Iron deficiency anaemia in pregnancy: an observational study of tolerability, compliance with oral iron therapy and effects on haematological/biochemical markers

TIAP
Treatment of Iron deficiency Anaemia in Pregnancy study

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SPONSOR: TBC

1. SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the non-interventional study in compliance with the approved protocol and will adhere to the principles outlined in GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

EU Clinical Trials Directive (2001/20/EC), specifies a non-interventional study is one in which:

...the medicinal product(s) is (are) prescribed in the usual manner, in accordance with the terms of the marketing authorization. The assignment of the patient to a particular therapeutic strategy is not decided in advance by a trial protocol, but falls within current practice, and the prescription of the medicine is clearly separated from the decision to include the patient in the study. No additional diagnostic or monitoring procedures shall be applied to the patients, and epidemiological methods shall be used for the analysis of collected data.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor
I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies and serious breaches of GCP from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: ..... Date: ...../...../...

Name (please print):
.....

Position: .....

Chief Investigator:

Signature: ..... Date: ...../...../...

Name: (please print):
.....

2. KEY STUDY CONTACTS

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3. LIST OF CONTENTS

1. SIGNATURE PAGE 2
2. KEY STUDY CONTACTS 3
3. LIST of CONTENTS 4
4. LIST OF ABBREVIATIONS 5
5. STUDY SUMMARY 6
6. STUDY FLOWCHART 7
7. THE NEED FOR A STUDY: BACKGROUND AND RATIONALE 8
    1. Anaemia 8
    2. Treatment 9
    3. Diagnostic tests to identify anaemia 9
8. OVERARCHING PURPOSE AND OBJECTIVES 11
9. Aims & Objectives 12
10. STUDY DESIGN AND SETTING 12
11. PARTICIPANT ELIGIBILITY CRITERIA 12
    1. Participant inclusion criteria: 13
    2. Participant exclusion criteria: 13
    3. Study restrictions 13
12. STUDY PROCEDURES, AND ASSESSMENT SCHEDULES 13
    1. Identification and recruitment 13
    2. Consenting 14
    3. Data collection: Baseline clinical data 15
    4. Blood Sampling 15
    5. Symptom and Tolerability Assessment 16
    6. Participant follow-up and assessments 17
    7. Follow up and pregnancy outcome data 18
    8. End of study 19
13. STUDY TREATMENT 19
14. ADVERSE EVENT REPORTING 20
    1. Definitions of Adverse Events 20
    2. Expected Drug-Related Adverse Events / Reactions 20
    3. Adverse Events that Require Expedited Reporting 21
    4. Investigators Assessment of SAE 21
    5. Notification and Reporting of Serious Adverse Events 22
    6. Overview of the Safety Reporting Process/Pharmacoviligance responsibilities 22
    7. Statutory Reporting 22
15. Ethical and Regulatory Issues 22
    1. Compliance 22
    2. Ethical Compliance 22
    3. Data Protection and Patient Confidentiality 23
    4. Indemnity 23
    5. Amendments 24
    6. Access to the Final Study Dataset 24
    7. Peer Review 24
    8. Patient & Public Involvement 24
16. STATISTICS AND DATA ANALYSIS 25
    1. Primary outcome measure 25
    2. Other Outcomes 25
    3. Statistical Analysis and Overview Analysis Plan 25
17. DATA MANAGEMENT 26
    1. Participant Confidentiality and Data Access 26
    2. Archiving 27
18. DATA MONITORING AND INSPECTION 27
19. PUBLICATION POLICY 28
20. Protocol Amendments 28
21. REFERENCES: 29

