role of monocytes was beyond the scope of my study. The phagocytic function of monocytes has been previously demonstrated in pregnancy and in hypertension.[236] Reduced elimination of factors causing cardiovascular dysfunction may be a mechanism contributing to the evolution of pregnancy complications.

It was interesting to observe the monocyte count in women with prior hypertension being

higher than in unaffected women at baseline, but reduce during the pregnancy, whilst for women without prior hypertension the opposite was true with the count starting lower and rising to reach a level similar to that of the baseline of Group 1. It is possible that women in Group 1 mount an increased inflammatory response during pregnancy, and this accounts for their previous pregnancy complication. It could also be true that having had a pregnancy complication last time, the innate immune system shows increased activity in the subsequent pregnancy. These data cannot explain why the counts are increased or delineate cause and effect.

6.6 Strengths and limitations

Because of the paucity of existing literature on the roles of the monocyte subsets in pregnancy, the potential for confounding is greater. I acknowledge the potential for confounding due to as yet unrecognised cofactors, the identification of which requires further work.

Appointments were routinely made in the mornings to standardize the sample collection time. On rare occasions if a patient could only attend in the afternoon then in order to maximise the completeness of follow up a minority of appointments were made later but conditions were standardised in accordance with the protocol. Since the number of afternoon appointments was few, the influence of diurnal variation is likely to be small, but may represent a limitation of the study.[252]

Studies with a small sample size are more susceptible to random errors. The care taken during data acquisition, checking and analysis should minimise this risk. The small number

of participants in the study increases the chance of a false negative findings, the so-called Type 2 error. The repeated measures of the same participants in the longitudinal study introduces the possibility of Type 1 error. In order to reduce the risk of false positive findings, post hoc testing was employed in order to raise the threshold for significance levels as previously described.

I measured monocytes in peripheral blood, and these levels might not reflect the counts of their counterparts in the tissues, such as macrophages in the placenta or myocardium. These observational, in vivo, data can be used to generate hypotheses but more experimental data is required to test them.

Differences between the groups (and indeed aspects in which they are similar) could be attributable to variables which are not controlled for. An example is aspirin use in the study group. It was not possible to exclude women taking aspirin as aspirin is recommended for all women with previous hypertension in pregnancy. There is emerging evidence that the so-called "low dose" aspirin (75mg) is insufficient and a higher dose is more effective in reducing pregnancy complications.[89, 289] Antiplatelet therapy has been previously shown to reduce MPAs.[241, 242] Recent evidence from a study which assured compliance with aspirin therapy showed no significant difference in MPA count with low dose aspirin.[290] In my study MPAs were higher in Group 1 in which aspirin consumption was higher. There was no difference in platelet count between the groups. Sensitivity to aspirin and treatment response varies.[291] Patients whose platelet reactivity remains high despite antiplatelet treatment are at increased risk of major adverse cardiac events and this might be related to the pro-inflammatory effects of MPA formation.[292] It is likely that other factors in Group 1 cause

platelet activation and their complex formation with monocytes despite being on treatment. I did not assess compliance with aspirin treatment and additionally there are numerous mechanisms of aspirin resistance including poor absorption, accelerated platelet turnover producing new unaffected platelets and platelet interactions with other cells such as monocytes.[293]

The fact that all women with previous pregnancy hypertension were by definition parous introduced another difference between the groups. Matching for parity would have necessitated the exclusion of primigravidae from the study. Restricting recruitment to multiparous women would have reduced rate of recruitment and the ability to compare findings with other published work.

I did not perform any work on the levels of pro- or anti-inflammatory cytokines which would have been useful data support hypotheses about the function roles of the monocyte subsets. Since these are observational data, the biological importance of the differences seen between groups is debatable, and one can only speculate at this stage. This substudy was not powered to evaluate the effect of individual monocyte subsets on clinical outcomes.

6.7 Conclusions

I have described for the first time the differences in monocyte subsets between women with previous gestational hypertensive disease and healthy pregnant women. These data are enhanced by comparison with a group of healthy non-pregnant women, and by use of the most up-to-date nomenclature to define the monocyte subsets. These preliminary data demonstrates the importance of separate analysis of the three distinct monocyte subsets.

CHAPTER 7: ECHOCARDIOGRAPHIC STRUCTURE AND FUNCTION, MONOCYTE SUBSETS AND PREGNANCY OUTCOMES

7.1 Introduction to chapter

In **Chapter 5** I presented data from the cardiovascular studies and in **Chapter 6**, results from the monocyte substudy were shared. The aim of this chapter is to integrate these data in order to assess the relationship between echocardiographic structure and function and monocyte heterogeneity in pregnancies with and without current and past hypertension. Maternal and fetal outcomes will also be discussed.

7.2 Aims and hypothesis

My aims and hypotheses are stated in **Chapter 3**. To briefly recap, the hypotheses are that:

1) abnormal ventricular-arterial interaction will be found in women with a history of hypertension in pregnancy;

2) changes in cardiac structure and function may be predictive of recurrent gestational hypertensive disease;

3) levels of circulating monocytes would be related to the ventricular-arterial interaction.

7.3 Methods and materials

7.3.1 Postnatal data collection

Data pertaining to delivery, obstetric complications and blood pressure was obtained from the electronic patient record, information from General Practitioners and verification with patients by telephone follow up.

7.3.2 Statistical analysis

The statistical analysis plan is described in **section 4.9**. Normally distributed data are expressed as mean±SD and non-normally distributed data as median±IQR. The Chi squared test was used to compare outcomes between groups when they were categorical. The Student's t test was used when the data was continuous and normally distributed, whilst the non-parametric Mann Whitney test was used for continuous data which were not normally distributed. A *P* value of <0.05 is considered statistically significant.

For correlation and regression analyses, data were checked carefully to ensure that assumptions are met and that the tests are valid. Bivariate Pearson correlation was performed to assess the association between monocyte subsets and cardiovascular parameters. Variables which are significant in univariate analysis, and clinically important factors, are then used in multivariable analysis. Binomial logistic regression was used to analyse variables associated with the development of hypertension in pregnancy. Multivariable linear regression was used to identify independent associations in the dataset.

Where statistical analysis is not appropriate due to small numbers, maternal and fetal outcomes are described without quantitative analysis.

7.4 Results

7.4.1 Obstetric outcomes

Some 9 women (12%) delivered at another hospital, having transferred their care during the pregnancy for various reasons relating to maternal choice/convenience. There were no

transfers of care for obstetric or fetal indications. All the women who delivered elsewhere gave permission for their data to be used in the study and for the collection of outcome data. A complete set of outcome data was collected.

The mean gestation at delivery was 39^{+0} weeks (273±10 days) for Group 1 and 39^{+5} weeks (278±11 days) for Group 2 (*P*=0.08). In Group 1 there were 14 vaginal deliveries, of which 1 was assisted. In Group 2 there were 41 vaginal deliveries of which 6 were assisted. There were 3 emergency and 8 elective Caesarean sections in Group 1, and 9 Caesarean sections in Group 2, of which 4 were emergencies and 5 planned. The increased Caesarean section rate in Group 1 is most likely due the number of these parous women with a previous Caesarean section, who had a repeat Caesarean section in this pregnancy.

There was no significant difference in birthweight between groups with a mean birthweight of 3198±568g in Group 1 and 3330±586g in Group 2 (*P*=0.36). Birthweight was under the 10^{th} customised centile for gestation for 7 babies overall, 3/25 in Group 1 and 4/50 in Group 2 (*P*=0.58). At the other end of the spectrum for growth, there were 10 babies with birthweight above the 90^{th} customised centile, 2/25 in Group 1 and 8/50 in Group 2 (*P*=0.34).

The only known case of fetal abnormality was a baby with Down syndrome that was not diagnosed antenatally. The mother was the 40-year-old multipara who developed gestational hypertension. She had declined screening for trisomies 13, 18 and 21 and there had been no anomalies detected on antenatal scans. Other obstetric complications included one case of spontaneous preterm labour and delivery in each group, both after 35 completed weeks. Some 5 women developed gestational diabetes during the study. There were 3 cases of

postpartum haemorrhage. There were no cases of placenta praevia or abnormally invasive placenta in the study participants. There was one case of term stillbirth in a woman from Group 2 with normal blood pressure. She attended for elective repeat Caesarean section and reported reduced fetal movements and scan diagnosed late intrauterine fetal death. The birthweight was normal and consistent with antenatal scans. Placental histology and post mortem was declined by the parents, and routine investigations failed to reveal any cause, therefore the stillbirth is unexplained. None of these adverse obstetric outcomes were related to the conduct of the study or the patient's participation in my research. There were no complications with any of the study procedures.

7.4.2 Hypertension in pregnancy

Overall 9 women (12%) developed hypertension in pregnancy, of which 6 were in Group 1 (prior hypertension) and 3 from Group 2. The incidence of hypertension in pregnancy was significantly different between groups (6/25 vs 3/50, P=0.024). Of those in Group 1 who developed hypertension in pregnancy, there were 4 cases of gestational hypertension, of which all were late onset at term. Two women developed recurrent preeclampsia. One case was in a patient who had early onset preeclampsia in her first pregnancy, who developed hypertension and proteinuria with a PCR of 96 from 37 weeks of gestation. She was induced then had a vaginal delivery of a 4920g baby complicated by shoulder dystocia. The other case of recurrent preeclampsia was a woman who had late onset PET last time and presented with a booking blood pressure of 137/75mmHg. At her research appointment at 19 weeks of gestation her blood pressure was 140–145/69–72mmHg with no proteinuria. She developed proteinuria from 23 weeks of gestation, with PCR of 63 and normal renal function throughout the pregnancy. This is most likely to be a case of essential hypertension with superimposed

preeclampsia. The preeclampsia remained a mild case controlled with labetalol monotherapy and delivery was at 38 weeks with normal birthweight. Blood pressure settled into the prehypertension range after the cessation of antihypertensives in the postnatal period.

In Group 2 there were 3 women who developed hypertension for the first time. One was 40 years old, now Para 3. She had a BMI of 40 and Type 2 diabetes requiring insulin in pregnancy. She developed hypertension postnatally which then resolved with no requirement for antihypertensives 6 weeks after delivery. The other two cases were in primiparae, one of whom developed gestational hypertension at 36 weeks, the other mild preeclampsia with a PCR of 129 at 38 weeks.

The women who developed hypertension had a significantly higher booking weight compared to those women who remained normotensive (P=0.04). The ratio E/e' was significantly higher at 20±1 weeks of gestation in women who developed gestational hypertensive disease (P=0.005) and affected women also had a reduced ejection fraction at the midtrimester time point (P=0.01). No other significant differences were observed, accounting for the small number of cases.

7.4.3 Correlation and regression analyses of experimental data

Correlation

There was a significant, positive correlation between Mon1 and total vascular resistance index, end-systolic elastance and arterial elastance index (**Table 41**). For total monocyte count, there was also a significant positive correlation with ventricular (Ees) and arterial (EaI) elastance parameters. For augmentation index adjusted to heart rate 75 beats per minute,

Mon1, Mon3 and total monocytes were all positively correlated ($P<0.05$ for all). Mon1 was significantly and negatively correlated with left ventricular volume index, as was total monocyte count.

