A PERIPARTUM ASSESSMENT OF NAILFOLD CAPILLARY DENSITY IN WOMEN WITH PREECLAMPSIA

by

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Abstract

Preeclampsia (PE) is characterized as new-onset hypertension in pregnancy (>140mmHg systolic or >90mmHg diastolic), along with proteinuria and is a leading cause of maternal mortality and morbidity worldwide. Despite the etiology of PE remaining elusive, it is thought that placental trophoblast cells do not adequately invade into the inner myometrium layer of the uterus during placentation, resulting in the incomplete remodelling of the uterine spiral arteries. As a result, the placenta becomes hypoperfused and releases pathogenic factors in response. Pathogenic factors, such as reactive oxygen species (ROS) and soluble fms-like tyrosine kinase one (sFlt-1), cause systemic endothelial dysfunction, ultimately leading to hypertension and accompanying symptoms of PE. While symptoms typically resolve after delivery, those with a history of PE are at an increased risk of developing cardiovascular disease (CVD) later in life. Despite this, many individuals with a history of PE in pregnancy are not offered cardiovascular risk surveillance or intervention to prevent CVD postpartum. Since CVD and increased risk of CVD have both been associated with declined nailfold capillary density, nailfold video capillaroscopy (NVC) could be used as a postpartum assessment tool to identify those at increased risk of CVD. The objective of the current study was to determine if the nailfold capillary density (capillaries/mm^2) of women with PE is reduced when compared to controls both antepartum and postpartum. Previous literature supports our hypothesis that nailfold capillary density will be reduced in preeclamptic participants when compared to controls and that this decline in density will persist postpartum. The nailfold capillaries of 32 control and 15 preeclamptic participants were assessed using NVC within 2 weeks antepartum and 48-hours postpartum. No difference in the average nailfold capillary densities was found between groups before or after delivery. Prior evidence of structural and functional changes to the microvasculature after a pregnancy complicated by PE suggests that further investigation to clarify these results is necessary. Further awareness of the lasting impacts of PE on the

microvasculature may provide support for the surveillance of PE patients postpartum and to mitigate their increased risk of CVD later in life with pharmaceutical and lifestyle interventions.

Co-Authorship

Jennifer Armstrong, Logan Barr, and Dr. Graeme N Smith developed the experimental design. Jennifer Armstrong completed all recruitment and experimental work with assistance from Michelle Rody, Jessica Pudwell, and Kira King. All writing was completed by Jennifer Armstrong.

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List of Abbreviations

AMDA	Asymmetric Dimethylarginine
APGAR	Appearance Pulse Grimace Activity and Respiration
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CVD	Cardiovascular Disease
CVR	Cardiovascular Risk
DDAH	Dimethylaminohydrolase
eNOS	Endothelial nitric oxide synthase
HELLP	Hemolysis, Elevated Liver enzymes, and Low Platelet count
HIF-1α	Hypoxia-inducible Factor 1-alpha
IUGR	Intrauterine Growth Restriction
KHSC-KGH	Kingston Health Sciences Centre – Kingston General Hospital
NO	Nitric Oxide
NVC	Nailfold Video Capillaroscopy
PE	Preeclampsia
ROS	Reactive Oxygen Species
sFlt-1	Soluble Fms-like Tyrosine Kinase-1
sGC	Soluble Guanylate Cyclase (sGC)
SGA	Small for Gestational Age
LGA	Large for Gestational Age
TLR	Toll-like Receptor
uNK.	Uterine Natural Killer
VEGF	Vascular Endothelial Growth Factor

Chapter 1

Introduction

1.1 Overview of preeclampsia

Preeclampsia (PE) is characterized as new-onset hypertension in pregnancy (>140mmHg systolic or >90mmHg diastolic), along with proteinuria of 0.3g per 24 hours (Cunningham & LaMarca, 2018; de Jager et al., 2017; Enkhmaa et al., 2016). This disorder is the 3rd most likely cause for maternal morbidity and mortality worldwide and affects 3-7% of all pregnancies (Enkhmaa et al., 2016). If PE goes untreated, it can develop into eclampsia, which is a critical and life-threatening maternal disease characterized by one or more tonic-clonic convulsions (Fishel Bartal & Sibai, 2020). A tonic-clonic convulsion is a seizure classification where the individuals muscles undergo tonic contractions, defined as muscle stiffening, followed by clonic contractions, characterized by a jerking motion (Realfsen et al., 2015) In addition to the most common signs of new-onset hypertension and proteinuria, affected individuals might experience systemic organ dysfunction. Additional signs include thrombocytopenia, renal insufficiency, and impaired liver function, along with symptoms such as edema, headache, and visual disturbances (Ives et al., 2020).

The lack of available treatments for PE makes clinical management challenging. Currently, therapeutic management may include: (1) pharmaceuticals to manage high blood pressure and (2) magnesium sulfate to prevent seizures. However, delivery of the neonate and placenta is the only method available to resolve symptoms and stop disease progression (Amaral et al., 2017).

The etiology of PE remains elusive, however, it is thought that early-onset PE may be associated with shallow trophoblast cell invasion into the endometrium layer of the uterine wall

rather than into the inner myometrium layer (Enkhmaa et al., 2016). The inadequate trophoblast invasion leads to incomplete remodeling of spiral arteries, which causes ischemia of the placenta (Enkhmaa et al., 2016). Late-onset PE may be caused by underlying maternal vascular dysfunction (Possomato-Vieira & Khalil, 2016). Regardless of the mechanism, PE is a systemic disease as it is associated with general endothelial dysfunction (Rafii Tabrizi et al., 2021). The endothelium is a layer of single cells that line the vascular system and detect changes in blood composition to control vascular function, while serving as a physical barrier to regulate the transfer of water, nutrients, and waste into the vascular system (Boeldt & Bird, 2017). Endothelial dysfunction is defined as any alteration in the structure or function of the endothelium (Kolka & Bergman, 2012).

Hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome is characterized as either a progressed form of PE or an independent disorder affecting individuals during pregnancy (Kinay et al., 2017). The risk of developing eclampsia, the most severe form of pre-eclampsia, is higher in HELLP syndrome patients, especially at gestational ages past thirty-four weeks (Kinay et al., 2017).

In addition to the impacts on maternal health, pregnancies complicated by PE can negatively impact the neonate. Intrauterine growth restriction (IUGR) is a pregnancy complication defined as reduced fetal growth and can occur as a result of PE (Backes et al., 2011). Contrary to small for gestational age (SGA), where neonates are below the 10^{th} weight percentile for their gestational age but otherwise healthy, infants who experience IUGR do not reach their growth potential (Peleg et al., 1998). Infants born to mothers who experienced IUGR are at a higher risk of mortality when compared to infants of the same gestational age whose mothers did not experience IUGR (Aucott et al., 2004). PE increases the risk of intrauterine fetal demise, particularly in severe PE. In mild PE, however, the risk of intrauterine fetal demise declines by more than 50% when compared to severe PE (Backes et al., 2011). Pregnancies complicated by PE can also have long-term consequences for the offspring, primarily those that

were impacted by IUGR. Offspring are at a higher risk of developing CVD, hypertension, and type-2 diabetes (Cunningham & LaMarca, 2018). It is thought that this increased risk in offspring is a result of the adverse in-utero environment created by PE and the resulting epigenetic changes to offspring DNA (Cunningham & LaMarca, 2018).

PE was previously understood as a disease that occurs only during pregnancy. However, it is now well recognized that there are postpartum consequences of the disease. Individuals who have had a pregnancy complicated by PE are twice as likely to develop CVD and three times more likely to develop hypertension later in life when compared to those who experienced uncomplicated pregnancies (Enkhmaa et al., 2016). Additionally, the time of onset of PE during pregnancy has been correlated with the risk of developing CVD, with women who experienced early-onset PE being at higher risk than those diagnosed with late-onset PE. Whether PE is a cause of CVD later in life, a result of poor cardiovascular health pre-pregnancy, or a combination of both remains unknown. CVD is the leading cause of mortality for women in Canada and the United States (Enkhmaa et al., 2016). As such, it is necessary to further investigate the impact of PE on women's CVD risk to provide evidence that supports the surveillance and early intervention of women who have had a pregnancy complicated by PE.

1.2 Types of preeclampsia

Prior to 34-weeks, PE is considered early-onset and at or beyond 34-weeks is late-onset (Raymond & Peterson, 2011). One thought is that early-onset PE is a result of placental abnormalities, while late-onset PE is a result of other maternal factors. This is supported by the finding that the prevalence of placental lesions resulting from underperfusion is significantly higher in patients with early-onset PE when compared to those with late-onset PE (Ogge et al., 2011). Additionally, asymmetric dimethylarginine (AMDA) has been found in significantly higher concentrations in patients with early-onset PE than those with late-onset PE (Alpoim et al., 2013). It is possible that the increase in ADMA in PE is a result of a decrease in dimethylarginine

dimethylaminohydrolase (DDAH). DDAH typically converts ADMA to citrulline and dimethylamine to then be excreted in the urine. However, the increased oxidative stress observed in PE inactivates DDAH, thus preventing ADMA from being excreted. ADMA is a known inhibiter of nitric oxide (NO) production, which is associated with impaired placentation and endothelial dysfunction. As such, these results may suggest that early-onset PE is linked to placenta pathogenesis, while late-onset PE is not (Alpoim et al., 2013).

It is possible that the two subtypes are distinct in the mechanisms by which they cause clinical symptoms. After performing a transcriptome analysis of early-onset PE, late-onset severe PE, and late-onset mild PE samples, the amount of differentially expressed genes were found to be 2977, 375, and 42, respectively (Ren et al., 2021). Here, a larger amount of differentially expressed genes correlates with greater genetic involvement in PE. The differentially expressed genes in early-onset PE are associated with metabolism-related pathways, rather than immune-related pathways as found in late-onset severe PE (Ren et al., 2021). These findings are contradictory to a recent study that found alterations in the placental innate immune system in early-onset PE, but not late-onset PE. Specifically, placentas from early-onset PE have decreased expression of complement, Toll-like receptor (TLR) associated genes, mast cells, and macrophages when compared to healthy placentas (Broekhuizen et al., 2021). Overall, further investigation is required to determine the molecular mechanisms responsible for the development of early- and late-onset PE.

Various biomarkers may serve as potential predictors of the subtypes of PE. For instance, maternal serum leptin levels are significantly higher in women with PE than controls, and significantly higher in women with early-onset PE when compared to late-onset PE (Salimi et al., 2014). Hypoxia-inducible factor 1-alpha (HIF-1α) levels have been found to be significantly higher in patients with PE when compared to controls at or beyond 20-weeks gestation and higher in those with early-onset PE than late-onset PE. This protein is responsible for inducing the

transcription of vascular endothelial growth factor (VEGF) and does so in response to hypoxia (Sriyanti et al., 2019). Identifying biomarkers specific to the subtypes of PE may be useful to classify patients' signs and symptoms and ultimately, provide more specific treatment and care to those who are at risk of and are diagnosed with PE.

1.3 Risk factors for preeclampsia

There are a range of factors that put individuals at higher risk of developing PE in pregnancy. Goetzinger et al., (2014) developed a "multi-parameter risk-based scoring system" to be used to predict preeclampsia in the first trimester. After assessing a cohort of 1200 women, they identified the most significant risk factors for preeclampsia to be chronic hypertension, previous preeclampsia, diabetes, and body mass index above 30kg/m^2 (Goetzinger et al., 2014). A history of IUGR– small for gestational age (SGA) has also been found to increase a mother's risk (Ayala-Ramirez et al., 2020). Maternal age contributes to PE risk, with mothers aged <25 years being at an increased risk of developing severe PE at term and eclampsia at all gestational ages. Mothers aged ≥35 years have been found to have a higher risk of severe preeclampsia and HELLP syndrome (Lisonkova et al., 2021). Overall, there are several factors that can increase the risk of developing PE in pregnancy.