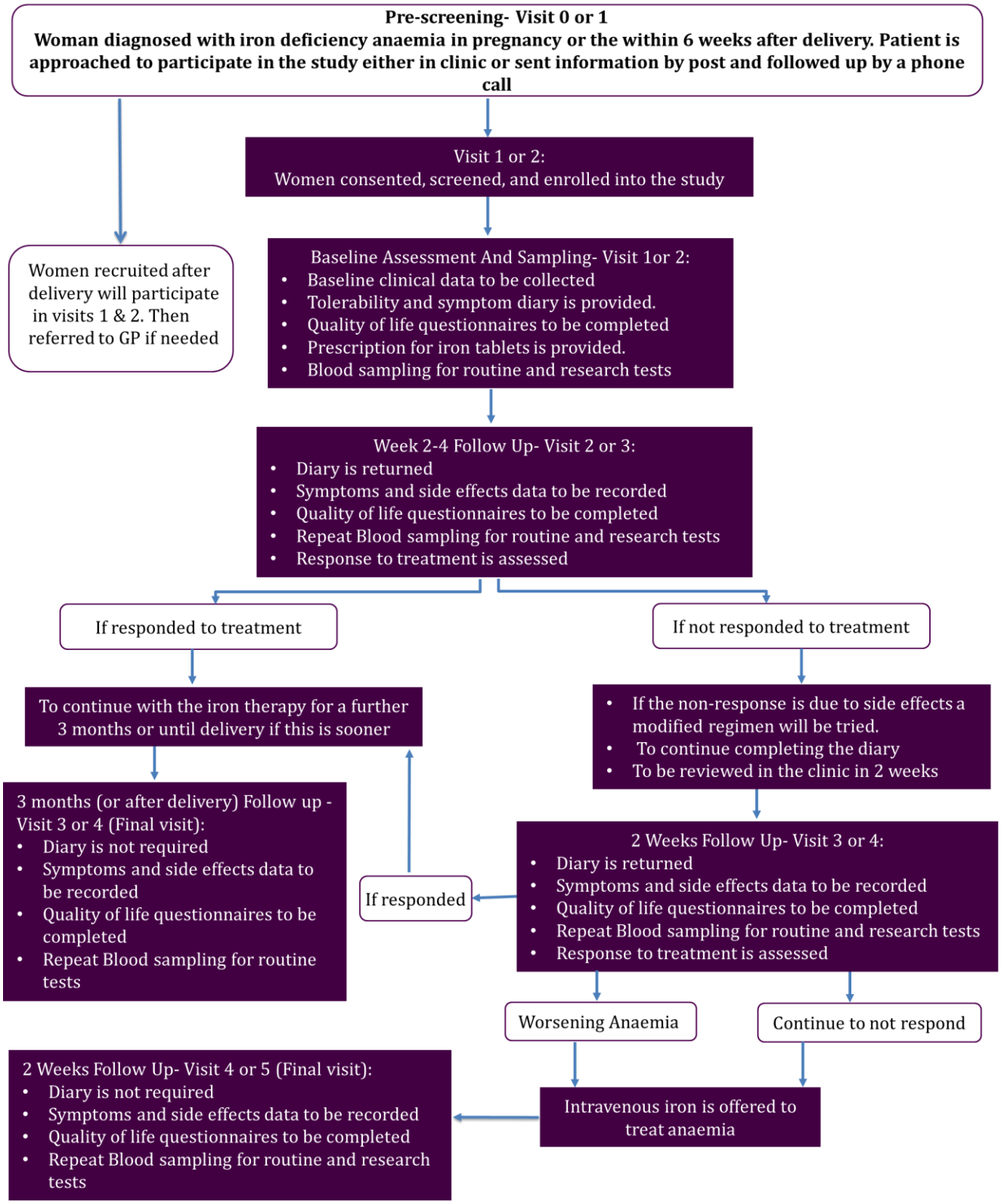
4. LIST OF ABBREVIATIONS

aOR	adjusted odds ratio
BCSH	British Committee for standards in Haematology
BSH	British Society Haematology
CI	Confidence Interval
CI	Chief investigator
CRP	C-reactive protein
ELISA	enzyme linked immunosorbant assay
FBC	Full blood count
GCP	Good Clinical Practice
GDM	Gestational diabetes
GP	General Practitioner
Hb	Haemoglobin
IFN-g	Interferon-gamma
IL	Interleukin
IQR	Interquartile range
ISF	Investigator Site File
IV	Intravenous
MA	Marketing authorisation
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean corpuscular volume
NHS	National Health Service
PI	Principal Investigator
PIL	Participant Information Leaflet
REC	Research Ethics Committee
RWT	Royal Wolverhampton NHS Trust
TDF	Theoretical Domains Framework
TGF-β	Transforming growth factor-beta
SMG	Study Management Group
TNF	tumor necrosis factor
WCC	White cell count
WHO	World Health Organisation
WHO-5	The World Health Organisation- Five Well-Being Index
WIMM	Weatherall Institute of Molecular Medicine
WiP	Well-being in Pregnancy

5. STUDY SUMMARY

Study title	Iron replacement in pregnant women: an observational study on tolerability, compliance with oral iron and effects on haematological/biochemical markers of anaemia
Short title	TIAP - Treatment of Iron deficiency Anaemia in Pregnancy study
Clinical Phase	Observational and mechanistic
Study Design	Prospective cohort study
Study Participants	Anaemic (iron-deficient) pregnant women receiving oral iron replacement therapy
Planned Sample Size	120
Treatment duration	Initial treatment duration is up to 4 weeks for the assessment period of the study. Depending on the response, the iron treatment may continue throughout pregnancy or until 3 months after delivery if the anaemia is diagnosed in the third trimester, as per local guidelines and under the direction of the supervising obstetrician.
Follow up duration for data collection and sampling	4 weeks for the treatment period and 6 weeks post-delivery for obstetric outcome data
Planned Study Period	2 years- recruitment period will be 18 months with a 6 month follow up period
Objectives	1. To describe the symptoms at presentation with anaemia during pregnancy 2. To assess the correlations between symptoms and degree of anaemia at presentation 3. To quantify the clinical symptom response, side effects, tolerability, compliance and acceptability with oral iron treatment (using recommended national dosing schedules) 4. To document the haematological changes in haemoglobin, ferritin, transferrin saturation, serum hepcidin, other research biomarkers of iron metabolism and pro-inflammatory cytokines during a treatment course of oral iron 5. To investigate whether biomarkers, such as serum hepcidin, can predict the response to oral iron
Primary outcome Secondary Outcomes	An increase in haemoglobin of at least 10g/L • Tolerability and concordance with treatment • Quality of life assessments in response to treatment
Treatment	Oral elemental iron as per standard care (ferrous sulphate)
Formulation, Dose, Route of Administration	Ferrous sulphate 100-200 mg up to three-times daily as per standard guidelines

6. STUDY FLOWCHART



7. THE NEED FOR A STUDY: BACKGROUND AND RATIONALE

1. Anaemia

The burden of anaemia remains unacceptably high during pregnancy. Over a third of women are anaemic by their third trimester of pregnancy. The most common cause is iron deficiency (Friel, 2014). One key factor is rising iron requirements throughout pregnancy. By the third trimester, body iron demand outstrips the amount that can be provided by diet alone. Therefore, iron stores must be sufficient at the start of pregnancy (≥300 mg) to make up for that shortfall (Bothwell, 2000). In addition, the start of pregnancy is often characterised by sub-optimal iron stores (Bothwell, 2000). There are risks associated with anaemia for the mother and infant. Anaemia in the first and second trimester has been significantly correlated with low birth weight and pre-term birth (Haider, 2013), and is associated with impaired neurological development of the baby (Congdon, 2012). It also increases the risk of intrauterine fetal death (Lone, 2004), and the likelihood of the mother requiring blood transfusions during or after delivery (Patterson, 2014).

Our own work is consistent with these findings (Nair et al, 2017). A retrospective cohort of anonymised data from 14,001 women was analysed. All were singleton pregnancies ≥24 weeks' gestation giving birth between 2013-15 (7,175 from Royal Wolverhampton National Health Service (NHS) Trust, 2013-14 and 6,826 from Guy's and St Thomas' NHS Foundation Trust, 2014-15). Multivariable logistic regression was used to quantify associations between haemoglobin and categories of anaemia (no anaemia ≥110g/L; mild anaemia 100-109g/L; moderate-severe anaemia <100g/L) at booking and 28 weeks with stillbirth and perinatal death, adjusting for 11 confounders (age, ethnicity, BMI, parity, smoking, gestational diabetes mellitus, antepartum haemorrhage, hypertensive disorders, and pre-existing diabetes mellitus, haemoglobinopathies, and other medical comorbidities).