Table 41: Correlation analysis between monocyte subsets and cardiovascular parameters at 13±1 weeks of gestation

	Mon1		Mon2		Mon3		Total monocytes	
	r	***P***	**r**	***P***	**r**	***P***	**r**	***P***
Haemodynamics								
Stroke volume index (ml/m^2)	–0.17	0.21	–0.02	0.90	–0.01	0.97	–0.15	0.27
Cardiac index (L/min/m^2)	–0.03	0.80	–0.08	0.55	0.13	0.34	–0.01	0.92
Total vascular resistance index (dyne.s/cm^5/m^2)	0.27	0.04	0	1.0	–0.02	0.86	0.19	0.15
Ventricular parameters								
Left ventricle volume index (ml/m^2)	–0.33	0.02	–0.24	0.08	–0.14	0.31	–0.34	0.01
Left ventricular mass index (g/m^2)	–0.05	0.72	0.11	0.38	–0.05	0.69	–0.03	0.84
End-systolic elastance	0.34	0.008	0.04	0.74	0.14	0.29	0.32	0.01
End-diastolic elastance	0.25	0.06	0.06	0.64	–0.07	0.58	0.21	0.11
Diastolic function								
E/A ratio	–0.16	0.22	–0.01	0.95	–0.11	0.42	–0.17	0.19
E/e'	–0.02	0.88	0.04	0.76	–0.09	0.49	–0.01	0.92
Arterial parameters								
Arterial elastance index	0.43	<0.001	–0.10	0.46	0.06	0.67	0.35	0.007
AIx75	0.29	0.03	–0.02	0.90	0.27	0.04	0.31	0.02
Aix75MAP	0.10	0.50	–0.02	0.91	0.11	0.48	0.14	0.36
Ventricular-arterial coupling								
Arterial-ventricular interaction	–0.03	0.83	–0.08	0.52	–0.15	0.27	–0.05	0.69

AIx75, augmentation index adjusted to standardise for heart rate 75 beats per minute; Aix75MAP, Aix75 adjusted for mean arterial pressure; r, Pearson correlation coefficient.

Regression

In this final section I present some exploratory analyses of my experimental data, to assess the association between the cardiovascular parameters (presented in **Chapter 5**) and the monocyte subsets (presented in **Chapter 6**).

Table 42: Multivariable logistic regression of predictors of hypertension – blood pressure at 13±1 weeks of gestation

Variable	OR	95% CI	*P*
Age	1.04	0.90–1.21	0.58
Parity	0.81	0.13–4.97	0.82
SBP (mmHg)	**1.11**	**1.02–1.20**	**0.02**

CI, confidence interval; OR, odds ratio; SBP, peripheral systolic blood pressure.

These variables statistically significantly predict development of hypertension in the current pregnancy P=0.03, R^2=0.16. Systolic blood pressure was the only variable adding significantly to the prediction.

Table 43: Multivariable logistic regression of predictors of hypertension – blood pressure at 13±1 weeks of gestation

Variable	OR	95% CI	*P*
Age	1.04	0.89–1.23	0.59
Parity	0.82	0.13–5.10	0.84
SBP (mmHg)	**1.11**	**1.02–1.21**	**0.02**
White ethnicity	1.12	0.21–6.04	0.90

Aspirin	0.74	0.11–5.04	0.76

CI, confidence interval; OR, odds ratio; SBP, peripheral systolic blood pressure.

This model is not statistically significant for the prediction of hypertension in the current pregnancy but demonstrates that systolic blood pressure remains a significant predictor for hypertension later in pregnancy after adjustment for age, parity, ethnicity and aspirin consumption.

Table 44: Multivariable logistic regression for association with left ventricular mass index at 13±1 weeks of gestation

Variable	Coefficient	Standard error	*P*
Age	–0.003	0.007	0.03
Parity	0.037	0.073	0.61
White ethnicity	0.045	0.071	0.53
Systolic blood pressure	**0.011**	**0.003**	**0.001**
Mon1	–0.15	0.11	0.19

Systolic blood pressure is statistically significantly associated with left ventricular mass index, after adjustment for age, parity, ethnicity and Mon1 count. This model has $F(4,70)= 2.7$, $P=0.03$, $R^2=0.13$ with systolic blood pressure the only significant association.

Table 45: Multivariable logistic regression for association with total vascular resistance index at 13±1 weeks of gestation

Variable	Coefficient	Standard error	*P*
Age	0.008	0.006	0.22
Parity	–0.14	0.064	0.03
White ethnicity	–0.69	0.063	0.27

Systolic blood pressure	–0.0006	0.0027	0.84
Mon1	**0.21**	**0.10**	**0.04**

These variables have a statistically significant association with total vascular resistance index, F(5,53)=2.42, *P*=0.047, R^2=0.11. Mon1 count and parity were the only variables adding significantly to the model.

Table 46: Multivariable logistic regression for association with left ventricular volume index at 13±1 weeks of gestation

Variable	Coefficient	Standard error	*P*
Age	–0.003	0.006	0.62
Parity	0.041	0.061	0.50
White ethnicity	–0.034	0.059	0.57
Systolic blood pressure	0.003	0.003	0.18
Mon1	**–0.26**	**0.092**	**0.007**

This model does not statistically significantly predict left ventricular volume, F(5,49)=1.77, *P*=0.14, R^2=0.07. The association between left ventricular volume index and Mon1 count remains statistically significant after adjustment for age, parity, ethnicity and systolic blood pressure.

Table 47: Multivariable logistic regression for association with end systolic elastance at 13±1 weeks of gestation

Variable	Coefficient	Standard error	*P*
Age	0.0004	0.011	0.97
Parity	–0.17	0.12	0.17

White ethnicity	–0.18	0.12	0.14
Systolic blood pressure	0.003	0.005	0.54
Mon1	**0.45**	**0.19**	**0.02**

Mon1 is significantly associated with end systolic elastance, after correcting for age, parity, ethnicity and systolic blood pressure. This model statistically significantly predicts end systolic elastance, F(5,53)=2.49, *P*=0.04 , R^2=0.11.

Table 48: Multivariable logistic regression for association with arterial elastance index at 13±1 weeks of gestation

Variable	Coefficient	Standard error	*P*
Age	0.001	0.005	0.80
Parity	0.0001	0.051	0.99
White ethnicity	–0.08	0.05	0.10
Systolic blood pressure	0.0008	0.002	0.73
Mon1	**0.23**	**0.077**	**0.004**

Mon1 remains significantly associated with arterial elastance index after correction for age, parity, ethnicity and systolic blood pressure. For this model F(5,53)=3.23, *P*=0.01 , R^2=0.16.

7.5 Discussion

I have shown an association between monocyte counts, especially Mon1, and measures of arterial and ventricular elastance in pregnant women. Mon1 monocytes are large, highly phagocytic cells involved in resolving inflammation and repairing tissue injury, but their adherence and infiltration into vascular walls at the sites of endothelial injury can also be

detrimental. These data suggest that monocytes may have a role in the pathogenesis of hypertension in pregnancy. Mon1 may be involved in the development of hypertension, or may be part of the (mal)adaptation to raised blood pressure. I described in my systematic review (**Chapter 2**) that cardiac structural and functional changes are present before hypertensive disorders of pregnancy manifest clinically. More work is required to discover if differences in monocyte subsets are associated with these findings in women destined to develop hypertension in pregnancy.

Of the 9 women who developed hypertension in pregnancy, all but one had a BMI greater than 25 at booking. Some 4 were overweight (BMI 25-29.9), 1 was obese (BMI 30-30.9), and 3 were severely obese (BMI>40). It is proposed that the presence of metabolic syndrome, low-grade inflammation and endothelial dysfunction lead to the increased incidence of preeclampsia in obese women.[294] In a recent review, Arena *et al* describe an obesity-hypertension phenotype in the midst of the "non-communicable disease crisis" posed by the obesity epidemic.[295] Accumulation of adipose tissue leads to increased sympathetic nervous system activity, changes in endocrine function (particularly blood glucose regulation and sodium homeostasis via hyperinsulinaemia and insulin resistance) and altered renal and endothelial function.[295] There are proponents of the placental origin hypothesis, as well as those who advocate for the cardiovascular origins of preeclampsia. It is possible that PET represents a common phenotypic expression of two distinct processes, the first being a predisposition to cardiovascular disease and the second being impaired trophoblastic invasion.[296]

There are several reasons why incidence of hypertension in pregnancy is a risk factor for

future cardiovascular disease. PET and cardiovascular disease share the same risk factors. Pregnancy can be regarded as a 'stress test' whereby a problem is unmasked by the demands of pregnancy. It is also possible that hypertension itself, and PET in particular, causes endothelial damage and dysfunction of which the legacy is future cardiovascular disease. It would be interesting to compare the long-term outcomes of women with an increased latency from PET diagnosis to delivery, with those whose pregnancy shortened by early planned delivery, to determine whether duration of exposure makes a difference to outcome.

7.6 Strengths and limitations

In sections **5.6** and **6.6** of the preceding chapters I have discussed the limitations of my methodology. This previous critical appraisal of the study is relevant also to this chapter. The number of patients in this study is relatively small and this must be borne in mind when interpreting the results, as there is a chance of bias. In striving for a thorough analysis of the data in order to explore any plausible relationship between measurements, multiple comparisons have been made. This increases the risk of false positive findings. If 20 null hypotheses were rejected during a thesis where the threshold for significance is $P<0.05$, it is likely that one difference quoted as significant would be a chance finding. With this in mind, I have described throughout when post hoc tests have been employed, and when the *P* value to achieve statistical significance has been adjusted to reduce the risk of Type 1 error.

Changes in cardiac function happen in early gestation. The first trimester should not be ignored and recently innovative studies have been able to follow women from before conception to early gestation and throughout pregnancy. Pre-conceptual can determine a woman's baseline cardiac function, rather than using non-pregnant or postpartum indices as a

control. More studies starting before pregnancy will help to answer whether women affected by hypertension in pregnancy have alterations even before the pregnancy begins. Preconceptual studies are methodologically challenging. Women accessing obstetric and gynaecological services prior to pregnancy tend to have medical problems requiring preconceptual counselling or subfertility issues. In my study over 10% of women who booked their antenatal care at City Hospital delivered their baby elsewhere. Studies beginning in the preconceptual period are likely to require either a more stable population (for example in more remote, rural areas) or a commitment from or incentive for women to attend follow up appointments.

7.7 Conclusions

I have demonstrated an association between monocytes and arterial and ventricular stiffness. The importance of recognising 'high normal' blood pressure as 'not normal' has been reiterated, since increasing blood pressure in early pregnancy is related to development hypertension later. More prospective studies involving large numbers of women, ideally starting before conception and following women through pregnancy, are required investigate further.

CHAPTER 8:
SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

8.1 Summary of findings

My systematic review identified that echocardiography is a valuable tool to stratify risk and can guide management in the preclinical and clinical phases of gestational hypertension and preeclampsia. Increased vascular resistance and LV mass were the most consistent findings in hypertensive disorders of pregnancy. Differentiating features from normal pregnancy were LV wall thickness ≥1.0cm, exaggerated reduction in E/A and lateral e' <14cm/s. There was disagreement between studies with regard to cardiac output due to the timing of echocardiography, although reduced stroke volume was an indicator of adverse prognosis. Diastolic dysfunction and left ventricular remodelling are most marked in severe and early-onset PET, but are also markers of PET before clinical manifestation, and are associated with adverse outcomes.

Women with prior hypertension are more likely to have blood pressure in the 120–139/80–99mmHg (prehypertension) range, and their blood pressures were higher than other pregnant women throughout. There is an association between blood pressure level at the beginning and end of second trimester and later development of hypertension.

