Pregnancy-specific reference intervals for infection markers: unblurring the limits of normality

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Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It was composed by myself and has not been submitted in any previous application for any degree. The work presented (including data generated and data analysis) was carried out by myself in collaboration with the authors listed below. Parts of this thesis have been published:

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Dockree S, Brook J, Shine B, James T, Vatish M. Pregnancy-specific reference intervals for BNP and NT-pro BNP – changes in natriuretic peptides related to pregnancy. *J Endocr Soc* 2021;5:bvav091.

Dockree S, Shine B, Impey L, Mackillop L, Randeva H, Vatish M. Improving diagnostic accuracy in pregnancy with individualised, gestational age-specific reference intervals. *Clin Chim Acta* 2022;527:56-60.

Dockree S, O'Sullivan J, Shine B, James T, Vatish M. How should we interpret lactate in labour? A reference study. *BJOG* 2022 Available at: `https://doi.org/10.1111/1471-0528.17264`

Dockree S, Shine B, Pavord S, Impey L, Vatish M. White blood cells in pregnancy: reference intervals for before and after delivery. *EBioMedicine* 2021;74:103715.

Dockree S, Knight M, Kennedy S, Vatish M. The role of procalcitonin in the diagnosis of histologically confirmed chorioamnionitis: a systematic review. *BJOG* 2019;126(S2):160-1.

Dockree S, Brook J, Shine B, James T, Vatish M. Is there a role for C-reactive protein during and after labour? *Ann Clin Biochem* 2021;58:671-2.

My contributions to each paper are stated in the Bibliography, and all co-authors have signed this document to confirm their agreement.

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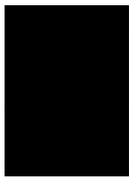

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Abstract

Background

Maternal sepsis is a major global cause of maternal death, and bacterial infections in pregnancy are responsible for a huge proportion of maternal and neonatal adverse outcomes. A lot of time and resources are dedicated to investigating suspected infection in pregnancy and around the time of delivery, and blood tests are ubiquitously used in UK obstetric practice to objectively investigate the likelihood and severity of disease. However, it is poorly understood how infection/inflammatory markers behave in pregnancy, and how (or whether) they should be interpreted to inform clinical decisions.

Methods

Over the course of three years, I have authored a series of peer-reviewed articles, letters and conference abstracts, as part of an active working group. We have defined 95% reference intervals (RIs) for the major infection markers used in current clinical practice: C-reactive protein (CRP), white blood cells (WBC) and its subtypes, procalcitonin (PCT) and lactate. In several cases we have evaluated these new reference limits in other cohorts to investigate their added value for improving diagnostic accuracy in pregnancy.

Results

The 95% RI for CRP is substantially and consistently elevated from the first trimester of pregnancy, and using this upper reference limit (19 mg/L) rather than that which is currently recommended (7 mg/L) confers a significant improvement in the overall diagnostic accuracy for chorioamnionitis. WBC are also elevated from early in pregnancy (5.7-15.0 x10^9/L), with subtype-specific changes in the differential counts. Both CRP and WBC are so dramatically and unpredictably elevated in labour and the postnatal period that they cannot reliably be interpreted to investigate infection and, while lactate in pregnancy is similar to non-pregnant women (<2 mmol/L), a much higher limit should be used to investigate the severity of sepsis in labour (4 mmol/L). In contrast, levels of PCT are stable throughout pregnancy, and the existing upper limit (0.25 ng/mL) is valid throughout labour and the puerperium.

Discussion

These publications constitute a substantial body of novel evidence in the field of obstetrics. Some of this may be applied directly to influence policy and clinical practice, while other findings may form the basis of important future research. By drawing reference to these publications and associated works, I present this thesis as an investigation of how we must re-evaluate our clinical practices to improve diagnostic accuracy and prevent morbidity and mortality from infections in pregnancy.

Abbreviations

BMI	Body mass index
BNP	B-type natriuretic peptide
COVID-19	Coronavirus infectious disease 2019
CRP	C-reactive protein
GLOSS	Global Maternal Sepsis Study Research Group
GP	General practitioner
ICD	International Classification of Diseases
IFCC	International Federation of Clinical Chemistry
IL-6	Interleukin-6
IQR	Interquartile range
NCCLS	National Committee for Clinical Laboratory Standards
NT-pro BNP	N-terminal pro-B-type natriuretic peptide
PCT	Procalcitonin
PPROM	Premature preterm rupture of membranes
RCOG	Royal College of Obstetricians and Gynaecologists
RI	Reference interval
SIRS	Systemic inflammatory response syndrome
TNF-α	Tumour necrosis factor alpha
UK	United Kingdom
WBC	White blood cells
WHO	World Health Organisation

Introduction

Every year around 300,000 women die during pregnancy or shortly after childbirth.[1] More than one in ten maternal deaths are caused by sepsis, constituting the third leading cause of maternal mortality worldwide, and a substantial proportion of all other maternal deaths are associated with infections and their complications. It is increasingly clear that the burden of sepsis has been underappreciated; the WHO Global Maternal Sepsis Study Research Group (GLOSS) recently reported that more than half of all inpatient maternal deaths were associated with some degree of infection.[2] Importantly, this was published before the emergence of the novel Coronavirus disease (COVID-19), which confers a disproportionate burden on maternal death and serious morbidity,[a] with or without associated secondary bacterial infections.[3] Most maternal deaths are preventable with appropriate recognition and treatment (particularly those from sepsis) and, although there has been a significant reduction in the global maternal mortality ratio in recent years, the rate of change in most parts of the world is slower than anticipated.[4] There are myriad initiatives to address this at regional, national, and international levels, and sequential reports have described complex and persisting themes, which clearly require a multifaceted approach to make a meaningful change within our lifetimes.[5–7]

Delivering the optimal treatment for sepsis (including the timely and appropriate use of antibiotics) relies on the ability to make an accurate diagnosis, often using clinical tests to inform clinical decisions. Failing to recognise pregnant women as high-risk individuals with unique physiological profiles risks the lives of mothers and their babies, but it is unclear

[a] Covid-19 is also associated with adverse perinatal outcomes, often driven by prematurity, complications during pregnancy and delivery, and childhood infections, although this is beyond the scope of this project.

how this may be achieved in the absence of robust evidence-based guidance.