Apart from a higher proportion of women from ethnic minorities reflecting local demographics the study population compared well to the general population in England. The prevalence of all grades of anaemia at the first visit was 7% and the prevalence of moderate / severe anaemia (i.e. <100g/L) increased from 2% to 7%, between the first and 28 week visits. Additionally, 26% of women with normal haemoglobin at the first visit became anaemic by 28 weeks. In total 46% of women had anaemia at some point during their pregnancy. The risk of stillbirth and perinatal death decreased linearly per unit increase in haemoglobin concentration at first visit (adjusted odds ratio (aOR) stillbirth=0.70, 95% confidence interval (CI) 0.58–0.85; aOR perinatal death=0.71, 95%CI 0.60–0.84). Thus, in terms of the population, for every 10g/l increase in haemoglobin there was a 30% reduction in the risk of stillbirth. Compared with women with normal haemoglobin concentrations, the risk of both stillbirth and perinatal death was four-fold [aOR 4.34 (2.11-8.92)] and three-fold higher [aOR

2.91 (1.46 - 5.81)] in women with moderate-severe maternal anaemia at first and at 28 weeks visits, respectively.

2. Treatment

Current approaches to management of anaemia are described in national guidelines, which focus on case finding (detection/treatment) of low haemoglobin concentrations (British Committee for Standards in Haematology, 2012), and are underpinned by low quality evidence. There is evidence that current strategies for anaemia management are not effective. For instance, in a national descriptive study of 638 women with anaemia in early pregnancy, it was found that 281 (44%) of these women had anaemia at or after 28 weeks (Barroso et al, 2011). Furthermore; in a recent large local audit over two years (2015-16, in Wolverhampton), 343 women had anaemia before 16 weeks and of these, 64% still had anaemia at 28 weeks. Current testing to establish a diagnosis of iron-deficiency involves additional blood tests (ferritin) and it may be several weeks before women are counselled and started on oral iron treatment.

In addition, a significant proportion of women who receive oral iron therapy may go on to receive intravenous (IV) iron infusions which is costly and requires administration in an observed setting. The main indications for receiving iron infusions are when: oral iron therapy fails to improve haemoglobin levels after two weeks; the oral iron is not well tolerated (gastro-intestinal side effects); the patient is still defined as anaemic after four weeks of oral iron therapy; or when the patient is diagnosed as anaemic within four weeks of the estimated delivery date. In addition, women closer to term and delivery would be expected to benefit more from IV iron infusion.

Patients with severe anaemia (such as a haemoglobin level < 70g/L) need to be immediately referred to secondary care (Pavord, 2012) for assessment as parenteral iron may be required. Blood transfusion is reserved for extreme circumstances such as imminent cardiac compromise, severe anaemic symptoms which require urgent treatment, or if there is a high risk of further haemorrhage (Pavord, 2012).

3. Diagnostic tests to identify anaemia

Anaemia has traditionally been defined by World Health Organisation (WHO) thresholds, which are gestation dependent, to reflect the greater proportionate expansion of the plasma volume during pregnancy compared to the increase in red cell mass. Further tests for the investigation of iron deficiency include: blood film; red cell indices such as mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration; alongside iron studies such as serum ferritin, serum iron and total iron binding capacity/transferrin saturation.

All have their limitations and none has proven capable of predicting the response to oral iron therapy during pregnancy. These parameters have been analysed for their predictive value in anaemic, non-pregnant patients. An additional parameter, the iron-regulatory hormone hepcidin, which is not routinely tested, has also been included in these analyses. There is now some evidence to suggest that in haemodialysis patients, mean cell volume, serum ferritin and hepcidin levels can help to predict oral iron therapy responses (Takasawa, 2015). Furthermore, in a wide range of patients diagnosed with iron deficiency anaemia, serum hepcidin performed exceptionally well as a predictor of response to oral iron therapy, particularly when compared to transferrin saturation and serum ferritin levels (Bregman, 2013).

Unfortunately, of the three more promising candidates: mean cell volume, serum ferritin and hepcidin, there are still drawbacks to their use in pregnant patients. For example, mean cell volume increases during pregnancy and can therefore offset the reduction in volume associated with iron deficiency anaemia (Pavord, 2012). These influences may impair its predictive value for response to oral iron therapy.

Furthermore, whilst serum ferritin can accurately reflect iron stores in a healthy patient, this relationship is lost in acute and chronic infection and inflammation, as serum ferritin is an acute phase reactant. Its measurements are further confounded by day-to-day variation, which can be as great as 25% (Beard, 1994), and by factors specific to pregnancy such as haemodilution. Serum ferritin variation at each trimester of pregnancy also suggests that serum ferritin can dissociate from its relationship with iron stores, with one study reporting a rise in serum ferritin levels in the third trimester of pregnancy (Asif, 2007). In short, serum ferritin may be a poor informer of body iron status in pregnancy.

As a result, interest has been directed towards hepcidin as a possible predictor of response to oral iron therapy in pregnant patients. This protein has emerged as a major regulator of iron status. Its expression is increased in states of inflammation and iron overload and repressed in iron deficiency, hypoxia, and erythroid expansion. Hepcidin binds to ferroportin causing its internalisation and degradation. Ferroportin is the sole iron exporter and is expressed in macrophages and duodenal cells, allowing iron recycling and absorption. Hepcidin can therefore inhibit iron release from macrophages leading to an 'iron-restricted' erythropoiesis or from duodenal cells, leading to dietary iron deficiency.

Stimulation of immune responses in anemia of inflammation is often associated with upregulation of ferritin resulting in intracellular excessive storage of iron. In addition, Interleukin (IL)-6 production during these inflammatory conditions stimulates the release of hepcidin which consequently inhibits the iron absorption by the enterocytes and downregulates ferroportin therefore increases

intracellular storage of iron and leading to a decrease in iron bioavailability. Other cytokines such as IL-22 may have the same effect independent of IL-6. Furthermore, inflammatory cytokines down-regulate transferrin receptors on the cell surface. Reduction in bioavailable iron is an important step in limiting iron supply to pathogens but can contribute to the anaemia of inflammation and the upregulation of proinflammatory cytokines. These cytokines may also have a role to play in pregnancy disorders such as stillbirth.