1.4 Pathophysiology of preeclampsia

1.41 Healthy implantation and placentation

The placenta serves to exchange gases and nutrients between the fetus and mother, while removing waste from the fetus (Okada et al., 2018). Given the placenta's critical role in supporting the growth of the fetus, its proper development is important for the health of the neonate. The first step in developing a healthy placenta is preparing the endometrium for possible pregnancy through decidualization. Specifically, decidualization involves the conversion of endometrial stromal fibroblast cells into decidual stromal fibroblast cells (Okada et al., 2018).

This reprogramming downregulates pro-inflammatory markers and increases the expression of genes that promote cellular proliferation, tolerance, and tissue invasion. These characteristics are critical to promote growth of the fetus and prevent the mother's immune system from rejecting the fetus (Carter, 2021). Now, the endometrium is ready for the possible implantation of an embryo.

Implantation involves the apposition, attachment, and invasion of the endometrium by the blastocyst, all of which occurs between 6 and 10 days after conception (Ng et al., 2020). Once the blastocyst invades the endometrium, the cells that contact maternal cells are called the synsytiotrophoblast and the remaining blastocyst cells are referred to as cytotrophoblasts. A subset of cytotrophoblast cells then invade into the endometrium, through trabeculae created by the synsytiotrophoblast cells, and differentiate into extravillous cytotrophoblast cells (EVTs) (Mendes et al., 2019). At about 10 weeks' gestation, the EVTs completely invade the endometrium into the inner third of the myometrium (Ng et al., 2020).

Next, the placenta-uterus connection is made through the remodeling of uterine spiral arteries (Mendes et al., 2019). Spiral artery remodeling is initiated by cytokines released by uterine natural killer (uNK) cells and macrophages that serve to loosen the smooth muscle layer of the spiral arteries (Carter, 2021). The EVTs invade through two different routes: (1) intravascularly from the basal plate into the lumina of the spiral arteries and (2) interstitially through the decidua towards the spiral arteries (Carter, 2021). When the EVTs invade the maternal spiral arteries, they destroy and replace the muscular layer of the arteries with an endothelium-like layer that is of fetal origins (Ng et al., 2020). The ideal placenta allows for a high volume of blood to be circulated with minimal resistance. As such, it is critical that the spiral arteries are altered to have thin walls and a wide, funnel shape (Ng et al., 2020). The result of spiral artery remodeling is depicted in Figure 1, where EVTs can be observed invading a spiral artery. Figure 1 demonstrates the directionality of the invasion and resulting blood flow. Overall,

the process of implantation and the development of the placenta is a complex, yet critical, beginning to a healthy pregnancy.

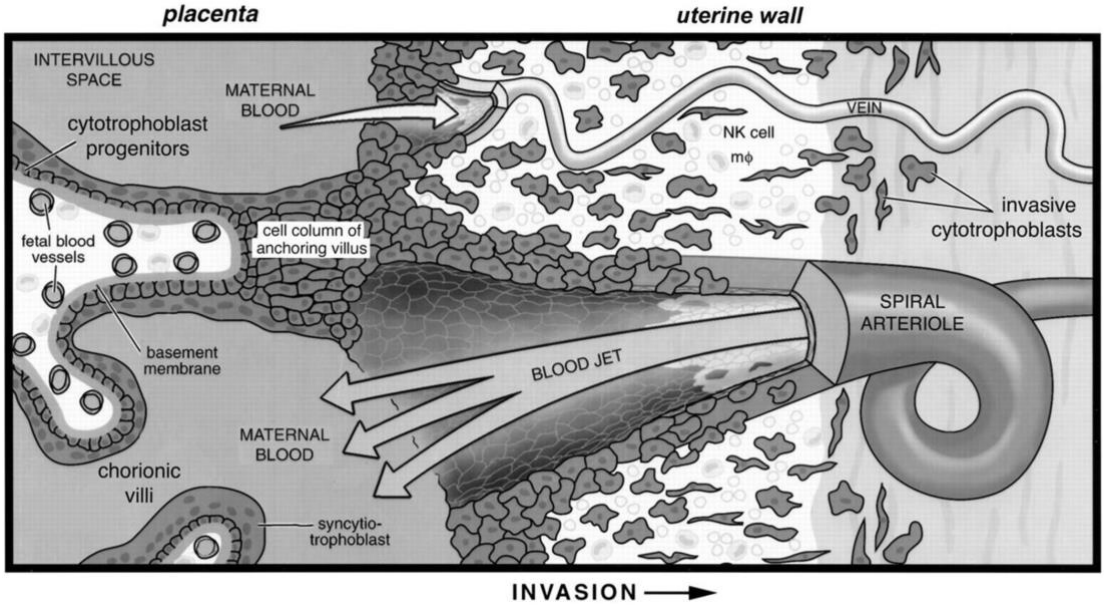

Figure 1: The cytotrophoblast invasion in a normal pregnancy. The EVTs invade through the uterine wall to remodel spiral arteries. This establishes the utero-placental connection, which is essential for the passage of nutrients, gases, and waste between the mother and fetus (Red-Horse et al., 2005).

1.42 Improper placentation in PE

Since the development of the placenta is critical for a healthy pregnancy, issues in this important process can lead to pregnancy complications. During placentation in pregnancies that develop into preeclampsia, placental cytotrophoblast cells have been found to incompletely undergo the transformation from an endothelial to vascular-like phenotype (Zhou et al., 1997). As a result, the EVTs invasion only spreads to the decidua layer, not into the deeper, myometrial portions of the spiral arteries. This lack of depth is demonstrated in comparison to the invasion of cytotrophoblasts in a healthy pregnancy in Figure 2. Thus, in placentas linked to PE later in pregnancy, the spiral arteries are incompletely remodeled, leaving many vessels with their original, endothelial lining. The lack of widened and thinned spiral arteries, as depicted in Figure 2, leads to hypoperfusion of the placenta (Chaiworapongsa et al., 2014).

In response, the placenta releases pathogenic factors that are associated with the clinical manifestations of PE. Anti-angiogenic factors including soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin become imbalanced with pro-angiogenic factors such as vascular endothelial growth factor and placental growth factor. As a result, the release of proinflammatory factors including cytokines, hypoxia-inducible factor (HIF), reactive oxygen species (ROS), and angiotensin AT_1 are released (Yu et al., 2018).

In summary, it is well supported that the development of PE beginning with abnormalities in placentation triggers abnormal levels of angiogenic factors, which lead to the systemic symptoms of PE. However, the initial cause of abnormal placentation remains poorly understood.

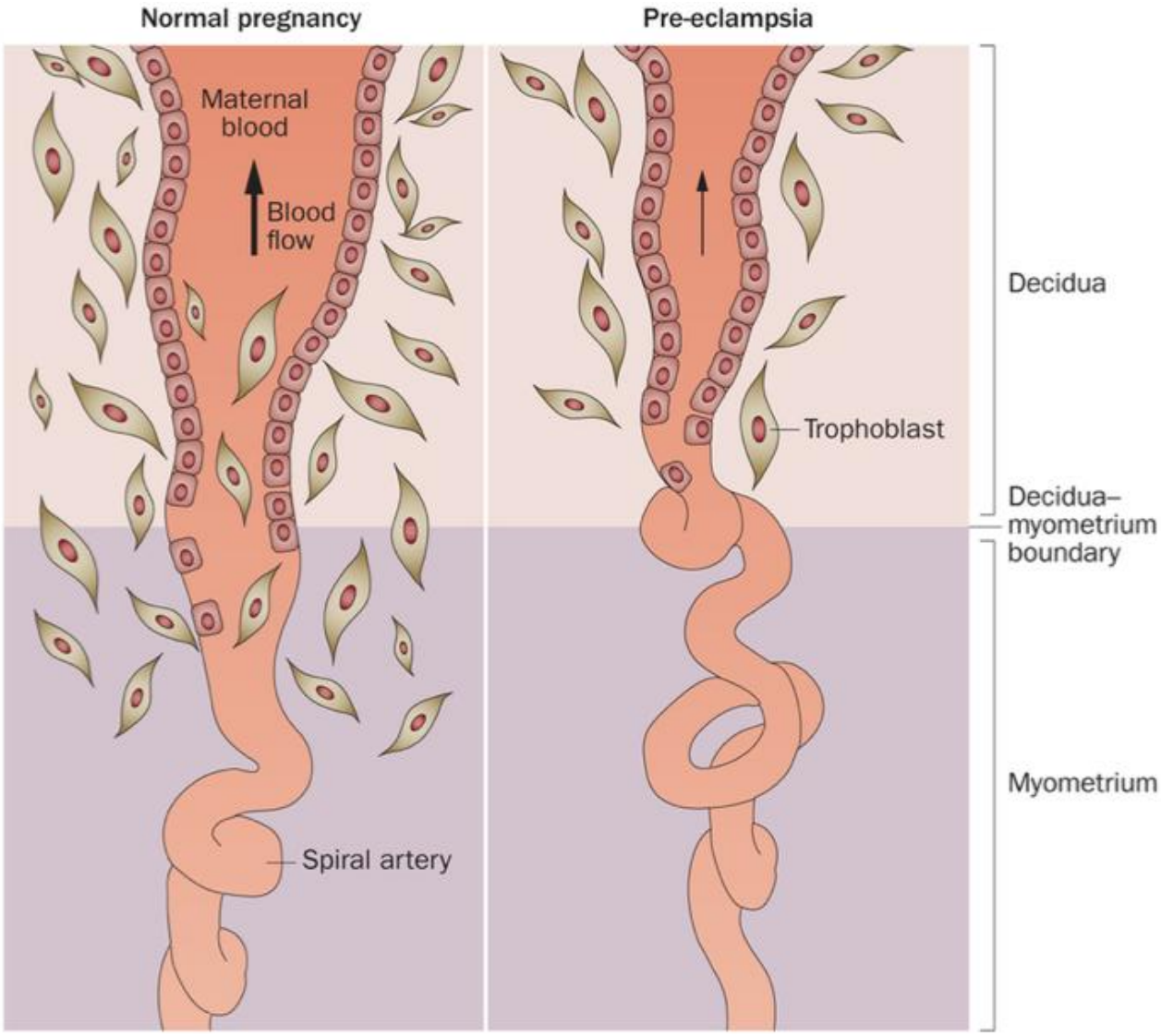

Figure 2: Spiral arteries in normal and preeclamptic pregnancies. The placentas of individuals who go on to develop PE in pregnancy are compromised. EVTs do not fully invade into the myometrium of the uterus and, as a result, maternal spiral arteries are incompletely remodeled. This prevents spiral arteries from taking on the widened shape that improves blood flow to the placenta during pregnancy, causing hypoperfusion of the placenta (Chaiworapongsa et al., 2014).

1.5 Preeclampsia and Endothelial dysfunction

Endothelial cells line the entirety of the cardiovascular system and function to support homeostasis through several functions. Endothelial cells can filter fluid, alter the tone of blood vessels, and respond to and adjust hormones (Rajendran et al., 2013) in response to changes in

blood contents (Boeldt & Bird, 2017). Generalized endothelial dysfunction has been consistently identified as a key component in the development of PE (Tomimatsu et al., 2019). The imbalance of placental antiangiogenic proteins is a well-supported cause of the resulting endothelial dysfunction seen in PE (Yu et al., 2018). Most prominently, there is an increase in sFlt1, which antagonizes VEGF (Maynard et al., 2003). VEGF is important in inducing prostacyclin synthesis, which activates adenylyl cyclase to increase cyclic adenosine monophosphate (cAMP) synthesis. As a result, cellular Ca^{2+} concentration decreases, causing the relaxation of smooth muscle and vasodilation. Similarly, VEGF increases NO concentrations by stimulating endothelial nitric oxide synthase (eNOS) expression in endothelial cells. NO then activates soluble guanylate cyclase (sGC), to increase cyclic guanosine monophosphate (cGMP) synthesis, ultimately decreasing Ca^{2+} concentration and causing vasodilation (Tomimatsu et al., 2019). However, in PE, since the excess of sFlt1 leads to a decline in VEGF activity, there is a subsequent loss of cAMP and NO, and thus, a lack of vasodilation.