I recognised the physiological adaptation of the heart to pregnancy in many of the parameters studied at baseline (13 weeks of gestation). When comparing pregnant women to non-pregnant controls, the former had increased cardiac output, reduced total vascular resistance and increased left ventricular volumes. Increased left ventricular filling pressures were observed in pregnancy.

In the study group of pregnant women with previous hypertension in pregnancy some

differences were observed. There was a trend towards increased left ventricular mass index. Late diastolic transmitral flow velocities (A wave) were increased compared to other pregnant and non-pregnant women and the E/A ratio was lower. Augmentation index (adjusted for heart rate and blood pressure) was increased compared to both other groups. Women without prior hypertension demonstrated more compliance (reduced EaI and Ees) compared to the non-pregnant controls, but this adaptation was not seen in pregnancy after hypertension where increased arterial stiffness was observed.

In the longitudinal analysis of cardiovascular parameters, some changes were seen irrespective of history of hypertension. There was a reduction in E/A and e' in the third trimester. An increase in Ees resulted in a reduced EaI/Ees (ventriculo-arterial coupling) as a measure of cardiovascular performance by the third trimester.

CD14++CD16–CCR2+ (Mon1) and CD14+CD16++CCR2– (Mon3) subsets and their aggregates with platelets were increased in pregnant women with previous hypertension at baseline. Total monocyte counts, and Mon1 in particular, were significantly and positively correlated with ventricular (Ees) and arterial (EaI) elastances. Mon1 was also positively correlated with augmentation index and total vascular resistance index.

The women who developed hypertension during the study had a significantly higher booking weight compared to those women who remained normotensive. Worse myocardial compliance in association with higher left ventricular early filling pressures (significantly higher E/e') were observed at 20±1 weeks of gestation in women who developed hypertension later in pregnancy.

8.2 Conclusions

Elevated arterial elastance occurs in pregnant women with a past history of gestational hypertension or preeclampsia, despite similar ventricular elastance to women without prior hypertension. Mon1 monocytes are large, highly phagocytic cells involved in resolving inflammation and repairing tissue injury, but their adherence and infiltration into vascular walls at the sites of endothelial injury can also be detrimental, and may be responsible increased arterial stiffness in women with a history of hypertension in pregnancy. Changes in cardiovascular performance and their relationship to monocyte heterogeneity may potentially be mechanisms leading to increased long term cardiovascular risk following pregnancy hypertension. Echocardiography is a valuable tool to stratify risk and can guide management and counselling for women with current or past history of hypertension in pregnancy.

8.3 Implications for clinical practice

The Rapid Obstetric Screening Echocardiogram (ROSE) has been developed by Alicia Dennis and colleagues in Melbourne.[297] They advocate its use for acutely unwell pregnant women and its clinical utility will be tested in prospective studies. In a recent systematic review and validation study, transthoracic echocardiographic assessment of cardiac output was found to have excellent agreement with pulmonary artery catheterization in pregnant women in the critical care setting. The authors therefore suggest that echocardiography be considered the new gold reference standard.[97] As the use of echocardiography in obstetric care increases, there will be more potential for large scale collaborative studies. More data is required regarding added clinical value in terms of improving outcomes, in order to make recommendations for the use of echocardiography in routine clinical practice.

Since the publication of my systematic review in which I suggested that haemodynamic assessment could be used to guide choice of antihypertensive medication, Stott *et al* have published exciting results from a study in which they demonstrated that the implementation of haemodynamic monitoring reduced the rate of severe hypertension in women starting antihypertensive therapy.[298] The haemodynamic insight enabled informed decisions to provide labetalol monotherapy to women with low vascular resistance and dual therapy with a vasodilator for those with high vascular resistance.[299] Prospective clinical studies have been made more possible by the advent of non-invasive monitors.

Several non-invasive monitors for haemodynamic assessment have emerged on the market during the lifetime of this research. The Ultrasound Cardiac Output Monitor (USCOM®, USCOM Ltd, Sydney, Australia) uses continuous wave Doppler to analyse transthoracic blood flow. The Non-Invasive Cardiac Output Monitor (NICOM®, Cheetah Medical, Boston, USA) uses probes applied to the chest wall to measure thoracic bioreactance and as such is operator-independent. In a recent study the USCOM and NICOM devices were compared with transthoracic echocardiography and there was good agreement for measurement of cardiac output in the third trimester, although due to differences in precise values the authors recommend the development of device-specific reference ranges.[282] I will consider such devices when developing future research protocols since the learning curve for their use is shorter than that for echocardiography, facilitating more widespread training amongst the research team. Further work is required to define the use of such devices in clinical practice.

8.4 Future directions and suggestions for future study

It is important to delineate which factors are responsible for irreversible changes in cardiac structure and function. If modifiable risk factors for pathological remodeling can be identified then interventions can be targeted towards preventing maladaptation with its adverse long-term consequences.

Cardiac magnetic resonance imaging (MRI) is superior to echocardiography in terms of the amount and quality of data it provides about cardiac structure and function. It is an accurate and precise imaging modality but is less available and acceptable to pregnant women. Expertise is required to perform and interpret the MRI findings. Cardiac MRI in maternal medicine is an exciting prospect.

Whilst research investigating cardiac output is important, it is crucial that increased attention is given to diastole in future research. Pregnant women are different to the older population who comprise majority of cardiologists' patients. In younger patients, diastolic dysfunction will precede systolic heart failure for considerable time, in contrast to the elderly where there is increased myocardial fibrosis. Diastolic dysfunction should be at the forefront of future research.

Obstetricians and their patients require a better screening test for the hypertensive disorders of pregnancy and their complications in order to improve maternal and fetal outcomes. If women could be triaged more accurately into high and low risk groups then resources (more regular monitoring, growth surveillance, planned delivery) could be targeted where they are most needed. Maternal haemodynamics, besides mean arterial pressure and uterine artery

Doppler, do not yet have a defined role in prediction of pregnancy hypertension. These parameters are likely to be used in screening models for PET, including that superimposed on chronic hypertension or developing from GH, and its complications. To justify screening, whether it be in the first trimester or later in pregnancy, further research into effective interventions is also required in order to improve outcomes for 'screen positive' women and their babies.

The results of a recent randomised controlled trial show that aspirin prevents preeclampsia.[89] The Pre-eclampsia in Hospital: Early Induction or Expectant Management (Phoenix) Study is a randomised trial, due to report later in 2019, which investigated whether a decision for delivery or expectant management, when PET is diagnosed between 34^{+0} and 36^{+6} weeks, leads to the better maternal and fetal outcomes.[300] Objective measures are required for decision tools in clinical practice since randomisation is only justified in the research setting. Availability of haemodynamic assessment after PET diagnosis may help to stratify women into a high risk group requiring early delivery and a low risk group suitable for standard management. The extent to which the heart is affected by preeclampsia, or by non-proteinuric hypertension, might depend on the latency between diagnosis, delivery and resolution of the condition. It is possible that expectant management may be detrimental to the woman, due to prolonged and excessive exposure of a heart that has failed to adapt to pregnancy to further stress. In this sense, obstetric management may contribute to the excess of cardiovascular morbidity in later life following preeclampsia. Data relating to length of exposure of the cardiovascular system to raised blood pressure, or to the multisystem syndrome of preeclampsia, would be useful in future studies. This would be methodologically difficult in terms of definition of start and end points and acquisition of the data.

This thesis has studied cardiac structure and function in relation to hypertensive disorders in pregnancy. Cardiac dysfunction is linked to placental dysfunction, since the placenta is an organ requiring adequate perfusion. Placental pathology secondary to cardiovascular pathology results in disordered fetal growth. Screening, diagnosis and management of fetal growth restriction focusses on reducing stillbirth and developmental handicap. Further work is required to determine if the failure of the maternal heart to meet the demands of pregnancy is related to disorders of fetal growth and their sequelae, and which techniques will enable such cases to be identified. If cardiac dysfunction can be identified near term, this may help to identify the fetuses at risk. The key problem with growth restriction at term is detection as most are not small. Many of the babies at risk have an estimated fetal weight in the normal range, but they are growth restricted because they had the potential to be bigger. Cardiac dysfunction could be a new indication for a third trimester fetal growth scan for women who would otherwise not qualify for this examination, which is more sensitive than the routine measurement of symphysis-fundal height.

How to follow up women with a history of hypertension in pregnancy has not been defined and as such practice varies. It is important to identify what sort of surveillance these women require and which healthcare professional should oversee this. Strategies to reduce the risk of long term cardiovascular morbidity might include lifestyle modification, structured exercise programmes or medications such as aspirin or angiotensin-converting-enzyme inhibitors even in asymptomatic patients.

Prehypertension has not yet received much recognition from obstetricians and warrants

further study. Future work must clarify the effect of prehypertension preconceptually and during pregnancy. It is important to ascertain whether reducing the length of time the heart is exposed to blood pressure in the prehypertensive range will improve long term outcomes. In the maternal medicine sphere the effect of lowering blood pressure on maternal and fetal outcomes is also of great interest. Although contraindicated during pregnancy, the use of angiotensin-converting enzyme (ACE) inhibition for cardioprotection may prevent pathological remodelling in prehypertensive women of childbearing age, and their use might be considered before conception and after delivery.

Appendices

1. Publications arising from this thesis

Castleman JS, Ganapathy R, Taki F, Lip GYH, Steeds RP, Kotecha D. Echocardiographic structure and function in hypertensive disorders of pregnancy: A systematic review. *Circulation Cardiovascular Imaging* 2016 Sep; 9(9) doi: 10.1161/CIRCIMAGING.116.004888

Ganapathy R, Grewal A, Castleman JS. Remote monitoring of blood pressure to reduce the risk of preeclampsia related complications with an innovative use of mobile technology. *Pregnancy Hypertension* 2016 Oct; 6(4):263-265 doi: 10.1016/j.preghy.2016.04.005

2. Abstracts arising from this thesis

Castleman JS, Shantsila A, Brown RJ, Ganapathy R, Kotecha D, Shantsila E, Lip GYH. Monocyte subsets and arterial elastance at 13 weeks gestation and the effections of prior hypertension in pregnancy.
Presented at the 3rd International Congress on Maternal Hemodynamics, Cambridge, 2018

Castleman JS, Srinivasan M, Newport F, Cochran D, Ganapathy R. Reduced preoperative fibrinogen is associated with increased blood loss at caesarean section.
Presented at the British Maternal and Fetal Medicine Society Conference, Brighton, 2018
Published in: *BJOG* 2018 Apr; 125(S2): 41

Castleman JS, Ganapathy R, Kotecha D, Lip GYH, Shantsila E. Increased CD14++CD16-CCR2+ (Mon1) monocytes in pregnant women with previous hypertension.