The role of infection markers in pregnancy

The old adage goes that clinicians must "treat the patient, not the numbers". Most will agree that pertinent information should be gathered from a range of sources; useful information may be obtained from blood tests, clinical imaging, and medical technology, although the results rarely stand alone and should be interpreted in the context of the whole clinical picture. Clearly, the importance of accurately diagnosing and treating infection in pregnancy is not only to prevent death; pregnancy poses many diagnostically challenging situations, and weighty decisions (carrying potentially severe consequences) must often be made with little evidence available. Therefore, it is reasonable to consider more objective measures to help with the difficult questions, such as:

A woman is tachycardic in labour with raised white bloods cells of 22 $\times 10^9$/L, does she need antibiotics?

C-reactive protein is elevated at 14 mg/L after prolonged preterm rupture of membranes at 22 weeks' gestation, is it safe to continue pregnancy?

How do you investigate suspected infection one hour, one day, or one week after delivery?[b]

Although these are common situations in real-world clinical practice, there have been very few large studies investigating the application and accuracy of infection markers in pregnancy. Until recently, there were no established pregnancy-specific reference intervals (RI), and results were often interpreted by extrapolating from those derived from non-pregnant populations. In part, this may be because there is no consensus on how

[b]This question is often managed by junior clinical staff in the first instance, and it is asked equally in labour wards and non-obstetric settings, such as GP surgeries and emergency departments.

pregnancy-specific reference studies should be performed. I will briefly outline the concept of defining RIs, including essential considerations for how the methods should be adapted for use in pregnancy, which I will then apply and evaluate in this thesis.

Reference intervals: adapting the method

An RI defines the distribution of values expected in a healthy population. Variation is driven by underlying physiological, genetic and/or environmental factors, and the extent of this variation must be understood to interpret clinical investigations. A seminal series of papers from the International Federation of Clinical Chemistry (IFCC) and the National Committee for Clinical Laboratory Standard (NCCLS) outline the rationale and recommended methods for defining RIs.[8–14] While there have been substantial technological, statistical, and methodological developments since their publication (which have been well summarised in recent reviews),[15–17] these recommendations continue to underpin most reference studies.

Population selection

Reference individuals are selected based on a definition of relative health. This definition is subjective so pragmatic, relevant selection criteria should be chosen for the test in question. Individuals may be selected prospectively (*a priori*), using selection criteria informed by previous studies in similar populations. While this approach is convenient, it may not be possible for novel tests about which little is known, and so often there is little evidence available for tests used in pregnancy. Alternatively, a retrospective approach may be used (*a posteriori*), particularly for novel tests about which the physiological profiles are incompletely understood. These principles are broadly relevant in pregnancy-specific reference studies, but I propose that some important adjustments and additions are required.

At first glance, one may assume that pregnant women are more likely to be healthy than the non-pregnant population. Fewer than one fifth of

pregnant women in the UK are obese,[18] and the vast majority have their first baby before 40 years of age,[19] so there should be a relatively low risk of serious disease in most women of childbearing age. However, partly due to the uptake of assisted reproductive therapies and advancing maternal age, it is increasingly common for pregnancies to be complicated by pre-existing diseases and/or new pregnancy complications. In some of the studies included in this thesis (those which used prospectively collected data and well-documented participant characteristics) there was enough information to refine the population to include only healthy women with good perinatal outcomes, and to exclude those with incident diseases. Gathering such detailed information is sometimes unfeasible, for example when using retrospective data from tens of thousands of women. As I will demonstrate, indirect approaches may be cautiously employed to address this, such as using proxy markers and routinely collected clinical codes. Overall, given the dynamic nature of pregnancy, it is advantageous to employ aspects of *a priori* and *a posteriori* sampling to obtain an objectively healthy population.

It is widely accepted that reference populations should consist of at least 120 participants, to allow reference limits to be calculated precisely.[14,20] Furthermore, if it is hypothesised that RIs may differ according to certain characteristics (e.g., sex or age), populations may be selected to be representative of clinically relevant subgroups of at least 120 participants, in case partitioned RIs are indicated.

There are two further major considerations in pregnancy:

i) Pregnancy is a dynamic physiological state with marked changes in all major body systems throughout gestation.[21] It is often pragmatic to categorise gestational age into trimesters or smaller intervals of days or weeks, in which case serial measurements are valuable.[c] Further

[c]When investigating shorter time intervals, the desired population size can quickly escalate beyond what is feasible in most prospective studies, and the role of retrospective "big" data becomes important.

sub-classifications may include antenatal, intrapartum and postnatal measurements, as there are often important physiological changes between these periods.

ii) Pregnancy is associated with a marked, progressive expansion in the circulating plasma volume, and the degree of this increase is approximately proportional to maternal mass. Therefore, on average, circulating markers may be subject to a greater degree of haemodilution with advancing gestational age, and this may be more marked in women with a higher body mass index (BMI).[d] BMI is not a key consideration in most non-pregnant reference studies, and some major pregnancy-specific studies have even excluded obese women altogether, which potentially limits their generalisability.[22] Wherever it was possible, we have investigated pregnancy-specific RIs in the context of maternal BMI, and I propose that it should be considered as an essential selection and/or partitioning criterion.