Fluctuations between ischemia and reperfusion seen with the decreased uteroplacental blood flow associated with PE can cause the release of pro-inflammatory cytokines like TNFα and IL-6 (Yu et al., 2018). IL-6 contributes directly to endothelial dysfunction by damaging the tight junctions of endothelial cells and disrupting cell signaling (Yu et al., 2018). TNFα indirectly effects the endothelium by downregulating eNOS and mitochondrial biogenesis, causing mitochondrial dysfunction and the subsequent release of ROS (Yu et al., 2018). ROS then causes direct physical damage to the endothelium. It is thought that TNFα and IL-6 may work in tandem to increase endothelian-1 (ET-1), a known marker of endothelial dysfunction (Yu et al., 2018).

The observed endothelial dysfunction leads to the systemic vascular disease that is a hallmark of PE. Since the endothelium cannot adequately function, the physiological and anatomical vascular changes that occur in a healthy pregnancy are compromised (Boeldt & Bird, 2017). In healthy pregnancies, angiogenesis along with the widening and lengthening of vessels

occurs to compensate for the significant increase in blood volume associated with pregnancy. The net result of these changes is an increase in the surface area of the vasculature, and thus, a decrease in resistance to accommodate the increased volume (Boeldt & Bird, 2017). In PE, however, angiogenesis and vasodilation are impaired by the systemic endothelial dysfunction that accompanies the disease (Tomimatsu et al., 2019). As a result, the maternal system is not able to adequately compensate for the increase in blood volume that comes with pregnancy, which increases systemic resistance and ultimately leads to hypertension (Boeldt & Bird, 2017).

1.6 Future risk of CVD

The relationship between PE and future CVD is well established (Chen et al., 2014; Cunningham & LaMarca, 2018; Lazdam et al., 2012). Individuals who have experienced a hypertensive pregnancy are at twice the risk of developing CVD later in life when compared to normotensive pregnancies (Cunningham & LaMarca, 2018). Similarly, the risk of ischemic heart disease and stroke increases by two-fold after experiencing a hypertensive pregnancy (Chen et al., 2014). Furthermore, developing chronic hypertension, a common risk factor for CVD, is three- to four-times more likely after experiencing PE (Chen et al., 2014). Lazdam et al., (2012) reported that women who were diagnosed with PE prior to 34 weeks saw a greater increase in their blood pressure over a 6–13-year period postpartum than those with late-onset PE. Thus, the onset of PE may be important in assessing the future risk of CVD.

Continuing to learn more about the lasting impacts of PE on the microvasculature will contribute to a better understanding on whether PE increases one's risk of CVD later in life, or if PE resulted due to preexisting CVD risk factors, which are also risk factors for PE.

1.7 Microvascular structure and disease

The structure of the systemic microvasculature has been found to be altered in various disease states. In particular, capillary rarefaction, a decline in capillary density, has been associated with hypertension (Rizzoni et al., 2011). It has not yet been concluded whether the

microvascular changes are a result or cause of hypertensive disease (Rizzoni et al., 2017). However, de Moraes and Tibirica (2017) found microvascular rarefaction in patients with borderline hypertension and normotensive young adults at high risk of developing hypertension. This suggests that microvascular alterations, including rarefaction, may be an early indication of and potential treatment target for hypertension or CVD. In these patients, capillary rarefaction is seen in the distal portion of microvascular networks. This decline in capillary density was seen both under basal conditions and while inducing venous congestion (Agabiti-Rosei & Rizzoni, 2017). Similarly, retinal capillary rarefaction has also been observed in patients with hypertension and type 2 diabetes mellitus (Jumar et al., 2016). Beyond hypertensive diseases, capillary rarefaction has been associated with chronic kidney disease in which peritubular capillaries are specifically impacted (Kida et al., 2014). Overall, there is building evidence that changes to microvascular structure, primarily capillary rarefaction, may be an indication or outcome of cardiovascular diseases. As such, microvascular structure may provide insight into the risk of CVD later in life in patients with PE.

1.8 Microvascular structure and Preeclampsia

Changes in microvascular structure have also been linked to hypertensive disorders in pregnancy. Common parameters to evaluate microvascular structure are functional and structural capillary densities. Functional capillary density refers to the number of capillaries per area that are filled with red blood cells at baseline and structural capillary density describes the total number of capillaries per area including those that are not perfused at baseline (Antonios et al., 2013). In order to assess structural capillary density, venous occlusion is induced to prevent backflow of blood from the capillaries to the heart. Doing so fills all existing nailfold capillaries with red blood cells so that they can be viewed and assessed using nailfold capillaroscopy.

In general, hypertensive pregnancy is associated with functional capillary rarefaction, a decline in capillary density, both during pregnancy and for years after (Boardman et al., 2020).

Women diagnosed with PE have also been found to have reduced capillary density (Antonios et al., 2013; Hasan et al., 2002). Antonios et al., (2013) reported only a decline in structural nailfold capillary density during pregnancies complicated by PE, while Hasan et al., (2002) found a reduction in both functional and structural nailfold capillary densities in those with PE.

The development of capillary rarefaction occurs in a healthy pregnancy between 27 and 32 weeks but resolves naturally within six weeks of delivery (Nama et al., 2012). In women with PE, the development of capillary rarefaction occurs earlier, between 20 and 24 weeks, is more severe, and continues past the puerperium period (Nama et al., 2012). The evidence of capillary rarefaction development prior to the onset and diagnosis of PE suggest that these microvascular changes could be involved in the pathogenesis of the disease (Nama et al., 2012). Capillary rarefaction has also been found to have an inverse relationship with blood pressure during pregnancy, regardless of whether the individual has been diagnosed with PE. It has been suggested that the decline in capillary density associated with PE is an indication of a weak cardiovascular system that is not able to properly adapt to pregnancy (Hasan et al., 2002). Another microvascular alteration seen in women experiencing PE is a higher percentage of tortuous and dilated capillaries. However, this difference disappeared three months after delivery (Houben et al., 2007). Women with PE have reduced perfusion of their capillaries when compared to healthy controls. The decline in perfusion is characterized as a significant decrease in the number of capillaries filled with red bloods cells ≥50% of the time (Weissgerber et al., 2019). Compared to participants diagnosed with pregnancy complications that do not affect the microcirculation, those with gestational hypertension and PE had a significant reduction in capillary recruitment (Rusavy et al., 2015). A reduction in capillary recruitment is defined as the difference between the number of structural and functional capillaries per area in the individual and suggests a weakness in the functional abilities of the microvasculature in women with gestational hypertension and PE (Rusavy et al., 2015).

1.9 Rationale

It is well established that PE is associated with systemic endothelial dysfunction caused by the release of pathogenic factors from the hypoxic placenta. TNFα, a proinflammatory mediator found in excess in PE, has been found to cause a loss of endothelial cell-lined capillaries (Koller et al., 2020). This finding along with the association of a decline in capillary density with CVD, which individuals with a history of PE are at a significantly higher risk for, suggests that individuals with PE may experience a reduction in nailfold capillary density. However, it remains uncertain whether nailfold capillary density is affected in individuals who are experiencing a pregnancy complicated by PE both during pregnancy and postpartum. Understanding how the microvasculature responds to a pregnancy complicated by PE both prior to and after delivery may provide support for postpartum surveillance and early pharmaceutical or lifestyle intervention for individuals who experienced PE and thus, are at a higher risk of developing CVD.

1.10 Hypothesis

We hypothesize that PE is associated with a decline in the number of nailfold capillaries per $1mm^2$ that persists postpartum. This microvascular change may help to identify women who are at greatest risk of future CVD and may benefit from CVD screening, lifestyle modification and/or pharmaceutical intervention.

1.11 Objective

To determine if PE is associated with a decline in the number of nailfold capillaries per $1mm^2$ both antepartum and postpartum using nailfold video capillaroscopy (NVC).

Chapter 2

Methods

2.1 Participants

This study obtained ethics approval from the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (#6028542). Participants recruited for the study were between the ages of 18 and 40 with a singleton pregnancy and a plan to deliver at Kingston Health Sciences Centre – Kingston General Hospital (KHSC – KGH). Participants that had a medical history of high blood pressure, diabetes, lupus, kidney disease, gestational diabetes, coronary heart disease, Raynaud's syndrome, scleroderma, arthritis, collagen-vascular diseases, or extensive manual work were excluded. To be included as a participant with PE, a clinical diagnosis by the care team at KHSC - KGH was required.

2.2 Recruitment

To begin recruitment, the list of booked Cesarean sections found on the Labour and Delivery ward at KHSC – KGH was reviewed. The names of patients booked for a Cesarean section between January 2021 and January 2022 were recorded weekly. The charts of these individuals were then reviewed to assess eligibility. If eligible, the patient was approached at the obstetrics and gynecology clinic at KHSC – KGH. Obstetrical clinic charts at KHSC – KGH were reviewed to identify possible controls with an uncomplicated, singleton pregnancy. When initially approached, patients were asked if they had an interest in learning about the study and if so, were walked through a detailed description of the study and provided with written consent. The Labour and Delivery ward at KHSC – KGH was consulted regularly to check for admitted patients with a PE diagnosis. These women were then approached and consented on the Labour and Delivery ward after screening their medical charts for eligibility.

2.3 Study visits

Most participants underwent study visits both antepartum and postpartum, with some, particularly those with PE, only completing one of the visits. For control participants, the antepartum study visit was conducted at one of their appointments at the obstetrics and gynecology clinic at KHSC – KGH. This first study visit was conducted in the Maternal Fetal Studies room on Kidd 5 at KHSC – KGH. For participants diagnosed with PE, the first study visit was conducted after they had been admitted to the hospital and while they were awaiting delivery. Prior to both study visits, all participants were asked to avoid caffeine, as capillary perfusion has been found to be higher in women who consumed caffeine within the past 6 hours compared to those that have not (Weissgerber et al., 2019).

If in the Maternal Fetal Studies Room, participants were seated upright in a chair, with their hand resting on a table beside the chair, at elbow height. For study visits conducted in hospital rooms, participants remained in a comfortable seated position in their bed. The time it took for participants to fill out the study questionnaire allowed for them to acclimatize to the room temperature (Ciołkiewicz et al., 2010; Mazzotti et al., 2014). After filling out the study questionnaire, participants had their blood pressure taken using the BPtru automated machine. This non-invasive, automated machine takes 6 blood pressure readings and omits the first reading when calculating an average of the subsequent 5 blood pressures. The remainder of the study visit involved conducting the nailfold assessment as described below.

For all participants, postpartum visits were scheduled between 12 and 48 hours postpartum. Study visits with all postpartum visits were carried out in the participant's hospital room. Participants remained in their hospital beds in a comfortable seated position with their hand at or below heart level. At the postpartum visit, the diastolic blood pressure used to induce venous occlusion at the antepartum visit was used again for consistency.

2.4 Equipment

The apparatus in Figure 3 was on an Ergotron workstation that could be transported between the Maternal Fetal Studies Room and participant hospital rooms. The workstation depicted in Figure 3 includes the INSPECTIS CAP Pro Nailfold Capillaroscopy Software, the INSPECTIS digital capillaroscope, immersion oil, and the Hokanson rapid cuff inflation system. The digital capillaroscope has 5.0MP image resolution and connects with the INSPECTIS CAP Pro Nailfold Capillaroscopy Software through hi-speed USB 3.0. This tool was equipped with a 200x magnification lens and built-in illumination to produce high quality images of the nailfold. For clear images, immersion oil provided by INSPECTIS was placed on the nailfold prior to imaging. To induce venous occlusion, a Hokanson rapid cuff inflation system was used. The cuff, as shown in Figure 3, is connected to the system that inflates the cuff to a selected pressure and maintains the pressure for the indicated time.