Presented at the Birmingham and Midland Obstetrical and Gynaecological Society Autumn Meeting, Nuneaton, 2017

Castleman JS, Ganapathy R, Lip GYH, Shantsila E. Increased CD14++CD16-CCR2+ monocyte-platelet aggregates in pregnant women with previous hypertension.
Presented at the 16th World Congress in Fetal Medicine, Ljubljana, 2017.
Published online: https://fetalmedicine.org/abstracts/2017/var/pdf/abstracts/2017/2173.pdf

Castleman JS, Ganapathy R, Taki F, Lip GYH, Steeds RP, Kotecha D. Is there a role for echocardiography in the management of hypertensive disorders of pregnancy? Results from a systematic review.
Presented at the European Congress of Perinatal Medicine, Maastricht, 2016.
Published in *The Journal of Maternal-Fetal & Neonatal Medicine* 2016; 29(S1): 272

Castleman JS, Ganapathy R, Kotecha D, Lip GYH, Shantsila E. Increased CD14++CD16-CCR2+ (Mon1) monocytes in pregnant women with previous hypertension.
Presented at the European Congress of Perinatal Medicine, Maastricht, 2016.
Published in *The Journal of Maternal-Fetal & Neonatal Medicine* 2016; 29(S1): 273
Winner of the Young Investigator Award from European Association of Perinatal Medicine.

Castleman JS, Ganapathy R, Taki F, Lip GYH, Steeds RP, Kotecha D. Is there a role for echocardiography in the management of hypertensive disorders of pregnancy? Results from a systematic review.
Presented at the West Midlands Obstetrics and Gynaecology Trainees Committee Scientific

Meeting, Birmingham, 2016.

Castleman JS, Miti C, Ajibona OO, Lip GYH, Ganapathy R

Cardiopulmonary resuscitation with a different slant: the importance of perimortem caesarean delivery.

Presented at the European Society of Cardiology Congress, Barcelona, 2014.

Published in *European Heart Journal* 2014; 35(S1): 1208-9

3. Standard operating procedures (SOPs) used in this thesis

SOP: General Operation of the Flow Cytometer

N.B. Use of the flow cytometry is forbidden without having been officially trained.

1. Introduction

This is the general purpose SOP on

- start up
- calibration, and
- clean up of the flow cytometer.

Blood sample analysis for specific population will be dealt with by other SOPs

Health and Safety / COSHH issues

Beware of….

- Lasers
- Danger of electric shocks
- assume bloods may be biohazardous
- Bleach is toxic – do not eat/drink.

Contact details:

2.1 BD "FACS Flow" Running solution [Becton Dickinson Catalogue No. 342003]10L containers. Source: Becton Dickinson (UK) Between Towns Road Cowley Oxford , Oxfordshire,OX4 3LY Fax: 01865 781578

2.2 BD "FACS Clean" Cleaning Solution [Becton Dickinson Catalogue No. 340345]

2.3 Three ml BD Falcon tubes [Becton Dickinson, Catalogue No. 352054]

2.4 Clear pipette tips [Alpha Laboratories Limited Catalogue No **FR1250** 1250ul Fastrak Refill NS] 40 Parham Drive Eastleigh,Hampshire SO50 4NU UK Tel – 02380483000 Fax – 02380643701

2.5 Yellow pipette tips [[Alpha Laboratories Limited Catalogue No **FR1200** 200ul Fastrak Refill NS] 40 Parham Drive Eastleigh, Hampshire SO50 4NU UK Tel – 02380483000 Fax – 02380643701

2.6 Calibration beads from Becton Dickinson – these live in the top shelf of the door of the fridge.

3. Start up procedure

Part 1 – restoring reagents and preparation

1. Switch on Flow Cytometer by pressing the green switch on the right hand side. The Apple Macintosh computer must also be switched on, but only 15 secs **after** the Flow Cytometer, or the link will not be recognised. Open the reagent panel on the left hand side (LHS) by pulling the lid towards you. On the left is the sheath fluid reservoir, in the middle are switches and tubes, on the right is the waste reservoir.

2. Carefully unscrew the top of the sheath fluid reservoir and fill with sheath fluid (in large box on shelf at head height – use plastic tube) to the level indicated on the top right hand corner of the reservoir (little plastic bar).

3. Carefully disconnect/unscrew the waste container and empty contents down sink with plenty of water. Add approximately 40ml concentrated household bleach along with 360 ml of distilled water and reconnect container (plastic tubes available).

4. Pressurise the unit (takes about 20-30 sec) by moving the black toggle switch "Vent Valve" switch to the down/front position. It is located at the rear of the middle section between the sheath fluid tank and the waste container.

5. Air must be excluded from the tubing system by flushing it out. Any excess air trapped in the sheath filter can, if necessary, be cleared by venting through the bleed tube (the dead-ended rubber tube with a cap).

6. Close the drawer

Part 2 - cleaning the machine

7. Ensure that a 3 ml Falcon tube (labelled 1) approximately 1/3 full of distilled water is positioned over the sample injection port (SIP) – a needle sheath - and that the swing arm is positioned under this tube. Press the prime button on the panel. When the system enters "standby" with in 30 seconds then press the "prime" button again. When the standby and low buttons comes on again then remove tube 1. We will re-use tube 1 in the shut down procedure.

8. Prepare a second falcon tube (labelled 2) with FACS-clean (should contain about 2750 microliters so that when inserted on to the sip it doesn't touch the **O** ring). This is a

smaller box on a shelf at above head height and above the bigger box of sheath flow fluid

9. Present tube 2 to the SIP and place support arm underneath it. Press the buttons "run" and "high" on the panel at the same time, and run the FACS-clean in falcon tube 2 with supporting arm to left or right open for one minute. Then return the supporting arm to underneath the tube and "run-high" five minutes. This process ensures the machine is clean prior to running samples and helps minimise blockages.

10. Return to falcon tube 1 with distilled water. Repeat the above step 9 with this distilled water tube.

11. Press the '**STANDBY**' and '**LOW**' button on the system.

12. The machine is now ready to calibrate.

4. Calibration

Whoever calibrates the machine at the beginning of the day, carries the responsibility for ensuring correct function.

He/she will sign in the log book that the machine is calibrated.

The calibration sheet itself must be signed by the operator and placed in the folder on top of the printer.

Mr Balakrishnan is responsible for keeping these sheets in order and responding to any problems. He will periodically remove 'old' calibration sheets to his office for long term storage.

This procedure must be done right after the start up procedure so that the system is calibrated.

The regents (beads with known characteristics) can be found in the fridge behind the door.

Check the expiry dates and prepare the reagents as advised.

1. Take two 3 ml falcon tubes and mark one of them as A and the other one as B.

2. Aliquot 1 ml (clear tip micropipette) of sheath flow (big box head height on shelf) in tube A and 2 ml in tube B.

3. Take the box of Calibrite bead from the top rack of the fridge door.

4. Gently mix the five tubes by inverting upside down ten times so that the beads are suspended uniformly before using them

5. Prepare the tube A by very gently squeezing out a single drop of unlabelled beads (i.e. no flurochrome) (white top) and the labelled APC beads (blue). Be sure to mix the contents of tubes before inserting it onto the needle of the SIP.

6. Prepare the tube B by pouring a single drop of each of five beads; Unlabelled (white) APC beads (blue), Per-CP Beads (red), PE Beads (pink) and FITC Beads (green).

7. On the computer, click on the FACS-comp icon (with a picture of a test tube) in the icon menu bar and the FACS-comp window will appear on the computer screen displaying the user ID and other details. Click 'accept', which proceeds on to the product code/lot number. Ensure that the bead lot numbers are accurate and then carry on with the procedure.

8. Replace the beads back into their box and then into the fridge

9. Click the "run" button on the screen which indicates to insert the tube labelled A on to the SIP. Mix gently before inserting and press the buttons "high" and "run" on the control panel of the FAScalibur. Then press start.

10. The system then sets the photo-multiplier tube voltage settings (takes about 2 minutes) based on the tube A beads, and then tells you to insert the Tube B. Insert the tube B after mixing it gently, then press start.

11. The system again analyses the compensations and, if satisfied, gives the result that it is Ok to proceed.

12. Once this result is satisfactory, the results of this set up will be printed out. Write your name on the print out and place it in the FACS comp results folder. If the machine is not satisfied, it will tell you and you will seek assistance from Scientific Staff.

13. The machine is now ready to run blood samples according to the specific SOP

5. Shut-down procedure by the last operator of the day

Each operator must decide if he/she is the last operator of the day. This may be achieved by talking to colleagues. Shutting down procedure must be signed off in the log book

1. In this section we re-use tubes 1 and 2 with distilled water and FACS clean respectively.

2. Install FACS Clean tube 2 over the SIP needle. Press button '**High'** and '**Run'** on the panel. Leave the support arm out at 90 degrees for approximately 1 minute. This cleans the outer portion of the aspiration sheath. The fluid will be rapidly aspirated, so ensure that the tube doesn't empty completely.

3. Now replace the side arm under the Falcon tube and allow to run for approximately 5 minutes. This cleans the inner portion of the aspiration sheath and the FACS machine itself.

4. Repeat steps 2 and 3 with the distilled water tube 1. Once step 3 is complete, Leave the sheath in falcon tube 1 containing distilled water and press '**STANDBY'.**

5. Open the reservoir draw and depressurise the machine by moving the "Vent Valve" toggle switch to the up/rear position. The machine will hiss as it depressurises.

6. Leave the machine on for a further 5 minutes to allow the laser lamp to cool. Turning the machine off prematurely will result in the lamp cracking.

7. Finally power down the FACScalibur (green button) and Apple Mac, by clicking on the apple icon and clicking on the shut down option in the drop down menu.

8. Clean up !

9. Note: * IF THE SYSTEM IS TO BE USED AGAIN ON THE SAME DAY…

   LEAVE THE SYSTEM ON STANDBY and then

   DEPRESSURISE THE SYSTEM.

SOP: Absolute count of monocyte subsets and assessment of surface marker expression on them by Flow Cytometry

N.B. Use of the flow cytometry is forbidden without having been officially trained

Required pre-training

1. SOPs on venepuncture, good clinical practice

2. SOP 195 – general operation of the flow cytometer

1. Introduction

Monocytes are circulating blood cell participating in innate immunity, inflammatory response as well as other processes such as angiogenesis, formation of tissue macrophages and dendritic cells, etc. Monocyte include several subsets that can be discriminated on the basis of surface expression of CD14 (lipopolisacharide receptor) and CD16 (Fc gamma receptor III.

This method describes enumeration and characterisation of:

- CD14+CD16- monocytes (about 85%),

- CD14+CD16+ monocytes (also CCR2+, about 5%) and

- CD14lowCD16+ monocytes (also CCR2-, about 10%),

2. Materials and Supplier contact details:

1) BD "FACS Flow" Running solution [Becton Dickinson, Catalogue No. 342003] 10L containers.