At present there is a paucity of research into the relationship between clinical symptomatology, hepcidin, iron status and pregnancy. In a recent review, Koenig *et al* (2014) suggested that there is growing evidence that maternal hepcidin correlates with maternal iron status during pregnancy. However, the data is far from clear-cut. In one recent study focusing on healthy pregnant women, there appeared to be a clear reduction in hepcidin concentration during pregnancy, in line with falling iron stores, and recovery of hepcidin levels following delivery (van Santen, 2013). However, other authors have reported that there is no association between serum hepcidin, body iron status and inflammation in similar cohorts (Simavli, 2014). Of further interest is the possible correlation between hepcidin and inflammatory conditions such as pre-eclampsia and obesity. In a small study, it was found that pre-eclamptic patients had higher plasma hepcidin and ferritin concentrations, and lower transferrin, total iron binding capacity, and mean cell haemoglobin concentrations compared to healthy pregnant women (Toldi, 2010). Possible associations between maternal obesity, raised hepcidin and C-reactive protein (CRP), and reduced fetal iron status have been reported in other studies (Dao, 2013), but larger studies are required.

Outside the small field of obstetric research, hepcidin has been heralded as a biomarker for distinguishing oral iron therapy "responders" from "non-responders". It was found that, within the subgroup of anaemic African children, hepcidin was superior to serum ferritin in terms of assessing anaemia, and for distinguishing between anaemia due to iron-deficiency from anaemia due to inflammation, particularly if the two coexist in the same patient. (Pasricha, 2014). In light of accumulating and inconclusive evidence for hepcidin assessment during pregnancy, further studies are required.

8. OVERARCHING PURPOSE AND OBJECTIVES

To better define the natural history and understand how to use oral iron therapy for iron deficiency anaemia in pregnant women. Specifically, the study will document the impact of treatment on symptomatology, the induction of side effects, and the utility of several haematological indices; haemoglobin, ferritin, transferrin saturation, reticulocyte haemoglobin concentration, alongside

changes in new markers of iron homeostasis (hepcidin), which may better predict the success of treatment with iron.

9. AIMS & OBJECTIVES

1. To describe the symptoms at presentation with anaemia during pregnancy.
2. To assess the correlations between symptoms and degree of anaemia at presentation.
3. To quantify the clinical symptom response, side effects, tolerability, and compliance with oral iron treatment (using recommended national dosing schedules).
4. To document the haematological changes in haemoglobin, ferritin, CRP, transferrin saturation, hepcidin and pro-inflammatory cytokines during a treatment course of oral iron.
5. To investigate whether biomarkers of iron metabolism, such as serum hepcidin, can predict the response to oral iron.

10. STUDY DESIGN AND SETTING

This protocol describes a prospective cohort study where pregnant women identified as anaemic are offered treatment and follow up through a dedicated anaemia clinic. Mothers will be invited to participate in this study aiming to assess response and tolerability to doses of oral iron, using a treatment schedule as described in national guidelines. Additional blood samples will be taken for subsequent detailed analysis of pathways of iron metabolism.

Standard practice is 'reactive' to anaemia occurring in pregnancy, and will be followed for the purpose of this study. All women are offered a full blood count (FBC) to screen for iron deficient anaemia at booking (usually 8-12 weeks' gestation) and 28 weeks' gestation. Maternity units have a system in place to ensure that all routine blood results, including the full blood counts, are reviewed promptly, and acted upon. Women identified with iron deficiency anaemia will be offered oral elemental iron or IV iron supplementation (and preferably within 28 days). Women will receive written information on the correct administration of iron and how to maximise absorption. A full blood count will be repeated 2 - 4 weeks following the start of therapeutic iron treatment.

This is a single center study at The Royal Wolverhampton NHS Trust (RWT) supported by the laboratory at Weatherall Institute of Molecular Medicine (WIMM), University of Oxford and the University of Wolverhampton. The clinical assessment of treatment and collection of samples will be carried out at RWT and the hepcidin/specialist iron analyses will be performed in WIMM and the University of Wolverhampton.

11. PARTICIPANT ELIGIBILITY CRITERIA

Participants will be considered eligible for enrolment in this study if they fulfil all the inclusion criteria and none of the exclusion criteria detailed below.

1. Participant inclusion criteria:

- Pregnant women (any stage during pregnancy up to 36 weeks) and women in the puerperium (within 6 weeks post-delivery) with anaemia as defined by WHO criteria and described in British Society Haematology (BSH)/ British Committee for standards in Haematology (BCSH) guidelines.
    - First trimester < 110g/l
    - Second and third trimester < 105g/l
    - Puerperium < 100g/l
- Age: 18-45 years
- Agreement to participate in the study with consenting

2. Participant exclusion criteria:

- Anaemic women presenting at or after 36 weeks as there may be insufficient time to delivery to assess responses to oral iron)
- Anaemic women affected by a (major) haemoglobinopathy e.g. B thalassaemia major sickle cell disease
- Women with overt clinical signs of sepsis
- Allergies to iron
- Hyperemesis Gravidarum / persistent vomiting
- Women with inflammatory conditions such as Crohns, ulcerative colitis, Systemic lupus erythematosus, Rheumatoid arthritis.
- Women with chronic renal failure

3. Study restrictions

Women can be enrolled in other studies that do not impact on the aims and methods of this study and vice versa. All potential participants will be asked and their care records scrutinised to discover if they are participating in any other research projects or programmes. If yes, they will then be assessed whether it would be appropriate to include them in this study or not.

12. STUDY PROCEDURES, AND ASSESSMENT SCHEDULES

1. Identification and recruitment

Eligible women with iron deficiency anaemia will be identified in 3 ways:

- Women diagnosed de novo in the hospital antenatal clinics and antenatal maternity ward with iron deficiency anaemia who have yet to receive treatment.
- Women on the postnatal ward who are found to be anaemic post-delivery.
- From a daily download of blood results from samples taken either at the booking or the 28 week visit to the antenatal clinics either in the hospital or community setting.