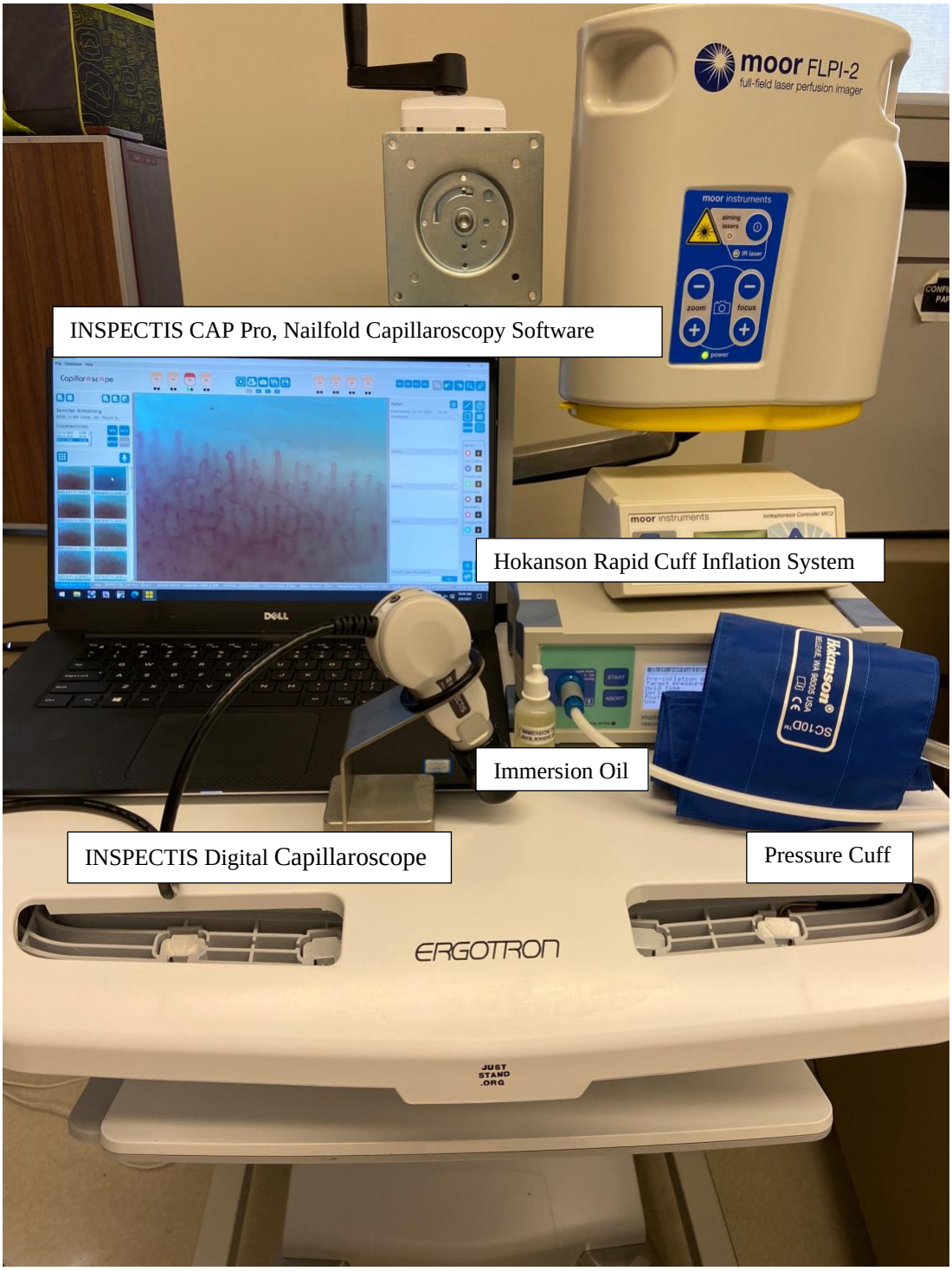

Figure 3: Nailfold Video Capillaroscopy equipment. The Ergotron workstation transported the INSPECTIS CAP Pro Nailfold Capillaroscopy Software, INSPECTIS Digital Capillaroscope, immersion oil, Hokanson Rapid Cuff Inflation System, and a pressure cuff.

2.5 Questionnaire

Participants were asked to fill out a questionnaire at their first study visit. This questionnaire allowed for the collection of information on the participant's past family and medical history along with other personal information such as age, height, weight, race, and income. Information related to hand health, such as previous trauma, was also collected. Behavioral questions, such as smoking, drinking, and exercise habits and any complications that have occurred during their current or previous pregnancies were recorded. Thirty minutes of moderate activity per day was considered physically active.

2.6 Chart Review

Participant charts were reviewed postpartum to collect information on their health during their pregnancy, labour, and delivery. Parameters including red blood cell count, white blood cell count, and platelet count measured through blood tests taken during hospital admission were recorded. Other information that was obtained from patient charts included time and type of delivery. The Appearance Pulse Grimace Activity and Respiration (APGAR) score of the neonate and their weight was recorded to give insight into their overall wellbeing. The neonate's weight was categorized as small for gestational age (SGA), normal, or large for gestational age (LGA) if their weights were below the 10^{th} percentile, between the 10^{th} and 90^{th} percentile, and beyond the 90^{th} percentile, respectively (Kramer et al., 2001). The participant was notified that their chart would be referenced for these additional details.

2.7 Nailfold video capillaroscopy (NVC)

Figure 4: The INSPECTIS Nailfold Video Capillaroscope. This digital capillaroscope has a 5.0MP image resolution and a 200x magnification lens with built-in illumination.

NVC is a non-invasive technique that allows for the visualization of capillaries in the nailfold. The handheld scope is shown in Figure 4.

Since the advancement from x12 as the standard technique to x200 magnification in 1990, users have been able to measure and characterize individual nailfold capillaries. Investigating the nailfold capillaries is particularly easy using NVC as it is a non-invasive tool

(Rimar et al., 2019). NVC has primarily been used in the diagnosis and treatment of Raynaud's syndrome, systemic sclerosis, and other connective tissue diseases. There is evidence that this technique also has clinical cardiovascular applications (Cheng et al., 2015). The nailfold is particularly useful for imaging because the capillaries are parallel to the skin making them easier to visualize than when they are perpendicular in other areas of the body (Emrani et al., 2017). NVC allows for the quantification of numerous measurements and morphological variations. Some examples include, density, length, tortuousity, crossed, or dilated (Emrani et al., 2017). Figure 5 shows both the typical hairpin loop structure of nailfold capillaries and a tortuous capillary.

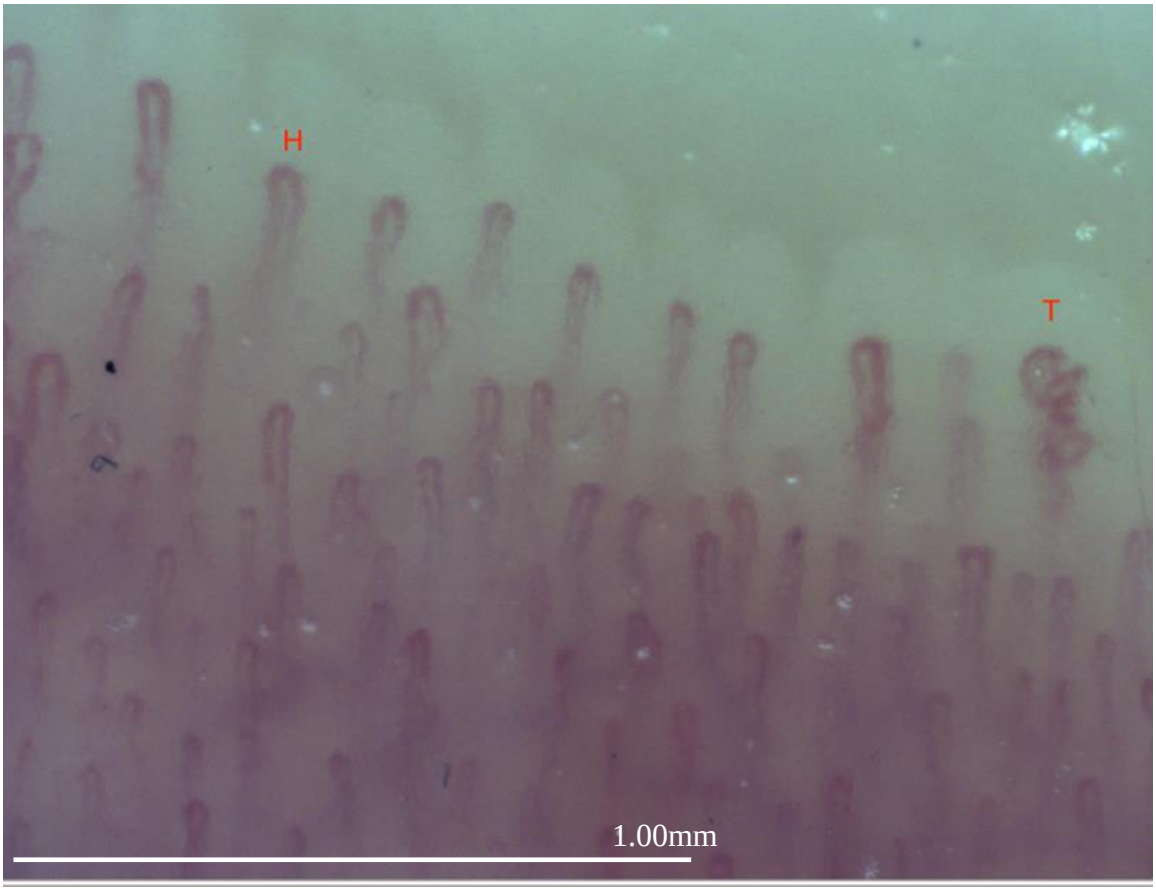

Figure 5: An image of the nailfold capillary bed of a study participant after inducing venous occlusion for 2-minutes. (H) indicates the typical, hairpin loop structure, while (T) is an example of a tortuous capillary. Both are found in healthy nailfolds.

NVC's ability to visualize and measure various parameters of capillary beds makes it a useful tool in assessing the microvasculature, which can give insight into an individual's vascular health.

2.7.1 The Nail Unit

The nail unit consists of the nail plate, distal finger tissues, innervating nerves, and microvasculature (de Berker, 2013). Beneath the nail plate is the nail bed, which includes all soft tissues directly beneath the nail plate (Zook et al., 1980). The nail plate is secured by the cuticle to the proximal nail fold, which is an indentation on the dorsum of the finger (Zook et al., 1980). Here, the nail plate is embedded under the skin (Fleckman et al., 2013). At the nailfold, there is a network of capillaries, which can be assessed using NVC. Figure 6 shows these nail structures relative to each other (de Berker, 2013).

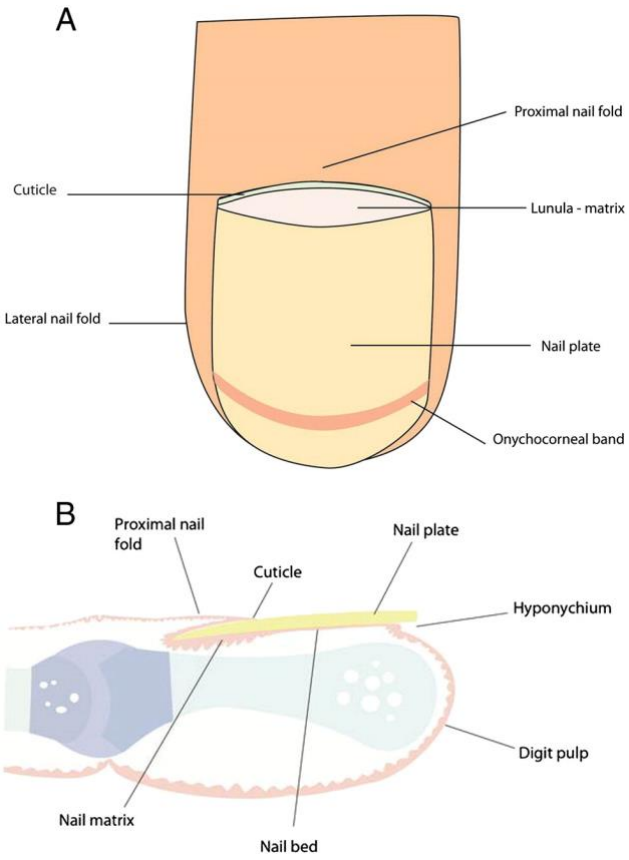

Figure 6: The nail unit, labeled, from top (A) and side (B) views. Image from: de Berker, D. (2013). Nail anatomy. Clinics in dermatology, 31(5), 509-515. The proximal nailfold is identified in images (A) and (B) in relation to other key structures of the nail unit. The proximal nailfold is the location of nailfold capillary assessment.