2) BD "FACS Clean" Cleaning Solution [Becton Dickinson, Catalogue No. 340345]

3) 3 ml BD Falcon tubes [Becton Dickinson, Catalogue No. 352054]

4) BD Lysing solution [Becton Dickinson Catalogue No. 349202]

5) Sterile Phosphate Buffered Saline solution, 0.5L bottles [Invitrogen Ltd, Catalogue No 20012-068]

6) BD TruCount™ tubes [Becton Dickinson Catalogue No. 340334]

7) CD16-Alexa Fluor 488-conjugated monoclonal antibody [AbD Serotec, Oxford, UK, Cat No. MCA2537A488]

8) CD14 -PE conjugated monoclonal antibody [R&D Systems Europe Ltd, Cat No. FAB3832P]

9) CD14-PerCP-Cy5.5 conjugated monoclonal antibody [Becton Dickinson Catalogue No. 550787]

10) CCR2-APC conjugated monoclonal antibody [R&D Systems Europe Ltd, Cat No. FAB151A]

11) CD42a- conjugated monoclonal antibody [Becton Dickinson, Catalogue No. 340537]

12) VEGF R2 (KDR)-APC conjugated monoclonal antibody [R&D Systems Europe Ltd Catalogue No. FAB321A]

13) CD34-PE conjugated monoclonal antibody (Becton Dickinson, Catalogue No. 555822)

14) CD34-PerCP conjugated monoclonal antibody (Becton Dickinson, Catalogue No. 345803)

15) TLR4 (CD284)-PE conjugated monoclonal antibody [R&D Systems Europe Ltd Catalogue No. FAB14781P]

16) IL-6 receptor α-APC conjugated monoclonal antibody [R&D Systems Europe Ltd Catalogue No. FAB227A]

17) Mac-1-PE conjugated monoclonal antibody (CD11/CD18, receptor for ICAM-1) [R&D Systems Europe Ltd Catalogue No. FAB1730P]

18) Integrin α4/CD49 complex-APC conjugated monoclonal antibody [R&D Systems Europe Ltd Catalogue No. FAB1354A]

19) CD163-APC conjugated monoclonal antibody [R&D Systems Europe Ltd Catalogue No. FAB1607A]

20) CXCR4-PE conjugated monoclonal antibody (CD184) [R&D Systems Europe Ltd Catalogue No. FAB170P]

21) VEGFR1-APC conjugated monoclonal antibody (R&D Systems Europe Ltd Catalogue No. FAB321A)

22) Clear pipette tips [Alpha Laboratories Limited Catalogue No FR1250 1250ul Fastrak Refill NS]

23) Yellow pipette tips [[Alpha Laboratories Limited Catalogue No FR1200 200ul Fastrak Refill NS]

24) Pipettes required – 2-20ul (gray), 5-40ul (red), 40-200 ul (yellow), 200-1000ul (purple), 10-100ul and 100-1000ul digital

3. Detailed method

3.1 General Preparation

3.1.1 Lysing solution.

Make from 50ml concentrate 10x FACS Lysing Solution (kept at room temperature). Dilute with 450ml distilled water in ½ litre bottle. This solution should not be used if it is older than a month (kept at room temperature).

3.1.2. Mastermixes

Mastermixes are made by responsible person in clearly labelled tubes from dark glass. Estimated number of samples to be done from one mastermix should not exceed monthly number of samples done.

3.1.3. Time from blood sample collection to beginning of sample preparation should be less 30 min, and must not be more than 60 min.

3.2 Blood sample preparation

3.2.1. Place EDTA blood sample on rotator.
3.2.2. Label tubes 1-5 and additional tube for absolute count (AC)
3.2.3. Place Antibodies from 'mastermixes':
Tube 1 - 22.5ul (red)
Tube 2 - 22.5ul (red)
Tube 3 - 22.5ul (red)
Tube 4 - 27.5ul (red)
Tube 5 - 22.5ul (red)
AC tube - 12.5ul (gray) - place on opposite side to count beads. Do not touch the!!!! If this happened – change the tube.
3.2.4. Add blood to tubes
a) Absolute count tube:
- Take 10-100ul digital pipette
- Set to 2 x 50ul
- Withdraw blood then expel by pressing 'RESET', take up blood once again
- Wipe pipette tip to remove excess blood
- Eject 50ul into AC tube – keep the tip well above the metal greed. Do not touch count beads!!!
- Replace tube top
b) Tubes 1-5
- Take 100-1000ul digital pipette
- Set to 10 x 100ul
- Withdraw blood then expel by pressing reset, take up blood once again
- Wipe pipette tip to remove excess blood
- Eject 100ul into tubes 1-5 (1 press)
- Replace rest of blood into EDTA blood tube (keep it in case you need to repeat the procedure)
3.2.5. Vortex all samples (level 3)
3.2.6. Place samples in dark for 15 minutes (taken from when last sample prepared)

3.3 Start up procedure [See SOP 195 on General Operation]

- Turn on FC and after few seconds computer.
- Empty waste (right container) and refill with 360ml distilled water and 40ml bleach (ensure no bleach on gloves)
- Fill machine with left container by FacsFlow to appropriate level (level of indentation)
- Pressurise
- Press LOW/PRIME with the arm closed (distilled water in place)- once PRIME light goes off press once more
- Remove distilled water and place 'top right' on rack

- Insert FacsClean tube (2ml of FacsClean) – opened arm 1 minute, closed 5 minutes - then remove and place 'top left' on rack- set FC on 'high/run'
- Insert a tube with sterile PBS - 1 minute open arm, 5 minutes closed arm

3.4. Further sample preparation

3.4.1. Red blood cell lysing
- Use purple pipette(200-1000ul)
- Put 2mls of FacsLyse in Tubes 1-5
- Put 450ul in AC tube
- Place tops on tubes and vortex
- Place AC tube in dark for 15 minutes- after which add 1.5 mls of PBS, vortex - sample ready to run
- Place tubes 1-5 in centrifuge- leave for 10 minutes before spinning for 5 minutes (300RPM)
- Remove tubes 1-5 from centrifuge , decant, add 3 mls of PBS, replace tops, vortex, spin again - 300RPM for 5 minutes
- Decant supernatant, resuspend in 100 ul of PBS – sample ready to run

3.5. Sample acquisition
3.5.1. Absolute count
- Run FacsComp if you are first user of the flow cytometer during the day
- Press CellQuest icon
- 'FILE' – 'Open document' – 'Data 1' – 'Mon protocols' – 'Mon no wash'
- ACQUIRE – 'Connect to cytometer'
- CYTOMETER – 'Choose instrument settings' – 'Mon no wash' – 'Set' – 'Done'
- Change appropriate directory to save flow data
- Change file name
- Place AC into cytometer and press 'Acquire' (press 'Acquire' on top bar, press 'Counters') - once finished, print
3.5.2. Expression of surface markers
- 'FILE' – 'Open document' - choose – 'Mon wash Luke/Ben'
- CYTOMETER – 'Instrument settings' – 'Open' - choose 'Mon wash Luke/Ben' – 'Set' – 'Done'
- 'WINDOWS' – 'Show browser'
- Change directory and file name as before
- Vortex and run tubes 1-5, changing file on each sample (1, 2, 3 etc)- print after each

3.4 Shut-down procedure [See SOP 195 on General Operation]

1. In this section we re-use tubes 1 and 2 with distilled water and FACS clean respectively.

2. Install FACS Clean tube 2 over the SIP needle. Press button '**High'** and '**Run'** on the panel. Leave the support arm out at 90 degrees for approximately 1 minute. This cleans the outer portion of the aspiration sheath. The fluid will be rapidly aspirated, so ensure that the tube doesn't empty completely.

3. Now replace the side arm under the Falcon tube and allow to run for approximately 5 minutes. This cleans the inner portion of the aspiration sheath and the FACS machine itself.

4. Repeat steps 2 and 3 with the distilled water tube 1. Once step 3 is complete, Leave the sheath in falcon tube 1 containing distilled water and press '**STANDBY'.**

5. Open the reservoir draw and depressurise the machine by moving the "Vent Valve" toggle switch to the up/rear position. The machine will hiss as it depressurises.

6. Leave the machine on for a further 5 minutes to allow the laser lamp to cool. Turning the machine off prematurely will result in the lamp cracking.

7. Finally power down the FAC-Scalibur (green button) and Apple Mac.

8. Clean up!

9. Note: * IF THE SYSTEM IS TO BE USED AGAIN ON THE SAME DAY…

   LEAVE THE SYSTEM ON STANDBY and then

   DEPRESSURISE THE SYSTEM.

SOP: Monocyte subsets and monocyte platelet aggregates by flow cytometry

SOP written by Eduard Shantsila and Andrew Blann

N.B. Use of the flow cytometry is forbidden without having been officially trained

Required pre-training

1. SOPs on venepuncture and on good clinical practice

2. SOP 195 – General operation of the flow cytometer

1. Introduction

Monocytes are large mononuclear cells (MNCs) derived from the bone marrow but on transit to the tissues where they seem likely to become semi-resident macrophages. Traditionally, they have been defined by glass-slide morphology, size, and scatter, but we now have the ability to define monocytes by cell surface molecules, using the FACS. For example, CD14 is a receptor for LPS present on monocytes, macrophages and neutrophils. CD16 is an antigen found on the Fc receptors and is present on natural killer cells, neutrophil polymorphonuclear leukocytes, monocytes and macrophages. So leukocytes populations can be further classified by the density of the expression of these markers, for example….

- M1 = CD14 strong CD16 negative
- M2 = CD14 strong CD16 strong
- M3 = CD14 weak CD16 strong

A further characteristic of monocytes in chemotaxis, such as to the chemokine monocyte chemoattractant protein-1 (MCP-1), a cytokine involved in monocyte infiltration in inflammatory diseases such as rheumatoid arthritis as well as in the inflammatory response against tumors. CCR2, short for chemokine (C-C motif) receptor 2, is a chemokine receptor for MCP-1 CCR2 has also recently been designated CD192.

Platelets are anucleate fragments of the cytoplasm of the megakaryocyte. They form thrombi when self-aggregating but more so in the presence of fibrin. However, platelets may also bind to monocytes. Cell surface markers of platelets include CD42a, also known as GpIX. It follows that dual labelling of blood with a monocyte marker (CD14/CD16/CCR2) and a platelet marker (CD42a) will identify monocyte-platelet aggregates (MPAs).

This SOP describes enumeration of monocyte subsets (dependent on expression of CD14, CD16 and CCR2) and their participation in the formation of MPAs. And of course you will need a platelet count for the project, derived from the full blood count, from the Advia (see

SOP 171).

2. Materials and Supplier contact details:

Micro-reagents are kept in the fridge behind the door or on nearby shelves. Bulk fluids in boxes on other shelves and beneath the benches.

1) BD "FACS Flow" Running solution [Becton Dickinson, Catalogue No. 342003] 10L containers.

2) 3 ml BD Falcon tubes [BD Catalogue No. 352054]

3) BD"FACS Clean" Cleaning Solution [BD Catalogue No. 340345]

4) BD Lysing solution [BD Catalogue No. 349202]

5) Sterile Phosphate Buffered Saline solution, 0.5L bottles [Invitrogen Ltd, Catalogue No 20012-068]

6) CD14 -PE conjugated monoclonal antibody - 100 tests [BD Catalogue No. 555398]

7) CD16 – Alex-flour 488 conjugated monoclonal antibody - 100 tests [ABD Serotec, Cambridge]

8) CD42a-PerCP conjugated monoclonal antibody [BD Catalogue No. 340537]

9) CCR2-APC conjugated monoclonal antibody [R&D Systems Europe Ltd, Cat No. FAB151A]
   [n.b. this combination of antibodies constitute a **Mastermix**: See ADB, ES]

10) Clear pipette tips [Alpha Laboratories Limited Catalogue No FR1250 1250ul Fastrak Refill NS]

11) Yellow pipette tips [[Alpha Laboratories Limited Catalogue No FR1200 200ul Fastrak Refill NS]

12) Count beads [BD (Trucount™ tubes)]. This is a crucial aspect as it will give us the number of monocytes/ml of venous blood. The product tube has a statement of the number of beads in each tube and so from this you can work out beads/mL.

Remember to dispose of all material thoughtfully.