All eligible women will be approached by the research staff (who are also the clinical care team), usually the clinical research fellows or research midwives, and given information (verbal & written) about the project. Consent may then be obtained after allowing sufficient time for the woman to consider the information and have all her questions answered to her satisfaction. This will be depending on each individual's needs and assessed by health professionals obtaining consent, which could vary between a few hours or a few days. In addition, eligible participants may be invited to take part in the study via mail by directly mailing them the study cover letter and Participant Information Leaflet (PIL). Participants will be followed up by phone and if interested patients they can either be consented in their clinic visit. All participants will be invited to attend a dedicated anaemia clinic for the initial visit.

We aim to recruit women into the study spread evenly throughout three time periods of pregnancy/post-partum:

1. Early pregnancy up to 24 weeks gestation
2. Mid – late pregnancy from 24+1 to delivery
3. Postnatally, up to 6 weeks after birth of the baby.

2. Consenting

The Chief investigator (CI), who is also the Principal Investigator (PI) retains overall responsibility for the conduct of research at the site, and this includes the taking of informed consent of participants at their site. The PI can delegate this process to medically qualified and midwife delegates. The PI must ensure that any person with delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, UK Policy Framework for Health and Social Care Research, principles of International Conference on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki.

The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the study without giving reasons and without prejudicing her further treatment and must be provided with a contact point for the local study team (i.e. PI and research fellows/midwives) from whom participants may obtain further information about the study. Consent will be established for the use of samples and data collected up to the point of withdrawal.

Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the study (as described below), and are out-with standard routine care at the participating site. Participants who agreed to take part will sign a Consent Form, which will also be signed by the PI or an appropriately delegated professional. The informed consent process will be documented in patients' medical records. The original Consent Form will be stored in the Investigator Site File (ISF), one copy will be filed in the participant's medical record and a second copy will be given to participant.

3. Data collection: Baseline clinical data

Baseline clinical characteristics will be collected from medical notes and participant interviews. These include as routine:

- age of the participant
- parity and timing of previous pregnancies
- gestation or post-partum time
- current use of vitamin supplements, make and frequency of administration
- If taking vitamin supplements details on who or what prompted them to take supplements.
- previous use of oral iron supplements
- current use of oral iron supplements
- previous experience of intravenous iron infusions
- number of infusions received if applicable
- number of infusions at the time of blood sampling if applicable
- history of recent red cell units transfused
- significant co-morbidities

Follow up Data Collection

- Routine clinical data will be collected on each participant on the pregnancy outcome including the condition of the neonate. See section 7.
- Blood sampling data and data collected through the questionnaires and diary will be prospectively collected as the study progresses.

4. Blood Sampling

Blood samples to be collected at baseline are:

- Ferritin, transferrin saturation, reticulocyte haemoglobin concentration, CRP.

- Full blood count indices - Haemoglobin (Hb), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), white cell count (WCC), platelet count
- Renal function – sodium, potassium, urea and creatinine
- Liver function tests – Bilirubin, Albumin
- Total B12
- Hepcidin and biomarkers of iron metabolism (research assay, not part of standard care)
- Cytokine profile (research assay, not part of standard care)

All samples of blood that are not part of standard care will be spun down into 2 aliquots within an hour of venesection and the plasma stored at -80 degrees Celsius prior to transporting to the relevant laboratory. Apart from the Hepcidin and Cytokine profile the other blood tests are part of routine clinical practice. The Hepcidin level and Cytokine profile will be undertaken at the University of Wolverhampton and University of Oxford (see below) and are exploratory tests (not used for diagnostic or monitoring purposes). Hepcidin levels will be determined before and after iron treatment using enzyme linked immunosorbant assay (ELISA) technique. The pro-inflammatory cytokines including (IL1, IL-2, IL-6, IL-7, IL-12p70, IL-17, IL-22, IL-12p40, tumour necrosis factor (TNF), Interferon-gamma (IFN-γ)) and the anti-inflammatory cytokines including (IL-4, IL-5, IL-10 and Transforming growth factor-beta (TGF-β)) will be assessed in the same patients using cytokine multiplex bead assays.

Samples of sera will be stored in a -80 degree freezer at the Royal Wolverhampton Hospitals NHS Trust. At the end of the study these samples will be transported and analyzed at the Weatherall Institute in Oxford, then returned to Royal Wolverhampton Hospitals NHS Trust. At the end of the ethically approved timeline of the study all samples will then be destroyed in accordance with the Human Tissue Authority's Code of Practice.

5. Symptom and Tolerability Assessment

At each visit, participants will be asked to complete Quality of Life questionnaires (The World Health Organisation- Five Well-Being Index (WHO-5) (Beck, 2004) and the Well-being in Pregnancy (WiP) questionnaire (Alderdice et al. 2017). The frequency and severity of symptoms associated with anaemia in pregnancy and the side effects experienced by the women will be assessed using a pre-piloted and adapted version of a published pregnancy symptomatology tool (Foxcroft 2013). The 'Anaemia in Pregnancy Assessment Questionnaire' has been adapted to investigate the barriers and enablers to taking oral iron therapy using the Theoretical Domains Framework (TDF) (Michie et al. 2005).

At the follow up visits the frequency of side effects, if any, induced by the iron treatment will be assessed by examining the pre-piloted tolerability and symptom diary adapted from Peirera (2014).

The diary will record the time at which participant takes the iron medication, the symptoms of anaemia participant experiences at the time and the side effects participant had suffered since the last tablet was taken. Participants will be asked to keep this diary for the period from initiation of treatment to the follow up appointment 2 to 4 weeks later. The completed diary will be returned to confirm compliance with treatment, symptoms of anaemia, and side effects experienced from the treatment itself. The data from the diary will be transcribed ready for analysis.

6. Participant follow-up and assessments

The following sections consider those aspects of care that are standard care and research for this study.

Groups 1-2 Women recruited in the antenatal period.

Visit 1 (T0)

- Baseline clinical data collection (routine care)
- Blood sampling for routine clinical assessment and research sampling
- Quality of life questionnaires (research)
- Explanation of the tolerability and symptom diary and completion of page 1 as a baseline pre-treatment assessment (research)
- Prescription given for oral iron (standard care).

At this (and subsequent) visits, the women will have a routine full antenatal check of both their health and that of their fetus. After discussion with the mother, these clinic visits may replace any scheduled visits to either the consultant or community antenatal clinic so that any inconvenience is kept to a minimum. The research clinic will be overseen by a consultant obstetrician with a special interest in maternal and fetal medicine and the management of the pregnancy will be based upon the clinical need of the woman.

Visit 2 (T0 + 2-4 weeks- routine visit)

After the initial visit the participant will return for a follow up visit or review appointment between 2 to 4 weeks after commencing oral iron.

- Clinical data recorded as per normal antenatal care
- Completion of the Quality of life (QOL) questionnaires (research).
- Retrieve tolerability and symptom diary (research)
- Repeat Blood Sampling as above for visit 1 including research samples
- In addition, they will be asked to complete the Anaemia in Pregnancy Assessment Questionnaire that further examines the symptoms and side effects they experienced over the

initial treatment period and asks about the barriers and enablers to taking the iron treatment using the Theoretical Domains Framework (Michie et al. 2005) (research).

- At this visit women will be assessed for response as detailed next (as per standard care):

Responders

For women who respond to treatment, as per routine practice, they will be asked to continue with the iron therapy at the dose at which they are tolerating side effects for a further 3 months or until delivery if this is sooner as per standard care. They will be reviewed again at the end of this period. (During the 3 months' period the diary will not be required but the symptomatology and side effects profile will be formally assessed at a final visit. Also QOL questionnaires and research blood samples will be required at this visit (research)).

Non-Responders (women who do not have the expected rise in haemoglobin)

The reasons for non-responding will be explored with these women. If the non-response is due to side effects a modified regimen of twice daily administration will be tried. They will be asked to continue with modified treatment (as per standard care) and the diary (research) and then given an appointment to be reviewed in the clinic in a further 2 weeks (Visit 3) when the same process will be followed as Visit 2. As per routine practice, women who continue to be non-responders, when assessed at visit 3, will be offered intravenous iron to treat their anaemia.

Worsening anaemia

Women, whose anaemia worsens between any of the visits, will be offered intravenous iron as per standard care. All women who receive intravenous iron will be asked to return to the clinic after 2 weeks for repeat blood sampling (including research blood samples and QOL questionnaires) to ensure that they have responded and their haemoglobin has increased. The number of visits will depend upon clinical need in these patients as per national BSH guidelines.

Group 3 Women recruited in the postnatal period.

These women will participate in visits 1 & 2, as described above.

For those women who are post-delivery and remain anaemic at the second visit a referral will be made to their General Practitioner for further follow up and on-going investigation as they feel appropriate and as per local guidelines.

7. Follow up and pregnancy outcome data

Haematological and tolerability data will be collected in line with the detailed schedule described above and extracted from the data collections systems at the end of the study.

Pregnancy outcome data will also be collected 8 weeks after birth to allow for complete data collection in the puerperium. These data are collected routinely for this group of women. Broad headings for the items are shown below:

Maternal

- Haemoglobin (Hb) concentration, MCV and MCH nearest to the point of delivery
- Hb, MCV, MCH in the puerperium
- Antenatal Obstetric complications e.g. pre-eclampsia, intra-uterine growth restriction, gestational diabetes mellitus, infections and sepsis, etc.
- Induction process if required
- Mode of delivery
- Complications associated with the delivery process. e.g. haemorrhage, perineal trauma, intra-operative trauma at caesarean section,
- Complications in the puerperium, e.g. secondary postpartum haemorrhage, sepsis, psychological disorders, etc.

Fetal

- Birth weight
- Gestation at delivery
- Condition at birth, e.g. 5 minutes Apgar score, cord blood gases, need for resuscitation and techniques used.
- Admission to higher level of neonatal care
- Neonatal complications and diagnoses.

8. End of study

The study will be closed 30 days after 8 weeks post-delivery of the last participant enrolled, to allow sufficient time for completion of baby outcomes data collection. The Chief Investigator will notify the REC of the end of the study or earlier if the study ended prematurely.

13. STUDY TREATMENT

Standard practice will be followed and enrolled women will be issued with a prescription for oral iron therapy as per the BSCH guidelines for iron deficiency anaemia in pregnancy. The women will be given verbal instruction on how to take the treatment. This will be backed up with written information. The BCSH guideline recommend 100-200mg elemental iron per day; this is traditionally

prescribed as 200mg ferrous sulphate three times a day (65mg elemental iron per tablet x 3 = 195mg), and local RWT policies describing this will be followed.

Iron has a marketing authorisation (MA) in the UK and is being used in its marketed presentation and packaging bearing the MA number.

14. ADVERSE EVENT REPORTING

The principles of ICH GCP require to follow specific procedures when notifying and reporting adverse events in clinical trials. These procedures, relevant to this non-interventional study, are described in this section.

1. Definitions of Adverse Events

The definitions to be applied to adverse events recorded in this study are given in Table 9a below.

Table 14a: Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence affecting a trial participant during the course of a clinical trial.
Adverse Reaction (AR)	Any adverse event when there is at least a possibility that it is linked to a trial drug or intervention.
Unexpected Adverse Reaction (UAR)	An unexpected occurrence of an adverse reaction
Serious Adverse Event (SAE)	A serious adverse event that: • results in death* • is life-threatening** • requires hospitalisation or prolongation of existing hospitalisation*** • results in persistent or significant disability or incapacity • is a congenital anomaly/birth defect • other important medical event(s)****
Serious Adverse Reaction (SAR)	An SAE that is thought to be causally linked to a trial drug or intervention.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	An unexpected occurrence of a SAR; there need only be an index of suspicion that the event is a previously unreported reaction to a trial drug or a previously reported but exaggerated or unexpected frequent adverse reaction.