2.7.2 Nailfold Video Capillaroscopy Protocol

The INSPECTIS digital capillaroscope was used to non-invasively image the nailfold capillaries of participants. During the nailfold assessment, images were taken both with and without inducing venous occlusion.

At the antepartum visits, control participants had images taken of their third, fourth, and fifth fingers on both hands. A drop of immersion oil supplied by INSPECTIS was placed on each of the fingers being imaged to allow for better visualization of the capillaries and a smoother glide against the nailfold. At each nailfold, the INSPECTIS capillaroscope was placed on the middle of the nail and slowly glided upwards towards the nailfold. The placement of the capillaroscope on the nailfold is shown in Figure 7.

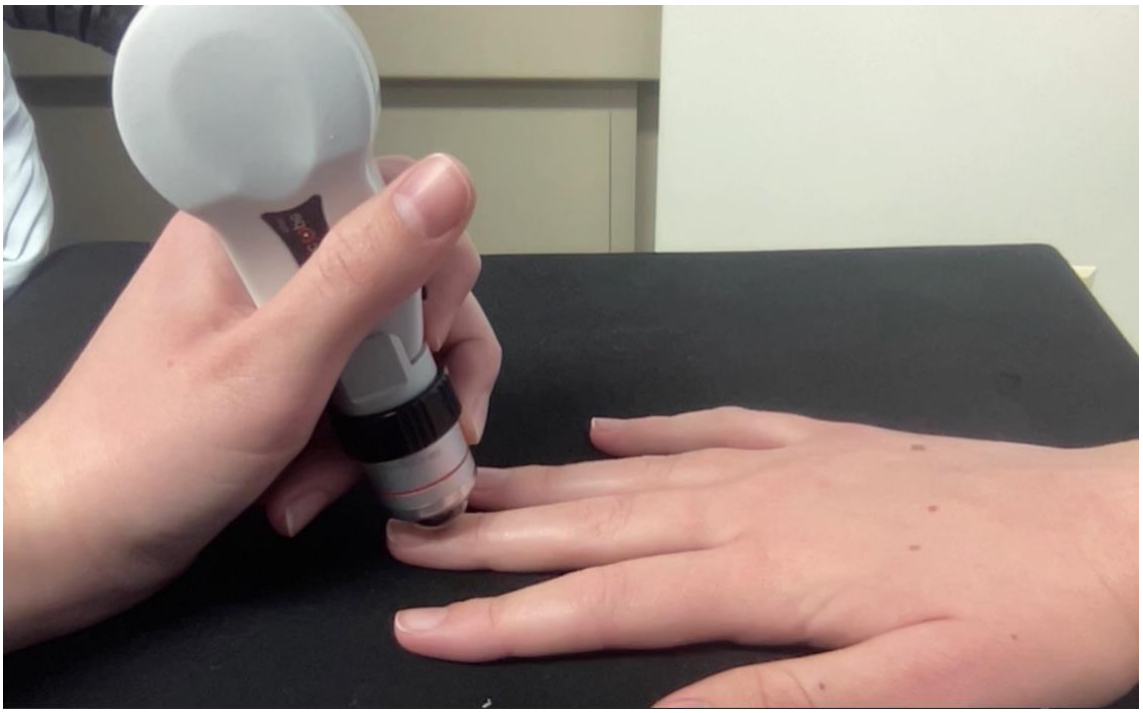

Figure 7: The INSPECTIS digital capillaroscope positioned on the nailfold of the third finger to take images of the nailfold capillaries.

Once the capillary bed was in view, five images were taken on the fifth and fourth fingers of the right hand. These fingers were selected as they have been found to have good transparency (Emrani et al., 2017). The thumb was not imaged due to poor visualization of the nailfold (Ruaro et al., 2017). With a pressure cuff on, but not inflated, five images were taken on the third finger of the right hand. The pressure cuff was inflated to the participant's previously measured average diastolic pressure for two-minutes. At two-minutes, five images were taken on the third finger. The third finger was chosen for assessment with venous occlusion as recommended in the literature (Antonios et al., 2013; Nama et al., 2012; Rusavy et al., 2015). After repeating the venous occlusion protocol twice more, the entire process was repeated on the participant's left hand.

At the visits with participants diagnosed with PE, only one hand was assessed. Given their medical state, participants often had fluids or medications being administered through IV in one of their hands. This hand was excluded to avoid occluding the delivery of the fluid when using the pressure cuff and to dismiss any impact of fluid delivery on the microvasculature. Similarly, at the postpartum study visits with control participants, the hand that did not have an IV was evaluated. Otherwise, the identical protocol as described above was followed on the available hand at the postpartum visits.

2.7.3 Microvascular Measures

The images taken during the clinic visits were used to determine the average capillary density of each participant. Calculating capillary density was done by superimposing a virtual $1mm^2$ square over the image and counting the capillaries within it. Figure 8 shows the placement of the $1mm^2$ square over an example image. When placing the $1mm^2$ square, the area with the most capillaries, that also contained at least one distal capillary, if possible, was chosen when analyzing images. Distal capillaries are those in the final row of nailfold capillaries, furthest away from the center of the body. The area chosen for analysis was required to include at least one

distal capillary to reduce bias in selecting areas within each image. It is possible that different areas of the nailfold could naturally have variance in the number of capillaries per area. Thus, ensuring each image analyzed was at the distal edge of the nailfold reduced some variance that may exist between different areas of the nailfold. Capillaries were then manually counted. If any portion of the capillary was inside of the $1mm^2$ square, then it was counted. Additionally, any capillary with a portion of its ascending and descending loops visible were counted. Figure 8 shows an example of how capillaries were counted.

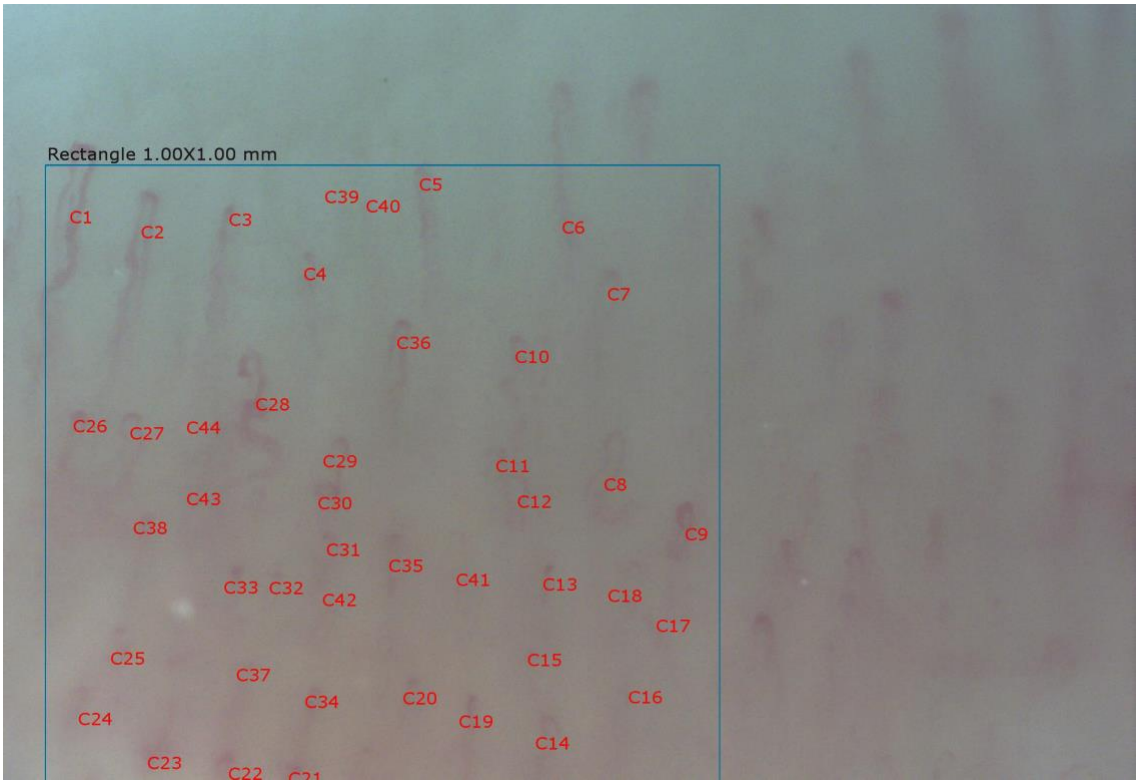

Figure 8: An image of nailfold capillaries taken using the INSPECTIS capillaroscope and counted mannually on the associated software. This image was taken with a lens of 200x magnification.

2.8 Statistical Analysis

GraphPad Prism, version 9.0, was used for all statistical analysis. Unpaired and paired t-tests were used to compare nailfold capillary density between groups and time points,

respectively. When normality could not be assumed, a two-tailed Wilcoxon matched-pairs rank test was used for matched data and a Mann-Whitney test was used for unmatched data. For categorical data, a Fischer's exact contingency test was used. A p-value of <0.05 was considered significant.

A power analysis was conducted to estimate sample size using data from Boardman et al., (2020) (N=173), which compared capillaries per mm^2 between 70 controls and 103 individuals with a history of hypertensive pregnancy. The assessment was conducted 5-10 years postpartum. Using a significant of 0.05 and a power of 80%, the minimum sample size calculated was 172 individuals divided evenly into control and preeclamptic groups.

Chapter 3

Results

3.1 Pilot Project: Developing a venous occlusion protocol (VOP)

Eight individuals participated in the pilot project. The average nailfold capillary densities before and after the VOP at 60mmHg were significantly different (P=0.0021). The average nailfold capillary densities were also significantly different (P=0.0031) before and after the VOP using the participant's previously measured diastolic pressure.

3.2 Patient Demographics

Between January 2021 and February 2022, 32 control and 15 preeclamptic participants took part in the primary study. An overview of the characteristics of the 47 participants, broken down into control or preeclamptic, are presented in **Table 1**. Information about participant and neonate characteristics at the time of delivery are shown in **Table 2**.

Participant groups did not differ in age (P=0.1241), race (P=0.7204), education level (P>0.9999), marital status (P=0.4592), history of smoking (P>0.9999), or hand dominance (P>0.9999) (**Table 1**). However, preeclamptic participants had higher systolic (P=<0.0001) and diastolic (P=0.0058) pressures. The average diastolic blood pressures of control and preeclamptic participants were 70.53mmHg and 77.54mmHg, respectively. Controls had an average systolic blood pressure of 105.2mmHg, while preeclamptics had an average systolic blood pressure of 123.9mmHg. Sixty-nine-point seven percent of control participants indicated being physically active, while 33.3% of those diagnosed with PE reported being physically active (**Table 1**). Preeclamptic participants had, on average, a greater yearly household income than controls, with 53.3% and 33.3% of participant households earning more than >$90,000 per year, respectively (**Table 1**).

Participant groups did not differ in parity (P=0.7422) or blood platelet count (P=0.5196) at the time of delivery. The gestational age of control participants at delivery was higher (P=0.004) than that of preeclamptic participants, with a mean gestational age of 39.3 and 37.4, respectively (**Table 2**). Neonates born to control participants weighed more on average than those born to preeclamptic participants, with a mean weight of 3471.9g and 2719.2g, respectively (**Table 2**). Six-point seven percent of control neonates were SGA, while no neonates born to preeclamptic participants were SGA (**Table 2)**. For 29% of the control participants, the index pregnancy was their first, while it was the first pregnancy for 40% of preeclamptic participants (**Table 2**).

Table 1: Maternal characteristics. Continuous data presented as mean (SD) and categorical data presented as number (%).