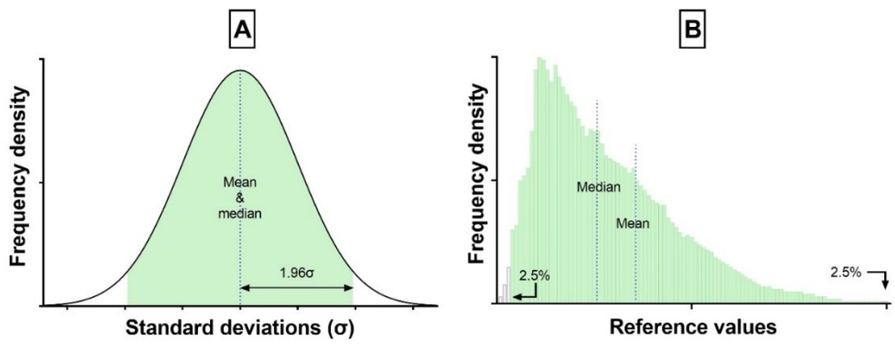
Distributions and outliers

The parametric 95% RI is estimated as the mean ± 1.96 standard deviations (although any central RI may be obtained by making appropriate adjustments). Alternatively, the limits of the corresponding non-parametric 95% RI are defined as the 2.5^{th} and 97.5^{th} centiles for ordered reference values (see Figure 1).

Outliers are inevitable, despite rigorous participant selection. Studies should include all reference values unless there is compelling evidence of a small number of extreme outliers, in which case they should be identified and excluded using a justifiable method.[12,23] Several options are available (e.g., those described by Dixon and Horn),[24–26] which are summarised elsewhere.[27] Reference distributions may change with advancing gestation (for example, starting as non-Gaussian in the first trimester but demonstrating a normal

[d] And other factors, like multiple pregnancy.

Figure 1: Reference distributions. Example data demonstrating central 95% reference intervals using parametric (A) and non-parametric (B) methods.



distribution by the end of pregnancy).[28] Therefore, for the investigation of outliers and the definition of reference limits, one must select a single distribution-appropriate method.

Aims of this thesis

In this thesis I will pose and address the following questions:

i) How should the conventional methods for defining RIs be adapted to be made specific to pregnancy?

ii) What are the RIs for key clinical tests for investigating suspected infection in pregnancy, and which maternofetal characteristics affect their limits?

iii) Are RIs the same at different stages of pregnancy (before, during, and after labour)?

iv) Do pregnancy-specific RIs improve diagnostic accuracy over the non-pregnant standards?

A note on form, structure, and terminology

This document is a brief accompaniment to a series of published, peer-reviewed articles, which is intended to summarise key findings and present them in context. A number of similar terms and definitions are used to

illustrate key concepts, which are clarified below. Further definitions are provided in the relevant manuscripts.

The term "reference interval" is preferable to "normal range", as *a)* reference data are not necessarily normally distributed, *b)* "normal" results do not always denote a completely healthy state, and *c)* the interval is not usually limited by the arithmetic range (minimum-maximum values), but another portion of the distribution. RIs are bound by upper and/or lower reference limits (also known as cut-offs, thresholds, or bounds).

I have used the term "marker" (e.g., infection marker) to refer to laboratory-based tests quantifying the circulating concentration of a substance in maternal blood. The results are usually interpreted as high or low (or cautiously as normal or abnormal) by drawing reference to an RI. I acknowledge that there are similarities and nuanced differences between "diagnostic" and "predictive" tests, and similar phrases are used throughout this project to describe objective investigative tools for identifying individuals with (or with the increased potential of developing) disease.

"Infection marker" is used to describe laboratory tests that are measured to investigate suspected infection. While they are often more accurately termed "inflammatory markers" due to their non-specific nature, they are commonly used for infection and will be referred to in this context. Infection may be assumed to be bacterial, unless otherwise stated.

Considering gestational age

Study 1: Pregnancy-specific reference intervals for BNP and NT-pro BNP - changes in natriuretic peptides related to pregnancy.

Study 2: Improving diagnostic accuracy in pregnancy with individualised, gestational age-specific reference intervals.

An essential prerequisite for defining RIs in pregnancy is a robust investigation of gestational age as an influential factor. Therefore, before investigating infection markers, two papers are presented to clearly highlight the clinical importance of gestational age in other domains (haematology and cardiology).

In Study 1 we defined RIs for B-natriuretic peptide (BNP) and its inactive fragment (NT-pro BNP), using our systematic method based on the aforementioned principles.[29] A key finding was that the RI for NT-pro BNP should be considered in the context of gestational age (e.g., trimester-specific limits). As well as considering the effect of advancing gestational age, the investigation of these temporal changes unveiled interesting underlying physiological processes, including the demonstration of haemodilution and cardiac strain as competing physiological mechanisms, which we demonstrated using proxy markers.

In Study 2 we modelled the trajectory of maternal platelets in pregnancy, which showed that interpreting results in the context of very precise measurements of gestational age conveys a significant benefit over using fixed limits for the prediction of gestational thrombocytopaenia.[30] As the RI for platelets has already been defined and the limits are well established in clinical practice, this was intended predominantly as an early methodological paper, and (for the purposes of this thesis) to introduce regression-based models for defining pregnancy-specific RIs. I will now demonstrate how we used these principles to define pregnancy-specific RIs for four of

the most commonly used diagnostic markers for infection, CRP, WBC, lactate, and PCT.

Summary of outcomes	**Impact and contribution to the field**
The definition of novel, trimester-specific RIs for natriuretic peptides in pregnancy	These RIs have recently been used in a prospective randomised trial in obstetric anaesthesia, which acted to validate our RIs in a separate healthy cohort.[31] They have also been used and investigated in a high-impact study on NT-pro BNP and hypertension. While the clinical impact of these limits has yet to be tested (over a single, fixed limit), NT-pro BNP levels below our first trimester limit are significantly associated with subsequently developing chronic hypertension.[32] Our findings have recently been incorporated into clinical guidelines from the American College of Cardiology.[33]
A multifaceted demonstration of the impact of gestational age and BMI on NT-pro BNP	Study 1 highlights gestational age (and other factors affecting circulating biomarkers) as clinically relevant factors, warranting further investigation in Study 2.
The definition of gestational age-specific RIs for platelets in pregnancy	This is the largest known study to have investigated platelets in pregnancy. We demonstrated the novel finding that gestational age-adjusted RIs can confer significant improvements in predictive accuracy over fixed limits. Unlike closely related subspecialties (e.g., fetal medicine), individualised, centile-based investigations are not currently used in maternal medicine, and this could represent an important paradigm shift in the way we approach novel biomarkers.