3. Detailed method

3.1 General Preparation

3.1.1 Lysing solution.

Make from 50ml concentrate 10x FACS Lysing Solution (kept at room temperature). Dilute with 450ml distilled water in ½ litre bottle. This solution should not be used if it is older than a month (kept at room temperature)

3.2 Blood sample preparation

1. Add 12.5µL of Mastermix Absolute Monocyte Count (which includes CD14 2.5 µL, CD16 2.5µL, CD42a 5µL and CCR2 2.5 µL fluorochrome labelled antibodies) with an electronic micropipette. Just place into the tube below a metal grid without touching the pellet.

2. Gently vortex the EDTA blood sample. Take 0.05 mL (=50 µL) of whole blood with electronic pipette and add to a Trucount™ tube.

3. Do not touch the pellet (this is critical!). Mix the tube gently with the vortex (3 sec). Incubate for 15 minutes in the dark, room temperature, shaking with horizontal shaker (set at 500 units). Add 0.45 ml (=450 µL) pre-diluted BD FACS Lyse solution (see 3.1.1) with a clear tip using the 1ml pipette. Incubate for 15 minutes on shaker as above.

4. Add 1.5 ml of PBS solution without touching the sample, followed by gentle vortex to ensure thoroughly mixed

3.3 Start up procedure [See SOP 195 on General Operation]

Part 1 – restoring reagents and preparation

13. Switch on Flow Cytometer by pressing the green switch on the right hand side. The Apple Macintosh computer must also be switched on, but only 15 secs **after** the Flow Cytometer, or the link will not be recognised. Open the reagent panel on the left hand side (LHS) by pulling the lid towards you. On the left is the sheath fluid reservoir, in the middle are switches and tubes, and on the right is the waste reservoir.

14. Carefully unscrew the top of the sheath fluid reservoir and fill with sheath fluid (in large box on shelf at head height – use plastic tube) to the level indicated on the top right hand corner of the reservoir (little plastic bar).

15. Carefully disconnect/unscrew the waste container and empty contents down sink with plenty of water. Add approximately 40ml concentrated household bleach along with 360 ml of distilled water and reconnect container (plastic tubes available).

16. Pressurise the unit (takes about 20-30 sec) by moving the black toggle switch "Vent Valve" switch to the down/front position. It is located at the rear of the middle section between the sheath fluid tank and the waste container.

17. Air must be excluded from the tubing system by flushing it out. Any excess air trapped in the sheath filter can, if necessary, be cleared by venting through the bleed tube (the dead-ended rubber tube with a cap).

18. Close the drawer

Part 2 - Cleaning the machine

19. Ensure that a 3 ml Falcon tube (labelled 1) approximately 1/3 full of distilled water is positioned over the sample injection port (SIP) – a needle sheath - and that the swing arm is positioned under this tube. Press the prime button on the panel. When the system enters "standby" with in 30 seconds then press the "prime" button again. When the standby and low buttons comes on again then remove tube 1. We will re-use tube 1 in the shut down procedure.

20. Prepare a second falcon tube (labelled 2) with FACS-clean (should contain about 2750 microlitres so that when inserted on to the sip it doesn't touch the **O** ring). This is a smaller box on a shelf at above head height and above the bigger box of sheath flow fluid

21. Present tube 2 to the SIP and place support arm underneath it. Press the buttons "run" and "high" on the panel at the same time, and run the FACS-clean in falcon tube 2 with supporting arm to left or right open for one minute. Then return the supporting arm to underneath the tube and "run-high" five minutes. This process ensures the machine is clean prior to running samples and helps minimise blockages.

22. Return to falcon tube 1 with distilled water. Repeat the above step 9 with this distilled water tube.

23. Press the '**STANDBY**' and '**LOW**' button on the system.

24. The machine is now ready to run samples.

3.4 Running blood samples.

Note. This must be learned from an experienced operator and you must seek scientific staff support to clear queries as the intricacies of the Cell Quest software are complex.

1. Open CellQuest Pro software
2. Click 'File' – 'Open'
3. Click on the 'Monocyte Protocols' folder within 'Data 1' folder.
4. Click on the 'Monocyte Absolute Count'. This will open study protocol.

5. Click '**Connect to Cytometer**', located under the 'Acquire' menu.

6. Under the '**Cytometer**' menu, click '**Instrument Settings**'. The window appears displaying the compensations and threshold. Change settings by clicking on the **open** icon on the window which displays the folders select 'Monocyte Protocols' folder with in the 'Data 1' folder and click on the 'Monocyte Absolute Count' instrument settings in this folder. This will update the system settings to the preferred settings for the acquisition. Click '**Set'** on the window and by clicking '**Done'** the windows disappears. Make sure to click '**Set**' prior to clicking '**Done**'.

7. Click the '**Acquire'** menu once more and click '**Show browser**'.

8. Click directory-'**Change'** in order to specify the location folder.

9. Initial user must create new folder by clicking on '**New folder'** and by entering the title of the folder and choose that folder.

10. Change the custom suffix to the preferred title and number for data and click '**OK'**.

11. Untick the setup box (by clicking on it) in the browser Acquisition window. Now insert your sample and press "RUN" and "HIGH".

12. Open swing arm at bottom right of the cytometer and replace the Falcon tube with the sample to be run. Replace the swing arm under the Falcon tube.

13. Press the buttons '**Run**' and '**High**' on the control panel of the cytometer.

14. Click '**Acquire'** on the browser menu. The sample will now run for ~ 12 mins. Cell events will be displayed on the screen throughout the process (n.b. the higher the cell density, the more rapidly the cells will be acquired).

15. Click on '**Counters'** under the '**Acquire'** and observe the events per second which varies from 1000 to 8000 depending on various factors. The objective is to acquire 10,000 count beads for analysis.

16. Observe the acquisition closely since the system may get blocked (which happens very rarely) and the plots may not show any progress and the counters may not show any events per second.

17. Click pause on the acquisition window and replace the sample from the SIP with sterile PBS and run for 20/30 seconds minutes (clicking acquire wouldn't change the results) and then continue acquisition with your sample on the SIP. If the problem still persists please inform the senior scientific staff and seek assistance.

18. After attaining the target events the analysis stops and the file number changes automatically. Click on '**print'** under the '**files'**. Confirmation window appears again click on print.

19. Vortex your next sample gently. Re-programme the software with a new sample number, and repeat the step 11.

20. If the cytometer is not ready message appears open the drawer and check the fluids level which may need refilling or emptying. The system may run out of Sheath fluid if there are more samples.

21. Be absolutely sure you have downloaded your results on to paper. Keep this paper safe. Do not assume the computer will keep the results safe, even if you have directed it to do so. Obtain all the raw data (cell numbers) and apply them into the specific spreadsheet you have designed for your project. The same spreadsheet should have the WBC and platelet count results from the Advia

3.5 Shut-down procedure [See SOP 195 on General Operation]

3. In this section we re-use tubes 1 and 2 with distilled water and FACS clean respectively. Install FACS Clean tube 2 over the SIP needle. Press button '**High'** and '**Run'** on the panel. Leave the support arm out at 90 degrees for approximately 1 minute. This cleans the outer portion of the aspiration sheath. The fluid will be rapidly aspirated, so ensure that the tube doesn't empty completely.

4. Now replace the side arm under the Falcon tube and allow it to run for approximately 5 minutes. This cleans the inner portion of the aspiration sheath and the FACS machine itself.

5. Repeat steps 2 and 3 with the distilled water tube 1. Once step 3 is complete, Leave the sheath in falcon tube 1 containing distilled water and press '**STANDBY'.**

6. Open the reservoir draw and depressurise the machine by moving the "Vent Valve" toggle switch to the up/rear position. The machine will hiss as it depressurises.

7. Leave the machine on for a further 5 minutes to allow the laser lamp to cool. Turning the machine off prematurely will result in the lamp cracking.

8. Finally power down the FACScalibur (green button) and Apple Mac, and then clean up!

9. Note: * IF THE SYSTEM IS TO BE USED AGAIN ON THE SAME DAY…

LEAVE THE SYSTEM ON STANDBY and then DEPRESSURISE THE SYSTEM.

4. Interpretation of plots

For the first couple of analyses you will need to have all this explained to you by Dr Blann or Dr Shantsila. These numbers refer to the illustrative plot and nine individual plots…

TOP THREE PLOTS

1. The top left initial plots show the FSC/SSC plot (forward and side scatter, all in green). This is needed to gate the presumed monocytes. Be generous at this stage, include all monocytes. Contamination by granulocytes and lymphocytes will be removed during the next stage.

2. Immediately to the right (i.e. centre) is a plot of the cells stained with CD14 (light blue) which further gates the monocytes to separate them from granulocytes. Note a large residual proportion of granulocytes at the top of the SSC index.

3. Top right is plot of CD14/CD16 events (red/brown). Four gates have been drawn to define different populations of monocytes. M1 defines CD14strong/CD16-ve, whilst M4 defines cells expressing a lot of CD16. The latter will be sub-typed shortly.

CENTRE THREE PLOTS

4. Centre left is a plot of the Count beads (green), which are sampled at a concentration of, for example, 50,000 beads/tube. From this you will get monocytes/mL and thus MPAs/mL. The CD14-PE horizontal axis is irrelevant.

5. Centre middle is (green) plot of CD16 versus CD14, which allows you to gate and exclude lymphocytes from analysis. Note that pattern is a bit like the upper right box, but with CD14-ve/CD16-ve events present.

6. Centre right is a plot derived from Gate 4. It shows events (cells) that express high and low levels of CCR2 according to side scatter. There is a gating line down the middle of this plot to give cells staining high and low staining for CCR2. Gate 5 is cells staining weakly for CCR2 (=M3) whilst Gate 6 is cells staining strongly for CCR2 (=M2).

LOWER THREE PLOTS (all CD42a versus CCR2)

7. Lower left is a plot of CD42a versus CCR2 on population M1. MPAs are to the right of the line

8. Lower middle is a plot of CD42a versus CCR2 in M2. MPAs are to the right of the line

9. Lower right is a plot of CD42a versus CCR2 in M3. MPAs are to the right of the line

Other numbers on the sheet (1- 12) refer to mathematical analyses, not to plots, as, follows….

5. Interpretation of results (numbers)

This is complicated, so pay attention. There are 12 analyses – the first 4 are raw data:

1. The total number of events counted and the acquisition date are given top left of the numbers section (i.e. 60,964 on 08-Apr-10).

2. On the far right is number of count beads (9127) used to quantify events to cells/µL

3. On the left is some maths from the opening plots showing number of total events collected in this particular analysis and the proportion that are monocytes.

4. Below this is the maths from Gates 5 and 6 (SSC and CCR2, middle right plot). So there are 667 M2 events and 871 M3 events, giving you relative proportions. This data is used to calculate the absolute count of subsets M2 and M3.

From these analyses numbers 1 – 4 the machine works out for you (given the count bead number in analysis 2 i.e. 9127) the percentage and numbers of monocytes and monocyte subsets, and these are given as numbers 5 – 12 as follows….

5. Mon is the total number of monocytes per µl, i.e. 582.95 cells/µL.
6. Mon 1 is the number of M1 monocytes per µl, i.e. 409.5 cells/µL.
7. Mon 2 is the number of M2 monocytes per µl, i.e. 98.23 cells/µL
8. Mon 3 is the number of M3 monocytes per µl, i.e. 75.22 cells/µL

The machine has also worked out the % of each subset immediately below.