	Control (N=32)	PE (N=15)	P-value
Maternal age			
Mean (SD)	31.8 (3.5)	30.5 (4.9)	0.1241
Diastolic Blood pressure			
Mean (SD)	70.5 (7.0)	105.2 (9.1)	0.0058
Systolic Blood Pressure			
Mean (SD)	77.5 (9.6)	123.9 (8.7)	<0.0001
Race, n (%)			
Caucasian	26 (81.3)	11 (84.6)	0.7204
Asian	2 (6.3)	1 (7.7)	
Black	2 (6.3)	0	
Other	2 (6.3)	1 (7.7)	
Unknown	1	2	
Education, n (%)			
University/College complete	27 (84.4)	11 (91.7)	>0.9999
University/College incomplete	3 (9.4)	0	
High school or less	2 (6.3)	1 (8.3)	
Unknown	1	3	
Income, n (%)			
>$90,000	11 (36.7)	8 (80.0)	0.0281
$60-89,000	14 (46.7)	2 (20.0)	
$30-59,000	4 (13.3)		
<$30,000	1 (3.3)		
Unknown	3	5	
Marital Status			
Married	21 (65.6)	10 (83.3)	0.4592
Common-Law	11 (34.4)	1 (8.3)	
Single		1 (8.3)	
Unknown	1	3	
Previous smoking, n (%)			
Yes	9 (31.0)	4 (36.4)	>0.9999
No	20 (69.0)	7 (63.6)	
unknown	4	4	
Physically active, n (%)			
Yes	23 (76.7)	5 (41.7)	0.0288
No	6 (20.0)	7 (58.3)	
Unknown	3	3	
Dominant Hand, n (%)			
Right	27 (96.4)	12 (100.0)	>0.9999
Left	1 (3.6)		
Unknown	5	3	

Table 2: Maternal characteristics and neonatal birth weight. Continuous data presented as mean (SD) and categorical data presented as number (%).

	Control (N=32)	PE (N=15)	P-value
Gestation age at delivery			0.0021
Mean (SD)	39.4 (1.3)	37.4 (3.7)	
Unknown	2	1	
Gestation, n (%)			
One	9 (29.0)	6 (40.0)	0.5139
Two	12 (38.7)	3 (20.0)	
Three	6 (19.4)	3 (20.0)	
Five	4 (12.9)	0	
Seven	0	2 (13.3)	
Eight	0	1 (3.03)	
Unknown	1	0	
Neonate weight			
Mean (SD)	3459 (517.9)	2719.2 (980.7)	0.0016
Unknown	2	2	
Birth weight for gestational age, n (%)			
SGA (<10%ile)	2 (6.9)	0	>0.9999
Normal	23 (79.3)	12 (92.3)	
LGA (>90%ile)	4 (13.7)	1 (7.7)	
Unknown	3	2	
Platelets			
Mean (SD)	206.5 (60.8)	211.8 (80.0)	0.4886
Unknown	4	3	
Medications, n (%)			
Magnesium Sulphate	0	3 (20)	0.076
Labetalol	0	3 (25)	0.0489
Adalat	0	2 (16.7)	0.1419

Table 3: Categorization of preeclamptic group. Categorical data presented as number (%).

	PE (N=15)
Time of onset, n (%)	
Early onset (<34 weeks)	3 (20.0)
Late onset (=/>34 weeks)	11 (73.3)
Postpartum	1 (6.7)
Severity, n (%)	
Mild	11 (73.3)
Severe	4 (26.7)

3.3 Average nailfold capillary densities of control and preeclamptic participants before and after delivery

Five preeclamptic participants had their nailfolds assessed both before and after delivery. Figure 9 shows no differences in the average nailfold capillary densities before and after delivery were observed (P=0.5582). No trend was observed when nailfold capillary densities for each PE participant before and after delivery were plotted (Figure 10).

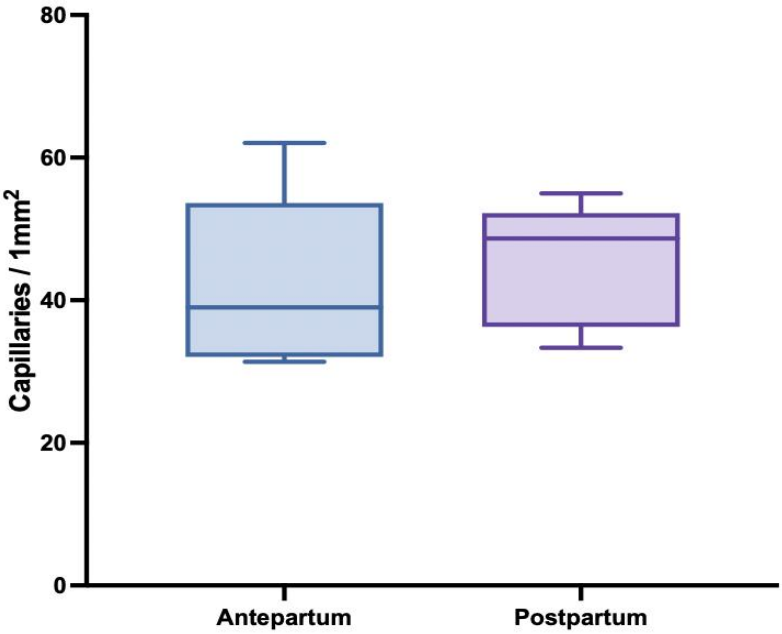

Figure 9: Preeclamptics antepartum and postpartum. The average nailfold capillary density (capillaries/1mm^2) of the 5 preeclamptic participants that were assessed both antepartum and postpartum. Time point differences assessed using a paired two-tail t-test (P=0.5582). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number of capillaries per 1mm^2.

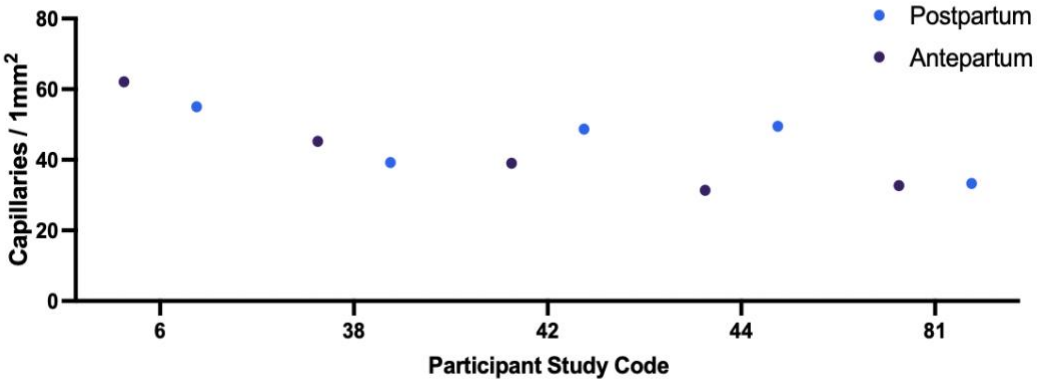

Figure 10: Individual preeclamptics antepartum and postpartum. The average nailfold capillary density (capillaries/1mm^2) in each individual preeclamptic participant that was assessed both antepartum and postpartum.

At each study visit, images of the nailfold were also taken after inducing venous occlusion for two minutes. Despite the induction of venous occlusion in preeclamptic participants, nailfold capillary density did not differ before and after delivery (**Figure 11**; P=0.4375).

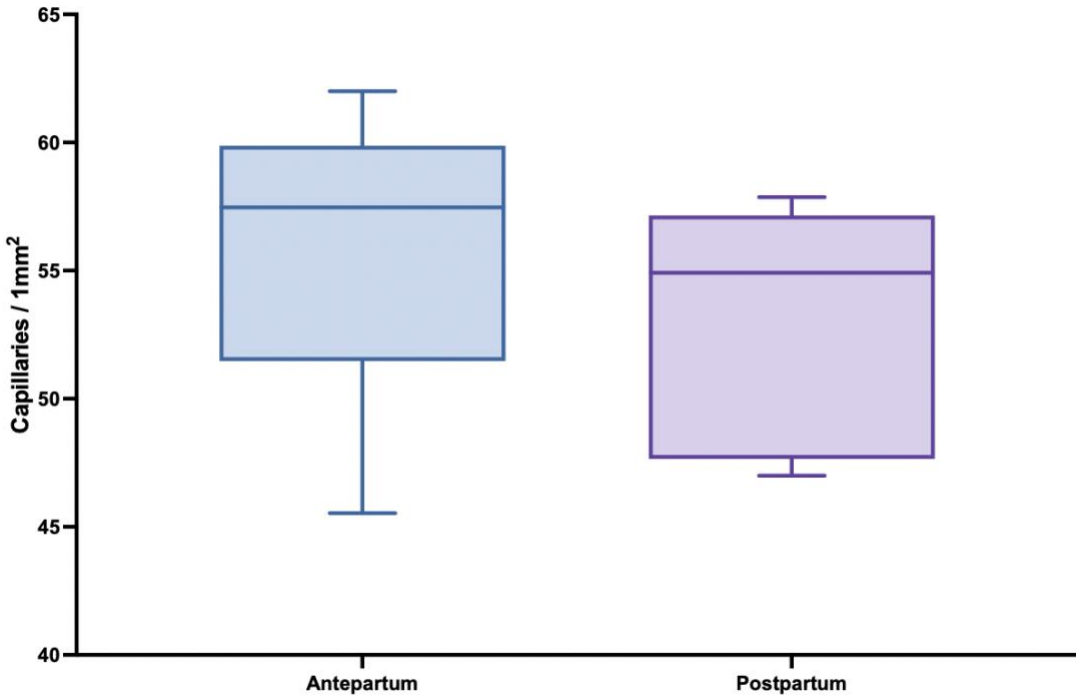

Figure 11: Preeclamptics antepartum and postpartum while inducing venous occlusion. The average nailfold capillary density (capillaries/1mm^2) after inducing venous occlusion in the 5 preeclamptic participants that were assessed both before and after delivery. Time point differences assessed using a Wilcoxon test (P=0.4375). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number

Twenty-four control participants had their nailfolds assessed both antepartum and postpartum. No difference was observed in their average nailfold capillary density before and after delivery (**Figure 12;** P=0.1920). No difference was found when comparing average nailfold capillary density of controls when venous inclusion was induced for two-minutes before and after delivery (**Figure 13;** P=0.5581).

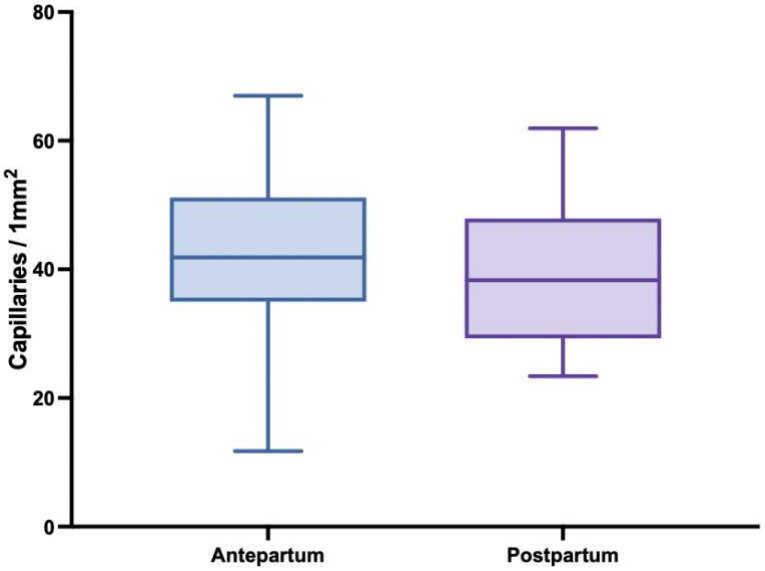

Figure 12: Controls antepartum and postpartum. The average nailfold capillary density (capillaries/1mm^2) of 24 control participants assessed antepartum and postpartum. Time point differences assessed using a paired two-tail t-test (0.1920). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number of capillaries per 1mm^2.