C-reactive protein

Study 3: Pregnancy-specific reference intervals for C-reactive protein improve diagnostic accuracy for infection: a longitudinal study.

Letter 1: Is there a role for C-reactive protein during and after labour?

CRP is probably the most widely used blood test for investigating suspected infection and inflammation. Until recently there was no established RI for CRP in pregnant women, although it is widely reported that levels are elevated from early pregnancy,[34] and the most promising previous studies investigating the diagnostic accuracy of CRP for pregnancy-related infections have used much higher cut-offs (>18.7 mg/L) than would usually be employed in non-pregnant populations (>7 mg/L).[35,36] It is also widely reported that CRP, IL-6, and other pro-inflammatory markers (TNF-α) are elevated in association with a raised BMI in non-pregnant adults.[37] This association is more marked in women than men, which is presumably driven by the relatively high proportion of active secretory pro-inflammatory adipose tissue in women.[38]

It is generally accepted that using CRP to investigate suspected (localised) infection is more reliable than using clinical features alone; an excellent review of women with histological chorioamnionitis demonstrated low sensitivities for maternal pyrexia (median 42%), maternal tachycardia (38%), and fetal tachycardia (36%). While the specificities were higher (86%, 85%, and 90%, respectively), the median sensitivity and specificities for CRP were 72% and 79% when using even relatively low reference limits.[36]

In Study 3, we investigated trimester-specific levels of CRP in 322 healthy pregnant women with good perinatal outcomes. As expected, CRP was substantially elevated in pregnancy (95% RI 1-19 mg/L), but there were no significant differences in either the mean CRP values or the upper reference limits in any trimester. To evaluate this, we identified a second co-

hort of women undergoing amniocentesis for suspected intrauterine infection, for whom the outcomes were known and cases could be discerned accurately. The overall diagnostic accuracy was significantly greater when using the pregnancy-specific upper reference limit than with the lower limit currently recommended by the RCOG.[39]

Before this study, we initially considered two opposing hypotheses with regards to BMI:

i) Compared with pregnant women with a low-normal BMI (<25 kg/m^2), women who were overweight-obese (≥25 kg/m^2) have a higher average CRP, because of the higher proportion of pro-inflammatory adipose tissue

ii) Women with a higher BMI will have a relatively greater degree of plasma expansion, thus greater haemodilution, so overweight-obese women will have a lower CRP than those with a low-normal BMI.

We reported that the former mechanism *(i)* was predominant; CRP was higher in overweight-obese women, as in non-pregnant adults, demonstrating a highly significant log-linear association in all trimesters. Interestingly, the BMI-specific CRP upper reference limits demonstrated at least marginal significance in all trimesters but, as partitioning was only clearly justified in the second trimester (14 *vs.* 28 mg/L), the overall recommendation is to use a unified cut-off of 19 mg/L regardless of gestational age and BMI. Rather than dividing groups by 25 kg/m^2, it is possible that 30 kg/m^2 may reveal significant group differences for which partitioning may be justified, and this cut-off is more commonly used as a clinically relevant threshold in other areas of clinical practice.[18] However, our study was not powered to investigate this, and it may be an interesting focus for future research, with the potential to optimise diagnostic accuracy even further.

Shortly after we defined and published the antenatal pregnancy-specific RI, a small study by Joyce, *et al.* reported a similar distribution of CRP

in term pregnancy before labour or delivery (<35 mg/L, with 86% of results ≤20 mg/L).[40e] Interestingly, this study also investigated CRP values in the first few days after delivery, sub-classified by the mode of birth. Overall, there was a huge range in CRP on the first postnatal day for vaginal deliveries and Caesarean sections in healthy women without evidence of infection (up to 167 and 152 mg/L, respectively), which persisted for at least the next two days. This prompted us to undertake a new investigation, and to publish Letter 1.[41] We selected a cohort of pregnant women who had been investigated for suspected infection in labour (those having CRP measured on the labour ward). This was then restricted to 95 adults with negative urine, genital, and blood cultures, whom we defined as those having no evidence of intrapartum infection.[f] CRP in the peripartum period varied widely (median 27 mg/L, IQR 11-47), and in more than half the CRP was elevated above the antenatal RI. This is broadly consistent with the findings reported by Joyce, *et al.*, and our study had the added benefit of using objective microbiological evidence to support the population selection.

Conclusions on CRP

In these studies we present objective evidence that using a pregnancy-specific RI for CRP significantly improves its diagnostic accuracy for infections before the onset of labour. Our higher pregnancy-specific upper reference limit has a significantly greater overall diagnostic accuracy, driven by a substantially better specificity, without materially compromising sensitivity. The benefits of this are likely to include less unnecessary intervention, including iatrogenic delivery, and better antibiotic stewardship.

[e]Likely reasons for the difference in the upper limit between studies are suggested in Letter 1 (CRP/PCT), and only some of these points were partly addressed in a response to the Editor.

[f]Clearly there remains a high risk of selection bias based on these selection criteria but, while it is difficult to define controls who are definitively not infected, this group of women with suspected infection is exactly the population for whom infection markers are usually measured.

However, after the onset of uterine contractions or Caesarean delivery, CRP is frequently elevated even further. We concluded that the range of CRP values in the intra- and postpartum periods is so wide that it cannot feasibly be interpreted for the purposes of investigating infection, and that it may have limited added value over clinical signs (or a superior infection marker). Furthermore, attempting to do so is unreliable, and the consequences may be harmful. I have not evaluated the value of using sequential/repeated measures to investigate trends in CRP (or any other marker) as it is beyond the scope of this project, but this would be an interesting focus for future studies.

Summary of outcomes	**Impact and contribution to the field**
The definition of a pregnancy-specific RI for CRP Evaluation of this RI in a second cohort	The RI has been adopted in clinical practice at the local and regional levels. It is recommended as part of the assessment of women with suspected chorioamnionitis, particularly for making decisions about tertiary referrals at peri-viable gestations.

Procalcitonin

Abstract 1: The role of procalcitonin in the diagnosis of histologically confirmed chorioamnionitis: a systematic review.

Study 4: A pregnancy-specific reference interval for procalcitonin.

Procalcitonin (PCT) is a highly specific marker for bacterial infection. It is used in several medical specialties to help discern between bacterial and viral infections or other causes of systemic inflammation, including the differentiation between uncomplicated COVID-19 viral infection and complicated bacterial co-infection.[42,43] Several randomised controlled trials have reported that PCT-guided pathways reduce mortality by supporting clinicians in the prompt diagnosis and management of bacterial infections and sepsis.[44,45] Furthermore, given its short induction time and rapid response to the change in clinical status, it can be used to monitor disease progression or resolution, and to guide when to commence or discontinue antibiotics (in non-pregnant populations). However, there is only one study in the known published literature (an abstract of 52 women with pyelonephritis) which has investigated using PCT for this purpose in pregnancy.[46g] While these pathways are well-established in respiratory, adult and neonatal intensive care, and respiratory units worldwide,[47,48] PCT has rarely been investigated in pregnancy. Until recently its behaviour in pregnancy was not well-defined, and consequently its use is not established in any known clinical guidelines.

In 2019 we published a succinct systematic review of studies investigating PCT in pregnancy, in which we found that the very few published studies mostly focused on the detection of chorioamnionitis after PPROM.[49] The

[g]The findings from this small study were positive; using a limit of 0.05 ng/mL to exclude infection and stop antibiotics early was not associated with adverse outcomes.

accuracy with which PCT identified histological chorioamnionitis was variably reported (sensitivity 14-92% and specificity 45-95%), which is likely driven by the absence of an established reference limit (range 0.05-1.9 ng/mL). Importantly, there was little available information on the levels expected in healthy pregnancy, or whether these changed with gestational age, childbirth, or surgical intervention. One notable study reported that a limit of 0.125 ng/mL was optimal for diagnosing pregnancy-associated sepsis with moderate sensitivity (68%) and good specificity (83%),[50] and a subsequent study from this group recommended using serial PCT measurements to assess the disease trajectory.[51] We concluded that there was an unmet need for an established RI for PCT, as an essential prerequisite to undertaking a diagnostic accuracy study of its use in pregnancy. To address this, we defined the pregnancy-specific RI for PCT in Study 4, using the same samples and similar methods used to investigate CRP. Unlike CRP (which increased in pregnancy, particularly in overweight women), the RI for PCT was unchanged in pregnant women at any stage of pregnancy (0.01-0.05 ng/mL) and was not associated with BMI or any other observed characteristic.

While levels of PCT are very low in healthy pregnant and non-pregnant adults alike, a higher reference limit is usually used to discriminate those with significant bacterial infection for whom antibiotics should be considered (>0.25 ng/mL).[52] Ours was not a diagnostic accuracy study, and we had not investigated its accuracy when using a higher limit, but we hypothesised that if PCT is unchanged in pregnancy then it may reasonable to use the same limits as for the non-pregnant population. To date I have found no evidence to the contrary, but this should be proved definitively before it can be implemented safely.

After having published Study 4, it remained uncertain to what extent PCT is affected by labour and delivery. In the aforementioned study of term pregnancies by Joyce, *et al.*, PCT was also measured on the first and third days after delivery in women without infection.[40] As with CRP, PCT is somewhat elevated in association with both vaginal and Caesarean de-

livery (with a statistically but not clinically significant further elevation in the latter), but the vast majority of results were below 0.25 ng/mL. Again, we confirmed this with our own findings in Letter 1: in 95 women with a full complement of negative cultures with 24 hours after delivery, 94% of PCT values were "normal" (IQR 0.05-0.1 ng/mL).

Conclusions on PCT

The RI for PCT is the same in pregnant and non-pregnant women. Given that there does not appear to be a physiological basis to justify using it differently in pregnancy (and considering the large body of evidence from other specialties), it seems prudent to use the existing limit (<0.25 ng/mL) for diagnosing bacterial infection and improving antibiotic stewardship, and previous studies have reported excellent sensitivities and specificities using this limit to detect various sources of sepsis in pregnant women.[53–55] We have demonstrated that most values in healthy pregnant women fall within the RI at all stages of pregnancy and the puerperium, regardless of the mode of delivery, which would constitute a significant improvement on CRP.

Summary of outcomes	**Impact and contribution to the field**
The definition of a pregnancy-specific RI for PCT, and an evaluation of generalisability for use before, during and after labour	The RI for PCT (and our associated recommendations) have been applied in high-impact prospective studies on the management of intrapartum sepsis.
An evaluation of PCT as a test for infection	We will apply our findings imminently in a large prospective study in the UK, in which we intend to implement the first guidelines for PCT-guided antibiotic therapy in pregnancy.

White blood cells

Study 5: White blood cells in pregnancy: reference intervals for before and after delivery.