* Death due to the underlying disease or associated conditions will not be reported as an SAE.
**The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
***Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition (including elective procedures that have not worsened) do not constitute an SAE.

2. Expected Drug-Related Adverse Events / Reactions

Ferrous Sulphate tablets is a marketed medicinal product in licensed indication with a well-known safety profile, therefore adverse reactions which are expected (assessed against the Summary of Product Characteristics (https://www.medicines.org.uk/emc/medicine/27767 and not serious do not require recording, as these will not improve the knowledge of the safety profile of the drug.

3. Adverse Events that Require Expedited Reporting

Serious Adverse Events (SAEs)

All adverse events which meet the definition of serious, occurring from the start of study treatment until 28 days post-study treatment must be recorded on the Serious Adverse Event Form and sent to the Sponsor within 24 hours of the research staff becoming aware of the event.

4. Investigators Assessment of SAE

The Principal Investigator is required to consider seriousness (according to the definitions given in table 14a), causality in relation to the study treatment and expectedness of any SAE. Causality will be assessed according to the following categories:

Table 14b: Definitions of Causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial drug or intervention). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial drug or intervention). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
Probable	The evidence is clearly in favor of attributing the adverse reaction to the trial drug or intervention
Definitely	There is conclusive evidence beyond reasonable doubt attributing the adverse reaction to the trial drug or intervention.

The Investigator must assess the expectedness of each SAE using the ferrous sulphate SmPC – see Section 14.2

Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5. Notification and Reporting of Serious Adverse Events

Serious Adverse Event (SAEs) that are considered to be 'related' and 'unexpected' (SUSAR) are to be reported to the sponsor within 24 hours of learning of the event and to the Research Ethics Committee (REC) within 15 days in line with the required timeframe.

6. Overview of the Safety Reporting Process/Pharmacoviligance responsibilities

The Serious Adverse Event Form must be completed by the Investigator (the Consultant named on the delegation of responsibilities log who is responsible for the patient's care).
The SAE should be reported to the CI within 24 hours of becoming aware of aware of the event
The Investigator must follow-up all reported SAEs until resolution or the event is considered stable.

The CI has the overall pharmacovigilance oversight responsibility and has a duty to ensure that pharmacovigilance monitoring and reporting is conducted in accordance with the sponsor's requirements. All reported serious adverse events will be reviewed by the Chief Investigator (or a medically qualified delegate).

7. Statutory Reporting

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the Yellow Card Scheme at: www.mhra.gov.uk/yellowcard

15. ETHICAL AND REGULATORY ISSUES

1. Compliance

This study complies with the Declaration of Helsinki 2013. It will also be conducted in compliance with the approved protocol, the principles of GCP, the UK Data Protection Act and the NHS UK Policy Framework for Health and Social Care Research

2. Ethical Compliance

- Before initiation of the study, the protocol, informed consent, participant diary, questionnaires and any information to be provided to the prospective participant will be submitted to an NHSREC for ethical approval. Any subsequent amendments will be submitted to, and approved by, the same REC.
- The protocol and study documents will be submitted by those delegated to do so to the Health Research Authority (HRA) for approval.

- For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor, will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with site (R&D department) so it can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.
- Substantial amendments that require review by NHS REC will not be implemented until that review is in place and other mechanisms are in place to implement at site.
- All correspondence with the REC will be retained.
- The Chief Investigator will produce the annual reports as required.
- The Chief Investigator will notify the REC of the end of the study.
- An annual progress report will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.
- If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.
- Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

3. Data Protection and Patient Confidentiality

- All study team must comply with the requirements of the Data Protection Act 1998 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.
- The confidentiality standards will be maintained by coding each participant enrolled in the study through assignment of a unique participant identification number.
- Consent will be sought from participants to inform their General Practitioner (GP) of their enrolment in the study. Medical information may be given to a participant's GP or other appropriate medical personnel responsible for the participant's welfare, for treatment purposes.
- Data generated by this study must be available for inspection upon request by representatives of the REC and other relevant national and local health authorities, as appropriate.

4. Indemnity

This is an NHS-sponsored research study, and the NHS indemnity scheme therefore applies. If there is negligent harm during the study when the NHS body owes a duty of care to the person harmed, NHS indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the study. The NHS indemnity scheme does not cover non-negligent harm. The liability

of the manufacturers of ferrous sulphate is strictly limited to those claims arising from faulty manufacturing of the commercial product and not to any aspects of the conduct of the study.

5. Amendments

The sponsor will decide whether an amendment is substantial or non-substantial for the purposes of submission to the REC. If the sponsor wishes to make a substantial amendment to the REC application or supporting documents, the sponsor will submit a valid notice of amendment to the REC for consideration. The REC will provide a response regarding the amendment within 35 days of receipt of the notice. Amendments will also be notified to the participating organisation (R&D department and local research team), to assess whether the amendment affects the NHS permission for the study site.

Once an amendment has been approved, the version number of the protocol will be updated. All superseded versions of the protocol will be kept in the Site File, scored through to indicate their replacement.

6. Access to the Final Study Dataset

The SMG will have access to the final study dataset. Custody of the final data set will reside with the CI and the Sponsor.

7. Peer Review

The study protocol has been subject to independent scientific peer review by two independent experts, by the sponsor (as part of sponsorship assessment), and by the NHDBT CTU research team. Feedback has been incorporated within the protocol and relevant study documents.

8. Patient & Public Involvement

This proposed study has been discussed at several meetings of the Nottingham Maternity Research Network (PPI network) to assess acceptability. PPI group who highlighted that anaemia often treated as ‘routine’ and not seen as significant; a need for anaemia to be resolved prior to labour due to the challenges of treating anaemia after birth; and the need to understand the follow up of the outcomes of anaemia. The PPI group will be consulted during the dissemination process and whether posters and other materials are required.

16. STATISTICS AND DATA ANALYSIS

1. Primary outcome measure

The proportion of pregnant women who meet the criteria for a clinical response, defined as an increase in haemoglobin concentration of 10g/l, 2-4 weeks after the onset of iron therapy.

2. Other Outcomes

1. The change in frequency and severity of symptoms associated with anaemia in pregnancy using a diary. (As assessed by our pre-piloted version of a published pregnancy symptomatology tool (Foxcroft 2013).
2. The frequency and severity of side effects induced by iron therapy to treat anaemia using a diary. (As assessed by a published tolerability tool (Peirera 2014), undertaken alongside the pregnancy symptomatology questionnaire)
3. A measurement of compliance with currently recommended oral iron regimens prescribed in pregnancy. (As assessed by patient reported compliance using a diary and the need for dose adjustments in line with BSCH guidelines)
4. To document the longitudinal changes in haemoglobin, red cell indices, iron, transferrin, ferritin, CRP, during a treatment course of oral iron.
5. To investigate the changes in research biomarkers, such as serum hepcidin, and whether they can predict the response to oral iron.

3. Statistical Analysis and Overview Analysis Plan

We aim to recruit approximately 120 patients into this study split equally between the three chosen time phases of pregnancy and the puerperium. This number divided between the cohorts represents a pragmatic decision based on estimated numbers of anaemic women seen in the antenatal period in Wolverhampton, and to support exploratory analyses to meet the objectives.

Data will be collected on paper Case Report Forms (CRFs), which will bear no patient identifiable data. Each patient will receive a unique CRF number for identification. Data will be stored using a system that complies with the requirements of the FDA CFR part 21 using a secure hospital server in accordance with the UK Data Protection Act 1998 and transferred onto statistical software for analysis.

Physiological recovery from anaemia will be defined in this study as in increase in Hb by $\geq$10 g/L by 2-4 weeks and these patients will be classed as 'responders'. Differences between responders and

non-responders will be explored by comparing baseline demographic data, haematological, biochemical and iron parameters. Data which do not follow a parametric distribution will be transformed. Geometric means will be calculated for logarithmically transformed data and mean values compared using the t-test. Statistical significance was defined as p value <0.05.

The primary outcome will be a response; an increase in the haemoglobin concentration of ≥10g/L, to iron treatment. This will be described as a percentage for the group as a whole and by each cohort, early pregnancy, late pregnancy and post-delivery.

Quantitative variables will be described using percentages and exact binomial 95% confidence intervals, continuous variables using median and interquartile range (IQR).

Logistic regression may be used to investigate associations of maternal anaemia and responses to treatment, as a binary outcome for the primary outcome.
Data from the study questionnaire and participants' diary will be analysed using descriptive statistical analysis.

Statistical analyses will be performed using statistical analysis software (SAS/STAT, Version 9 of the SAS System for Windows, SAS Institute, Inc., Cary, NC).

17. DATA MANAGEMENT

1. Participant Confidentiality and Data Access

Individual participant data for the TIAP study will be captured on a Case Report Form (CRF). Only the data required by the protocol will be captured in the CRF. All paper documents including the CRFs will be stored securely and only be accessible by study staff and authorised personnel. Electronic data will be stored on secure servers with secure back up arrangements. The servers, CRFs, and other data stores will be held in secure research office at RWT. Only clinical care teams will have access to patient identifiable data.

The CI/PI and delegated team at site will keep a record of all participating patients with sufficient information to link records, including CRFs, hospital notes and samples, and all original signed informed consent forms.

The study will comply with the Data Protection Act, which requires data to be anonymised before transferring to CRF and transferring outside clinical care team. Any electronic data processed off the hospital site will have been anonymised before leaving the site and stored in a password-protected system.

Data will be collected by staff trained in high quality handling and secure procedures. All other information and data will be stored electronically. The security of the database is maintained by the following principles:

- Access to clinical and personal data in the database will be limited to the local care team. Access will be secure and password protected. The research individuals will maintain confidentiality.
- The number of staff having access to all fields of the database will be limited.
- Direct access will be granted to authorised representatives from the Sponsor (i.e. host institution) and the regulatory authorities to permit study related monitoring, audits and inspections in line with participant consent. Responsible members of the University of Oxford, the Royal Wolverhampton NHS Trust Department of Research and Development or the University of Wolverhampton may be given access to data for monitoring and/or audit of the study to ensure the study is complying with regulations.

2. Archiving

Archiving will be authorised by the Sponsor following submission of the end of the study report. All study documentation will be archived for 5 years after completion of the study as per study Sponsor's policy and procedures. Destruction of these documents after the 5 years point will require authorisation from the Sponsor.

The site must keep the signed Informed Consent forms, all study documentation, and source documents collected during the study in a secure location (e.g. locked filing cabinets in a room with restricted access).

All data must be accessible to the competent authorities and the Sponsor with suitable notice for inspection. In addition, the Investigator must not discard or destroy any study specific materials unless otherwise instructed by the Sponsor.

18. DATA MONITORING AND INSPECTION

A Study Management Group (SMG) comprising the Chief Investigator, other lead investigators, and members of the research team. The TMG will be responsible for the day to day running and management of the study. It will meet at least four times a year, more often during set up and close down phases of the study. At least one face to face meeting will be held each year.

The safety data will be reviewed periodically by a nominated agent of the sponsor and discussed with sponsor to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis.

The host R&D department reserve the right to conduct site audits, either as part of its on-going audit programme, or in response to adverse observations occured.

19. PUBLICATION POLICY

- Reporting will be in compliance with CONSORT recommendations.
- The CI will approve any publications arising from this study
- No data may be made public before publication and never without agreement from the CI and Sponsor.
- Funder will be acknowledged in all publications/presentations.
- The final results of the study will be submitted to a peer-reviewed journal for publication. Authors will be the CI, the co-investigators and other members of the research team.
- A study identifier will be included on all presentations and publications. (ISRCTNxxxxx)
- For all papers or posters/presentations, we will use the International Committee of Medical Journal Editors of definitions of Authorship and the Contributorship (http://www.icmje.org/ethical_1author.html).

20. PROTOCOL AMENDMENTS

Revision History:

Protocol

Version	Author	Date	Reason for revision
1.2	Various	04/05/2018	Administrative changes

21. **REFERENCES:**

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