Next – for MPAs…

9. MPA is the total number of MPAs per µL, i.e. 102.86 cells/µL
10. MPA1 is the total number of MPAs in the M1 population, i.e. 71.29 cells/µL
11. MPA2 is the total number of MPAs in the M2 population, i.e. 19.28 cells/µL
12. MPA3 is the total number of MPAs in the M3 population, i.e. 12.29 cells/µL

From this you can work out the proportions given a calculator. It follows that since you have the platelet count from the Advia, you can also work out how many of the total platelet pool are bound to monocytes. But this is for a separate analysis.

Conclusion

Using this dataset as a template, the numbers that need to go into your spreadsheet are as follows…

Total monocyte count = 582.95 cells/µL

Subsets: M1 count = 409.50 cells/µL (70.25%)
M2 count = 98.23 cells/µL (16.85%)
M3 count = 75.22 cells/µL (12.9%)

Total MPA count = 102.86 cells/μL

Subsets: MPA1 count = 71.29 cells/μL
MPA2 count = 19.28 cells/μL
MPA3 count = 12.29 cells/μL

Once in the spreadsheet, you can easily do the arithmetic for conversion to %

6. Validation and quality control (all work done by a single operator)

The intra-assay reproducibility of the methods was assessed on six samples of blood; one set of three from a healthy male and second set of three from a woman with a history of renal and ovarian cancer. The inter-assay reproducibility was derived from 6 weekly samples from a healthy middle aged man. Intra-assay coefficients of variation (CV)(%) were as follows:

	Subject A	Subject B	Mean
Mon1	0.9	1.4	1.15
Mon2	10.6	9.9	10.25
Mon3	3.9	4.7	4.3
Total Mon	0.6	0.4	0.5
MPA1	3.4	5.2	4.3
MPA2	14.1	9.7	11.9
MPA3	8.2	6.8	7.5
Total MPA	3.2	4.5	3.85
		Median intra assay CV	**4.6%**

Inter-assay coefficients of variation (CV)(%) were as follows:

Monocytes	10.5%, then on a different occasion, 9.6%	
Monos 1	18.7%, ditto 17.2%	
Monos 2	39.3%, ditto 35.6%	
Monos 3	29.9%, ditto 27.5%	
Total MPAs	21.2%, then on a different occasion, 19.5%	
MPA1	31.8%, ditto 29.2%	
MPA2	28.4%, ditto 22.3%	
MPA3	31.8%, ditto 29.7%	**Mean inter-assay CV 26.4%, then 23.8%**

SOP: Arterial stiffness

Sern Lim November 2008

Updated by Nadya Kuzniatsova and Andrew Blann, September 2010

Contents

1. Background

Left ventricular (LV) contraction generates a forward travelling pressure wave. Some of this pressure wave will be reflected at certain sites (e,g. bifurcations and sites of constriction). Increased arterial stiffness results in earlier return of the reflected pressure wave in systole rather than diastole, and is generally believed to be a surrogate of poor blood vessel compliance. We can assess these aspects of vascular function with two methods:

(a) pulse wave analysis (PWA), which reports the reflected pressure wave as pressure augmentation and may be expressed as the augmentation index (AI), and

(b) pulse wave velocity (PWV, reporting in units of metres per second).

Increased arterial tone, such as that associated with endothelial dysfunction, also increases the reflected wave and the AI. PWA may therefore provide information on the functional properties of the arterial system. The end point index of PWA is the speed at which the pulse is transmitted along arteries, hence PWV, and is reported in metres per second. Both PWV and PWA can be done at the same time on the same subject.

The method may also be used to further analyse vascular function. The administration of salbutamol and GTN also allows assessment of endothelial-dependent and independent function as NO-mediated vasodilatation reduces arterial wave reflection. The relative change in augmentation index to salbutamol and GTN provides a measure of systemic endothelial function.

n.b. The SOP refers to radial and carotid artery assessment but can be adapted for the femoral.

2. Pulse wave analysis

1. Equipment

The patient, SphygmoCor with pressure transducer (Millar), Laptop computer with SphygmoCor software (for around 45 minutes), Power cables and USB connection cables, ECG monitoring dots, Omron BP recorder. If doing additional vascular function - salbutamol inhalers and GTN spray.

2. Preparation

    a. Allow the subject to rest in the room for about 10 minutes before scanning. The test must be done in a supine position.
    b. While the patient is resting, you should connect the power cables to the SphygmoCor machine (at the back) and the laptop computer. The computer should then be connected to the SphygmoCor machine (use the USB connection at the back). The ECG cables should be connected to the SphygmoCor machine (in front where it says 'ECG').
    c. Switch on the SphygmoCor machine first. The lights in front will blink. When the lights stop blinking and green light stays on 'ready', turn on the computer. This sequence will allow the software in the computer to recognise the SphygmoCor.
    d. The SphymoCor icon should be on the computer desktop. Double-click to activate the software. A warning will come on the screen, just click 'allow'.
    e. You will see PWA and PWV on the top left of the screen. Click on PWA for pulse wave analysis.
    f. Click 'create new' on the top right of the screen. You will need to enter the patient details in the relevant boxes (eg: date of birth) and click 'update'.
    g. Click on PWA on the top left for pulse wave analysis and then 'study' on the top left of the screen. Choose radial or carotid by ticking the box. Measure the patient's brachial blood pressure (Omron). Take 3 measurements and use the average of the last two readings.
    h. Enter BP measurements. Height and weight if you have the data.
    i. Place the ECG leads on the patient and click 'Capture data' on the top right of the screen. You are now ready to go! (NOTE: To ensure a stable, artifact free ECG, the skin should be properly prepared (hair removed at electrode site and skin cleaned with an alcohol wipe), and the electrodes positioned correctly. The leads can be placed either on the limbs or on the chest area if required for stronger QRS levels).

3. In the drawer of the tonometer machine, you will find the Millar tonometer with a plastic cap to protect the high fidelity tip (this cap is important, DO NOT lose it!). The tonometry should be connected already. DO NOT disconnect the tonometer. Remove the plastic cap and it is ready for use.

4. Feel for the radial or carotid pulse (depending on which you want to measure) and place the tonometer over the pulsation. You will see a pressure trace on screen. The

screen will automatically adjust the scale to accommodate the pressure trace. You need to make sure the trace is consistent for at least 12 seconds. To obtain the measurement, press the spacebar on the laptop. The machine will not take the last 2 seconds of recording to give you time to let go and press the spacebar.

5. The computer screen will then change to the data screen with the ensemble-averaged pressure trace (radial or carotid depending on the one you have chosen) shown and the derived central arterial pressure trace. First thing to do: check the quality control box on the top left of the screen. If this number is red, then the data is of insufficient quality and the measurement will need to be repeated.

6. The data will be available at the bottom of the screen. At the top right of the screen, click 'Export' to save the data in your folder. You can store the data as a text file or jpeg (the screen shot). I would suggest you store both. If you are not doing endothelial function assessment with the SphygmoCor, then your study is done!

3. Sub-method for endothelial function

1. If you want to assess endothelial function, you will need to assess endothelium-dependent and independent vasodilatation. Endothelium-*dependent* vasodilation is assessed by giving 2 puffs of salbutamol (inhaler). Blood pressure (the other arm) and PWA measurement should be repeated at 5, 10 and 15 and 20 minutes to look for maximal decrease in augmentation index.

2. This is then followed by 2 puffs of sublingual GTN (warn the patient they may get a mild headache / feel dizzy) to assess endothelium-*independent* vasodilatation. Similarly, BP and PWA measurement should be repeated at 5, 10, 15 and 20 minutes.

3. Make sure you label the files (eg: salbutamol and GTN) when you are storing the data. Figure 1 shows typical responses to these pharmaceuticals

Figure 1 shows differences in plots according to the use of these drugs

4. PVW measurement.

a. Return to the ‘Patient Screen’, select ‘PWV’ mode by either clicking on the arrow on the selection box (located next to the ‘Analysis’ button), or by pressing F6 on your keyboard to scroll through the mode options.

b. Open the Study Screen by clicking on the “Study” button or pressing F3 on your function keys on your keyboard. This screen will allow you enter the study details and to proceed to ‘Capture data’. Mandatory fields to be selected or entered:

- Sites from where the measurement is to be taken. This is to be done for both Site A and Site B. Site A is the site at which the first measurement is to be taken and Site B refers to the site at which the second measurement will be taken.
- When carotid is selected as the first site, enter the systolic and diastolic blood pressure values that have been obtained from the cuff sphygmomanometer or automatic blood pressure device.
- The Capture Time is set to a default of 10 seconds for both the Site A and Site B measurement. Alternative times can be selected for either or both Sites if required.
- The Distance should be measured and entered as follows: for carotid – femoral (if being done) and carotid – radial, the distance directly between the each artery location and the supra-sternal notch are entered in the distal and proximal boxes (NOTE: At present, there is no definitive way to measure the distance between the aorta and the femoral artery non-invasively. As a result there a number of methods that is used to measure the distance. If there is no existing methodology already in place, it is recommended that the measurement be taken in a direct line between the supra-sternal notch and the femoral artery
- The Medication, Notes, Operator and Anthropometric fields are optional.

c. To proceed to the capture data screen, click on the ‘Capture Data’ button or press ‘enter’ on your keyboard. Once the ECG trace is shown along the screen and is steady, proceed as follows:

- The tonometer should be placed at Site A location and adjustments to the position of the tonometer made until a strong, accurate and reproducible waveform is displayed in the ‘Signal Detail’ window. This signal will be automatically re-scaled and zoomed to fit the waveform within the signal detail window every 5 seconds. When you are satisfied that you have a good reading, press the ‘Space Bar’ on your keyboard or click the ‘OK’ button at the top of the screen (NOTE: You must have a minimum of 12 seconds of signal for the data to be captured (minimum of 22 seconds or 32 seconds if you have selected a capture time of 20 or 30 sec in the Study Screen). The last 2 seconds of waveforms will be deleted, allowing sufficient time to remove the tonometer from the wrist to activate the capture of data. A prompt window will appear confirming that the signals have been captured successfully.

- When you are ready to proceed to take the reading at Site B, click the OK button or press 'enter' on your keyboard. If you wish to take the reading again, click No and repeat the reading at Site A.
- Repeat the process by placing the tonometer at Site B and proceed with the capture when you have obtained a signal of satisfactory quality. A prompt box will appear to confirm that the signal was captured successfully. If you are satisfied with the reading at Site B and wish to proceed to the report, click OK. If you wish to repeat the reading at Site B, click No and repeat the reading at Site B (NOTE: remember that the signal representing the tonometer waveform for Site A should be the artery selected in the 'Study Screen').

d. Additional comments on performing carotid measurements. The patient should be lying on a bed. The patient's head should be tilted slightly to the back and to one side (either left or right). This is best achieved in the absence of a pillow. The operator should feel for the position for the strongest pulse and place the tonometer directly on the top of the skin at this point. The operator can be standing either behind the patient's head or to one side. A pillow may be placed across the shoulder of the patient to allow the operator to rest their forearm to ensure that the tonometer and wrist remain steady during the measurement.

e. Examine the report for quality control.

After you have completed the data capture, the 'Report Screen' will automatically be displayed. This can also be recalled at any time by selecting the patient in the 'Patient Screen' and pressing the 'Report button'. Before proceeding with the interpretation of results it important to check the quality control to ensure that your measurement has been recorded with sufficient quality. The SD (ms) in the statistical table should be below 6% of the mean time. If this is above 6% the number will appear in red. It is recommended that PWV should have a SD 10% or less. Between 10% and 15%, is borderline and careful examination of the waveforms should be done before making a decision as to whether to repeat the reading. For SD above 20% a repeat reading is recommended. The SD of the PWV and mean time provide an indication of how consistent the data is, and this may include some biological variation that is inherent in the reading. If you have performed more than one reading on a patient, the studies are listed chronologically in the 'Study Time' window and the most recent report will be at the bottom of the list. Each of the reports may be viewed by clicking on the study you wish to view. The report being displayed will correspond to the highlighted study in the 'Study Time' window.

5. Potential problems

1. The high fidelity tonometer is very sensitive to pressure (that is what it is meant to do!). You will need practise to achieve a consistent level of pressure in order to obtain reproducible readings. It is generally more difficult for carotid artery compared to the radial artery.

2. To avoid any problems with the computer, it should NOT be used for any other reasons (eg: surfing the internet). The computer and the tonometer (plus ALL the cables and ECG leads) should ALWAYS be kept together.

3. Every operator should create a folder for him/herself and store ALL the data in that folder. This should avoid confusion and the relevant data may be easily retrieved.

Intra-assay reproducibility

This was assessed on an apparently healthy 56 year old male at approximately 9.30 in the morning.

- PWV was 5.9, 7.1 and 6.6 m/s on three occasions in the same short session (15 minutes). This gives a mean PWV of 6.6 m/s with a standard deviation (SD) of 0.51. Hence intra-assay coefficient of variation (CV) = 7.7%.

- For PWA, the Augmentation index (AI) was 21, 24 and 25, giving mean (SD) of 23.3 (1.7) so that intra assay CV = 7.3%.

- Shortly afterwards, for comparison, blood pressure was measured six times using an Omcron machine, with the cuff being removed between each measurement. This data gave a mean/SD SBP of 117.3 (2.7) mm Hg. Hence intra assay CV is 2.3%. Similarly, the DBP was 71.2 (1.6) = 2.2%, and pulse rate was 52.8 beats/minute (1.34) = 2.5%.

Thus the SphygmoCor has a variance approximately three times that of the Omcron BP machine.

Inter-assay reproducibility (on different days)

25/8/2010: PVW = 8.4, AI = 27

26/8/2010: PVW = 6.6, AI = 23

1/9/2010: PVW = 7.5, AI = 21

2/9/2010: PVW = 7.4, AI = 21....on n=4, CV for PWV = 8.5%, for AI = 10.6%

3/9/2010: PVW = 5.1, AI = 22....on n=5, CV for PWV = 15.8%, for AI = 9.8%

4/11/2010: PVW = 6.9, AI = 21....on n=6, CV for PWV = 14.5%, for AI = 9.5%

Diurnal variation (DV, all done on 26th August 2010)

At 9.30 a.m., PVW = 6.6, AI = 23, SBP = 117, DBP = 71, pulse rate = 53

At 1.30 p.m., PWV = 7.3, AI = 20, SBP = 123, DBP = 61, pulse rate = 61

At 5.30 p.m., PWV = 7.0, AI = 24, SBP = 121, DBP = 74, pulse rate = 48

DV for PWV = 4.1%, AI = 7.6%, SBP = 2.1%, DBP = 8.1%, PR = 9.9%

SOP: Venepuncture

1.0 Introduction

Venepuncture is one of the most commonly performed invasive procedures (Peters et al, 1984) and is becoming more routinely performed by nursing staff (Jackson, 1996). The procedure requires knowledge of anatomy and physiology and the knowledge of health and safety issues and infection control pertinent to the patient and practitioner.

Venepuncture is necessary to obtain blood samples for diagnostic purposes and to monitor levels of blood components.

This policy should be read in conjunction with the Trust's Intravenous Therapy Training Programme Part B (Peripheral Cannulation and Venepuncture) and the following Infection Control Policies and Guidelines:

a. Hand Hygiene: http://swbhweb/upload/pdf/Hand_Hygiene.pdf

b. Personal Protective Equipment

c. Waste and sharps management

d. Equipment cleaning and decontamination

e. Control of blood and body fluid spillages

f. Specimen collection and transportation

2.0 Aim

To ensure all healthcare professionals involved in venepuncture carry out the procedure safely at all times.

3.0 Objectives

3.1 To ensure safe and effective collection of blood samples at all times.

3.2 To prevent infection due to microbial contamination.

3.3 To maintain the safety and comfort of the patient and staff member at all times.

3.4 To maintain safety of the staff member carrying out the procedure.

5.0 Venepuncture Procedure

5.1 Selection of site

- The superficial veins of the upper arms are most commonly chosen for venepuncture. These veins are numerous and accessible and the procedure can be performed with the minimal of discomfort (Marieb, 1998).

- Occasionally, the veins of the lower limb can be used but this should be avoided, as blood flow is less in this region and the risk of complications increase.

5.2 Selection of Vein

- The choice of vein must be individualised for each patient. The most prominent vein is not necessarily the best (Weinstein, 1997).

- Inspect the limb for infection, bruising and phlebitis. These areas should not be considered due to the risk of causing more damage. Areas of previous venepuncture should also be avoided as this can result in pain to the patient due to repeated trauma of the vein (Ahrens et al, 1991).

- Veins should feel soft, bouncy and refill when depressed. Veins which cross over joints and bony prominence should be avoided and those areas with little skin (inner aspect of the wrist), as this will cause added discomfort to the patient.

- The main veins of choice are (listed in order of priority)

a. Antecubital Veins- These veins are chosen for venepuncture because they are capable of providing copious and repeated blood specimens without damaging the vein providing a good technique is used (Mallet and Balley, 1996) a.1 Cephalic Vein a.2 Basilic Vein a.3 Median Cubital Vein

b. Median vein in the wrist

c. Dorsal metacarpal veins

- The femoral veins can only be accessed by medical staff for venepuncture.
- The metacarpal veins should only be used when the others are not accessible. 5.3 Improving venous access

a. Application of a tourniquet promotes venous distension. It should be tight enough to impede venous return but not restrict arterial flow.

b. Opening and closing of the fist ensures muscles force blood into the veins.

c. Lowering the arm below heart level.

d. Light tapping of the vein may be useful but can be painful and may result in the formation of haematoma specially in elderly patients and those with fragile viens (Dougherty and Lamb, 1999).

e. The use of heat, in the form of warm water or packs, encourages vasodilation and venous filling.

5.4 Preparation of equipment

Two devices commonly used for venepuncture are the straight steel needle (vacuum/closed system or syringe) and the winged steel infusion device (butterfly). The choice of device is dependent on the condition and accessibility of the patient's veins. The use of syringe is not recommended as it has many inherent problems (Dougherty and Lamb, 1999). The vacuum system is a safer system and has increased the efficiency of blood sampling (Dougherty and Lamb, 1999). This Trust recommends that practitioners undertaking this procedure use the vacuum (otherwise known as the closed-system) in venepuncture.

The package must be checked for integrity before use and the expiry dates checked on all devices and blood collection bottles.

5.5 Prepare patient/parents

Explain the procedure to the patient and provide opportunities for questions and discussion of the procedure, in order to reduce fears and anxiety and to encourage compliance in the patient.

5.6 Venepuncture technique

. 5.6.a Ensure lighting and privacy is adequate for the task being undertaken.

. 5.6.b Wash hands using an antibacterial soap or alcohol hand rub and ensure hands are thoroughly dry.

. 5.6.c Apply gloves.

. 5.6.d Apply a clean tourniquet to the chosen limb and select the most appropriate vein.

. 5.6.e Prior to venepuncture, the intended site should be cleansed with antimicrobial solution(s) using aseptic technique for 30 seconds (Elliot et all 1994; ICNA 2000; DOH 2001; CDC 2002). The antimicrobial preparation solution(s) should be allowed to air-dry completely before proceeding with the venepuncture (Dolan and Dougherty 2000; DOH 2001). A 70% alcoholic swab impregnated with 0.5% chlorhexidine gluconate solution should be used for skin disinfection (CDC Recommendation Category IA) unless the patient has known allergy to chlorhexidine.

. 5.6.f Do not re-palpate the vein or touch the skin after skin disinfection.

. 5.6.g Support limb with pillow or soft cushion.

. 5.6.h Inspect the device to be used and ensure its integr ity.

. 5.6.i Anchor the vein by applying manual traction a few centimetres below the proposed insertion point.

. 5.6.j Insert the needle smoothly at an angle of approximately 30 degrees.

. 5.6.k Reduce the angle when puncture of the vein is felt or there is a flashback of blood in the tubing of the winged infusion device (i.e butterfly safety blood collection set).

. 5.6.l Slightly advance the needle into the vein and withdraw the required amount of blood using a vacuumed blood collection system.

. 5.6.m Only attempt this procedure twice. If unsuccessful, contact a more experienced practitioner.

. 5.6.n Tourniquet should never be left on the arm for more than 1 minute due to the possibility of haemoconcentration. It should therefore be released after the first blood

bottle has been filled.

. 5.6.o Mix the blood bottle by inverting it 3-5 times. Do not shake the blood bottles.

. 5.6.p Place a low - linting sterile dressing over the puncture site but DO NOT apply pressure.

. 5.6.q Remove the needle fully, then apply pressure to the puncture site. Digital pressure should be applied on straight arm -not bent (Dyson and Bogod, 1987) by the practitioner and not the patient (Godwin et al, 1992), until bleeding has ceased.

. 5.6.r Discard the needle directly into the sharps container.

. 5.6.s The vacuum system must also be discarded as they are single use only.

. 5.6.t Mix well and ensure all bottles are labelled correctly at the patient's bedside .

. 5.6.u Inspect the puncture site before applying a sterile dressing.

. 5.6.v Ensure patient is comfortable.

. 5.6.w Send specimens to laboratory in specimen bags. (Policy SHC/COI/002- The Microbiology Laboratory)

. 5.6.x Discard all waste as per Trust policy (SHC/COI/013 – Waste Disposal)

6.0 Training

6.1 All practitioners undertaking venepuncture must be adequately trained and deemed competent on this skill.

6.2 Training for venepuncture may be completed during pre-registration period (i.e. medical staff) or post-registration (i.e. nurses, midwives, ODAs, ODPs, etc.).

6.3 For post-registration training (i.e. nurses, midwives etc.), practitioners must follow the approved training provided by the Trust and undertake assessment by an experienced practitioner following the Trust's Venepuncture Assessment package (See IV Therapy Part B: Peripheral Cannulation and Venepuncture Training Programme). This training can also be accessed and is covered in the Unit E8 of the Operating Department Practice Standard for theatre staff. THE TRAINING MUST ALWAYS PRECEED THE ASSESSMENT.

6.4 Practitioners trained in other Trusts (applies to qualified non- medical practitioners only)

should first undergo an Assessment of Prior Learning and Experience (APLE) prior to undertaking venepuncture in this Trust. Practitioners who wish to undertake this assessment must obtain an approved APLE form from one of the Clinical Skills Educators (Learning and Development Department: extension 5238-City Site). The assessment must be carried out by an experienced senior practitioner who is competent in venepuncture and is practicing the skill regularly.

6.5 All practitioners have a professional obligation to maintain their knowledge and skills (NMC 2002). It is therefore essential that practitioners keep abreast of clinical advances and changes in practice, and it is therefore recommended that training updates be attended (DHSS Regulations, 1976) at least every 3 years or earlier if the practitioner deem it necessary (RCN, 1999). This should be followed by and monitored by the ward/department manager/medical director as part of the personal development review process.

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