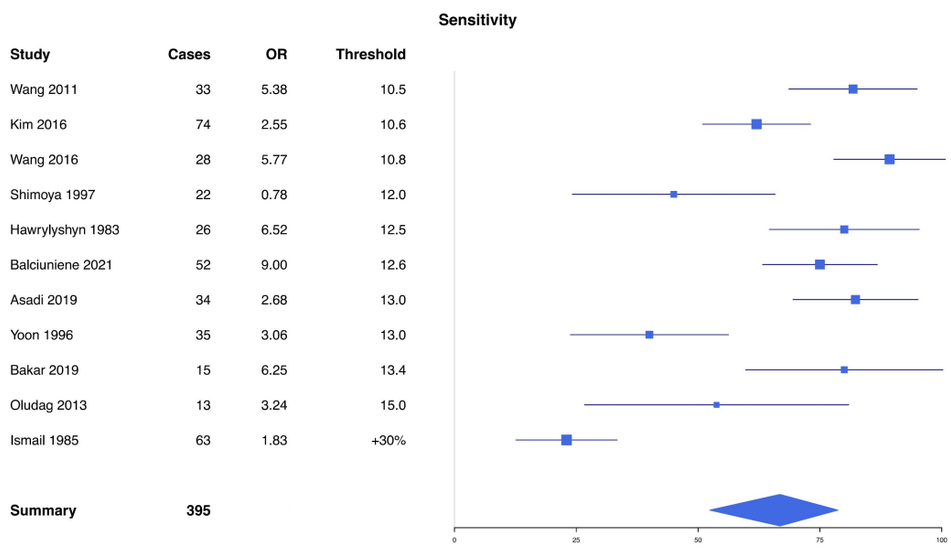
Alongside CRP, the WBC count is the other major infection marker used in all areas of modern clinical practice, including obstetrics. The RI for total WBC in non-pregnant adults is consistently reported between around 4-11 x10^9/L,[56] with well-described RIs for each of the constituent cell subtypes (neutrophils, lymphocytes, eosinophils, basophils, and monocytes). It is widely understood that WBC rise in pregnancy, but the extent of this is unclear, and the RCOG still advocate using only a slightly higher cut-off of 12 x10^9/L when investigating suspected infection in or after pregnancy.[39,57] As with CRP, the leading hypothesis is that using a (physiologically justified) higher upper reference might limit adverse consequences for women without infection.

Several studies have investigated the accuracy with which WBC can be used to investigate infection in pregnancy, most commonly after PPROM.[58–61] Furthermore, a few studies have investigated individual cell subtypes, including neutrophils and the neutrophil/lymphocyte ratio.[62,63] Importantly, there is no consensus on which threshold/s should be used. These studies are summarised in Figures 2 and 3, demonstrating the unsurprising finding that sensitivity increases with a lower reference limit, and specificity increases with a higher limit. Based on a yet unpublished meta-analysis, for the diagnosis of chorioamnionitis after PPROM, WBC had a modest pooled sensitivity and specificity of 67% and 63%, respectively, although the reference limits used varied widely.[h] Clearly, the RI for WBC should

[h] Submitted and awaiting review (shared with permission). Hyde E, Dockree S, Saker J, Vatish M. The diagnostic accuracy of white cell count in women with prolonged preterm rupture of membranes: a systematic review and meta-analysis. 2021 (submitted).

be defined, to enable further studies to choose reference limits based on a sound physiological basis.

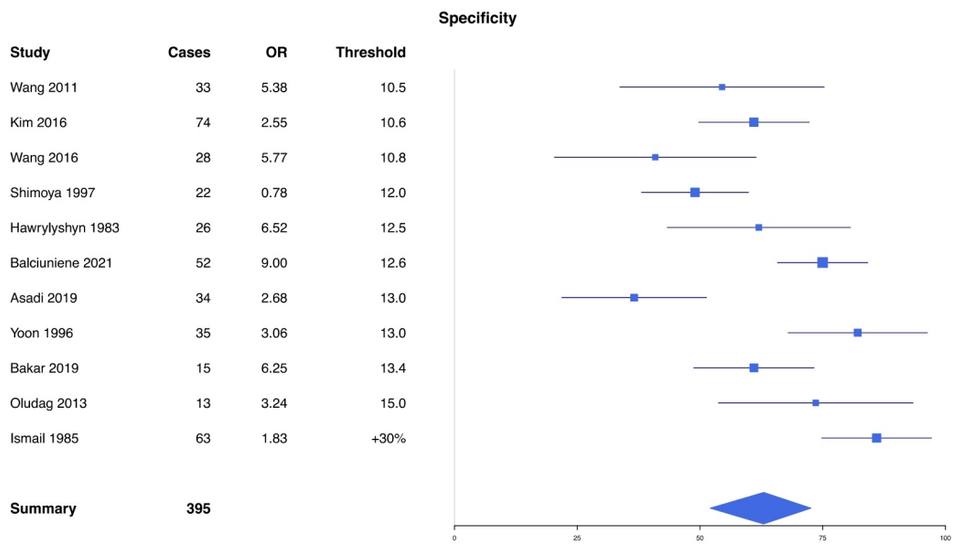
Figure 2: Sensitivity of total white blood cells for chorioamnionitis after prolonged preterm rupture of membranes.



Study 5 was, by far, the largest of those included in this project. In a retrospective population study of 24,318 women with good perinatal outcomes, we modelled the RIs for all WBC subtypes between 8-40 weeks of gestation, based on 80,637 measurements over five years, and in the first three weeks after delivery using a subset of 14,320 results from 9,271 women. Using such a large population enabled a detailed investigation into the nuanced changes between each gestational week, which has never been reported before. As expected, total WBC are elevated throughout pregnancy (5.7-15.0 x10^9/L), which is driven by a marked neutrophilia (3.7-11.6 x10^9/L). Interestingly, there were nuanced changes in cell subtypes throughout pregnancy, but overall, the reference limits stayed approximately stable throughout gestation.