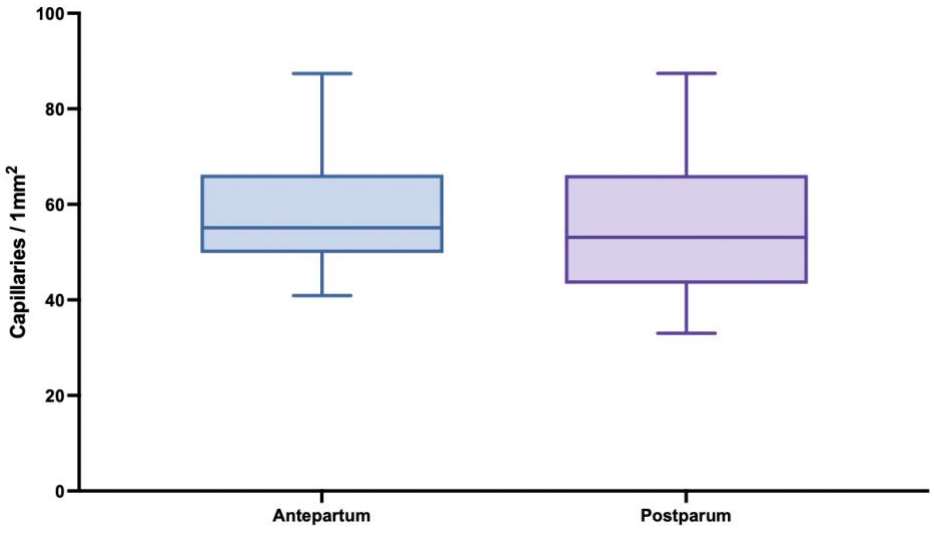

Figure 13: Controls antepartum and postpartum while inducing venous occlusion. The average nailfold capillary density (capillaries/1mm^2) after inducing venous occlusion in 24 control participants before and after delivery. Time point differences assessed using a paired two-tail t-test (P=0.5581). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number of capillaries per 1mm^2.

Prior to delivery of the neonate and placenta, no difference was found in the average nailfold capillary density when comparing preeclamptic (n=7) and control (n=32) participants (**Figure 14**; P=0.6979). This finding was consistent after inducing venous occlusion (**Figure 15;** P=0.5305).

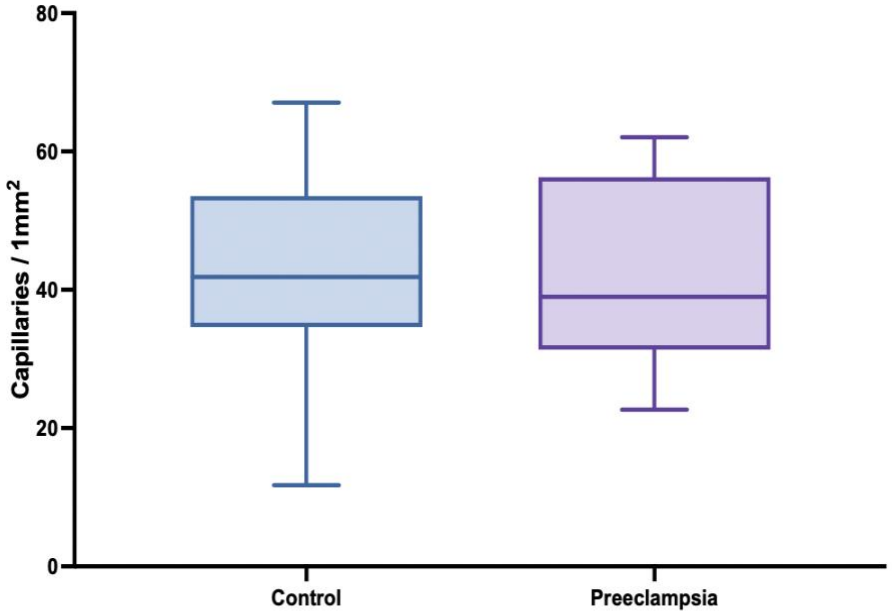

Figure 14: Controls and preeclamptics antepartum. The average nailfold capillary density (capillaries/1mm^2) in control and preeclamptic participants prior to delivery. Difference between mean was assessed using an unpaired two-tail t-test (P=0.6979). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number

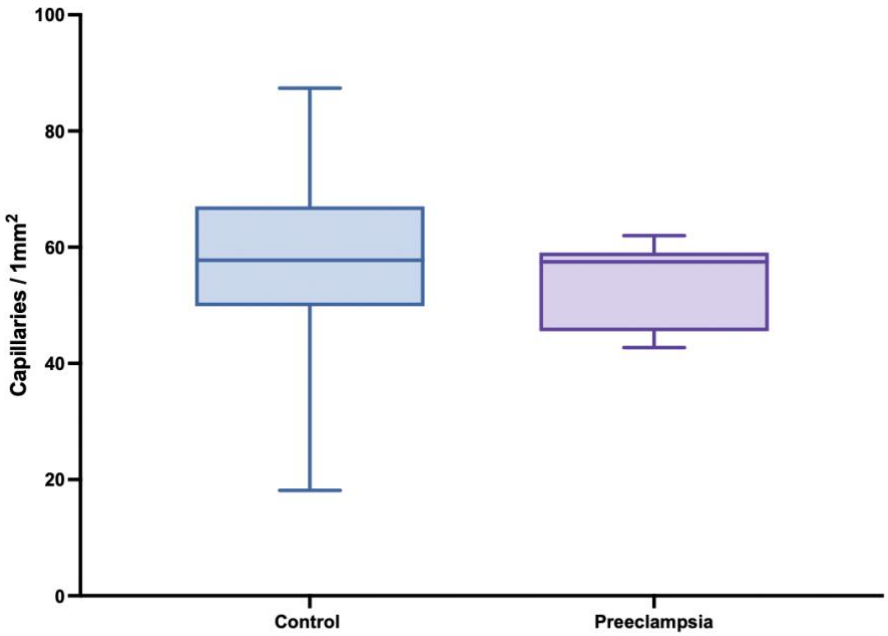

Figure 15: Controls and preeclamptics antepartum while inducing venous occlusion. The average nailfold capillary density (capillaries/1mm^2) in control and preeclamptic participants while inducing venous occlusion before delivery. The difference between means was assessed using a Mann-Whitney test (P=0.5305). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number of capillaries per 1mm^2.

No difference was found between preeclamptic (n=13) and control (n=24) participants postpartum either before (**Figure 16**; P=0.0570) or after (**Figure 17**; 0.9104) venous occlusion. However, there is a trend towards preeclamptic participants having a greater nailfold capillary density postpartum at baseline prior to inducing venous occlusion.

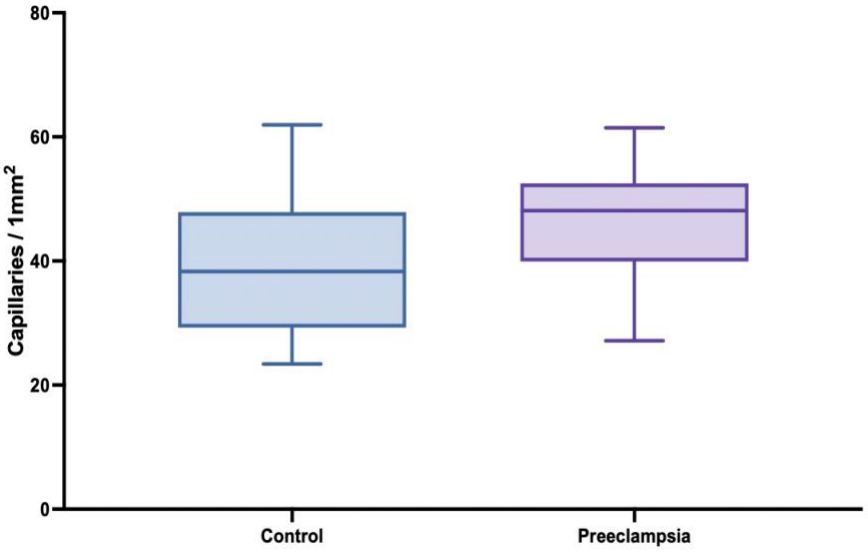

Figure 16: Controls and preeclamptics postpartum. The average nailfold capillary density (capillaries/1mm^2) in control and preeclamptic participants after delivery. The difference between means was assessed using an unpaired two-tail t-test (P=0.0570). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number of capillaries per 1mm^2.

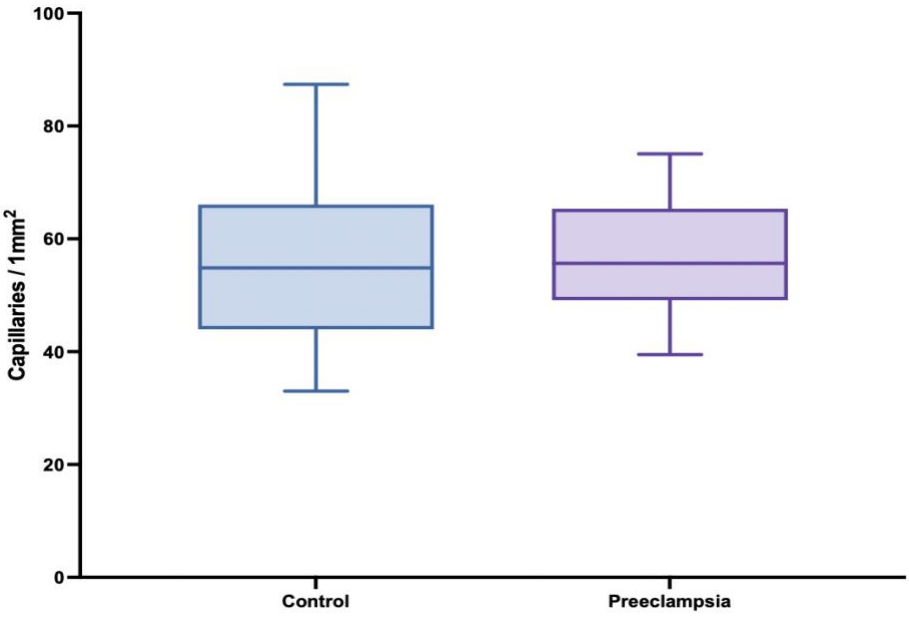

Figure 17: Controls and preeclamptics postpartum while inducing venous occlusion. The average nailfold capillary density (capillaries/1mm^2) in control and preeclamptic participants while inducing venous occlusion after delivery. The difference between means was assessed using an unpaired two tail t-test (P=0.9104). Whiskers represent minimum and maximum number of capillaries per 1mm^2. Line within the box represents the median number of capillaries per 1mm^2.

Chapter 4

Discussion

A pilot project was conducted to determine the pressure that would be used to induce venous occlusion to permit measurement of structural nailfold capillary density. Since inflating the pressure cuff on the participant's bicep to both 60mmHg and their individual diastolic pressures both significantly increased nailfold capillary density from baseline, it was decided that the participant's individual diastolic pressure would be used in the primary study. A pressure equal to or greater than individual diastolic pressure is required to induce venous occlusion and cause the temporary pooling of blood in the nailfold capillary bed. Considering that the population being studied has PE, it is likely that their diastolic pressure will be higher than 60mmHg and thus, 60mmHg may not adequately induce venous occlusion in the case group.

This study compared the microvascular structure of individuals with PE to healthy controls before and after delivery. Participants with PE had higher BPs prior to delivery, which is a defining symptom of PE (Cunningham & LaMarca, 2018; de Jager et al., 2017; Enkhmaa et al., 2016). Consistent with the literature, participants with PE delivered at early gestational ages and gave birth to lower weight neonates than the control group. PE is associated with prematurity (Amaral et al., 2017) and is a risk factor for and the most common cause of IUGR in pregnancy (Backes et al., 2011). However, none of the neonates born to preeclamptic participants were SGA, thus, it is likely that the smaller average weight of neonates born to the preeclamptic group was a result of being born at an earlier gestational age than the controls rather than growth restriction.

It is thought that PE impacts the microvasculature due to the systemic endothelial dysfunction associated with the disease (Rafii Tabrizi et al., 2021; Tomimatsu et al., 2019). It has

been proposed that the excess TNFα released in PE causes direct damage to endothelium-lined tubules, leading to the destruction of capillaries (Koller et al., 2020). Previously, reduced nailfold capillary densities have been identified in hypertensive pregnancy (Boardman et al., 2020; Hasan et al., 2002), CVD, and risk factors for CVD, such as obesity and diabetes (Agabiti-Rosei & Rizzoni, 2017; de Moraes & Tibirica, 2017; Jumar et al., 2016; Viljanen et al., 2019). Thus, the assessment of nailfold capillaries may provide insight into the impact of PE on the microvasculature prior to delivery and the risk of developing CVD postpartum.