RCOG guidelines advise that a "transient leucocytosis" (of unspecified magnitude and duration) can occur in labour, and it can be assumed that changes in WBC start on the day of delivery as a direct result of labour

Figure 3: Specificity of total white blood cells for chorioamnionitis after prolonged preterm rupture of membranes.



and/or surgical intervention.[57] In Study 5 (which relied on indirect measures, as with so many "big data" studies), we excluded blood results collected within 24 hours prior to delivery, as it is possible that these results contributed to the timing or mode of delivery itself. Therefore, WBC from the perinatal period start from the first postnatal day, and the trajectory can be inferred from the previous 24 hours. We reported that WBC are indeed elevated from the first day after delivery and that this shows a very slow resolution, only falling to (antenatal) pregnancy levels by around one week. This is strongly affected by the mode of birth, but overall average WBC values did not fall to pre-pregnancy levels until approximately three weeks later.

Conclusions on WBC

There are reasonable physiological grounds to justify using a relatively high reference limit for WBC from early pregnancy to term, where the main benefit is likely to be optimising specificity. To date, only one study is known to have investigated the diagnostic accuracy of WBC for in-

trauterine infection with a cut-off as high as 15 x10^9/L, and even this showed only a moderate specificity (74%) and a poor sensitivity (54%).[58] There must be further investigation into the extent with which the use of the new pregnancy-specific RI affects overall diagnostic accuracy (i.e., maintaining its ability to identify disease), to ascertain whether it can materially optimise the utility of WBC in the antenatal period, or whether it would be more beneficial to look at other markers.

As I have described with CRP, the range in WBC values is so broad in the first week of the puerperium that it is probably not feasible to define an RI, or to interpret meaningful results about the likelihood of infection. After this point, an appropriate RI may be used depending on the timing of sampling, which will also require further evaluation.

Summary of outcomes	**Impact and contribution to the field**
The definition of pregnancy-specific RIs for each WBC subtype An investigation of WBC changes in the postnatal period to assess generalisability and feasibility	The new RIs are currently being evaluated in practice, to investigate their added diagnostic value of the existing non-pregnant standards (as for CRP). It is now recommended that lone WBC measurements are not reliable for investigating suspected infection in labour and the puerperium, which has materially changes local guidelines. The new RIs for CRP and WBC will both be assessed in the pending study of PCT, for a direct comparison of their value.

Lactate

Study 6: How should we interpret lactate in labour?

Lactate is well-established as a fundamental part of the management of sepsis, and it is enshrined in international guidance and global campaigns.[64,65] Hyperlactataemia (>2 mmol/L) may indicate tissue hypoperfusion secondary to sepsis in non-pregnant adults, and high (or rising) levels are closely associated with adverse outcomes, including mortality.[66] However, as we explored in Study 6, lactate increases in association with several states of relative hypoxaemia, including labour, which introduces diagnostic uncertainty around its use in pregnancy around the time of delivery.

A recent meta-analysis reported that lactate levels in healthy pregnant women are similar to those in the non-pregnant population, and thus may be used similarly to investigate disease severity in women with sepsis.[67] However, this study concluded that "venous lactic acid levels can be used as a screening tool in pregnant women just as the test would be used in non-pregnant women, except that elevations may be seen during labo[u]r, especially later in labo[u]r when there is maximal skeletal muscle contraction". This is a curious statement for several reasons. Firstly, lactate is conventionally used as a marker of severity rather than for discriminating between those with and without infection (neither of which constitute a screening tool). Secondly, without a suitably large individual patient data study, it is important to clearly define an upper limit for labour, which this study did not report. Furthermore, it is unclear to what extent different factors affect lactate levels in pregnant women in the absence of infection, and how/whether it can be interpreted in the same way as other markers.

In Study 6 we collected lactate data from 1,279 women within 24 hours of delivery, to investigate the normative levels observed around the time of labour and delivery. We reported that approximately half of lactate results

were higher than the most commonly used limit (2 mmol/L), which significantly limits its specificity. In contrast, approximately 95% of results were less than 4 mmol/L (consistent with RCOG guidance),[39,57] which we support as meaningful limit based on a sound physiological basis. In sequential models, we demonstrated that lactate is associated with the mode of birth (partly driven by the length of labour) and other markers of haemoconcentration/dilution, which explains a large part of the lactate changes in women who do not have sepsis.

Conclusions on lactate

We concluded that lactate is often substantially elevated in labour and immediately after delivery in the absence of sepsis. Using the conventional (non-pregnant or antenatal) reference limit has the potential to adversely influence diagnostic and prescribing practices when inappropriately extrapolated for use in labour and the puerperium, which is consistent with the above findings on CRP and WBC. However, while its use as a screening tool is not recommended in current guidelines and lactate-guided decision making about antibiotics is not evidence-based, it is important to remember that lactate still has a role in the management of women being treated for sepsis and it has a key role in the assessment of severity. We have reported how this can be achieved, and by publishing it in a high-impact obstetric journal, we hope that the findings are likely to reach the intended readership, enabling its application directly into clinical practice.

Summary of outcomes	**Impact and contribution to the field**
The definition of a pregnancy-specific RI for lactate around the time of delivery	This article has been well-received in the obstetric community, with interest from researchers and clinicians, and a significant presence on social media. We will immediately review the existing sepsis "screening" tools used in regional tertiary centres, to investigate how these can be improved in light of the new evidence.

Applying infection markers

It is often difficult to decide who needs antibiotics, particularly in labour and the postnatal period. Normal physiological responses to exertion and surgery often mimic those associated with the systemic inflammatory response syndrome (SIRS), such as pyrexia and tachycardia. Given this uncertainty, and the potential for serious adverse consequences if sepsis is missed, it may be understandable why clinicians might give antibiotics "just in case". However, this has repercussions on postnatal care, neonatal treatment, and the risk of antibiotic resistance at local, regional, and even international levels. We have endeavoured to clarify how infection markers can be used to inform decisions about maternal infection, sepsis, and antibiotics, and our findings are summarised in Table 1.

It is important here to clarify the distinction between localised (bacterial) infection and sepsis. Infection, although it may have serious effects on a fetus, is not associated with systemic end-organ compromise, which limits the use of lactate, but reiterates the value of other sensitive markers (particularly CRP). On the other hand, in women with convincing clinical evidence of sepsis, CRP and WBC may have limited added value, but lactate remains an essential tool for investigating the severity of tissue hypoperfusion and response to treatment. PCT is possibly the most promising infection marker, but it has been underappreciated in obstetric practice (indeed, most large observational studies, randomised trials, and service evaluations have excluded pregnant women altogether). I believe that PCT has great potential for the investigation of infection around the time of delivery, and guiding safe antibiotic prescribing practices. My primary aim as a postdoctoral clinical researcher is to plan and undertake prospective studies of PCT, firstly to quantify the scope of the problem (i.e., how many inappropriate prescriptions may potentially be safely avoided) and then by trialing the first PCT-guided protocol in UK obstetric practice.

Table 1: Recommended reference limits for infections markers according to the stage of pregnancy.

Infection marker	Antenatal	Intrapartum	Postnatal
C-reactive protein	✔ 19 mg/L	✖	✖[ix]
White blood cells (total) [x]	✔ 15 x10^9/L	✖	✔ 15 x10^9/L from 7 days 11 x10^9/L from 3 weeks
Procalcitonin [xi]		✔ 0.25 ng/mL	
Lactate [xii]	✔ 2 mmol/L	✔ 4 mmol/L	

[ix] For at least three days
[x] Sensitivity requires further investigation
[xi] Warrants validation in pregnancy
[xii] As a marker of severity only

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First author articles			
Study	**Impact factor**	**Citations**	**Contribution**
Dockree S, Shine B, Pavord S, Impey L, Vatish M. White blood cells in pregnancy: reference intervals for before and after delivery. *EBioMedicine* 2021;74:103715.[68]	11.2	3	Conceptualisation, methodology, software, formal analysis, writing (original draft, review & editing), visualisation.
Dockree S, O'Sullivan J, Shine B, James T, Vatish M. How should we interpret lactate in labour? A reference study. *BJOG* 2022.[69]	7.331	-	Conceptualisation, methodology, formal analysis, writing (original draft, review & editing)
Dockree S, Brook J, James T, Shine B, Impey L, Vatish M. Pregnancy-specific reference intervals for C-reactive protein improve diagnostic accuracy for infection: a longitudinal study. *Clin Chim Acta* 2021;517:81-85.[70]	3.786	7	Conceptualisation, methodology, formal analysis, writing (original draft, review & editing)
Dockree S, Brook J, James T, Shine B, Vatish M. A pregnancy-specific reference interval for procalcitonin. *Clin Chim Acta* 2021;513:13-16.[28]	3.786	10	Conceptualisation, methodology, formal analysis, writing (original draft, review & editing)

First author articles			
Study	**Impact factor**	**Citations**	**Contribution**
Dockree S, Brook J, Shine B, James T, Vatish M. Pregnancy-specific reference intervals for BNP and NT-pro BNP – changes in natriuretic peptides related to pregnancy. *J Endocr Soc* 2021;5:bvav091.[29]	CiteScore 5.3	5	Conceptualisation, methodology, formal analysis, writing (original draft, review & editing)
Dockree S, Shine B, Impey L, Mackillop L, Randeva H, Vatish M. Improving diagnostic accuracy in pregnancy with individualised, gestational age-specific reference intervals. *Clin Chim Acta* 2022;527:56-60.[30]	3.786	2	Methodology, formal analysis, investigation, writing (original draft, review & editing)
Dockree S, Brook J, Shine B, James T, Green L, Vatish M. Cardiac-specific troponins in uncomplicated pregnancy and pre-eclampsia: a systematic review. PLoS One. 2021;16:e0247946.	3.752	2	Conceptualisation, data curation, formal analysis, investigation, methodology, project administration, software, writing (original draft, review & editing)
Conference abstracts			
Dockree S, Knight M, Kennedy S, Vatish M. The role of procalcitonin in the diagnosis of histologically confirmed chorioamnionitis: a systematic review. *BJOG* 2019;126(S2):160-1.[49]	7.331	-	Conceptualisation, methodology, formal analysis, writing (original draft, review & editing)
Dockree S, Sweetland S, Yang O, Beral V. MO6204 Breastfeeding and endometrial cancer risk in the Million Women Study: a cohort study. *BJOG* 2018;125:15-18.	7.331	-	Conceptualisation, methodology, formal analysis, writing (original draft, review & editing)
Douglas J, Dockree S, Theaker J, Hayes M. 704 Multi-focality of testicular germ cell tumours – single institution review of 100 UK cases. *J Urol* 2013;189:e289-90.	7.45	-	Formal analysis, writing (original draft, review & editing)

Letters			
Dockree S, Brook J, Shine B, James T, Vatish M. Is there a role for C-reactive protein during and after labour? *Ann Clin Biochem* 2021;58:671-2.	1.893	2	Formal analysis, writing (original draft, review & editing)
Co-authored articles			
Cauldwell M, Steer P, Curtis S, Mohan A, Dockree S, *et al.* Maternal and fetal outcomes in pregnancies complicated by Marfan syndrome. *Heart* 2019;105:1725-31.	5.994	23	Data collation, writing (review & editing)
Cauldwell M, Mackie F, Steer P, Henehghan M, Baalman J, Brennand J, Johnston T, Dockree S, *et al.* Pregnancy outcomes in women with primary biliary cholangitis and primary sclerosing cholangitis: a retrospective cohort study. *BJOG* 2020;127:876-84.	7.331	18	Data collation, writing (review & editing)
Cauldwell M, Steer P, von Klemperer K, Kaler M, Grixti S, Hale J, O'Heney J, Warriner D, Curtis S, Mohan A, Dockree S, *et al.* Maternal and neonatal outcomes in women with history of coronary artery disease. *Heart* 2020;106:380-86.	5.994	9	Data collation, writing (review & editing)
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