The objective of the present study was to determine if there was a difference in the nailfold capillary density between controls and participants diagnosed with PE, and if this difference persisted postpartum. We hypothesized that there would be a decline in the number of nailfold capillaries per $1mm^2$ associated with PE prior to delivery and that would persist into the early postpartum period. Our data showed no difference in the average nailfold capillary density of controls and PE participants, either before or after delivery. This finding was consistent with and without inducing venous occlusion, which indicate the structural and functional densities, respectively.

Our findings are consistent with those of Houben et al., (2007), who did not find a difference in structural or functional nailfold capillary densities in the 3rd trimester. Antonios et al., (2013) similarly found no difference in functional nailfold capillary density prior to delivery. However, a decreased structural nailfold capillary density was found at 27-32 and 34-38-week's gestation in women who went on to develop PE compared to those that did not (Antonios et al., 2013). Additionally, Hasan et al., (2002) identified a lower structural and functional capillary density at the dorsum of the middle phalanx of individuals with PE when compared to individuals with an uncomplicated pregnancy at a matched gestational age. There have been conflicting findings when investigating nailfold capillary density postpartum as well. No difference between individuals with PE and controls was found at 3 months postpartum (Houben et al., 2007).

However, a decline in functional nailfold capillary density in individuals with a history of hypertensive pregnancy at 5-10 years postpartum has been recorded (Boardman et al., 2020).

Despite a non-significant difference between control and preeclamptic participants postpartum, the functional nailfold capillary densities of preeclamptic participants trended towards being greater than control participants. Based on previous literature, this finding was unexpected. It is possible that the greater functional nailfold capillary density of preeclamptic participants is a result of the medications taken by the preeclamptic participants. Our study did not exclude participants that were given oxytocin to induce labour or medications to treat the signs of PE, such as magnesium sulphate and antihypertensives. Although significantly more of the preeclamptic participants were taking labetalol, a beta-blocker, than controls, it is unlikely that this impacted results as treatment of hypertension with beta-blockers has not been found to effect nailfold capillary density (Penna et al., 2008). However, both oxytocin and magnesium sulphate result in systemic vasodilation in large and small arteries in pregnant individuals (Rabow et al., 2018; Tang et al., 2018), which leads to increased perfusion of capillaries (Ramanlal & Gupta, 2022). Therefore, it is possible that oxytocin and magnesium sulphate could have led to the increased functional nailfold capillary density compared to controls postpartum. This hypothesis is supported by previous studies that have ensured that participants were not taking oxytocin or magnesium sulphate and did see a difference in nailfold capillary density between groups (Antonios et al., 2013; Hasan et al., 2002). However, Houben et al., (2007) failed to exclude individuals taking oxytocin and/or magnesium sulphate and did not find a difference between groups, similar to the results of the primary study. Therefore, it is possible that not controlling for oxytocin and magnesium sulphate administration could have affected our results.

Given the range of findings in the literature, gestation age or time since delivery could impact nailfold capillary density. In uncomplicated pregnancies, nailfold capillary density increases, with the maximum density reached in mid-gestation, and declining thereafter, but does not return to pre-pregnancy levels until 6 weeks postpartum (Hasan et al., 2002). This might

explain why at 5-10 years postpartum the nailfold capillary density differed between those with and without a history of hypertensive pregnancy but did not within 48-hours as observed in the present study.

With the follow-up assessment occurring within 48-hours postpartum, differences in participant labour and delivery experiences could influence results. For instance, hemorrhagic shock has been associated with decreased functional capillary density (Govender et al., 2021). Blood loss due to delivery was not controlled for and could affect the nailfold capillary densities measured in the early postpartum period.

Our imaging approach differed from previous studies. Investigators that found reduced capillary density in individuals with PE took video of each nailfold for 5 minutes to detect intermittently perfused capillaries (Antonios et al., 2013; Hasan et al., 2002). Our study captured 5 images across a 30 second window. Houben et al., (2007) also did not record for 5 minutes and found similar results to our study. Prolonged video may be better for observing functional nailfold capillary density as it would give a more accurate average density by permitting intermittently perfused capillaries to be observed and counted.

The present study's approach to induce venous occlusion differed from others in the literature. Sixty millimeters of mercury has been used for all participants in other studies, regardless of individual diastolic blood pressure or PE diagnosis (Antonios et al., 2013; Antonios et al., 1999; Hasan et al., 2002; Houben et al., 2007). The current study used individual diastolic blood pressure. If venous occlusion is not adequately induced, it could result in fewer nailfold capillaries being perfused with red blood cells. Given that diastolic pressure was significantly higher in those with PE than controls, it is possible that venous occlusion was not completely induced in previous studies, thus leading to an appearance of declined structural nailfold capillary density. In future studies, using a consistent pressure to induce venous occlusion may reduce

potential confounding variables. However, it should be confirmed that the selected pressure is sufficient to induce venous occlusion in all participants.

The current study has strengths. Though nailfold capillary density has been studied in PE previously, this study is the first to investigate this parameter within 48-hours of delivery. This timeline is significant as PE symptoms resolve quickly following delivery (Amaral et al., 2017). As such, our study sought to learn about the microvasculature close to the time that symptoms commonly disappear. To calculate average nailfold capillary density a total of 25 images per hand were taken and counted. Taking an average from a large set of images may account for intermittently perfused capillaries. To control for the impact of other disorders, participants were excluded if they had any metabolic diseases, such as diabetes, or cardiovascular diseases prior to or during pregnancy. Doing so allowed us to connect our findings with PE and limits confounding variables.

There are also limitations to this study that could be improved in future work. This study is underpowered. Power calculations estimated a sample size of 172 participants divided into 86 control and 86 preeclamptic participants. Given the small sample size of the current study, conclusions based on the findings should not be made. The primary study's preeclamptic group was not further broken down into early and late-onset PE. While still inconclusive, it is thought that early-onset PE may be a result of placental abnormalities and late-onset a consequence of preexisting maternal conditions (Ogge et al., 2011). The higher concentration of AMDA, a NO inhibitor, in early-onset PE compared to late-onset could lead to more pronounced endothelial dysfunction in early-onset PE and thus, a more severe effect on nailfold capillary density (Alpoim et al., 2013). Groups were also not split into mild and severe PE for analysis. Including the full range of PE severity in the preeclamptic group may not accurately represent the effect of PE on nailfold capillary density. Previous studies have not assessed the difference in nailfold capillary density between mild and severe PE, however, alterations to microvascular function have been found to be significantly higher in individuals with a history of severe PE compared to

individuals with a history of mild PE (Barr et al., 2021). It is possible that the variance in the effect of PE on microvascular function depending on severity could be reflected in the microvascular structure as well. However, further investigation is required to determine if nailfold capillary density is impacted by disease severity. Increasing the sample size of both the control and preeclamptic groups and further categorizing the preeclamptic individuals into subgroups may further clarifying the near significant increase in nailfold capillary density seen in preeclamptics when compared to controls postpartum.

The primary study included women of ages 18-40. Age has been found to be negatively correlated with skin capillary density (Li et al., 2006). While the mean age of the control and preeclamptic groups did not significantly differ in the current study, it is possible that the natural decline in skin capillary density associated with age impacted the results.

To address the study limitations the following alterations to the methodology would have been ideal. Control and PE groups were not matched for gestational age at the time of the nailfold assessments. Given that there are changes in nailfold capillary density associated with uncomplicated pregnancies (Hasan et al., 2002), gestational-age matching could control for the normal impacts of pregnancy on the microvasculature. Including a study group of age-matched women who are not pregnant may also provide insight into the relationship between microvascular changes in pregnancy and PE. Increasing recruitment would allow for the division of the preeclamptic group into early and late-onset PE and further into subgroups of mild and severe PE. Creating additional subgroups would provide a more wholistic picture of how various classifications of PE impact nailfold capillary density. Finally, performing the nailfold assessment on PE participants prior to administering medications to induce labour or manage PE signs could better represent the microvasculature in the preeclamptic state.

The risk of developing CVD is significantly higher for women with a history of PE in pregnancy (Enkhmaa et al., 2016). Therefore, assessing microvascular structure, function and density and correlating with other markers of cardiovascular risk (CVR) when assessed in the first

year postpartum, is required to better understand the role microvasculature may play in the development of future CVD. Since reduced capillary density is associated with CVD and increased risk of CVD (de Moraes & Tibirica, 2017), nailfold video capillaroscopy could contribute to a postpartum risk assessments for future CVD. If a reduction in nailfold capillary density is observed, nailfold video capillaroscopy could be used as a simple clinically relevant tool to quickly identify who should undergo further CVR screening and to perhaps monitor therapeutic interventions.

A future study using nailfold video capillaroscopy to assess nailfold capillary density at 6 months postpartum may be beneficial. Assessing nailfold capillary density in individuals with a history of PE in their previous pregnancy and controls may provide further insight into lasting impacts of PE on nailfold capillary density. Breaking each group into two subgroups, one group having identified CVRs and the other without, would be beneficial in determining if reduced nailfold capillary density is associated with other CVRs observed after a pregnancy complicated by PE. It would be important to assess women within a small age range such as 25-30, or match for maternal age, to account for the natural decline of capillaries per mm^2 with age (Li et al., 2006). A similar nailfold capillaroscopy protocol to the current study could be used. Assessing all fingers on both hands of each participant would make each individual average nailfold capillary density more accurate and would account for potential differences between non-dominant and dominant hands. Known risk factors for CVD such as high blood pressure and high BMI could be measured at the follow up study visit. The participant's measured blood pressure and BMI along with the diagnosis of other CVRs, such as diabetes, would determine which subgroup the individual would be placed into. If reduced nailfold capillary density is determined to be associated with increased risk of CVD, nailfold video capillaroscopy could be used as a non-invasive postpartum assessment of an individual's CVR after a pregnancy complicated by PE.

There is support in the literature of an association between reduced skin capillary density and PE although our data around term do not support this. Contradictory findings in previous

studies regarding nailfold capillary density in PE along with our findings suggest that the relevance of nailfold capillary density to PE is inconclusive. However, given the consistent association between reduced capillary density and increased CVD risk, nailfold video capillaroscopy as a tool for CVD risk assessment postpartum should be further investigated. While it is possible that nailfold video capillaroscopy is not useful in determining CVD risk at term, it could be a more accurate risk assessment tool further into the postpartum period. In the future, nailfold video capillaroscopy may be helpful in identifying those that are at risk of developing CVD later in life. Due to the semi-quantitative nature of nailfold video capillaroscopy, it would be best used as a component of a CVD risk assessment that considers multiple factors. Being able to determine who is at risk of developing CVD after a pregnancy complicated by PE would allow for pharmaceutical interventions, such as low dose aspirin, and lifestyle modifications, including changes to diet and exercise. If intervention can be offered, those at risk of developing CVD later in life may be able to reduce their risk of and prevent their development of CVD.

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Appendix A

Ethics Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD (HSREB)

HSREB Full Board Amendment to Ethics Clearance

December 14, 2020

Dr. Graeme Neil Smith
Department of Obstetrics and Gynaecology
Queen's University

TRAQ #: 6028542
Department Code: OBGY-364-20
Study Title: "OBGY-364-20 Intrapartum vascular assessment of women with pre-eclampsia"
Review Type: Full Board
Date of Full Board Meeting: December 7, 2020
Date Ethics Clearance Issued: December 14, 2020

Dear Dr. Smith:

The Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the amendment event form and is granting ethics clearance for the changes listed below:

Document Name	Comments	Version Date
Consent Form	OB-INT Consent 9DEC2020	2020/12/09
Questionnaire	Data Collection Form	2020/11/18

Documents Acknowledged:
- Other Document: Summary of Changes

Regards,

Albert F. Clark.

Albert F. Clark, PhD
Chair, Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, he requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the international Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Product Regulations; Part 3 of the Medical Devices Regulations, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federalwide Assurance Number: FWA#: