Anaemia and Iron Status Assessment in Maternal Health: From Local Guidelines to Global Context

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For my parents & their parents.

Abstract

Iron deficiency is the leading cause of anaemia globally and disproportionately affects women of reproductive age, particularly during and after pregnancy. Despite widespread screening programmes, iron supplementation strategies, and sustained international policy efforts, the burden of maternal anaemia and iron deficiency remains persistently high in many settings. This persistence may partly reflect ongoing challenges in assessing and monitoring iron deficiency and anaemia during pregnancy and the postpartum period. Although biomarkers such as haemoglobin, ferritin, and transferrin saturation are widely used in clinical practice and research, uncertainty remains regarding their interpretation in maternal health. Pregnancy-related physiological adaptations alter iron metabolism and biomarker behaviour across gestation, while different biomarkers reflect distinct aspects of iron status and may not provide equivalent information.

This thesis examines the use of iron status biomarkers to assess and monitor iron deficiency and anaemia in maternal health.

Chapter 2 evaluates variation in recommendations for the assessment, treatment, and monitoring of iron deficiency and anaemia across 49 NHS maternity guidelines in the United Kingdom. While haemoglobin thresholds for anaemia were broadly consistent, substantial variation was observed in ferritin thresholds, recommendations for intravenous iron, and approaches to monitoring treatment response.

Chapter 3 presents a systematic review of 31 randomised controlled trials of oral iron therapy during pregnancy and examines how haematological response has been assessed in clinical research. Haemoglobin was measured universally but rarely in isolation, with considerable variation in biomarker selection and assessment timing. Monitoring approaches also differed across healthcare settings.

Chapter 4 presents a secondary analysis of the PANDA trial and investigates the behaviour of key iron biomarkers under different prophylactic oral iron supplementation regimens during pregnancy. Ferritin, transferrin saturation, and hepcidin declined across gestation, while soluble transferrin

receptor increased. Observed biomarker trajectories appeared to be strongly influenced by physiological adaptation during pregnancy, complicating the interpretation of supplementation effects.

Chapter 5 examines postpartum anaemia and iron deficiency within a nested sub-study of the SMARThealth Pregnancy 2 trial in rural India. No clear differences in anaemia prevalence were observed between intervention and control groups. Anaemia remained common at 12 months postpartum and was frequently multifactorial, with overlapping iron, folate, and vitamin B12 deficiencies. Estimates of iron deficiency varied substantially depending on the biomarker definition used, while agreement between ferritin- and soluble transferrin receptor-based classifications was limited. Capillary point-of-care haemoglobin substantially over-classified anaemia compared with laboratory-based venous assessment, highlighting the influence of measurement approach on estimates of both anaemia prevalence and iron deficiency.

Across diverse maternal health settings, commonly used biomarkers reflected different aspects of iron biology and frequently produced different classifications of iron deficiency. Pregnancy physiology, inflammation, and measurement approach further influenced biomarker interpretation, contributing to variation in how iron deficiency and anaemia were defined, diagnosed, and monitored. Together, these findings show that iron status cannot be evaluated independently of the physiological and clinical context in which it is measured, and support more physiologically informed and context-sensitive approaches to the diagnosis and monitoring of iron deficiency and anaemia.

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And to Benny, for following me to this city, for making it your own, and for constantly reminding me that there is joy and adventure to be found in almost everything.

Statement of Authorship

I declare that this thesis is a presentation of my own original research and has not been submitted, either wholly or substantially, for any other degree or qualification at this or any other institution.

Where chapters include material arising from collaborative research or published or submitted manuscripts, the nature and extent of my contribution are described in the *'Statement of Contribution'* provided at the start of each chapter.

I confirm that this thesis does not contain any material previously published or written by another person except where duly acknowledged in the text.

Signed: 

Sarah Madeleine Haynes

Date: 25 June 2026

Statement on Use of Artificial Intelligence

In accordance with University of Oxford guidance on the responsible use of AI in research and academic work, generative artificial intelligence (AI) tools were used during the preparation of this thesis to support language refinement, proofreading, formatting assistance, and technical troubleshooting of code. Tools used included ChatGPT (OpenAI) and Claude (Anthropic). As these tools were used iteratively throughout the thesis preparation process, specific model versions are not reported.

All substantive academic content, including study design, data collection, statistical analysis, interpretation of findings, and scientific conclusions, was conceived, conducted, and verified by the author.

The author takes full responsibility for the content of this thesis.

Signed: 

Sarah Madeleine Haynes

Date: 25 June 2026

Publications and Presentations Related to this Thesis

Publications

- Haynes S, Armitage AE, Roy N, Morovat A, Drakesmith H, Churchill D, Stanworth SJ. *Impact of Oral Iron Dosing Regimens on Iron Biomarkers in Pregnancy: Insights from the PANDA Trial.* Blood Advances. 2026; bloodadvances.2026019740. doi:10.1182/bloodadvances.2026019740. (This work forms the basis of Chapter 4.)
- Haynes S, Balboula I, Collins P, Parry-Smith W, Fye H, Elsmore A, Collis R, de Lloyd L, Churchill D, Bell SF, Stanworth SJ. *Variation in Antenatal Anaemia Guidelines Across the NHS in the United Kingdom: A Cross-Sectional Study.* British Journal of Haematology. Published online 20 August 2026. doi:10.1111/bjh.70780. (This work forms the basis of Chapter 2)

Submitted Manuscripts

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Related Publications

- Maynard S, Wright Drakesmith C, Smith M, Haynes S, Katumba A, Maharajan V, Maurer K, Erone I, Shah A, Nigam G, Roy N, Bankhead C, Stanworth S. *Patterns of testing and the high burden of iron deficiency anaemia in cirrhosis: a national cohort study.* Blood Red Cells & Iron. 2026. doi:10.1016/j.brci.2026.100070.

- Smith MC, Wright-Drakesmith C, Haynes SM, Suzanne P, Shah A, Roy NB, Lee JJ, Maurer K, Stanworth SJ, Bankhead C. Prevalence and patterns of testing for anaemia in primary care in England: a cohort study using an electronic health records database. *British Journal of General Practice*. 2025;75(753):e232–e240.

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List of Abbreviations

Abbreviation	Definition
AGP	Alpha-1-acid glycoprotein
AI	Artificial intelligence
AIIMS	All India Institute of Medical Sciences
AMB	Anaemia Mukt Bharat
ANC	Antenatal care
ANOVA	Analysis of variance
aOR	Adjusted odds ratio
ASHA	Accredited Social Health Activist
BMI	Body mass index
BNF	British National Formulary
BRINDA	Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia
BSH	British Society for Haematology
B12	Vitamin B12
CENTRAL	Cochrane Central Register of Controlled Trials
CI	Confidence interval
COMET	Core Outcome Measures in Effectiveness Trials
CRP	C-reactive protein
DRG	DRG Instruments GmbH (assay manufacturer)
EIA	Enzyme immunosorbent assay
ELISA	Enzyme-linked immunosorbent assay
FBC	Full blood count
FIGO	International Federation of Gynecology and Obstetrics
Hb	Haemoglobin
HCT	Haematocrit
HIC	High-income country
HOW	Haematology in Obstetrics and Women's Health
ICC	Intraclass correlation coefficient
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDWA	Iron deficiency without anaemia
IFA	Iron and folic acid
IQR	Interquartile range
IV	Intravenous
LIC	Low-income country
LMIC	Low- and middle-income country
LMM	Linear mixed-effects model

LoA	Limits of agreement
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MeSH	Medical Subject Headings
NFHS-5	National Family Health Survey 5
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health and Care Research
OBS-UK	Obstetric Bleeding Study United Kingdom
OSF	Open Science Framework
PANDA	Primary prevention of maternal Anaemia to avoid preterm Delivery and other Adverse outcomes
PHC	Primary health centre
POC	Point-of-care
PPI	Patient and public involvement
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
RBC	Red blood cell
RCT	Randomised controlled trial
RCOG	Royal College of Obstetricians and Gynaecologists
RDW	Red cell distribution width
REC	Research Ethics Committee
REML	Restricted maximum likelihood
ROB-2	Risk of Bias 2
SD	Standard deviation
SHP2	SMARThealth Pregnancy 2
sTfR	Soluble transferrin receptor
SWiM	Synthesis Without Meta-analysis
TIBC	Total iron-binding capacity
TSAT	Transferrin saturation
UK	United Kingdom
UMIC	Upper-middle-income country
WHO	World Health Organization
WIMM	Weatherall Institute of Molecular Medicine

Note on Language

This thesis primarily uses the term “women” when referring to populations affected by iron deficiency, anaemia, pregnancy, childbirth, and the postpartum period, reflecting the terminology used in the cited literature, clinical guidelines, public health frameworks, and underlying research studies included in this work. The author recognises that not all people who experience pregnancy or related reproductive health conditions identify as women, and that transgender men and non-binary individuals may also experience the conditions discussed in this thesis.

Chapter 1 - Background and Rationale: The Global Challenge of Anaemia and Iron Deficiency in Maternal Health

Statement of Contribution

This chapter is my own work and has not been published elsewhere.

1.1 Chapter Overview

This chapter outlines the background and rationale for this thesis, which examines the use and interpretation of iron status and anaemia biomarkers in maternal health. It begins by describing the global burden of anaemia and iron deficiency, with a particular focus on women of reproductive age and pregnant women, before considering iron deficiency across its clinical spectrum and associated consequences.

Next, this chapter introduces the definitions of anaemia and iron deficiency, including current diagnostic thresholds and the uncertainty surrounding them. It describes iron metabolism and the biomarkers used to assess iron status, highlighting the limitations of these measures and their implications for diagnosis and monitoring. The impact of pregnancy physiology on iron metabolism and biomarker interpretation is then considered before reviewing current approaches to managing iron deficiency anaemia (IDA) in pregnancy and the international and local clinical guidelines that inform practice.

Finally, this chapter presents the thesis aim and objectives, outlines the structure of the subsequent chapters, and summarises research collaborations, patient and public involvement (PPI), and researcher positionality. The background presented in this chapter highlights several challenges in the assessment and monitoring of iron deficiency and anaemia in maternal health. While a range of iron status biomarkers are used across clinical practice and research, uncertainty remains regarding their application and interpretation during pregnancy and the postpartum period. These challenges provide the rationale for the studies presented in this thesis, which examine the use of iron status biomarkers across a range of maternal health settings and contexts.

1.2 Iron Deficiency Anaemia: A Global Burden

1.2.1 Anaemia as a Global Health Problem

Anaemia remains one of the most prevalent and persistent public health challenges worldwide. In 2021, an estimated 1.92 billion people were affected globally, with women of reproductive age, including pregnant women, most affected. Anaemia also disproportionately affects low- and

middle-income countries (LMICs) and is strongly associated with socioeconomic disadvantage more generally.[1] For these reasons, the prevalence of anaemia among women of reproductive age is widely used as an indicator of nutritional status and health system performance at the population level.[2,3]

Despite sustained global attention, progress towards reducing anaemia prevalence has been limited and inconsistent. While worldwide prevalence has declined modestly since 1990, the reductions have been substantially smaller among women and girls than among men and boys. During the reproductive years, anaemia affects approximately one-third of women compared with one in ten men, and the absolute number of affected women continues to rise with population growth.[4]

In 2012, the 65th World Health Assembly set an ambitious global nutrition target to halve the prevalence of anaemia in women of reproductive age, then estimated to be 27.6%, by 2025.[2] Despite efforts towards this goal, recent World Health Organization (WHO) estimates indicate that 35.5% of pregnant women and 30.7% of non-pregnant women of reproductive age were anaemic in 2023, with the highest burden concentrated in sub-Saharan Africa and South Asia. Recognising this lack of progress, the WHO extended the target deadline of a 50% reduction to 2030.[3] However, the 2025 Lancet Haematology Commission on anaemia has concluded that even this revised target is unlikely to be achieved under current strategies, and has called for more targeted research into the complex, context-specific causes and consequences of anaemia in women.[5]

Anaemia is a heterogeneous condition with many underlying causes that vary by geography, age, and sex. Dietary iron deficiency is the leading contributor globally, accounting for up to 66% of the total burden of anaemia, particularly among women of reproductive age.[4,6] Other causes include infectious diseases, inherited haemoglobin disorders, chronic disease, and deficiencies of other micronutrients such as vitamin B12 and folate.[4] In many low- and middle-income settings, anaemia reflects the convergence of multiple causes,[7] whereas in high-income settings, primary iron deficiency remains the dominant driver in women of reproductive age.[8,9] This thesis focuses

primarily on iron deficiency, both as an independent clinical entity and as the leading cause of anaemia in women globally, while acknowledging the multifactorial nature of anaemia, particularly in the final empirical chapter, which will examine causes of postpartum anaemia in rural India.

1.2.2 Iron Deficiency: The Leading Cause of Anaemia

Iron is essential for oxygen transport, energy metabolism, DNA synthesis, and immune function. Iron deficiency arises when the iron supply is insufficient to meet physiological demands and is the most common micronutrient deficiency worldwide, affecting more than two billion people.[10] Iron deficiency is particularly common in women of reproductive age due to cumulative iron losses across the life course (see Figure 1.1). Menstrual blood loss, especially in the context of heavy menstrual bleeding, often results in iron loss that exceeds dietary iron intake.[9,11] Pregnancy imposes substantial additional iron demands to support the expansion of maternal red cell mass, placental development, and foetal growth.[12] Peripartum blood loss may further deplete iron stores.[9,13] Short inter-pregnancy intervals may prevent full recovery of iron stores, contributing to cumulative depletion over time.[14,15] These accumulating demands are one rationale for the recommended inter-pregnancy intervals of 18–24 months and are particularly relevant in low- and middle-income settings, where dietary iron intake may be insufficient to replenish stores between pregnancies.[16,17]

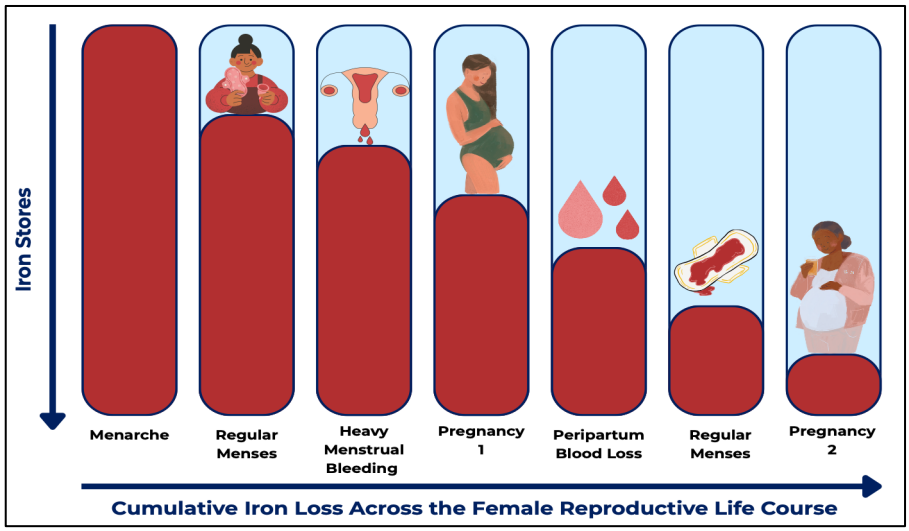


Figure 1.1 Cumulative iron losses across the female reproductive life course
Each stage of the reproductive years imposes iron demands that may exceed dietary intake, leading to progressive depletion. Created with Canva.

Iron deficiency exists across a spectrum ranging from iron deficiency without anaemia (IDWA) to IDA.[18] IDWA is characterised by depleted iron stores despite preserved haemoglobin concentrations, whereas IDA represents a later stage of deficiency in which iron availability becomes insufficient to support erythropoiesis and haemoglobin concentrations fall below diagnostic thresholds. As iron deficiency progresses, changes occur across multiple biomarkers of iron status and erythropoiesis before anaemia develops.[10,19] Figure 1.1 illustrates this progression from iron repletion to IDA. The biomarkers shown in the figure are introduced here to illustrate the spectrum of iron deficiency and are discussed in greater detail in Section 1.3.

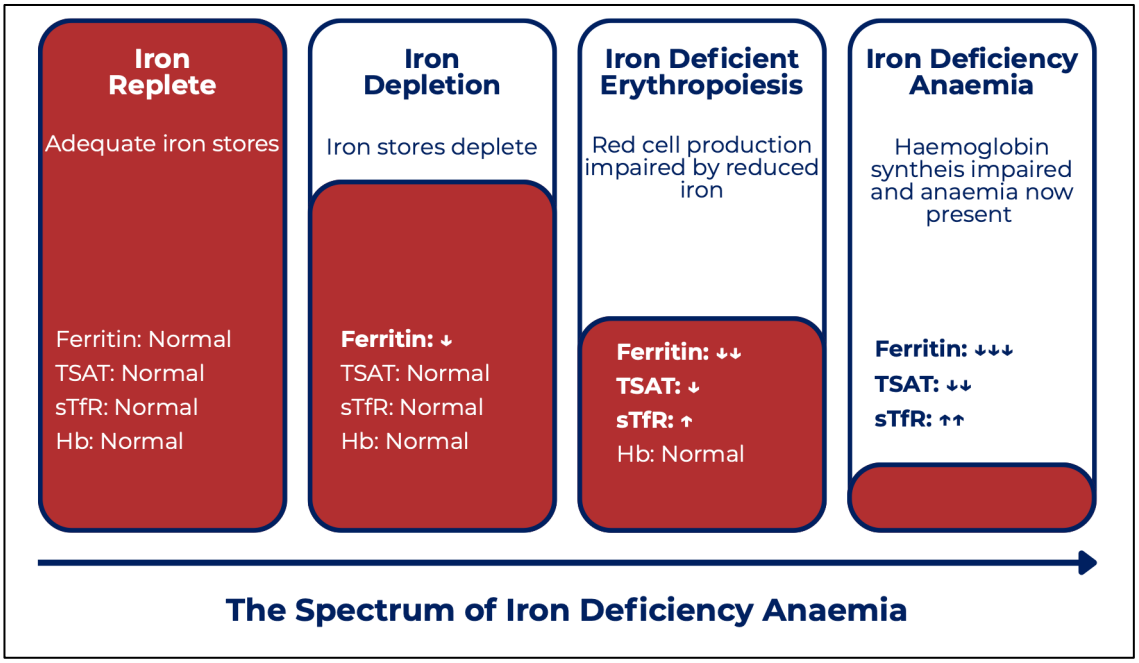


Figure 1.2 The spectrum of iron deficiency, from replete iron stores to iron deficiency anaemia
Serum ferritin declines earliest, followed by a fall in transferrin saturation (TSAT) and a rise in soluble transferrin receptor (sTfR); haemoglobin (Hb) and mean corpuscular volume (MCV) decline only at later stages.[10,19] Created with Canva.

There is increasing recognition that IDWA represents a clinically meaningful condition, rather than simply a precursor to anaemia.[18] This perspective is supported by physiological evidence suggesting that many of the consequences of iron deficiency arise before haemoglobin declines, reflecting the progressive redistribution of iron from tissues to maintain erythropoiesis as stores fall.[10,20] Evidence from randomised trials suggests that iron supplementation in non-anaemic women with low ferritin improves symptoms such as fatigue and exercise tolerance.[21,22]

IDA affects approximately 30-60% of pregnancies globally.[23] The burden of IDWA in pregnancy is less well characterised but appears to increase across gestation, with studies showing most women are diagnosed in later pregnancy,[24] with as many as 75% of women iron-deficient by the third trimester.[25] However, estimates of iron deficiency prevalence vary considerably according to the biomarkers and thresholds used to define deficiency. As routine antenatal screening frequently relies on haemoglobin measurement, women with IDWA may not be identified.[9] Consequently, the true burden of iron deficiency during pregnancy may be underestimated. This issue highlights the importance of biomarker selection and interpretation, which are discussed later in this chapter.

1.2.3 Clinical Consequences of Iron Deficiency and Anaemia in Maternal Health

Although IDWA and IDA lie along the same spectrum of iron depletion, the evidence linking them to adverse maternal and perinatal outcomes differs substantially. Most adverse outcomes associated with poor iron status have been demonstrated in women with established anaemia, whereas evidence linking IDWA to equivalent harm remains less consistent. This distinction is important when interpreting the literature, considering which women may benefit from intervention, and evaluating the outcomes used to assess treatment effectiveness.

Maternal anaemia: associations with adverse outcomes

IDA reduces oxygen-carrying capacity and may impair tissue oxygen delivery during a period of increased maternal and foetal physiological demand. In pregnancy, IDA has been consistently associated with increased risks of preterm labour, postpartum haemorrhage, hypertensive disorders of pregnancy, and higher levels of care admission.[23,26] Meta-analyses indicate that maternal anaemia is associated with a risk of preterm delivery approximately 1.3 to 2.0 times that of non-anaemic women, with greater risk observed when anaemia is moderate to severe or present in early gestation.[27] Intrauterine growth restriction and low birthweight are also more frequently reported among infants born to mothers with anaemia, potentially reflecting impaired placental oxygen delivery and altered nutrient transport.[28,29]

Gestational timing appears to influence the degree of risk. Anaemia arising in the first trimester has been more strongly associated with adverse outcomes, including stillbirth, than anaemia developing later in pregnancy, and evidence suggests that correction of haemoglobin in the second half of pregnancy may not fully reverse risks established earlier in gestation.[30–32] Analysis of data from India's fifth National Family Health Survey (NFHS-5), a nationally representative survey conducted between 2019 and 2021, identified an association between maternal anaemia and stillbirth, reinforcing the clinical importance of early identification.[33]

The consequences also extend beyond delivery. Women entering the postnatal period with established anaemia may experience impaired recovery, greater fatigue, and an increased risk of postnatal depression, mediated in part through iron's role in neurotransmitter synthesis and myelin production.[34,35] In the longer term, both iron deficiency and anaemia during pregnancy have been associated with impaired neurodevelopmental outcomes in offspring. The association is more consistently demonstrated for maternal anaemia,[36,37] while emerging prospective data suggest that iron deficiency in the absence of anaemia may also affect neonatal iron stores and neurodevelopment independently of haemoglobin level.[38] These effects are estimated to be greatest in low-resource settings where anaemia is more prevalent and frequently coexists with other nutritional deficiencies.[37,39]

Iron deficiency without anaemia: a less certain evidence base

IDWA is common, affecting a substantial proportion of pregnant women, particularly in the third trimester,[40] and has been associated with symptoms including fatigue, reduced exercise capacity, and restless leg syndrome despite preserved haemoglobin concentrations.[10,41] However, the relationship between IDWA and adverse obstetric and perinatal outcomes remains incompletely understood.

Prospective cohort data suggest that IDWA in early pregnancy is associated with an increased risk of subsequent progression to IDA and with lower neonatal iron stores, even when maternal haemoglobin is preserved.[30,40] Some studies have also linked low first-trimester ferritin

concentrations to modest increases in the risk of preterm birth, although effect sizes are generally smaller and confidence intervals wider than those reported for IDA.[31,32]

Interpretation of this literature is complicated by substantial methodological heterogeneity. Studies vary considerably in their definitions of iron deficiency, gestational timing of assessment, and degree of confounder adjustment.[18,42] In addition, many studies examining adverse pregnancy outcomes have used haemoglobin concentration or anaemia status as the primary exposure, rather than directly assessing iron deficiency using iron status biomarkers.[27,29] Consequently, it is often difficult to determine whether observed associations reflect the effects of iron deficiency itself or the effects of anaemia. Studies specifically evaluating iron deficiency in the absence of anaemia are fewer in number and have reported less consistent findings.[18]

Excess iron and high haemoglobin: the U-shaped curve

The clinical focus in pregnancy has understandably been directed towards the consequences of deficiency, but accumulating observational and mechanistic evidence suggests that iron status may carry risk at both ends of the spectrum. This pattern is sometimes described as a U-shaped relationship between iron status and adverse outcomes, reflecting two distinct phenomena: the risk associated with low iron status and anaemia, and the potential risks associated with high haemoglobin concentrations or excess iron exposure in women who are already iron-replete.[42,43]

Haemoglobin concentrations at the upper end of the pregnancy distribution have been associated with impaired placental blood flow and an increased risk of intrauterine growth restriction, low birthweight, and hypertensive disorders of pregnancy.[28,29] These associations are thought to reflect reduced plasma volume expansion and consequent impairment of uteroplacental perfusion rather than iron excess.

Separately, excess iron exposure in women with adequate iron status may carry its own risks. In a randomised controlled trial (RCT) restricted to non-anaemic pregnant women with haemoglobin concentrations of at least 132 g/L, daily supplementation with 50 mg elemental iron was associated with significantly higher rates of small-for-gestational-age infants and hypertensive

disorders compared with placebo, despite producing higher third-trimester haemoglobin concentrations.[44] Similarly, an observational study of more than 1,000 non-anaemic pregnant women in South India found that those in the highest third of supplemental iron intake had lower birthweight offspring and a greater risk of term low birthweight than those in the lowest third.[45] Although the mechanisms remain incompletely understood, excess iron may contribute to oxidative stress and placental dysfunction, providing biological plausibility for these observations.[46–48]

Although these findings raise important questions regarding iron supplementation in women with adequate iron status, the evidence remains considerably less developed than that supporting the harms of iron deficiency. Nonetheless, they have contributed to the ongoing debate about universal supplementation policies and the importance of distinguishing supplementation strategies for women with established deficiency from those for non-anaemic women with replete iron stores.

This clinical uncertainty is compounded by diagnostic uncertainty. The recognition of IDWA as a distinct clinical entity depends on reliable, consistently applied diagnostic criteria. Addressing this diagnostic uncertainty is therefore a prerequisite for generating the evidence needed to determine whether, and in whom, asymptomatic IDWA in pregnancy warrants treatment. The following section examines how anaemia and iron deficiency are currently defined, and the limitations of the thresholds used to identify them.

1.2.4 Definitions and Diagnostic Thresholds

Anaemia is defined in clinical practice using haemoglobin-based thresholds that vary by age, sex, and pregnancy status. For non-pregnant women of reproductive age, the WHO defines anaemia as haemoglobin <120 g/L.[49] In pregnancy, the 2024 WHO guideline defines anaemia using trimester-specific thresholds: <110 g/L in the first trimester, <105 g/L in the second trimester, and <110 g/L in the third trimester.[49] However, thresholds continue to vary across international and

national guidelines, with some applying <105 g/L across both the second and third trimesters, while others use a single threshold of <110 g/L across gestation.[49–53]

Iron deficiency is most commonly assessed using serum ferritin as a marker of iron stores, although the threshold used to define deficiency varies. The WHO defines iron deficiency as ferritin <15 μg/L in the general population, with higher thresholds recommended in the presence of inflammation.[54] Elsewhere, particularly in high-resource settings, a threshold of <30 μg/L is now used more commonly, following recent research in non-pregnant populations demonstrating that lower cutoffs may not detect clinically relevant iron depletion.[55–57] Ferritin concentrations may also vary modestly according to the analytical platform used, as differences in assay calibration and methodology can contribute to between-study and between-laboratory variation in reported values.[58,59]

To better understand current definitions of anaemia and iron deficiency, particularly in pregnancy, it is imperative to examine how they were developed. Diagnostic thresholds are derived from population-based reference distributions and clinical consensus and are therefore shaped by the populations studied, the methods used, and the assumptions underlying their interpretation.[60]

The haemoglobin thresholds currently in use, including those adopted by the WHO, were established in the 1960s and 1980s based on limited evidence.[49] Analyses of the evidence base underpinning these thresholds have highlighted limitations, including reliance on studies conducted predominantly in European and North American populations, with limited representation of diverse populations and age groups.[61] In pregnancy, haemoglobin cutoffs have largely been informed by expert consensus and observational data rather than clearly defined physiological inflexion points or associations with adverse outcomes.[49,61]

Recent efforts have sought to establish a stronger empirical basis for defining low haemoglobin concentrations during pregnancy. The INTERGROWTH-21st Project developed international gestational age-specific haemoglobin centiles using data from healthy women with uncomplicated pregnancies across eight geographically diverse countries, including the United Kingdom and

India.[62] Rather than proposing diagnostic thresholds for anaemia, INTERGROWTH generated a prescriptive reference standard, identifying values below the third centile (approximately 94–102 g/L, depending on gestational age) as unusually low. These values are lower than currently recommended pregnancy haemoglobin cut-offs and, if adopted, would substantially reduce the proportion of women classified as anaemic. However, INTERGROWTH was not designed to determine the haemoglobin concentrations associated with adverse maternal or perinatal outcomes. Consequently, uncertainty remains regarding the most clinically meaningful thresholds for defining anaemia during pregnancy. The Redefining Maternal Anaemia in Pregnancy and Postpartum (ReMAPP) study is addressing this question by seeking to derive outcome-based haemoglobin thresholds for pregnant and postpartum women, reflecting growing recognition of the limitations of existing definitions.[63]

Similar challenges arise in defining reference intervals for ferritin. Laboratory reference intervals are typically derived from the statistical distribution of a sampled population, with the lower limit of normal defined by the lowest 2.5% of values.[60] This approach assumes that the reference population represents optimal physiological health, an assumption that may not hold for iron status in women of reproductive age, among whom iron deficiency is highly prevalent. A systematic review of the origins of ferritin reference intervals found that many reference populations included individuals with undiagnosed iron deficiency, particularly menstruating women.[64] When iron deficiency is endemic within the reference population, the derived thresholds become artificially lowered and underestimate the true burden of deficiency. This has been demonstrated in bone marrow studies in women of reproductive age, which have demonstrated that a ferritin threshold of <15 µg/L identifies absent iron stores with only moderate sensitivity and may miss up to 50% of individuals with '*true iron deficiency*'.[10,65,66] Emerging evidence suggests that physiologically meaningful ferritin thresholds may be even higher, with findings suggesting iron homeostasis may not fully normalise until ferritin approaches approximately 50 µg/L.[67,68] Although these newer ferritin thresholds are being adopted for non-pregnant and pregnant individuals, this research has primarily been conducted on non-pregnant populations.

There is, however, growing interest in establishing physiologically informed, rather than statistically derived, thresholds for both non-pregnant and pregnant populations, with some proposing trimester-based physiologically informed ferritin thresholds in pregnancy.[67–69]

In pregnancy, where physiological changes in plasma volume and iron metabolism further complicate interpretation, these limitations are particularly important to consider. The following sections examine iron metabolism and the biomarkers used to assess iron status before considering how pregnancy physiology influences their interpretation.

1.3 Iron Metabolism and Biomarkers of Iron Status

1.3.1 Iron Homeostasis

Iron metabolism is tightly regulated to maintain systemic iron homeostasis. Although iron is essential for cellular function, excess iron is potentially toxic, and the body lacks a mechanism for active iron excretion. As a result, iron balance is tightly controlled, primarily through regulation of intestinal absorption and release of iron from stores.[70]

Hepcidin, a peptide hormone produced by the liver, is the central regulator of this process. It acts by binding to ferroportin, the sole cellular iron exporter, thereby preventing iron release from the cell into the circulation. This reduces iron absorption from intestinal enterocytes and limits the release of iron from macrophages and hepatic stores, thereby controlling circulating iron levels.[71]

Hepcidin production is regulated by four key physiological signals: iron status, inflammation, erythropoietic demand, and hypoxia. Hepcidin is upregulated in states of iron sufficiency and in response to inflammation, particularly through interleukin-6–mediated pathways, thereby reducing iron availability and leading to functional iron deficiency. In contrast, hepcidin is suppressed in iron deficiency, hypoxia, and increased erythropoietic demand, allowing greater iron absorption and mobilisation from stores to support red blood cell (RBC) production. Through this coordinated system, iron availability is dynamically matched to physiological need while limiting toxicity and restricting iron availability to pathogens during infection.[70–72] Although hepcidin is not yet routinely measured in clinical practice, it provides important mechanistic

insight into iron regulation and is included in the biomarker panels in Chapters 4 and 5 of this thesis.

1.3.2 Functional Compartments of Iron Metabolism

Understanding iron metabolism is essential for interpreting the biomarkers used to diagnose and monitor iron deficiency. As outlined in the previous section, current definitions rely heavily on haemoglobin and ferritin levels; however, a range of additional biomarkers is available to assess iron status. Each biomarker reflects a different stage of iron metabolism, and no single marker captures the full complexity of an individual's iron status.

Iron metabolism involves four main stages: absorption, transportation, storage, and utilisation. Biomarkers of iron status reflect each of these stages (see Figure 1.3).[73] Dietary iron is absorbed in the duodenum and proximal jejunum, then transported in serum bound to transferrin. It is subsequently delivered to the liver and spleen for storage or to the bone marrow for storage or erythropoiesis. Bone marrow aspirate is considered the gold standard for measurement of iron stores; however, it is invasive and provides limited information about circulating iron availability.[70,73] Therefore, surrogate biomarkers of iron status are used in both clinical practice and research to provide a non-invasive assessment of iron metabolism at its various stages.

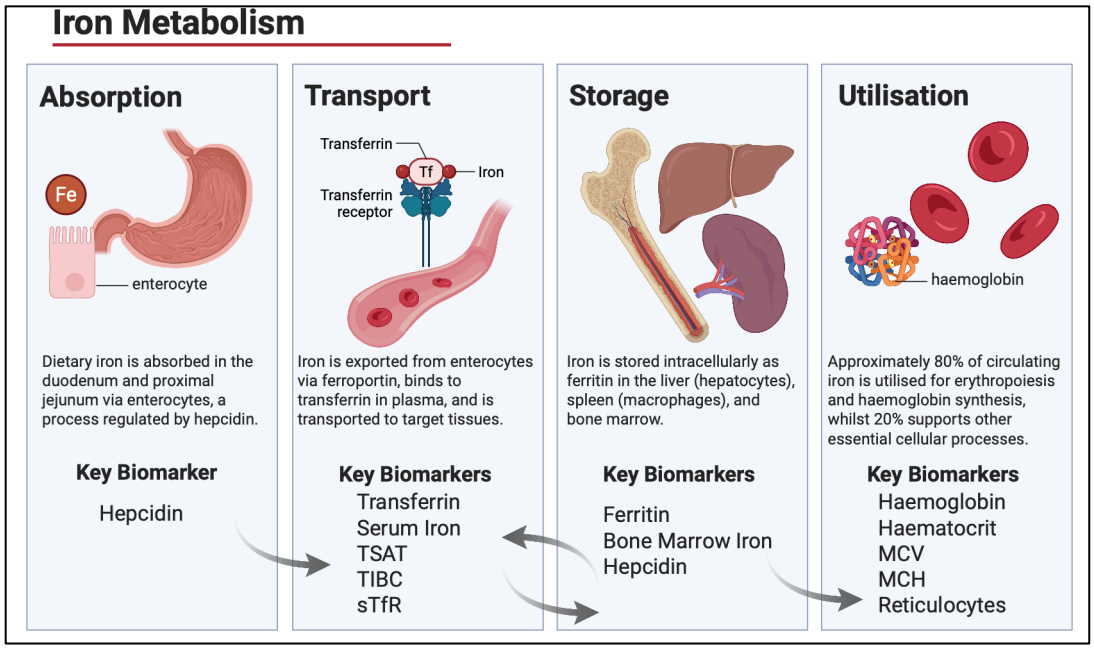


Figure 1.3 Iron metabolism: the four functional compartments
Key biomarkers at each stage reflect iron status and erythropoietic demand.[70,73] Created in BioRender (Haynes, 2026; https://BioRender.com/gayanu4).

1.3.3 Iron Status Biomarkers

Serum ferritin is widely used as a marker of iron stores and generally reflects intracellular storage iron, predominantly in the liver, spleen, and bone marrow. Ferritin is an iron-storage protein composed of 24 heavy (H) and light (L) subunits that form a hollow shell around an iron-containing core (see Figure 1.4). The H subunits facilitate iron oxidation, while the L subunits support nucleation and mineralisation of iron within the core. Ferritin therefore functions as a reservoir for cellular iron while limiting the accumulation of potentially reactive free iron. Importantly, the amount of iron contained within individual ferritin molecules can vary.

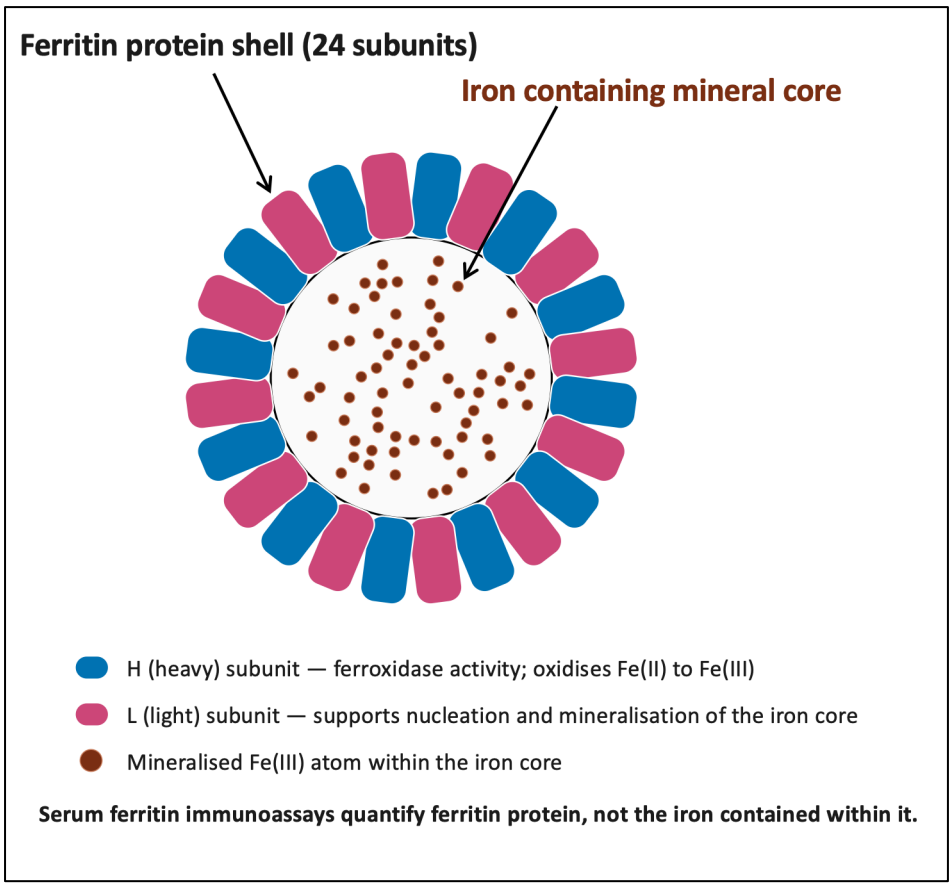


Figure 1.4 Schematic structure of ferritin and its iron-containing core
Ferritin comprises a 24-subunit protein shell surrounding an iron-containing mineral core. The amount of iron stored within individual ferritin molecules can vary; serum ferritin immunoassays quantify ferritin protein rather than the iron contained within it.[72,74]

Serum ferritin immunoassays quantify circulating ferritin protein rather than directly measuring the iron contained within each molecule.[72,74] Despite this limitation, serum ferritin remains a useful surrogate marker of iron stores under steady-state conditions. It is minimally invasive,

relatively inexpensive, and widely available, making it the standard first-line marker for iron deficiency across most clinical guidelines. Serum ferritin is, however, an acute-phase reactant, meaning its concentration increases in response to inflammation, infection, or tissue injury, independently of iron status. While low ferritin is highly specific for iron deficiency, normal or elevated ferritin does not exclude deficiency in the presence of inflammation. This can mask underlying iron deficiency and lead to misclassification, particularly in populations with a high burden of inflammation.[56,75] For this reason, higher ferritin thresholds have been proposed in the presence of inflammation;[54,76] however, these thresholds have not yet been robustly validated, and their application in pregnancy remains particularly uncertain.

Serum iron levels reflect circulating iron bound to transferrin, which is the primary iron transport protein in the plasma. Serum iron is reduced in iron deficiency; however, it exhibits substantial diurnal variation and is influenced by recent dietary intake, limiting its utility as a standalone measure.[10]

Transferrin, synthesised in the liver, binds and transports iron to tissues. Transferrin concentrations increase in iron deficiency to enhance iron transport but decrease in the presence of inflammation as a negative acute-phase reactant. In isolation, transferrin provides limited clinical information, as it reflects iron transport capacity rather than iron availability and is influenced by hepatic protein synthesis and inflammatory states; therefore, it is typically interpreted alongside serum iron as transferrin saturation (TSAT).[77]

TSAT represents the proportion of transferrin that is bound to iron and is calculated as the ratio of serum iron to total iron-binding capacity (TIBC). TIBC reflects transferrin concentration and the blood's iron-binding capacity. TSAT therefore provides an estimate of circulating iron immediately available for tissue uptake and erythropoiesis. In iron deficiency, TSAT is typically low due to reduced circulating iron and increased transferrin.[74]

In the setting of inflammation, the interpretation of common iron biomarkers becomes more complex. Hepcidin-mediated iron sequestration lowers serum iron, whilst transferrin decreases as

a negative acute-phase reactant. Although both components of TSAT decline, the fall is typically more pronounced for serum iron, resulting in a net reduction in TSAT that reflects reduced circulating iron availability. However, the reduction of TSAT can be less pronounced than in iron deficiency without inflammation, making it difficult to distinguish between true iron deficiency and inflammation-related functional iron deficiency.[78]

Soluble transferrin receptor (sTfR) is a circulating fragment of the membrane transferrin receptor, released into plasma following cleavage from the cell surface. It is predominantly derived from the cell surface of erythroblasts in the bone marrow and reflects erythropoietic/cellular iron demand, as well as early tissue-level iron deficiency. In iron deficiency, sTfR concentrations typically increase, reflecting upregulation of transferrin receptor expression to facilitate greater cellular iron uptake. Unlike ferritin and TSAT, sTfR is less influenced by inflammation and may therefore provide a more reliable indicator of iron status in inflammatory states. However, sTfR concentrations also increase with erythropoietic activity, complicating interpretation. As a result, elevated concentrations may not necessarily indicate iron deficiency alone, particularly in physiological states associated with increased erythropoiesis such as pregnancy.[74,79] Additionally, poor inter-assay standardisation limits comparability across platforms, as reference ranges and diagnostic thresholds vary between assays.[80]

The ferritin index (sTfR/$\log_{10}$ ferritin) has been proposed as a composite marker of functional iron deficiency, integrating measures of cellular iron demand and iron stores.[81] However, its clinical utility is constrained by the same issues of assay variability and lack of standardised thresholds, which limit interpretability and broader application.

1.3.4 Markers of erythropoiesis

Markers of erythropoiesis provide a functional assessment of iron availability by reflecting the adequacy of iron supply to the bone marrow for red blood cell production.[20] As discussed previously, iron deficiency exists along a spectrum, progressing from depletion of iron stores to impaired erythropoiesis and ultimately to the development of IDA. Biomarkers of erythropoiesis

therefore capture the later stages of this process, when iron availability becomes insufficient to support effective red cell production.

Haemoglobin remains the most widely used indicator of IDA and reflects the result of sustained iron-restricted erythropoiesis. It can be measured using laboratory-based venous samples or point-of-care (POC) devices, with venous samples considered the gold standard.[49] Haematocrit (HCT) measures red cell mass as a percentage of blood volume and decreases in anaemia. Red cell indices offer further insight into erythropoietic dynamics; MCV and mean corpuscular haemoglobin (MCH) decrease in iron deficiency, reflecting the production of smaller, hypochromic red cells, while red cell distribution width (RDW) increases due to greater heterogeneity in red cell size.[82]

Although these markers are routinely used in clinical practice, they reflect downstream consequences of iron deficiency and are therefore relatively insensitive to early changes in iron status. In addition, many of these parameters are influenced by factors other than iron availability, including inflammation, haemoglobinopathies, and deficiencies of other micronutrients.[82] As a result, while markers of erythropoiesis are essential for identifying and characterising anaemia, they provide limited information about earlier stages of iron deficiency and should be interpreted alongside biomarkers of iron stores and iron transport when assessing iron status.

In summary, no single biomarker provides a complete assessment of iron status. Instead, commonly used biomarkers reflect different aspects of iron metabolism and erythropoiesis, each with distinct biological and analytical limitations. These challenges become particularly relevant during pregnancy, when physiological adaptations affecting haematology and iron metabolism may alter biomarker concentrations independently of underlying iron status.

1.4 Pregnancy Physiology and the Diagnostic Challenge

Pregnancy introduces an additional challenge to the assessment of iron status. The physiological adaptations required to support maternal and foetal development alter both haematological parameters and iron metabolism throughout gestation, influencing many of the biomarkers used

to diagnose and monitor iron deficiency. Consequently, biomarker values measured during pregnancy cannot always be interpreted in the same way as those obtained in non-pregnant populations. Understanding these physiological changes is therefore essential for interpreting iron-status biomarkers during pregnancy and for evaluating the diagnostic thresholds used in clinical practice.

1.4.1 Haemodilution of Pregnancy

During pregnancy, plasma volume expands by approximately 40–50%, while red blood cell mass increases by only 25-30%, resulting in physiological haemodilution.[83] This expansion is not linear across gestation; plasma volume rises most rapidly during the second trimester, with the maximal dilutional effect occurring between approximately 28 and 32 weeks' gestation (see

Figure **1.5**).[84–87] Consequently, haemoglobin concentrations decline across pregnancy, even in women with adequate iron stores.[88] To account for these physiological changes, trimester-based haemoglobin thresholds (105 g/L in the second trimester; 110 g/L in the first and third trimesters) are typically used to define anaemia in pregnancy.[49]

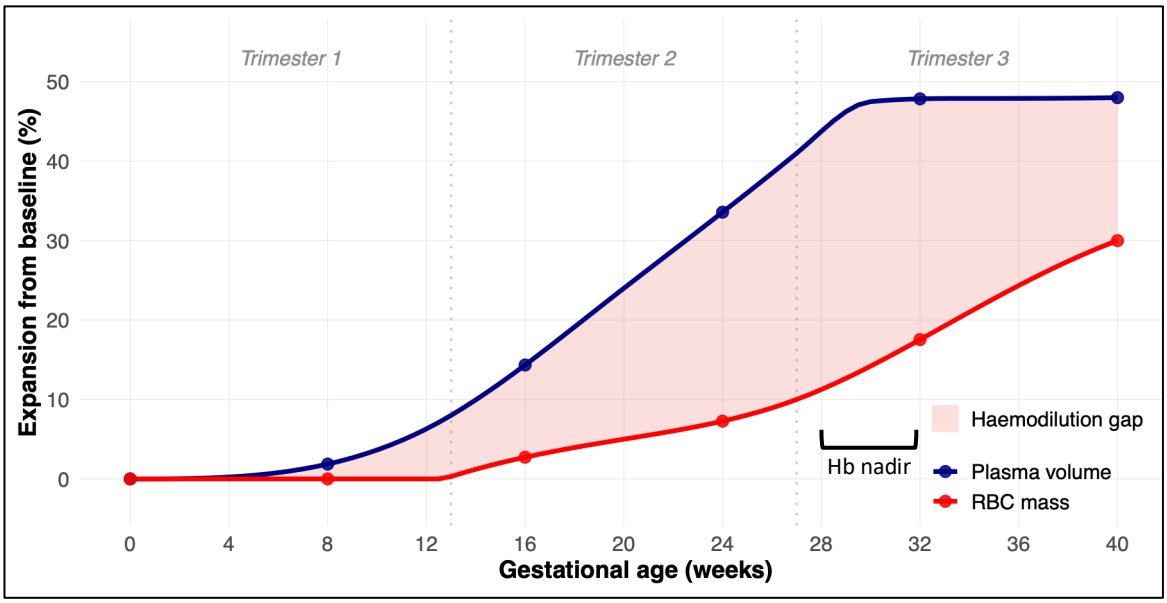


Figure 1.5 Plasma volume and red blood cell mass expansion across pregnancy
Trimester-specific trajectories of plasma volume and red blood cell mass, synthesised from published data and representing approximate physiological patterns. The haemoglobin (Hb) nadir occurs at 28–32 weeks' gestation.[84–87] Hb = haemoglobin.

Physiological haemodilution supports uteroplacental perfusion and may enhance maternal tolerance of blood loss at delivery. However, both low and high maternal haemoglobin

concentrations have been associated with adverse maternal and perinatal outcomes, suggesting that this adaptation operates within a relatively narrow physiological range.[29,88] Higher haemoglobin concentrations, often reflecting inadequate plasma volume expansion, have been linked to preeclampsia, impaired placental perfusion, and small-for-gestational-age infants.[29]

Serum ferritin may also be affected by haemodilution and changes in iron metabolism that occur in pregnancy. Ferritin concentrations decline across pregnancy, a pattern consistently observed in longitudinal studies.[12,67,89] This decline is believed to reflect a combination of plasma volume expansion, increased iron utilisation, and mobilisation of maternal stores, but it remains unclear to what extent the decline represents true depletion versus physiological adaptation.[12,67,89] Consequently, trimester-based ferritin cut-offs are being investigated and proposed, but have not yet been robustly validated.[69]

1.4.2 Iron Metabolism in Pregnancy

Pregnancy is associated with a substantial increase in iron requirements. Daily iron requirements rise from approximately 8 mg/day in non-pregnant women to around 27 mg/day in pregnancy, reflecting the demands of maternal red cell mass expansion, placental development, and foetal growth.[12] In early pregnancy, iron requirements are relatively modest, even decrease due to cessation of menstruation, but they substantially increase in the second and third trimesters, which coincides with the period when iron deficiency and anaemia are most frequently identified.[24]

To meet increased iron demands, pregnancy is accompanied by coordinated changes in iron regulation. Hepcidin concentrations are progressively suppressed during the second and third trimesters, facilitating increased intestinal iron absorption and mobilisation of maternal iron stores to support both maternal and foetal requirements.[12,90] This suppression is considered a key physiological adaptation, enabling efficient transfer of iron across the placenta. The mechanisms underlying hepcidin suppression are not yet completely understood, but are believed to involve placental, foetal or hormonal regulatory factors, given that suppression occurs even in the absence of any other evidence of iron deficiency.[91,92]

1.4.3 Biomarker Changes Across Pregnancy

These physiological adaptations of pregnancy not only result in characteristic changes in haemoglobin and ferritin but also affect other iron-status biomarkers and red cell indices.

Similar to ferritin, TSAT generally declines across gestation. This reflects both reduced circulating iron and increased transferrin concentrations, which rise in pregnancy to support enhanced iron transport and TIBC.[89]

In contrast, sTfR concentrations may rise throughout pregnancy, driven by increased erythropoiesis rather than by iron deficiency alone. This reduces the specificity of sTfR as a marker of iron deficiency in pregnancy, particularly in the absence of pregnancy-specific reference ranges.[93,94]

Haematocrit follows a similar pattern of decline to haemoglobin, with a nadir in the late second to early third trimester. Reticulocyte count increases due to heightened erythropoietic drive needed to expand maternal red cell mass. Reticulocytosis also leads to a modest increase in mean corpuscular volume (MCV), given the larger size of reticulocytes compared with mature red cells, and an increase in RDW, consistent with greater heterogeneity in red cell size.[95]

Declining ferritin and TSAT, rising sTfR, and altered red cell indices may all occur as part of normal pregnancy physiology, yet similar patterns are also seen in iron deficiency.[56] This overlap between normal physiological adaptation and iron deficiency creates a distinct diagnostic challenge for clinical practice. Many diagnostic thresholds for iron deficiency are largely derived from non-pregnant populations and do not account for pregnancy-specific changes. This uncertainty has contributed to variation in how iron deficiency is screened for and diagnosed across clinical settings. The next section of this chapter will examine clinical practice recommendations across international guidelines for the screening, diagnosis, and management of iron deficiency and anaemia in pregnancy. These changes are summarised in Table 1.1 below.

Table 1.1 Summary of key iron status biomarkers

Biomarker	Measures	IDA	Pregnancy	Inflammation	Key limitations
Serum ferritin	Iron stores	↓	↓ (typically declines across gestation)	↑	Acute-phase reactant; concentrations increase with inflammation and infection. Diagnostic thresholds vary across guidelines and populations.
Serum iron	Circulating iron	↓	↓ generally, decreases	↓	Marked diurnal and day-to-day variation; influenced by recent dietary intake and fasting status.
Transferrin	Iron transport capacity	↑	↑ (generally, increases)	↓	Influenced by inflammation, nutritional status, and liver function; indirect measure of iron availability.
TSAT (%)	Circulating iron availability (serum iron / TIBC)	↓	↓ (rising transferrin dilutes ratio)	↓	Derived from both serum iron and transferrin and therefore inherits limitations of both measures.
sTfR	Cellular iron demand / erythropoietic activity	↑	↑ (may increase due to erythropoiesis)	Typically, not affected	Influenced by erythropoietic activity as well as iron status; poor inter-assay standardisation limits comparability across platforms.
Ferritin index (sTfR/$\log_{10}$ ferritin)	Composite: iron demand relative to stores	↑	↑ (max increase)	↓	Combines limitations of ferritin and sTfR; thresholds and assay-specific reference ranges are not standardised.
Hepcidin	Iron regulatory hormone	↓	↓↓ (progressive suppression to facilitate absorption)	↑	Limited assay standardisation; no universally accepted diagnostic thresholds and not routinely available in clinical practice.
Hb	Oxygen-carrying capacity (late marker of ID)	↓	↓ (haemodilution; non-linear nadir 24–32 wks.)	Variable	Non-specific and relatively late marker of iron deficiency; influenced by plasma volume expansion, hydration status, and sampling method (capillary versus venous).

Iron status biomarkers, including their expected changes in iron deficiency and pregnancy and their key confounders. TSAT = transferrin saturation; sTfR = soluble transferrin receptor; IDA = iron deficiency anaemia; TIBC = total iron-binding capacity; Hb = haemoglobin.

1.5 Management of Iron Deficiency and Anaemia in Pregnancy

Management of iron deficiency and anaemia in pregnancy depends on the identification, interpretation, and monitoring of iron-status biomarkers. Clinical guidelines therefore provide

recommendations regarding screening, diagnosis, treatment with oral or intravenous (IV) iron supplementation, and assessment of treatment response.[96] However, considerable variation exists between national and international guidelines, reflecting differences in disease burden, healthcare infrastructure, resource availability, and interpretation of the evidence base.

Importantly, guidelines differ not only in their recommendations but also in their clinical focus. Some are primarily concerned with the diagnosis and management of iron deficiency, including IDWA, whereas others focus predominantly on the prevention and treatment of anaemia at a population level. Consequently, recommendations regarding screening strategies, diagnostic thresholds, treatment eligibility, and monitoring vary depending on whether the primary objective is the identification of iron deficiency itself or the prevention and treatment of established anaemia.

To explore the current recommendations for management of iron deficiency and anaemia in pregnancy, five clinical guidelines were selected for comparison:

(i) The British Society for Haematology (BSH) (2020),[51] reflecting the UK context central to this work,

(ii) Haematology in Obstetrics and Women's Health (HOW) (2025),[41] a recent international consensus developed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, emphasises iron deficiency,

(iii) The WHO (2016),[97] representing a global public health perspective,

(iv) The International Federation of Gynecology and Obstetrics (FIGO) (2025),[50] reflecting international obstetric consensus, and

(v) The Anaemia Mukt Bharat (AMB) strategy (2018),[98] relevant to the Indian setting examined in Chapter 5.

The selected guidelines differ in both scope and structure. Some provide dedicated recommendations for iron deficiency and anaemia (BSH and HOW), whereas others incorporate these into broader antenatal care guidance (WHO and FIGO). In India, national recommendations are delivered through the AMB public health strategy, launched in 2018 as part of the National

Health Mission. Key recommendations from international and national guidelines are summarised in Table 1.2

Table 1.2 Summary of national and international guideline recommendations

Domain	BSH (2020)	HOW (2025)	WHO (2016)	FIGO (2025)	India/AMB (2018)
Hb Screening	Booking & 28 wks.; additional for high-risk	Booking & 24–28 wks.	At least once; additional for high-risk	Booking & 28–30 wks.	POC capillary Hb at booking & ANC visits
Ferritin Screening	Risk factors or haemoglobinopathy	All pregnant women (GRADE 1C)	Not stated	If at risk	Not included in routine screening
Anaemia Diagnosis	<110 (1st); <105 (2nd/3rd) g/L	Not anaemia-specific; focuses on ID	<110 (1st); <105 (2nd/3rd) g/L	<110 g/L (all trimesters)	Mild: 100–109; Moderate: 70–99; Severe: <70 g/L
Iron Deficiency	SF <30 µg/L	SF <30 µg/L (GRADE 1D)	SF <15 µg/L	SF <30 µg/L	Not routinely assessed; Hb-based
Oral Iron Indication	Anaemia; prophylactic for high-risk	Iron deficiency	Prophylactic for all pregnant women	Universal supplement ation	Prophylactic IFA for all from 2nd trimester
Oral Iron Dose	40–80 mg daily	Not specified; alternate-day dosing considered	30–60 mg prophylactic; 120 mg treatment	30–60 mg daily	100 mg + 500 mcg folic acid daily
IV Iron Indication	Intolerance of or non-response to oral iron; after 34 weeks' gestation with Hb <100 g/L.	Intolerance/ refractory to oral or 3rd tri	Hb <70, late pregnancy, failure	Hb <70 or intolerance	Hb 70–89 (2nd tri); <70 transfusion
Monitoring (Oral)	Hb at 2-3 weeks; Hb rise expected but not defined	Not specified in consensus	Not specified; aim Hb >110 g/L	Hb rise ≥10 g/L at 2–4 wks. Hb at 2–4 wks.	At follow-up ANC visits

BSH = British Society for Haematology; WHO = World Health Organization; AMB = Anaemia Mukt Bharat; FIGO = International Federation of Gynecology and Obstetrics; SF = serum ferritin; IFA = iron and folic acid; POC = point-of-care; ANC = antenatal care; ID = iron deficiency; IDA = iron deficiency anaemia; IDWA = iron deficiency without anaemia; tri = trimester(s); wks. = weeks. Booking refers to the first antenatal visit.

1.5.1 Screening and Diagnostic Strategies Across Guidelines

International and high-income context guidelines (BSH, HOW, and FIGO) show broad alignment in their approach to screening and diagnosis. All recommend haemoglobin assessment at booking (the first antenatal visit) and in mid-pregnancy, use trimester-specific haemoglobin thresholds, and define iron deficiency using ferritin concentrations below 30 µg/L. One notable difference is that HOW recommends routine ferritin testing for all pregnant women, whereas BSH and FIGO reserve ferritin assessment for those with risk factors.

WHO recommendations differ in several respects. Most notably, WHO defines iron deficiency during pregnancy using a ferritin threshold of <15 μg/L rather than <30 μg/L and places greater emphasis on population-level approaches to prevention and supplementation.

In contrast, the India/AMB guidelines reflect the realities of high disease burden and limited resources. Anaemia is classified by severity rather than gestational age, ferritin is not routinely measured, and screening relies on point-of-care capillary haemoglobin rather than laboratory testing. In such settings, population-based approaches to anaemia management may be more feasible than individualised biomarker-based assessment. These differences in screening and diagnostic strategies have direct implications for treatment decisions, examined below.

1.5.2 Treatment of Iron Deficiency Anaemia in Pregnancy

Oral iron is the established first-line treatment for IDA in pregnancy, with IV iron typically reserved for women who are unable to tolerate oral therapy, fail to respond adequately, or present later in gestation when more rapid correction is required.[41]

Oral iron is widely accessible, inexpensive, and effective for many women, but tolerability remains a major limitation. Gastrointestinal side effects, including nausea, constipation, diarrhoea, dyspepsia, and abdominal discomfort, are common and often contribute to non-adherence and treatment discontinuation.[57,99] These adverse effects may be particularly problematic during pregnancy, when nausea and gastrointestinal symptoms are already common. While the benefits of treatment generally outweigh these risks in women with iron deficiency or anaemia, concerns remain regarding unnecessary treatment in women who are already iron replete. This has contributed to ongoing debate regarding universal versus screening-based supplementation strategies, particularly in higher-income settings where the prevalence of iron deficiency is lower and a greater proportion of women enter pregnancy with adequate iron stores.

In addition to uncertainty regarding who should receive treatment, questions remain regarding the optimal oral iron regimen. Latest evidence from non-pregnant populations suggests that alternate-day dosing may improve fractional iron absorption and reduce gastrointestinal side effects

compared with traditional daily regimens.[100] However, whether these advantages translate to pregnancy remains uncertain, as physiological adaptations during pregnancy may alter iron absorption and response to supplementation.

Additionally, a growing body of evidence, including systematic reviews of RCTs, supports the use of IV iron for the treatment of IDA in pregnancy.[99,101] These studies have demonstrated greater improvements in haemoglobin concentrations than oral iron; however, evidence of benefit for clinically meaningful outcomes, such as postpartum haemorrhage or transfusion requirements, remains limited.

These uncertainties in optimum management are reflected in the variation in treatment recommendations summarised in Table 1.2. High-income and international consensus guidelines (BSH, HOW, FIGO) generally restrict iron supplementation to women with anaemia or confirmed iron deficiency, whereas WHO and AMB recommend universal prophylactic oral iron supplementation. These differing approaches reflect variation in population needs, disease burden, and the feasibility of individualised screening and diagnosis. Recommended oral elemental iron doses vary across guidelines. BSH suggests 40–80 mg daily for treatment, WHO recommends 30–60 mg for prophylaxis and 120 mg for treatment, and AMB uses fixed-dose regimens combining iron and folic acid (IFA), with 60 mg elemental iron for prophylaxis and 100 mg elemental iron for treatment. HOW and BSH also note that alternate-day dosing may improve tolerability.

Regarding IV iron, BSH, HOW, and FIGO generally reserve use for severe anaemia or failure/intolerance of oral therapy, with use more commonly recommended later in pregnancy when there is limited time for oral treatment to be effective. In contrast, AMB recommends trimester-based haemoglobin cutoffs for initiating IV iron therapy.

Despite the failure of oral iron being a key indication for escalation to IV iron, there is no clear consensus on how to monitor the response to oral iron. BSH provides the most detailed guidance, recommending repeat haemoglobin assessment after initiation of therapy and advising that a rise

in haemoglobin should be demonstrable. It does not, however, define a pregnancy-specific target for an adequate response, noting that it varies according to baseline iron status, absorption, ongoing blood loss, and other contributing factors. Other guidelines provide minimal or no specific recommendations regarding monitoring response to either oral or IV iron. This uncertainty in assessing treatment response is examined in detail in Chapter 3.

Variation across international guidelines reflects both contextual differences in disease burden and healthcare infrastructure, as well as underlying uncertainty in pregnancy-specific iron management. Some of these differences are likely rooted in the physiological complexities described in the preceding sections. As iron biomarkers change dynamically across pregnancy and their interpretation remains uncertain, guideline recommendations necessarily rely on incomplete and, at times, extrapolated evidence.

1.6 Rationale, Aims and Structure of Thesis

1.6.1 Rationale

Iron deficiency and anaemia in maternal health remain major global challenges despite the widespread availability of diagnostic tools and treatment.[1–5] As the preceding sections have shown, progress in reducing their burden has been slower than anticipated, and the reasons likely extend beyond resource constraints or treatment availability alone.[2,3] As outlined in the preceding sections, assessment and management of these conditions are complicated by the physiological changes of pregnancy and limitations in the evidence underpinning current diagnostic and treatment approaches.

Although iron status biomarkers are widely used in maternal health, important questions remain regarding their role in assessing and monitoring iron deficiency and anaemia. Uncertainty persists regarding how iron deficiency should be defined, which biomarkers should be used, how treatment response should be assessed, and how biomarker results should be interpreted across pregnancy and different maternal health settings. These challenges are reflected in the variation among clinical guidelines and in the ongoing debate regarding screening, diagnosis, treatment, and monitoring strategies.

These knowledge gaps provide the rationale for the studies presented in this thesis. Through four complementary studies spanning clinical guidelines, pregnancy research, longitudinal pregnancy cohorts, and postpartum populations, this thesis examines the use of iron-status biomarkers for assessing and monitoring iron deficiency and anaemia in maternal health.

1.6.2 Thesis Aim, Structure, and Objectives

Aim:

To examine the use of iron status biomarkers in the assessment and monitoring of iron deficiency and anaemia in maternal health.

Structure:

To address this aim, the thesis comprises four empirical chapters (Chapters 2–5), followed by a final discussion and synthesis chapter (Chapter 6). Chapter 2 examines variation in recommendations for the assessment, treatment, and monitoring of iron deficiency and anaemia across UK maternity guidelines. Chapter 3 evaluates how the response to oral iron therapy during pregnancy has been assessed in the research literature. Chapter 4 investigates the behaviour of key iron biomarkers across pregnancy under different prophylactic oral iron supplementation regimens. Chapter 5 examines the prevalence and aetiology of postpartum anaemia and explores how biomarker selection and measurement approach influence the classification of iron deficiency and anaemia in a rural Indian population. Chapter 6 synthesises findings across the empirical chapters. The relationship between the thesis aim, objectives, research questions, and empirical chapters is summarised in Figure 1.6.

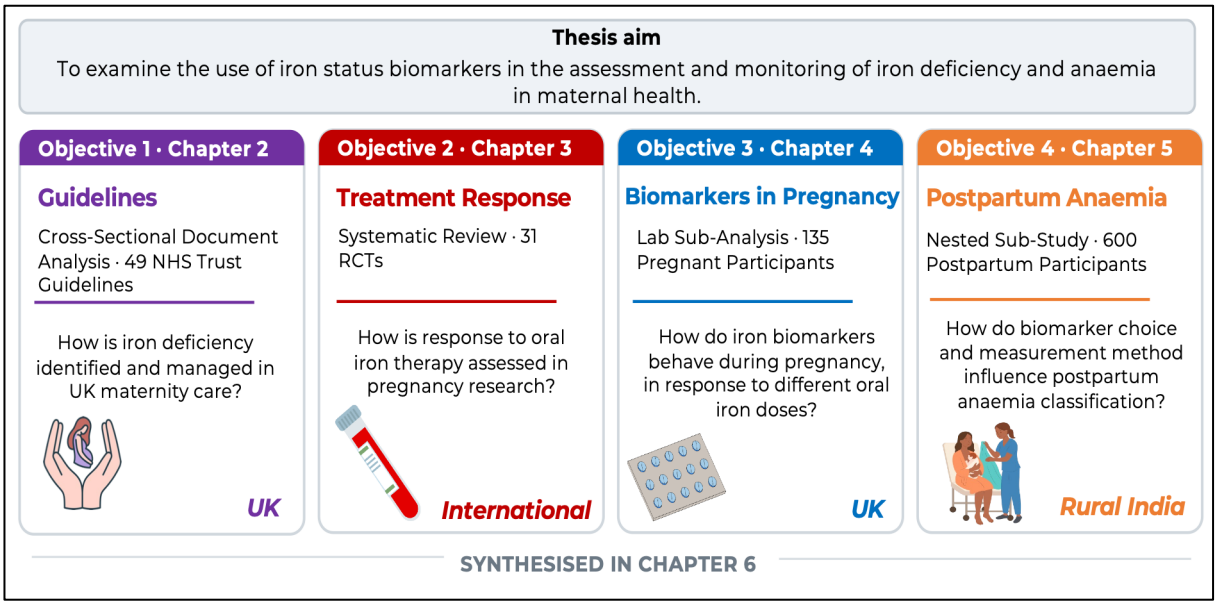


Figure 1.6 Overview of thesis structure, empirical chapters, and research questions
The relationship between the thesis aim, the four empirical chapters (Chapters 2–5), and the research questions addressed in each.

Objectives:

Objective 1 (Chapter 2)

To examine variation in local NHS maternity guideline recommendations for the screening, diagnosis, treatment, and monitoring of antenatal iron deficiency and anaemia, and their alignment with current British Society for Haematology (BSH) guidance.

Research questions:

1. What recommendations do local NHS maternity guidelines make regarding the screening, diagnosis, treatment, and monitoring of antenatal iron deficiency and anaemia?
2. To what extent are local NHS maternity guidelines aligned with the 2020 BSH guideline?

Objective 2 (Chapter 3)

To examine how the response to oral iron therapy during pregnancy has been assessed in clinical research.

Research questions:

1. Which haematological biomarkers are used to assess response to oral iron supplementation during pregnancy?
2. When is the response measured following initiation of oral iron therapy?
3. How do biomarker selection and assessment timing vary across healthcare contexts?

Objective 3 (Chapter 4)

To evaluate the effect of different prophylactic oral iron dosing regimens on key iron biomarkers during pregnancy.

Research questions:

1. Do daily, alternate-day, and three-times-weekly iron supplementation regimens produce different patterns of change in key iron biomarkers across pregnancy?
2. What do longitudinal changes in iron biomarkers suggest about the influence of supplementation regimen and physiological adaptation during pregnancy?

Objective 4 (Chapter 5)

To investigate the prevalence and aetiology of postpartum anaemia and evaluate the influence of biomarker selection and measurement approach on the classification of anaemia and iron deficiency amongst women in rural India.

Research questions:

1. Are there differences in the prevalence of postpartum anaemia between women enrolled in the intervention and control arms of the SMARThealth Pregnancy 2 (SHP2) trial?
2. What is the prevalence and likely aetiology of postpartum anaemia at 12 months, including the contribution of iron deficiency, folate deficiency, vitamin B12 deficiency, and inflammation in this population?

3. How closely do capillary point-of-care haemoglobin measurements captured under real-world field conditions agree with laboratory-based venous haemoglobin, and what are the implications for anaemia classification?

4. To what extent do ferritin- and sTfR-based definitions of iron deficiency agree, and how does inflammation influence this agreement in an Indian population?

1.6.3 Research Collaborations

This thesis draws on work conducted across multiple collaborative research programmes in the UK and India. Details of collaborators and individual contributions are provided within each empirical chapter and in the Statement of Authorship. While the empirical chapters arise from collaborative research, the integration, interpretation, and synthesis of findings presented within this thesis are my own.

1.7 Patient and Public Involvement and Positionality

PPI was integrated throughout the development of this thesis to ensure that the research questions and the interpretation of the findings reflected the experiences and priorities of women affected by iron deficiency and anaemia in pregnancy and the postpartum period. Contributors were recruited through the National Institute for Health and Care Research (NIHR) Blood and Transplant Research Unit in Data-Driven Transfusion Practice. They participated in structured online workshops facilitated by the PPI and Engagement Manager, Linda Von Neree. Participants represented a diverse cohort from across the UK, including individuals from racially and socioeconomically diverse backgrounds.



Figure 1.7 Community engagement and patient and public involvement in anaemia research: Islington Bangladeshi Association, London, April 2026
Photograph taken during a community engagement and patient and public involvement (PPI) event at the Islington Bangladeshi Association (IBA), London, in April 2026, and included with the permission of participants.

1.7.1 Key Themes from PPI Engagement

Several interconnected themes emerged from the PPI workshops. Participants described significant gaps in follow-up and monitoring after anaemia diagnosis, including limited repeat testing and lack of continuity of care between pregnancies. Anaemia was often experienced as an episodic condition and identified and treated within a single pregnancy, rather than managed longitudinally across the reproductive years. Participants also highlighted inconsistency in how anaemia was explained and managed, including limited guidance on iron supplementation and unclear expectations about treatment response. Broader structural barriers were identified, including time constraints within healthcare systems, difficulties accessing testing, and the cost of supplements.

These patient insights directly shaped the thesis focus. The emphasis on monitoring and follow-up is reflected in the systematic review (Chapter 3) and empirical analyses of biomarker trajectories and anaemia classification (Chapters 4 and 5). The importance of continuity of care across and beyond pregnancy aligns with the examination of anaemia at 12 months postpartum in

Chapter 5. Participant concerns regarding access and equity informed the interpretation of findings across UK and international contexts.

There are limitations to this PPI approach. Contributors were primarily based in the UK, which limits generalisability beyond the UK. Furthermore, although participants contributed at key stages, sustained involvement throughout the thesis was not feasible.

1.7.2 Positionality

I conducted this work as a clinician–researcher with early clinical experience in obstetrics, working primarily within an academic research setting. My broader personal context, including personal experience of recurrent IDA identified incidentally and managed without consistent longitudinal follow-up, inevitably informed my sensitivity to issues of recognition, monitoring, and continuity of care. To minimise potential bias, I interpreted findings in the context of the broader evidence base and through regular engagement with PPI contributors, clinicians, and researchers. These perspectives helped inform the interpretation of results and challenged assumptions that may have arisen from my own clinical and personal experiences.

Chapter 2 - Variation in Antenatal Iron Deficiency Anaemia Management Guidelines: A Cross-Sectional Analysis of Local NHS Guidelines in the United Kingdom

Publication Status

A version of this chapter has been published in the ***British Journal of Haematology***:

Haynes S., Balboula I., Collins P., Parry-Smith W., Fye H., Elsmore A., Collis R., de Lloyd L., Churchill D., Bell S., Stanworth S. Variation in Antenatal Anaemia Guidelines Across the NHS in the United Kingdom: A Cross-Sectional Study. **British Journal of Haematology.** Published online 20 August 2026. doi:10.1111/bjh.70780.

Collaborators

PANDA Trial Team: Prof Simon J Stanworth, Prof David Churchill

OBS-UK Trial Team: Dr Islam Balboula, Prof Peter Collins, Prof William Parry-Smith, Dr Haddy Fye, Dr Amy Elsmore, Prof Rachel Collis, Dr Lucy De Lloyd, Dr Sarah F Bell

Statement of Contribution

I conceived this study with S.J.S and D.C., and I led the coordination across two research programmes (OBS-UK and PANDA). I developed the data extraction framework and analysis plan; I conducted the data extraction and all analyses. IB contributed to data extraction. S.J.S and D.C. conceived the main PANDA trial. S.F.B led the OBS-UK study. All co-authors contributed to the implementation of the PANDA and OBS-UK programmes and provided input on the interpretation of findings and the revision of the manuscript.

2.1 Introduction

Chapter 1 highlighted ongoing uncertainty regarding the assessment and monitoring of iron deficiency and anaemia during pregnancy and demonstrated that recommendations vary across international clinical guidelines. Although UK national guidance is available through the BSH, little is known about how this guidance is interpreted and incorporated into local maternity guidelines across the UK.

This chapter presents a cross-sectional analysis of local NHS maternity guidelines across the UK. The study examines recommendations for the screening, diagnosis, treatment, and monitoring of antenatal iron deficiency and anaemia and evaluates their alignment with current BSH guidance.

By characterising variation in local guideline recommendations, this chapter provides insight into how national recommendations are interpreted within UK maternity services and establishes the context for the subsequent studies presented in this thesis.

2.1.1 UK National Guidelines for Antenatal Iron Deficiency and Anaemia

Several national and international guidelines provide recommendations for the assessment, treatment, and monitoring of iron deficiency and anaemia during pregnancy. As discussed in Chapter 1, these recommendations do not always align, reflecting differences in the underlying evidence base, clinical priorities, and healthcare settings for which they are intended.[96]

Within the UK, the BSH guideline[51] on iron deficiency in pregnancy is the most comprehensive national guidance on the management of iron deficiency and anaemia during pregnancy. Other guidance relevant to antenatal anaemia is available through the Royal College of Obstetricians and Gynaecologists (RCOG), particularly Green-top Guideline No. 47 on blood transfusion in obstetrics.[102] However, because the BSH guideline specifically addresses iron deficiency and anaemia during pregnancy, it was selected as the primary comparator for this study. The key BSH recommendations against which local NHS maternity guidelines were assessed are summarised in Table 2.1.

Table 2.1 Summary of key BSH guideline recommendations for antenatal anaemia management[51]

Domain	BSH Recommendation [51]
Routine Screening	
Haemoglobin	Booking (the first antenatal appointment) and 28 weeks. Additional screening for high-risk pregnancies.
Ferritin	Screening for women with known haemoglobinopathy or risk factors for iron deficiency.*
Diagnosis	
Anaemia	Hb <110 g/L (1st trimester), <105 g/L (2nd and 3rd trimesters), <100 g/L (postpartum).
Iron deficiency	Serum ferritin <30 µg/L.
Oral Iron Treatment	
Indication	Anaemia. Prophylactic iron may be given to women at increased risk of iron deficiency.
Preparation	Ferrous fumarate or other equivalent iron salts.
Dose	40–80 mg elemental iron daily. Alternate-day dosing may be considered to reduce gastrointestinal side effects.
Duration	Continue until haemoglobin normalised, then for a further 3 months and at least 6 weeks postpartum.
IV Iron Treatment	
Indication(s)	Intolerance of or non-response to oral iron (from 2nd trimester); or after 34 weeks' gestation with confirmed IDA and Hb <100 g/L.
Preparation	Ferric carboxymaltose, ferric derisomaltose, or iron sucrose. Cautions against iron dextran due to limited safety data in pregnancy.
Monitoring Post Oral Iron	
Biomarker	Haemoglobin.
Timing	2–3 weeks after commencing oral iron.
Expected response	Demonstrable rise in Hb by 2 weeks.

These recommendations were used as the reference standard against which local NHS site guidelines were assessed.[51] *Risk factors for iron deficiency were defined as twin or multiple pregnancy, vegan or vegetarian diet, known history of iron deficiency, multiparity, or an interpregnancy interval of less than one year. BSH = British Society for Haematology; IV = intravenous; NHS = National Health Service.

2.1.2 The NHS, Local Guidelines, and the Translation of Evidence into Practice

The NHS is a publicly funded healthcare system in which maternity care is delivered through more than 200 local organisational units: NHS Trusts in England, Wales, and Northern Ireland, and NHS Health Boards in Scotland.[103] These are referred to collectively as "NHS sites" throughout this chapter. Although national guidance is issued by professional bodies such as the

BSH and the RCOG, individual NHS sites are responsible for developing and maintaining local clinical guidelines that translate these recommendations into practice.

Antenatal care within the NHS is delivered through locally organised maternity services, typically led by obstetric and midwifery teams within individual NHS sites. Although national recommendations provide overarching guidance, decisions regarding laboratory testing pathways, referral criteria, intravenous iron services, and prescribing protocols are often operationalised at the local level. Access to specialist haematology services, day-unit facilities for intravenous iron administration, and laboratory reporting practices may also vary between sites.[51,104] Consequently, local guideline recommendations may reflect both interpretation of the evidence base and the practical realities of service delivery within individual maternity systems.

A recent national audit identified substantial variation in antenatal anaemia management across NHS maternity services, as well as variable adherence to BSH recommendations.[105] However, the extent to which this variation is reflected in local clinical guidelines is unknown. Examining local NHS maternity guidelines, therefore, provides an opportunity to assess how national recommendations are interpreted and incorporated into local guidance across the UK. It also allows exploration of whether variation is present before recommendations reach the level of individual clinical decision-making. Figure 2.1 illustrates the pathway from national guideline development through local adaptation to clinical practice.

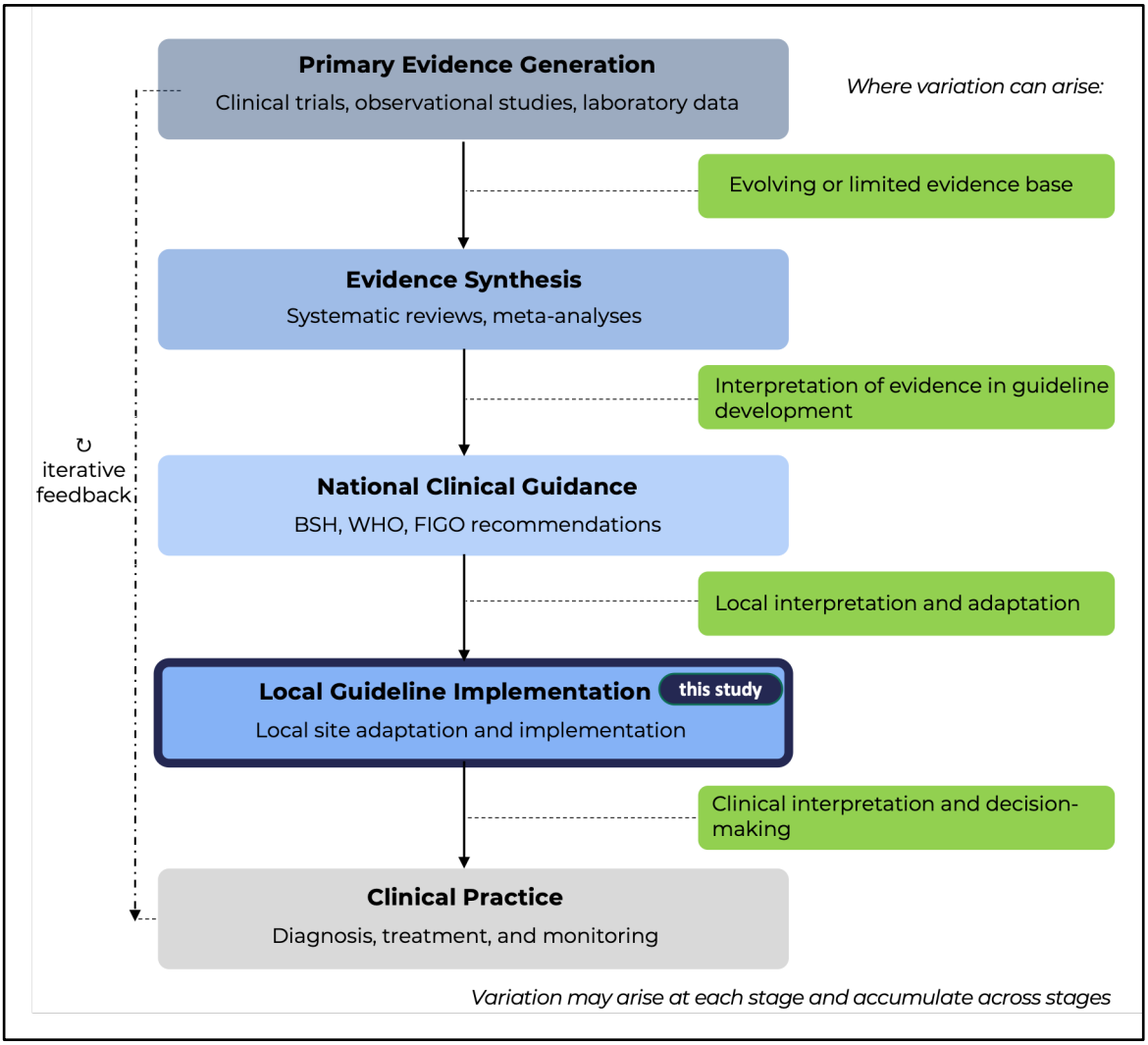


Figure 2.1 Translation of national antenatal anaemia guidance into local clinical guidelines and practice
The pathway from primary research evidence, through national guideline development, to local adaptation and clinical practice. Potential contributors to variation at each stage are shown on the left. The schematic is adapted from the Knowledge-to-Action framework and focuses on the knowledge-creation and local-adaptation stages relevant to this chapter.[106]

2.1.3 Unwarranted Variation in Healthcare

The possibility that local guidelines may differ despite being informed by the same national recommendations raises broader questions regarding variation in healthcare. Variation in care is not inherently problematic and may appropriately reflect differences in patient populations, service organisation, resource availability, or local clinical priorities.[106] However, variation may also occur where recommendations differ despite being derived from the same evidence base and healthcare system context.

The concept of unwarranted variation refers to differences in care that cannot readily be explained by patient need, clinical evidence, or patient preference.[107–109] Such variation has been associated with inequities in healthcare delivery and is recognised as a barrier to consistent, high-quality care.[107,110] Although this study does not seek to determine whether individual differences between guidelines are appropriate or inappropriate, characterising variation is an important first step in understanding how national recommendations are interpreted across healthcare settings.[111]

By examining local written guidance, rather than clinical practice alone, this study explores whether variation is present before recommendations reach individual clinical decision-making. In doing so, it provides insight into how national guidance for antenatal iron deficiency and anaemia is translated into local maternity care across the NHS.

2.1.4 Aim and Research Questions

Aim

To examine variation in local NHS maternity guideline recommendations for the screening, diagnosis, treatment, and monitoring of antenatal iron deficiency and anaemia, and their alignment with current BSH guidance.

Research Questions

1. What recommendations do local NHS maternity guidelines make regarding the screening, diagnosis, treatment, and monitoring of antenatal iron deficiency and anaemia?

2. To what extent are local NHS maternity guidelines aligned with the 2020 BSH guideline?

2.2 Methods

2.2.1 Study Design

This was a cross-sectional analysis of local NHS antenatal anaemia guidelines across NHS sites in the UK, with comparison to national recommendations. Local guideline recommendations were systematically extracted and compared with the 2020 BSH national guidelines.

This study was conducted as part of two broader research programmes: the Obstetric Bleeding Study UK (OBS UK) and the Primary prevention of maternal Anaemia to avoid preterm Delivery

and other Adverse outcomes (PANDA) trial. The OBS UK study is a nationwide initiative funded by the NIHR (HSDR-NIHR152057) aimed at improving the management of obstetric bleeding by implementing a standardised approach to recognising and managing postpartum haemorrhage across 36 NHS sites in the UK. The PANDA trial is a large-scale NIHR-funded trial (NIHR RP-PG-NIHR200869) investigating the effectiveness of routine iron supplementation for the prevention of antenatal anaemia, involving 16 NHS sites. Both studies provided access to participating NHS sites from which local iron deficiency or anaemia guidelines could be requested.

2.2.2 Setting and Participants

The study population comprised NHS sites (trusts and health boards) providing maternity services across the UK. The sampling frame was defined by sites participating in OBS UK and PANDA, supplemented by publicly available guidelines and one additional site approached directly. This approach was selected to achieve broad geographical representation across UK maternity services. No formal power calculation was performed, as the study was descriptive in nature and aimed to include as many guidelines as possible across all four UK nations.

2.2.3 Guideline Collection

Local guidelines were obtained through email requests to NHS sites participating in the OBS UK and PANDA research programmes. Each site was asked to provide its current local guideline(s) for the management of anaemia and/or iron deficiency in pregnancy. No date restrictions were applied; guidelines were included irrespective of publication or expiry date.

To ensure representation across all four UK nations, one additional site not participating in OBS UK or PANDA was approached directly. All responding sites consented to provide anonymised guidelines for research purposes. Guidelines were collected between September 2024 and June 2025. In addition, publicly available anaemia guidelines from three Welsh sites were obtained via the WISDOM NHS Wales website, a central repository of clinical guidelines.

Where guidelines were incomplete or unclear, follow-up requests were made to obtain clarification or further information. For guidelines embedded within broader obstetric documents (e.g. antenatal care guidelines), only anaemia and/or iron deficiency-specific content was extracted.

2.2.4 Inclusion and Exclusion of Guidelines

A total of 55 guidelines were collected. Four duplicate guidelines were removed where sites participated in both OBS UK and PANDA; in each case, the most recent or most complete version was retained.

Two guidelines were excluded because they consisted solely of single-page flow diagrams or algorithms and lacked sufficient detail to enable consistent data extraction across study domains. Follow-up contact with these sites confirmed that no additional detailed guidance was available. These documents did not contain sufficient information on screening, diagnostic thresholds, treatment recommendations, or monitoring and therefore could not be meaningfully compared with other guidelines or national standards. Excluding such guidelines ensured that the analysis was based on detailed, robust recommendations rather than oversimplified summaries that might omit critical details.

This resulted in a final sample of 49 guidelines for analysis. The included guidelines represented a geographically diverse sample across all four UK nations. A summary of the guideline selection process is presented in Figure 2.2.

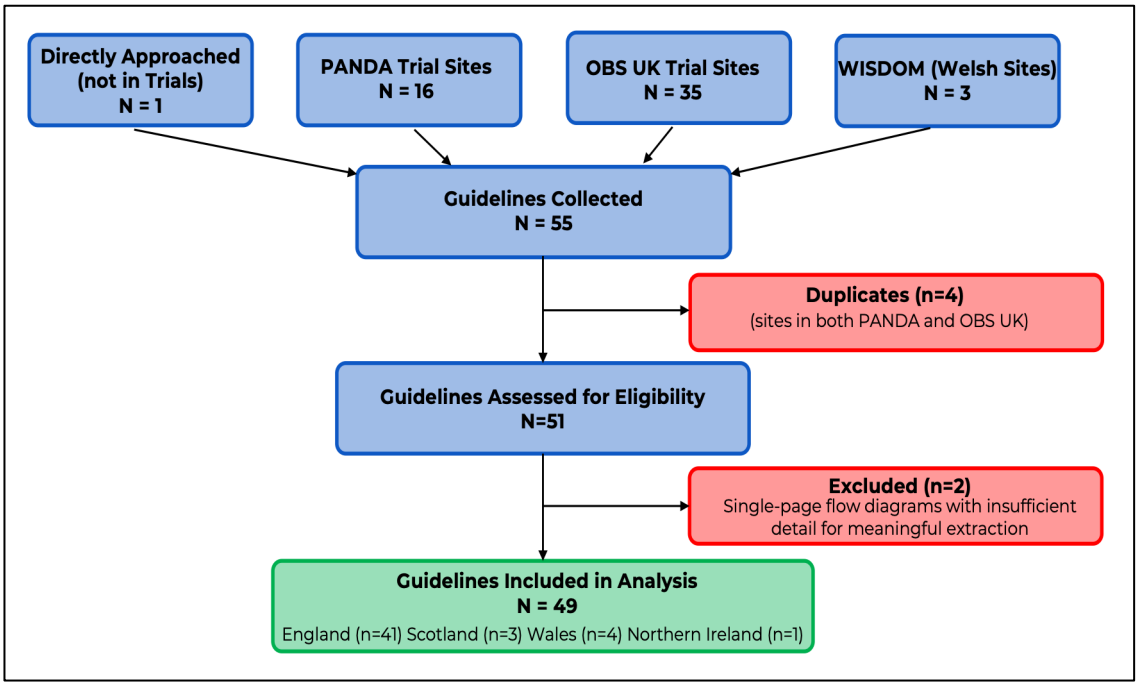


Figure 2.2 Flow diagram of guideline identification, screening, and inclusion
Guidelines were collected from three sources: the PANDA trial, the OBS UK study, and publicly available Welsh guidelines from the WISDOM NHS Wales website. Duplicates were removed where sites participated in both PANDA and OBS UK. Guidelines consisting solely of single-page flow diagrams were excluded owing to insufficient detail for meaningful data extraction.

2.2.5 Data Extraction

2.2.5.1 Extraction Tool

A structured data extraction form was developed in Microsoft Excel to ensure standardised and reproducible collection of data across all guidelines. The form was designed to capture detailed information across predefined areas of guideline recommendations, with fields corresponding to specific components of screening, diagnosis, treatment, and monitoring. The core domains and sub-domains included in the extraction tool are summarised in Table 2.2. The BSH 2020 national guidelines were entered into the extraction form as a reference standard, against which local guideline data could be systematically compared (see Supplementary Table S2.1) in Appendix 2 for the full data extraction form with the BSH guidelines entered as an exemplar).

Table 2.2 Domains and items included in the data extraction tool

Domain	Data Extracted
General Information	Guideline name; date of publication; expiry or review date
Screening	Whether haemoglobin and/or serum ferritin testing was recommended at booking, 28 weeks, and 32–36 weeks; indications for ferritin testing
Diagnostic Criteria	Haemoglobin thresholds for anaemia in the 1st trimester, 2nd/3rd trimester, and postpartum period; serum ferritin threshold for iron deficiency
Oral Iron Treatment	Indication for treatment; recommended preparation; dose; duration of treatment
IV Iron Treatment	Indications (haemoglobin threshold, gestational timing, failure of oral iron); recommended preparation(s); dose; contraindications
Monitoring Post-Treatment	Recommended test, timing, and expected response for both oral and IV iron treatment

The complete data extraction tool, with the 2020 BSH national guideline as an illustrative exemplar, is presented in Appendix 2 as Supplementary Table S2.1 BSH = British Society for Haematology; IV = intravenous.

2.2.5.2 Extraction Process

Two independent reviewers (SH and IB) extracted information from each guideline using the structured extraction form. To ensure accuracy and consistency, each independent reviewer cross-checked the other's extracted data in its entirety after the initial extraction was complete. Any discrepancies identified during cross-checking of the extracted data were resolved through discussion between the two reviewers (SH and IB), with reference to the original guideline document. Discrepancies were infrequent and primarily reflected minor data extraction or transcription errors rather than differences in interpretation.

2.2.5.3 Recording of Absent Recommendations

Where a guideline did not include information relating to a predefined domain in the extraction tool, this was recorded as "not stated." This indicated that no recommendation was provided in the guideline. The frequency of "not stated" responses was summarised for each domain and included in the overall assessment of guideline completeness.

2.2.6 Assessment of Guideline Alignment with National Standards

Overall compliance for each domain was determined by comparing local guideline recommendations with the relevant standards outlined in the 2020 national guidelines (BSH). To

provide a standardised assessment, each domain was classified according to a four-category framework developed for this study:

Aligned: the local guideline recommendation fully matched the corresponding BSH national guideline recommendation. For example, a guideline recommending Hb screening at booking (the first antenatal appointment) and 28 weeks was classified as "aligned" for Hb screening.

Aligned with additional measures: the local guideline met the core BSH recommendation but included additional actions or thresholds that exceeded, without contradicting, the national standard. For example, a guideline recommending routine ferritin screening at booking for all pregnant women (in addition to the BSH-recommended targeted screening) was classified as "aligned with additional measures."

Not aligned: the local guideline recommendation diverged from or contradicted the BSH national guideline. For example, a guideline using an anaemia threshold of Hb <120 g/L throughout pregnancy was classified as "not aligned."

Not stated: the guideline did not provide any recommendation on the domain in question.

This classification was applied independently to each domain (screening, diagnosis, oral iron treatment, IV iron treatment, and monitoring).

2.2.7 Estimation of Population Coverage

To estimate the proportion of UK births represented by the included guidelines, the annual number of births at each participating NHS site was obtained from publicly available national datasets. For English sites, data were sourced from the Office for National Statistics (ONS) birth summary tables and NHS Maternity Statistics.[112,113] For Scottish sites, data were obtained from the National Records of Scotland (NRS) Vital Events Reference Tables.[114] For Welsh sites, data were sourced from StatsWales maternity statistics.[115] For Northern Ireland, data were obtained from the Northern Ireland Statistics and Research Agency (NISRA) Registrar General Annual Report.[116] The combined annual birth figure across all 49 included sites was then compared with the UK's annual birth rate to estimate the percentage of UK births represented by the included guidelines.

2.2.8 Statistical Analysis

Given the study's descriptive nature, analyses were primarily descriptive. Data were collated and managed in Microsoft Excel. Frequencies and proportions were calculated for each domain and sub-domain to summarise the distribution of guideline recommendations and their alignment with national guidance across the 49 sites.

Analyses were conducted at both the domain level (e.g., screening, diagnosis, treatment, monitoring) and the sub-domain level (e.g., timing of haemoglobin testing, ferritin thresholds, oral iron dosing), as defined in the data extraction framework. No inferential statistical analyses were performed, as the aim was to describe variation rather than to test hypotheses.

All analyses and data visualisations, including heatmaps and summary graphs, were performed using RStudio (version 2024.12.0+467). Summary tables were produced in Microsoft Excel.

2.3 Results

2.3.1 Characteristics of NHS Sites

A total of 49 NHS site guidelines were included, with representation from England (n=41), Scotland (n=3), Wales (n=4), and Northern Ireland (n=1). Sixteen NHS sites provided guidelines through PANDA, and 36 NHS sites provided guidelines through OBS UK. The combined annual number of births across the 49 sites included was 216,449, approximately 33% of the UK's annual birth rate (see Figure 2.3).[112–116]

The median publication date of the guidelines was March 2023 (range: December 2010 – March 2025), with a median expiry date of November 2025 (range: November 2015 – August 2028). The median duration of guideline validity was 3 years. Most guidelines (n=43) were published after the updated national guidelines were published in late 2020. The general adherence of local site guidelines to national guidelines is summarised in Table 2.3.

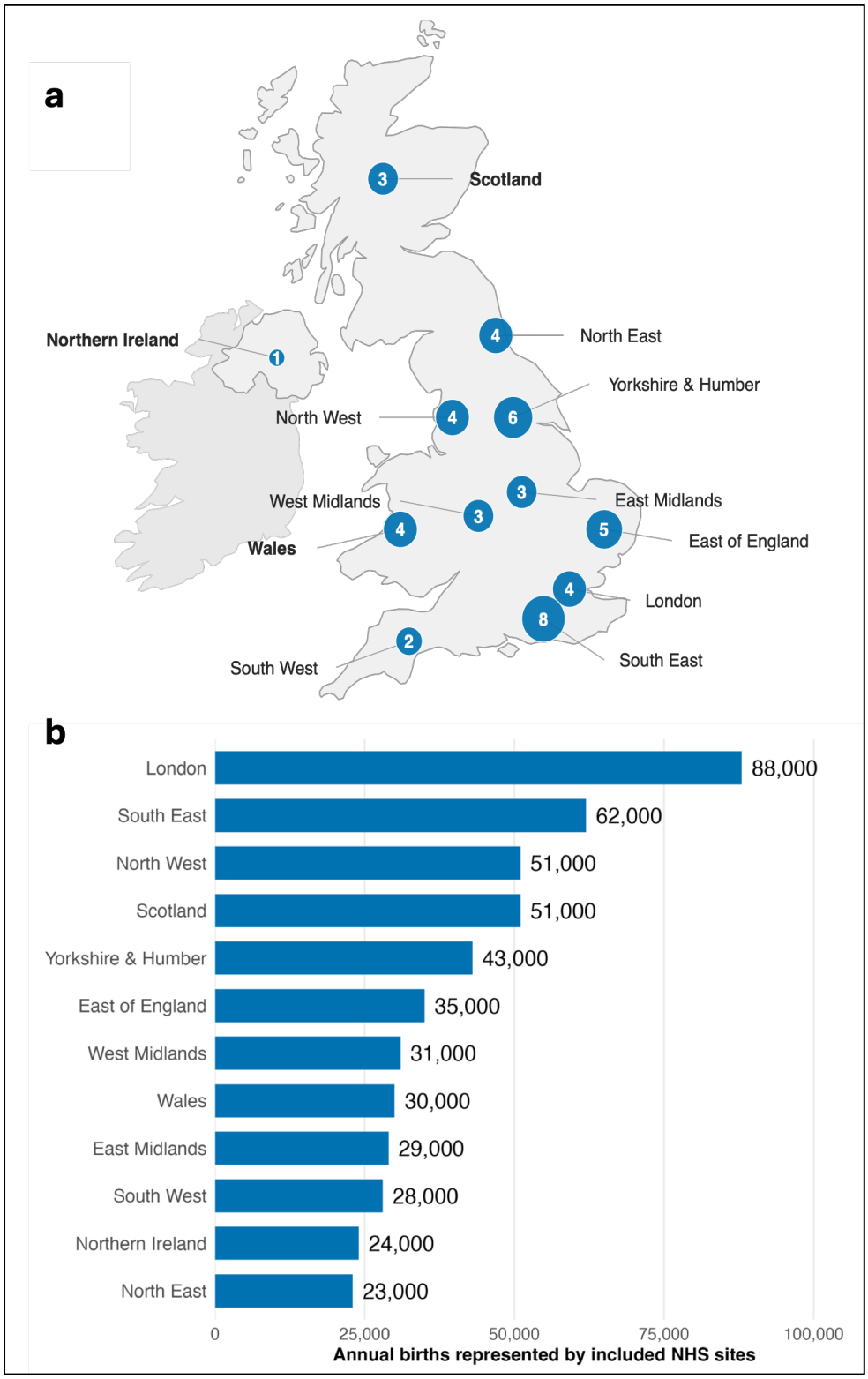


Figure 2.3 Geographic distribution of included NHS site guidelines and annual births represented across UK regions

(a) Number of local antenatal anaemia guidelines included from each UK region (n = 49). (b) Estimated annual births represented by the included NHS sites within each region, derived from publicly available national statistics. Together, the included sites represent approximately 216,000 births annually, around one-third of UK births. NHS = National Health Service.

Table 2.3 Summary of general alignment with national guidelines by NHS sites (n = 49)

Domain	Aligned	Aligned with Additional Measures	Not Aligned	Not Stated
Routine Screening				
Hb (Booking and 28 wks.)	41 (84%)	8 (16%)	0 (0%)	0 (0%)
Ferritin	41 (84%)	8 (16%)	0 (0%)	0 (0%)
Diagnosis				
Anaemia	47 (96%)	0 (0%)	2 (4%)	0 (0%)
Iron Deficiency	31 (63%)	0 (0%)	3 (6%)	15 (31%)
Oral Iron Treatment				
Indication	33 (67%)	3 (6%)	5 (10%)	8 (16%)
Preparation	46 (94%)	0 (0%)	0 (0%)	2 (4%)
Dose	34 (69%)	0 (0%)	13 (27%)	2 (4%)
Length of Treatment	10 (20%)	4 (8%)	18 (37%)	17 (35%)
IV Iron Treatment				
Indication	4 (8%)	30 (61%)	0 (0%)	15 (31%)
Preparation	43 (88%)	0 (0%)	1 (2%)	5 (10%)
Monitoring Post Oral Iron				
Biomarker	32 (65%)	15 (31%)	0 (0%)	2 (4%)
Timing	31 (63%)	6 (12%)	10 (20%)	2 (4%)
Response Definition	16 (33%)	16 (33%)	0 (0%)	17 (35%)

Each local guideline recommendation was assessed against the corresponding 2020 BSH national guideline recommendation and classified as aligned, aligned with additional measures, not aligned, or not stated. BSH = British Society for Haematology; NHS = National Health Service.

2.3.2 Screening Practices

Haemoglobin screening was universally recommended at booking and at 28 weeks, consistent with national guidance. Seven guidelines recommended an additional routine haemoglobin test at 32–36 weeks for all pregnant women; in contrast, national guidance recommends additional screening at this time point only in specific circumstances, such as multiple pregnancy.

Recommendations for ferritin testing were broadly aligned with national guidance. However, eight local guidelines (16%) recommended routine ferritin testing at booking for all pregnant women, of which seven also advised repeat testing at 28 weeks. National guidance does not

recommend routine ferritin testing in all pregnancies; instead, it advises testing in women with known haemoglobinopathy or other risk factors for iron deficiency. The geographic distribution of additional routine haemoglobin and ferritin screening across NHS sites is shown in Figure 2.4.

Among guidelines that did not recommend *routine* ferritin testing, the most commonly specified indications for testing were low haemoglobin (n=24), known haemoglobinopathy (including sickle cell disease or thalassaemia) (n=27), high risk of IDA (n=25), advanced gestational age (n=8), clinical judgement (n=4), and lack of response to oral iron therapy (n=3). Three guidelines did not specify any indications for ferritin testing.

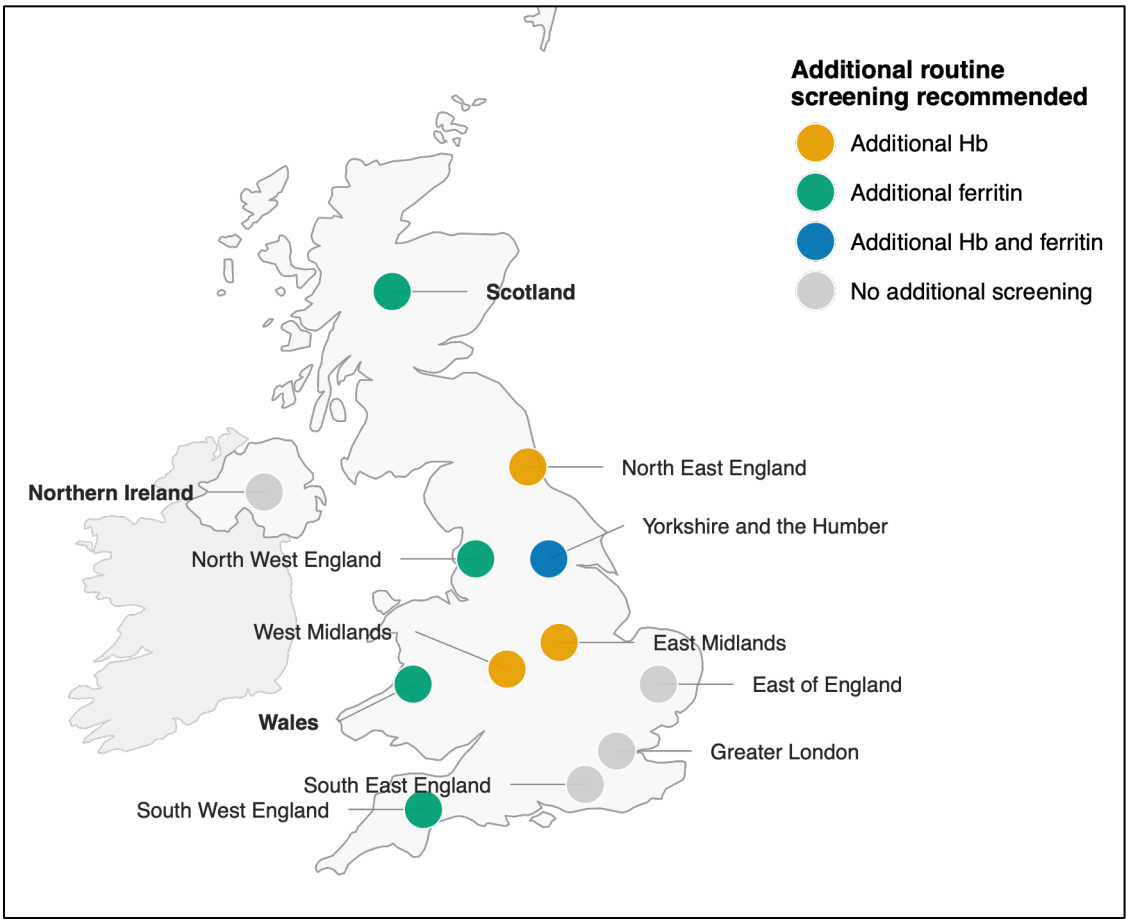


Figure 2.4 Geographic variation in recommendations for additional routine antenatal anaemia screening
Regions are classified according to whether any included local NHS guideline recommended additional routine haemoglobin screening, additional routine ferritin screening, both, or no additional routine screening beyond BSH recommendations. NHS = National Health Service; BSH = British Society for Haematology.

2.3.3 Diagnostic Criteria

Trimester-specific haemoglobin thresholds for anaemia were largely consistent across guidelines. Two recently published guidelines (2023–2025) differed in that they applied a single threshold of Hb <120 g/L throughout pregnancy.

Ferritin thresholds for diagnosing iron deficiency were also broadly consistent, with 31 guidelines (63%) using the <30 µg/L threshold recommended by national guidance. Ferritin thresholds were not specified in 14 guidelines (29%). Among those that differed, one guideline (published in 2022) used a threshold of <50 µg/L; another applied <30 µg/L in the first trimester and <15 µg/L thereafter; and a third used <15 µg/L throughout pregnancy.

2.3.4 Oral Iron Treatment

2.3.4.1 Indications for Oral Iron

Indications for oral iron treatment were varied. National guidelines recommend starting oral iron for any pregnant woman with anaemia (1st trimester: Hb <110 g/L, 2nd trimester onwards: Hb <105 g/L) or risk factors for IDA. The indications used by local sites are summarised in Table 2.4.

Table 2.4 Local NHS site guidelines' indications for oral iron, by trimester

Indication	Guidelines (N, (%))
1st Trimester Indications	
Hb <105 g/L	1 (2%)
Hb 80–105 g/L	4 (8%)
Hb <110 g/L	33 (67%)
Routine iron supplementation unless contraindicated	3 (6%)
2nd Trimester Indications	
Hb 80–105 g/L	4 (8%)
Hb <105 g/L	33 (67%)
Other Indications	
Haemoglobinopathy and low ferritin	8 (16%)
Ferritin <30 µg/L	27 (55%)
High risk women*	13 (27%)
MCV < 100 fL	3 (6%)

2.3.4.2 Oral Iron Formulation and Dose

Oral iron formulations were consistent, with most recommending ferrous sulphate or ferrous fumarate as first line. This aligns with the national guidelines and the British National Formulary (BNF), which guides the use of medicines in clinical practice in the NHS.[117]

Variability was noted in oral iron dosing recommendations. National guidelines recommend a daily dose of 40–80 mg of elemental iron, with the option to switch to alternate-day dosing to reduce gastrointestinal side effects.

Thirty-four guidelines (69%) recommended doses within the BSH-recommended range of 40–80 mg elemental iron daily. However, 13 guidelines (27%) recommended doses outside this range, with some recommending daily elemental iron doses of 100–200 mg. Many of these guidelines suggested oral iron tablets two to three times daily to achieve this dose.

2.3.4.3 Duration of Oral Iron Treatment

Recommendations for the length of oral iron treatment varied significantly between NHS sites. National guidelines recommend continuing oral iron until haemoglobin is normalised, then for a further 3 months, and for at least 6 weeks postpartum. Only 10 guidelines (20%) were fully aligned with this recommendation. 17 guidelines (35%) did not provide a recommendation on treatment duration, and 18 guidelines (37%) offered recommendations that were not aligned with national guidance.

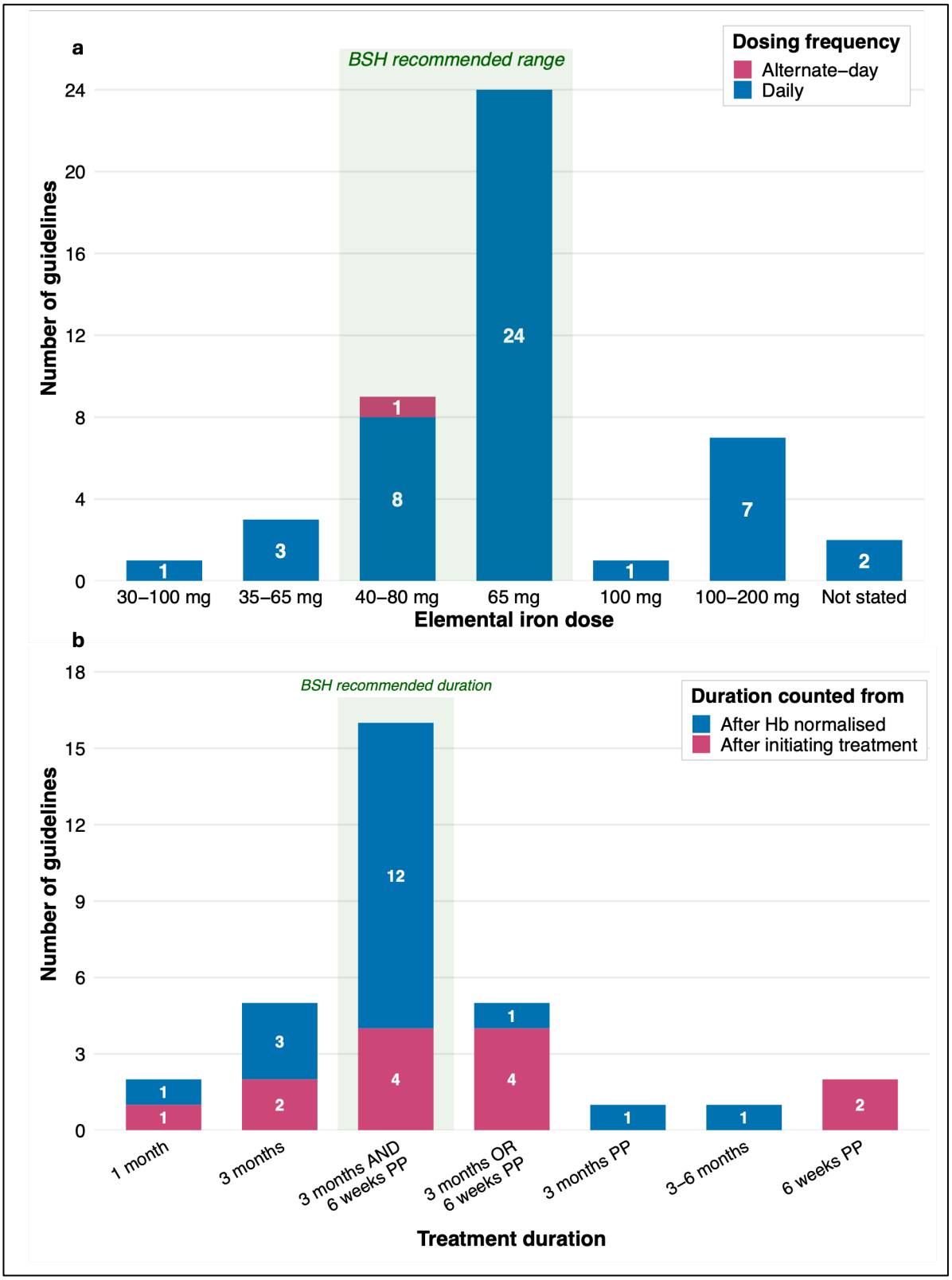


Figure 2.5 Local NHS site guidelines' recommendations for oral iron therapy in pregnancy
(a) Recommended elemental iron dose and dosing frequency. (b) Recommended duration of treatment, and whether duration was calculated from treatment initiation or from haemoglobin normalisation. Green shaded regions indicate the recommendations of the 2020 BSH guideline. PP = postpartum; BSH = British Society for Haematology; NHS = National Health Service.

2.3.5 Intravenous Iron Treatment

Forty guidelines (82%) recommended ferric carboxymaltose (Ferinject) or ferric derisomaltose (Monofer), consistent with national guidance. However, despite national guidance advising that there is insufficient evidence to support the use of iron hydroxide dextran (Cosmofer) in pregnancy, one guideline (published in 2012) recommended Cosmofer as a first-line option, and another (published in 2023) listed it as an alternative to ferric carboxymaltose.

There was substantial variation in haemoglobin thresholds used to initiate IV iron, ranging from <65 g/L to <110 g/L across guidelines (Figure 2.6). Some guidelines applied different thresholds based on clinical context, including the presence or absence of symptoms.

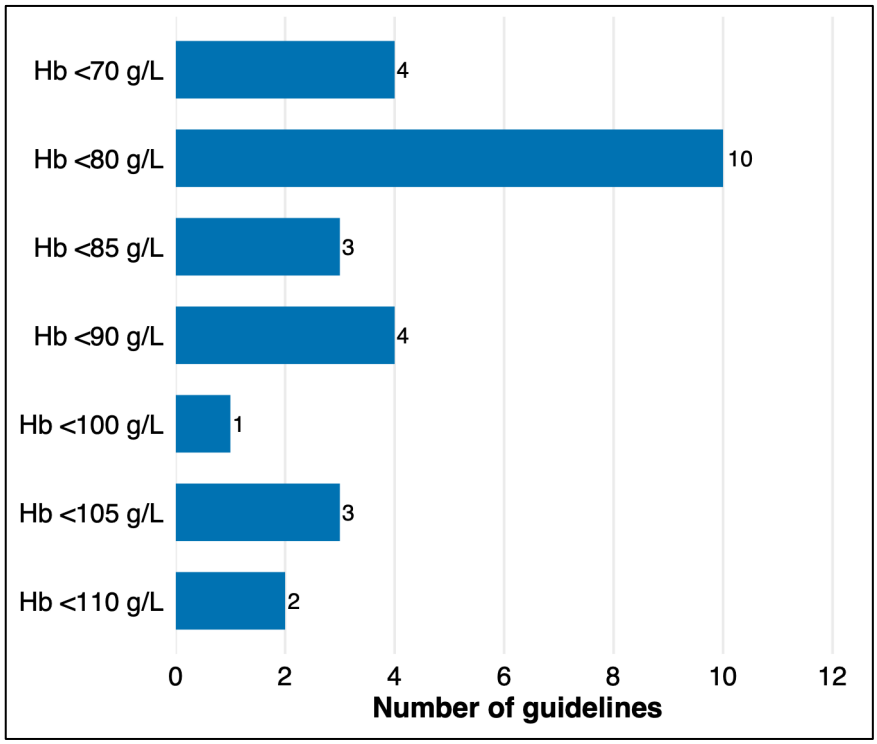


Figure 2.6 Haemoglobin thresholds for intravenous iron treatment
The number of local NHS site guidelines recommending intravenous iron at each haemoglobin threshold. NHS = National Health Service.

In addition to haemoglobin thresholds, IV iron was commonly recommended based on other clinical criteria. The most frequently cited indications were intolerance to oral iron (n=43, 88%), failure to respond to oral iron (n=29, 59%), malabsorption (n=27, 55%), and non-compliance (n=22, 45%).

Several guidelines also recommended IV iron in late gestation, often using gestation-specific thresholds (e.g. haemoglobin <90–105 g/L beyond 34 weeks) or in the context of a perceived need for rapid correction. Ferritin thresholds were less commonly specified, reported in 6 guidelines (12%) (Table 2.5).

Overall, IV iron was most positioned as a second-line treatment following oral iron intolerance or inadequate response. However, in some guidelines, particularly those specifying late gestation thresholds, IV iron appeared to be recommended without explicit instruction to trial oral iron first.

Table 2.5 Local NHS site recommendations for intravenous iron indications

Criteria for IV Iron	Guidelines (N, (%))
Serum Ferritin	6 (12%)
<10 µg/L	1 (2%)
<15 µg/L	1 (2%)
<30 µg/L	3 (6%)
<50 µg/L	1 (2%)
Late Gestation	30 (61%)
Hb <90 g/L at >34 weeks	3 (6%)
Hb <105 g/L at >34 weeks	4 (8%)
Hb <100 g/L at >28 weeks	7 (14%)
Low ferritin at >34 weeks	1 (2%)
Non-specific urgent correction	18 (37%)
Other Criteria	
Non-compliance	22 (45%)
Intolerance	43 (88%)
Failure to respond	29 (59%)
Malabsorption	27 (55%)
Significantly symptomatic	3 (6%)

IV = intravenous; NHS = National Health Service.

2.3.6 Monitoring Response to Iron Treatment

2.3.6.1 Monitoring Response to Oral Iron

Local NHS site guidelines showed substantial variation in recommendations for monitoring response to oral iron treatment. Haemoglobin alone was the most commonly recommended biomarker, used by 32 guidelines (65%) to assess treatment response. However, the recommended

timing of reassessment varied, ranging from 2 to 6 weeks (Figure 2.7). Fewer guidelines recommended additional biomarkers alongside haemoglobin, including ferritin (n=10), ferritin with folate and vitamin B12 (n=1), and RDW (n=1).

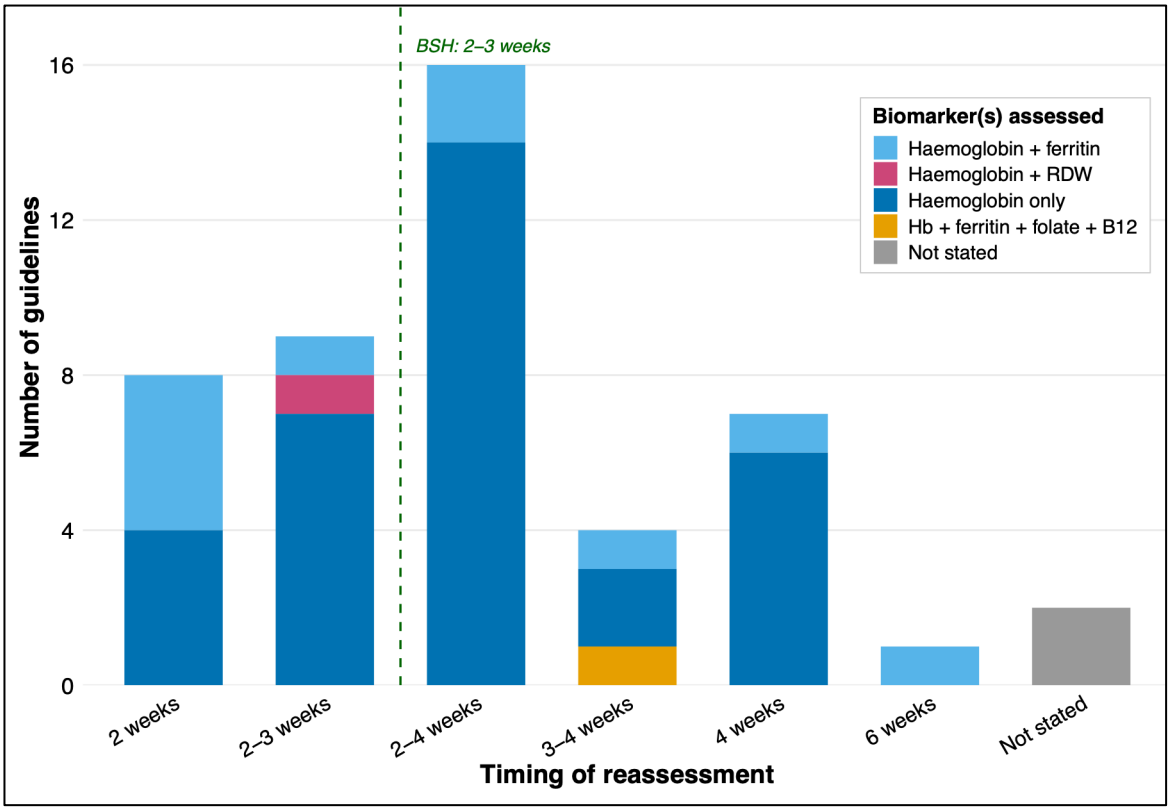


Figure 2.7 Recommendations for monitoring response to oral iron treatment
Timing of reassessment and the biomarker(s) recommended for monitoring response to oral iron therapy. The dashed line indicates the timing recommended by the 2020 BSH guideline (2–3 weeks). BSH = British Society for Haematology.

Definitions of treatment response were inconsistently specified. The BSH guideline recommends reassessment of haemoglobin after commencing oral iron treatment and refers to a demonstrable rise in haemoglobin as evidence of response, but does not define a pregnancy-specific numeric threshold. Sixteen guidelines (33%) defined response as any increase in haemoglobin. A further 15 guidelines (31%) specified a numeric increase in haemoglobin, with thresholds ranging from 10 g/L over 1 week to 20 g/L over 4–6 weeks. One guideline defined response using an increase in RDW. In contrast, 17 guidelines (35%) did not specify any criteria for assessing response. The definitions used are summarised in Table 2.6.

Table 2.6 Local NHS site guidelines' definitions of adequate response to oral iron treatment

Definition of Treatment Response	Time Frame (Weeks)	Guidelines (N, (%))
Any Hb increase	–	16 (33%)
10–20 g/L	4	2 (4%)
10 g/L	1	4 (8%)
10 g/L	2–4	5 (10%)
20 g/L	4–6	4 (8%)
Increase in RDW	–	1 (2%)
Not mentioned	–	17 (35%)

NHS = National Health Service.

2.3.6.2 Monitoring Response to IV Iron

Thirty-one guidelines (63%) included recommendations for monitoring response to IV iron. All advised repeat haemoglobin testing, while six also recommended ferritin measurement and two recommended reticulocyte counts.

The most specified interval for reassessment was 2–4 weeks (n=18). Only four guidelines defined an adequate treatment response. Comparison with national guidance is limited because current guidelines do not provide specific recommendations for monitoring after IV iron administration.

2.4 Discussion

2.4.1 Summary of Main Findings

This study found that despite the presence of national guidance, substantial variation existed across local NHS guidelines for the management of antenatal iron deficiency and anaemia. Although some recommendations were broadly consistent across sites, variation was most pronounced in four domains. First, ferritin testing varied both in whether it was recommended as a routine screening investigation and in the thresholds used to define iron deficiency. Second, oral iron treatment recommendations differed in indications, dosing, and duration, with several sites continuing to recommend higher daily doses that reflect older guidance rather than current evidence. Third, haemoglobin thresholds for initiating IV iron varied widely, with differing approaches to late gestation and symptom-based escalation. Fourth, monitoring of treatment response was one of the most heterogeneous domains, with variation in both the timing of

reassessment and the definition of an adequate response, and many guidelines providing limited or no guidance in this area.

2.4.2 Interpretation of Findings

Several factors may explain the variation observed across local guidelines. Some differences are likely to reflect appropriate adaptation of national recommendations to local healthcare contexts, including differences in population needs, service configuration, workforce capacity, laboratory pathways, and access to specialist services.[106,109] Such adaptation is an expected component of translating national guidance into local clinical practice. However, variation appeared greatest in areas where the evidence base remains limited, evolving, or open to interpretation. This was particularly evident for ferritin testing and diagnostic thresholds, oral iron dosing, intravenous iron use, and monitoring treatment response, where ongoing uncertainty may contribute to differences in how national recommendations are interpreted and translated into local clinical guidance.

Importantly, divergence from BSH recommendations should not necessarily be interpreted as evidence of poor adherence to national guidance. Given the evolving evidence base on maternal iron deficiency, some local guideline groups may have adopted alternative approaches informed by emerging evidence, local expert consensus, or alignment with other national or international recommendations. Variation may therefore reflect differences in how evidence is interpreted and prioritised, rather than inconsistency in guideline quality alone.

The following sections explore these areas of greatest variation in further detail, beginning with ferritin testing and diagnostic thresholds for iron deficiency.

2.4.2.1 Ferritin testing and diagnostic thresholds

The variation observed in ferritin testing and diagnostic thresholds reflects uncertainty regarding the role of ferritin in routine antenatal care. Current UK guidance does not recommend universal ferritin screening in pregnancy, and there is limited evidence to support its clinical effectiveness

or cost-effectiveness as a routine test.[51] The inclusion of routine ferritin testing in some local guidelines may reflect a growing recognition of the clinical implications of IDWA.[18,118]

Variation in ferritin thresholds further highlights this lack of consensus. Although many guidelines aligned with the <30 μg/L threshold recommended by the BSH,[51] the use of lower thresholds, such as <15 μg/L, is consistent with earlier UK guidance and current WHO definitions of iron deficiency.[54] In contrast, one guideline adopted a higher threshold of 50 μg/L. This approach may reflect emerging evidence from non-pregnant populations suggesting that ferritin concentrations below this level can indicate depleted iron stores and may warrant treatment.[119,120] However, the applicability of this evidence to pregnancy remains uncertain, as haemodilution and gestation-specific changes in iron metabolism may alter the relationship between ferritin concentration and iron status.[12,67,89]

Overall, variation in ferritin-related recommendations likely reflects both differences in the uptake of evolving evidence and ongoing uncertainty in how iron deficiency should be defined and identified in pregnancy.

2.4.2.2 Prophylactic oral iron and dosing

Prophylactic oral iron is not routinely recommended in current UK national guidelines because evidence of clinical benefit and safety remains unclear.[51] Associations between iron supplementation and maternal outcomes such as pre-eclampsia[121] and gestational diabetes[122] have been inconsistent, while concerns remain regarding side effects and tolerability in women who may not require treatment. These uncertainties likely contribute to variation in recommendations regarding prophylactic iron use across local guidelines.

Among guidelines recommending oral iron treatment, most were broadly aligned with current national dosing recommendations. However, some continued to recommend higher daily doses (100–200 mg elemental iron), likely reflecting older guidance and established prescribing practices. This variation persists despite more recent evidence suggesting that lower-dose

regimens may optimise iron absorption and improve tolerability without compromising effectiveness.[51,123,124]

2.4.2.3 Intravenous iron

The wide range of haemoglobin thresholds used to initiate IV iron, from <65 g/L to <110 g/L, suggests variation in clinical decision-making regarding treatment escalation. This is consistent with findings from Australia and New Zealand, where substantial variation in the increasing use of IV iron in clinical practice has been reported.[125]

IV iron results in a more rapid improvement in haemoglobin and iron indices compared with oral therapy.[126] Evidence regarding clinical benefit beyond haematological response has been less consistent. Earlier systematic reviews did not demonstrate clear improvements in most maternal or neonatal outcomes despite superior haematological responses with IV iron.[101,126] More recent trials, however, have reported improvements in selected birth outcomes in some populations. These include a reduction in low birthweight among women with moderate iron deficiency anaemia treated with ferric carboxymaltose,[127] and a higher mean birthweight among women with non-anaemic iron deficiency.[128] However, the latter finding should be interpreted cautiously, as birthweight was a secondary outcome and the study was not powered to detect differences in clinical endpoints. IV iron is also associated with higher direct and service-level costs. These findings highlight areas where stronger evidence and clearer guidance are needed.

2.4.2.4 Monitoring of treatment response

Monitoring treatment response was among the most heterogeneous domains assessed in this study. The BSH guideline recommends repeat haemoglobin assessment 2–3 weeks after initiating oral iron therapy and advises that a rise in haemoglobin should be demonstrable. However, it does not specify a pregnancy-specific numeric threshold for an adequate response, recognising that the expected haemoglobin rise may vary according to gestational age, and baseline haemoglobin concentration.[51]

Although many local guidelines adopted this general approach, substantial variation was still observed in the timing of reassessment, biomarkers used, and definitions of treatment response. Reassessment intervals ranged from two to six weeks, and others specified quantitative thresholds ranging from 10 g/L over one week to 20 g/L over four to six weeks. Seventeen guidelines provided no definition of treatment response.

This variation likely reflects the challenges of assessing treatment response during pregnancy, in which physiological changes across gestation may influence commonly used haematological and iron-status biomarkers. Response to oral iron is an important determinant of subsequent management, including consideration of IV iron therapy, yet there remains limited consensus on the optimal timing of reassessment and the criteria for defining an adequate response. Appropriate response to iron therapy is also associated with improved maternal and neonatal outcomes.[129]

The uncertainty surrounding treatment monitoring identified in this chapter provided the rationale for the systematic review presented in Chapter 3, which examines how treatment response is assessed in randomised controlled trials of oral iron supplementation during pregnancy.

2.4.2.5 Variation, Biomarker Interpretation, and Existing Literature

These findings extend previous work, which has largely focused on variation in clinical practice and guideline adherence, often attributing differences to implementation challenges, clinician behaviour, or local resource constraints.[96,130,131] Consistent with previous reports of variation in antenatal anaemia management,[53,105] the present study suggests that at least some of this variation may originate within local written guidance itself, before recommendations reach the level of individual clinical decision-making.

Notably, the greatest variation was observed in recommendations relating to ferritin testing and treatment monitoring. Variation in ferritin thresholds across sites, ranging from <15 μg/L to 50 μg/L, does not simply indicate that guidelines differ; it may also reflect differing interpretations of what a given ferritin value represents in pregnancy, where haemodilution, changes in iron metabolism, and acute-phase responses alter biomarker behaviour. Similarly, variation in

monitoring recommendations, including the timing of reassessment and definition of an adequate response, suggests limited consensus regarding how treatment response should best be assessed during pregnancy and which biomarkers are most informative in this setting.

These findings suggest that variation in recommendations may arise not only from differences in healthcare delivery but also from uncertainty regarding what commonly used iron biomarkers represent during pregnancy and how they should be interpreted.

2.4.3 Strengths and Limitations

A key strength of this study is the inclusion of many NHS sites across all four UK nations, providing a comprehensive overview of local guideline recommendations within a single healthcare system. The 49 included guidelines represent approximately 33% of the UK's annual birth rate, offering substantial population coverage. The use of a structured data extraction framework, applied independently by two reviewers with disagreements resolved through discussion, enabled systematic comparison across multiple domains of antenatal anaemia management.

The four-category alignment framework used to classify guideline recommendations against BSH guidance (aligned, aligned with additional measures, not aligned, not stated) is both a strength and a limitation of this study. As a strength, it provided a transparent and reproducible approach to summarising a large volume of guideline content and enabled systematic identification of areas of convergence and divergence. As a limitation, any classification of guideline content against a reference standard involves interpretive judgement, particularly where local guidelines used ambiguous or non-specific language. This was mitigated through independent extraction and cross-checking among reviewers, but the possibility that reasonable observers might classify individual recommendations differently cannot be ruled out.

Several further limitations should be considered. First, the sampling frame was based on sites participating in the OBS UK and PANDA research programmes, supplemented by publicly available guidelines, and may not represent all NHS maternity services. Sites willing to participate

in research programmes may differ systematically from non-participating sites, potentially in ways that affect the quality or currency of their guidelines. Second, the analysis reflects guideline recommendations rather than actual clinical practice, and variation in implementation is likely to exist beyond what is captured at the guideline level. Third, some guidelines lacked detail in specific domains, limiting the ability to fully assess the recommendations; the "not stated" category in the alignment framework captures this but cannot distinguish between a deliberate omission and an oversight. Fourth, this study did not assess the underlying reasons for local divergence from national guidance, nor did it extract data on haemoglobinopathy-specific recommendations, which may warrant further exploration.

A final limitation relates to the use of the 2020 BSH guideline as the reference standard. Although most included guidelines (n=43) were published after 2020, publication dates ranged from December 2010 to March 2025, meaning that some local guidelines were developed before the current BSH recommendations became available. In addition, guideline development and revision inevitably lag behind both emerging evidence and national guideline updates. Consequently, some observed divergence from BSH recommendations may reflect differences in publication and update cycles across organisations rather than true disagreement with national guidance.

2.4.4 Implications for Clinical Practice, Policy and Research

The impact of local guideline variation on clinical practice and patient outcomes is uncertain; however, it may contribute to differences in the diagnosis and management of antenatal anaemia, with potential implications for patient care. Inconsistencies in ferritin testing, treatment thresholds, and monitoring strategies create the potential for women with similar clinical profiles to be managed differently across NHS sites. These findings also highlight the importance of ensuring that local guidelines are clearly aligned with national recommendations and that any deviations are transparently justified. Local audit may provide a useful mechanism for evaluating adherence to guidelines and understanding the relationship between guideline content and clinical practice.

From a policy perspective, these findings highlight the challenges of translating national guidance into consistent local practice within a large national healthcare system. This is particularly relevant in the NHS context, where reducing unwarranted variation in care is a recognised priority within national operational planning frameworks.[110] The persistence of dosing recommendations in some guidelines that differ from current national guidance, alongside the absence of monitoring guidance in others, suggests that processes for reviewing and updating local guidelines in line with evolving evidence and national recommendations may require strengthening. At the same time, the development and maintenance of local guidelines require substantial time and resources, which may contribute to variation in their quality and currency.[132]

From a research perspective, the observed variation underscores important gaps in the evidence base. Two priorities emerge. First, studies linking guideline content to prescribing behaviour and patient outcomes are needed to determine whether the variation identified here has consequences for the quality and equity of antenatal anaemia care. Second, and more fundamentally, resolving the underlying evidence gaps in oral iron dosing, treatment response monitoring, and IV iron escalation criteria is essential for future national guidance to provide the specificity local implementation may require.

2.5 Conclusion

This study provides the first comprehensive analysis of local NHS guidelines for the assessment, treatment, and monitoring of antenatal iron deficiency and anaemia across the UK. Although broad agreement was observed in some areas of practice, important variation remained in recommendations regarding ferritin testing, oral iron dosing, IV iron use, and monitoring treatment response.

By demonstrating variation within local written guidance itself, this chapter highlights an important yet underexplored stage in the translation of evidence into clinical practice. The findings suggest that differences between guidelines may arise not only from local service delivery considerations but also from ongoing uncertainty regarding the evidence underpinning key aspects of antenatal management of iron deficiency and anaemia.

Variation was particularly evident in recommendations regarding treatment monitoring, including the biomarkers assessed, the timing of reassessment, and the criteria for defining response. Understanding the evidence underpinning these recommendations is therefore essential. The next chapter addresses this issue through a systematic review examining how treatment response to oral iron supplementation is assessed in pregnancy research.

Chapter 3 - **Assessing Response to Oral Iron in Pregnancy:** A Systematic Review of Randomised Controlled Trials

Publication Status

A version of this chapter was submitted to ***BMC Pregnancy and Childbirth*** and following external peer review, was revised and resubmitted in March 2026:

Haynes S., Younger A., Rosson S., Hirst J., Stanworth S. *Assessing Response to Oral Iron in Pregnancy: A Systematic Review of Randomised Controlled Trials*. Manuscript submitted to *BMC Pregnancy and Childbirth*, 2026.

Collaborators

Dr Ashley Younger, Ms Sarah Rosson, Prof Jane Hirst, Prof Simon Stanworth

Statement of Contribution

I conceived and designed the study and led the development and registration of the protocol. I conducted title/abstract and full-text screening, with AY and SR contributing as second reviewers. I independently developed the statistical analysis plan and performed all analyses. I led the interpretation of the findings and wrote the manuscript in full, with critical input and revision from co-authors.

3.1 Introduction

Chapter 2 demonstrated substantial variation in recommendations for monitoring response to oral iron therapy during pregnancy across NHS maternity guidelines. Variation was observed in the biomarkers used, the timing of reassessment, and the definitions of an adequate treatment response, suggesting limited consensus on how to assess response to oral iron in pregnancy.

This chapter examines the research evidence underpinning these monitoring practices through a systematic review of RCTs of oral iron therapy during pregnancy. The review investigates which haematological biomarkers are used to assess treatment response, when response is measured following treatment initiation, and how these approaches vary across healthcare settings.

Rather than evaluating the effectiveness of individual monitoring strategies, this chapter examines how the response to oral iron therapy is operationalised in clinical research. Characterising variation in biomarker selection and assessment timing provides context for the heterogeneity observed in clinical guidelines and contributes to the broader thesis aim of examining the use of iron status biomarkers for the assessment and monitoring of iron deficiency and anaemia in maternal health.

3.1.1 Monitoring Response to Oral Iron in Pregnancy

Oral iron supplementation is the first-line treatment for IDA in pregnancy because it is safe, accessible, and cost-effective.[51,133] However, its effectiveness is variable, and treatment is often limited by gastrointestinal side effects.[51,129,134,135] As a result, not all women respond adequately to oral iron, making the assessment of treatment response a key component of clinical management.

A poor response to oral iron therapy during pregnancy is commonly reported. It is often attributed to poor adherence or intolerance but may also reflect impaired absorption, inflammation-driven iron sequestration, or alternative causes of anaemia.[134,135] In a large cohort study involving more than 20,000 pregnant women, 17.8% were refractory to oral iron supplementation, and non-response was associated with adverse pregnancy outcomes.[129] Women whose anaemia was

corrected with oral iron had significantly lower odds of preterm birth and hypertensive disorders, whereas those with non-responsive anaemia experienced outcomes comparable to untreated women.[129]

These findings highlight the clinical importance of reliably identifying non-response to support timely investigation and appropriate treatment escalation. This is particularly relevant in the context of increasing use of IV iron in pregnancy, where decisions to escalate treatment are often based on perceived failure to respond to oral therapy.[51,133] Identifying those who do not respond to oral iron early in treatment is therefore critical to enable timely intervention with alternative therapies.

3.1.2 Limitations of Current Approaches to Monitoring Response

In current clinical guidelines, response to oral iron therapy during pregnancy is typically assessed using haemoglobin[51,133,136–138] (Table 3.1). This reflects the absence of a practical reference standard measure of iron status; although bone marrow iron assessment is considered the reference standard, it is not appropriate for routine care.[73] Haemoglobin therefore functions as a surrogate, end-stage marker of iron deficiency and treatment response.

Despite global reliance on haemoglobin, a recent review of national and international guidelines demonstrated limited consensus regarding both the timing of reassessment and the definition of an adequate response during pregnancy.[53] These haemoglobin-based recommendations appear largely extrapolated from non-pregnant populations, in which treatment response is commonly conceptualised as a relatively linear increase in haemoglobin following iron replacement. However, this assumption does not account for the substantial physiological changes that occur during gestation, including plasma volume expansion, altered iron trafficking, and inflammatory modulation.[12] Consequently, haemoglobin follows a non-linear trajectory across pregnancy, and observed changes in haemoglobin may reflect both treatment response and normal physiological adaptation, making interpretation more complex than in non-pregnant adults. The growing

recognition of IDWA as a clinically relevant condition further highlights the limitations of relying on haemoglobin alone.[18]

Table 3.1 Current guidelines for measuring response to oral iron in pregnancy

Guidelines	Location	Biomarker	Timing of Measurement	Expected Increase
Asia-Pacific[139]	Asia-Pacific	Hb	2 weeks after starting oral iron	≥ 10g/L at 2 weeks
BCG [138]	Canada	Hb	2-4 weeks after starting oral iron	≥ 10-20g/L at 4 weeks
CDC [136]	America	Hb or Hct	4 weeks after starting oral iron	Hb: ≥ 10g/L at 4 weeks Hct: ≥ 3% at 4 weeks
DSOG[140]	Denmark	Hb and SF	2-4 weeks after starting oral iron then, every 8 weeks for the duration of the pregnancy.	-
FEMECOG[141]	Mexico	Hb	Every 3 months for 1 year and annual from there on	-
FOGSI[137]	India	Hb	4 weeks after starting oral iron	-
NATA[142]	Belgium	Hb	2-4 weeks after starting oral iron	≥ 10g/L at 2 weeks ≥ 20g/L at 4 weeks
SAMNCP[143]	Australia	Hb and reticulocyte count	2 weeks after starting oral iron	-
Swiss Delphi[144]	Switzerland	Hb	Hb: 14 days after starting oral iron	Hb: ≥ 10g/L within 14 days
UK[51]	United Kingdom	Hb	2-3 weeks after starting oral iron	Demonstrable Hb rise by 2 wks.
WHO[133]		Hb	-	Aim Hb >110g/L

Adapted from results presented by Mintsopoulos et al. (2024).[53] Hb = haemoglobin; HCT = haematocrit; SF = serum ferritin.

3.1.3 Rationale, Aim and Research Questions

In the absence of pregnancy-physiology-informed recommendations, it remains unclear how to assess the response to oral iron therapy during pregnancy. Chapter 2 demonstrated substantial variation in monitoring recommendations across local NHS guidelines, including the biomarkers used, timing of reassessment, and definitions of an adequate response. This variation raises the question of whether the underlying research evidence demonstrates a consistent approach to assessing treatment response in pregnancy.

This chapter presents a systematic review of RCTs of oral iron therapy during pregnancy, focusing on how haematological response has been assessed in clinical research. Rather than evaluating the effectiveness of individual biomarkers or monitoring strategies, the review examines how

treatment response is operationalised across studies, including the biomarkers selected, timing of assessment, and definitions of response applied. By characterising variation in research practice, this chapter provides context for the heterogeneity observed in clinical guidelines and contributes to the broader thesis aim of examining the use of iron status biomarkers in the assessment and monitoring of iron deficiency and anaemia in maternal health.

Aim

To examine how the response to oral iron therapy during pregnancy is assessed in clinical research, including the biomarkers selected, timing of assessment, and definitions of response used across studies.

Research Questions

1. Which haematological biomarkers are used to assess response to oral iron supplementation during pregnancy?
2. When is the response measured following initiation of oral iron therapy?
3. How do biomarker selection and timing of assessment vary across healthcare contexts?

3.2 Methods

3.2.1 Study Design

This systematic review was conducted in accordance with the PRISMA 2020 guidelines.[145] The review protocol was prospectively registered with the Open Science Framework (OSF; ID: xys3e; registered 19 September 2024), accessible at https://osf.io/xys3e/, and the International Prospective Register of Systematic Reviews (PROSPERO; CRD420251014407), accessible at https://www.crd.york.ac.uk/PROSPERO/view/CRD420251014407.

Prospective registration was undertaken to ensure methodological transparency and to reduce the risk of selective reporting. Following registration, one modification to the protocol was made: restriction of eligible study designs to RCTs. This change was implemented during full-text screening and is described below.

3.2.2 Eligibility Criteria

Population

Studies were eligible if they included pregnant women with anaemia or iron deficiency, as defined by the investigators. Definitions of anaemia and iron deficiency varied across settings (e.g., haemoglobin thresholds or ferritin cut-offs) and were accepted as reported.

Studies were excluded if they included:

- Non-pregnant women
- Animal models

Intervention

The intervention of interest was oral iron supplementation during pregnancy, administered for at least five consecutive days. This minimum duration was selected because the earliest measurable treatment response, typically reticulocyte count, occurs within 5–7 days of initiating therapy.[146]

Studies in which oral iron was used as a comparator arm to IV or intramuscular (IM) iron were eligible if biomarker outcomes were reported separately for the oral iron group.

Studies were excluded if:

- Oral iron was combined with IV or IM iron
- Oral iron was combined with another co-intervention (e.g., folic acid or erythropoietin) where the independent effect of oral iron could not be isolated

Comparator and Context

No formal comparator was specified, as the aim of the review was to describe monitoring approaches rather than evaluate their relative effectiveness against a gold standard. However, studies that used placebo, IV, or IM iron as comparators were included if the oral iron arm met eligibility criteria.

Studies conducted across all healthcare settings (primary, secondary, tertiary) and countries were eligible. Countries were classified according to the World Bank income classification, a standardised and widely used framework, to enable comparison of biomarker selection and assessment timing across healthcare contexts.

Outcomes

Studies were included if the primary outcome was a change in any haematological biomarker (e.g., haemoglobin, serum ferritin, TSAT, sTfR, reticulocyte count) measured within 12 weeks of initiating oral iron therapy. The 12-week timeframe was selected to capture the initial response to oral iron therapy, rather than longer-term maternal or neonatal outcomes. Previous studies have shown that most treatment-related changes in haematological and iron status biomarkers occur within the first 12 weeks of therapy.[51,147,148]

Studies were excluded if:

- The primary outcome was non-biochemical (e.g., adherence, adverse effects, or clinical pregnancy outcomes such as preterm birth or postpartum haemorrhage)
- Biomarker assessment occurred more than 12 weeks after treatment initiation

This approach ensured that the haematological response remained the focus and minimised heterogeneity across studies designed to assess downstream clinical outcomes.

Study Designs

The original protocol permitted a range of study designs. During full-text screening, eligibility was restricted to RCTs.

This restriction was applied because enough RCTs were available, and RCTs represent the highest-quality evidence for intervention studies. Restricting inclusion to RCTs ensured that observed monitoring strategies reflected deliberate outcome selection within interventional study designs rather than incidental measurement in observational research. This approach was intended to capture practices most likely to inform guideline development and regulatory decision-making.

Quasi-RCTs, cluster-RCTs, and all non-randomised comparative studies were excluded. Non-primary research (e.g., reviews and commentaries) and non-English publications were excluded due to feasibility. The exclusion of non-English publications introduces potential language bias.

One trial (Abbas et al. 2019)[149] initially included in the review was retracted by the journal that published it while this chapter was being finalised. The retraction notice cited significant concerns regarding the integrity of the data and reported results, noting that the authors did not respond to enquiries or provide the original data.[150] The trial was therefore removed from the analysis, and all counts and figures presented in this chapter reflect the remaining 31 included studies. Removal of the study did not materially alter the findings or conclusions of the review.

3.2.3 Information Sources and Search Strategy

The search strategy was developed in collaboration with a specialist medical librarian. Initial scoping searches in MEDLINE informed the selection of Medical Subject Headings (MeSH) and free-text terms relating to pregnancy, iron deficiency, oral iron, and biomarkers of response. The strategy was peer-reviewed by two independent researchers prior to execution (Appendix 3 Supplementary Table S3.1).

The final search was conducted across five databases from inception to 14 March 2025:

- MEDLINE
- Embase
- Cochrane Central Register of Controlled Trials (CENTRAL)
- Web of Science Core Collection
- CINAHL

Grey literature sources and additional trial registries beyond OSF and PROSPERO were not searched, and no unpublished data were sought.

3.2.4 Study Selection

Search results were imported into Covidence (Veritas Health Innovation), and duplicates were removed. Three reviewers (SH, AY, SR) independently screened all titles and abstracts, followed

by a full-text review of eligible studies. Discrepancies were resolved through discussion or referral to a third reviewer. Reasons for full-text exclusion are recorded in the PRISMA flow diagram (Figure 3.1).

3.2.5 Data Extraction

Data extraction was conducted using a standardised tool developed in Covidence in accordance with the Joanna Briggs Institute (JBI) Manual for Evidence Synthesis[151] (Appendix 3 Supplementary Table S3.2). A second reviewer independently verified all extracted data, and discrepancies were resolved through discussion until consensus was reached.

Extracted variables included:

- Study characteristics (year, country, World Bank income classification[37], setting, methodology)
- Population characteristics (sample size, gestational age, baseline iron status)
- Oral iron regimen (type, dose, duration)
- Biomarkers measured and laboratory methods
- Timing of biomarker assessment

3.2.6 Risk of Bias Assessment

Risk of bias was assessed using the Cochrane Risk of Bias 2 (ROB-2) tool for randomised controlled trials.[152] ROB-2 evaluates bias across five domains: the randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result.

ROB-2 is designed to be applied at the level of a specific outcome. Because this review was descriptive, characterising how response to oral iron is assessed rather than pooling treatment effects, a single assessment was conducted per trial, anchored to the primary outcome used to evaluate treatment response in each study. This provided a consistent study-level summary of methodological quality that was proportionate to the review's aim. It was not intended to support

quantitative comparison of effect estimates between trials, and the resulting judgements should therefore be interpreted as an overall assessment of trial conduct rather than an outcome-specific appraisal. Assessments were conducted by one reviewer and independently verified by a second reviewer, with discrepancies resolved through discussion.

3.2.7 Sensitivity Analysis – Studies Conducted in India

Of the 31 included studies, 15 were conducted in India, representing a substantial proportion of both the total dataset and of studies conducted in LMICs (n = 18). Given this high representation, a prespecified sensitivity analysis was conducted to assess the robustness of the findings. Analyses were repeated, including only studies conducted in India, and compared narratively with the full dataset, supported by visualisation of biomarker use and timing patterns.

3.2.8 Data Analysis and Synthesis

This review was designed to describe how response to oral iron therapy is assessed during pregnancy, rather than to estimate treatment effects or to determine the superiority of individual monitoring strategies. Consequently, the primary objective was to characterise patterns in biomarker selection, assessment timing, and response definitions across studies. Given this descriptive objective and substantial heterogeneity in study populations, oral iron regimens, biomarkers measured, outcome definitions, and timing of assessment, a meta-analysis was not considered appropriate. Findings were therefore synthesised narratively in accordance with the Synthesis Without Meta-analysis (SWiM) reporting guidelines.[151] SWiM provides a structured framework for transparent reporting where meta-analysis is not appropriate.

Results were synthesised and reported by research question, with studies grouped by:

1. Biomarker Choice
2. Timing of Assessment
3. Healthcare Context

Descriptive statistics (counts and percentages) were used to summarise biomarker frequency, timing, and combinations. Results were stratified by World Bank income classification to examine

variation in monitoring approaches across settings. All statistical analysis and data visualisation were conducted using Microsoft Excel and RStudio (Version 2025.09.2+418).

3.3 Results

3.3.1 Study Selection

The literature search identified 4343 records for title and abstract screening. After deduplication and screening of titles, abstracts, and full texts, 31 RCTs were included in the final analysis (see Table 3.2). One additional trial (Abbas et al. 2019),[149] initially included, was retracted by the publishing journal during finalisation of this chapter and has been removed from the analysis. The most common reasons for full-text exclusion were non-randomised design, absence of oral iron as an intervention, or biomarker measurement not being the primary outcome. The study selection process is outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Figure 3.1).

Table 3.2 Summary of included studies

Study Id	Country / Income	Setting	Sample Size	Ga Incl. (Wks.)	Anaemia Incl. Criteria	ID Incl. Criteria	Intervention	Comparator(S)	Oral Iron Duration	Primary Biomarker And Timepoint	Risk Of Bias Judgement
Abdelmoety 2017[153]	Egypt / LMIC	Urban, Tertiary	150	14-18	Hb <110g/L	Ferritin <12ug/L	Iron amino acid chelate PO 15mg OD	Ferrous Fumarate PO 15mg OD	12 wks.	Hb: 12 wks.	Low Risk of Bias
Abhilashini 2014[154]	India / LMIC	Urban, Tertiary	100	30-34	Hb <110g/L	Peripheral Blood Smear**	Iron Sucrose IV	Ferrous Sulphate PO 60mg TDS	Until Delivery	Hb: 2 wks., 4 wks. and 37 wks. GA	Some Concerns
Al 2005[155]	Turkey / UMIC	Urban, Tertiary	90	26-24	Hb <105/L	Ferritin <13ug/L	Iron Sucrose IV	Iron Polymaltose Complex PO 100mg TDS	Until Delivery	Hb: 4 wks. and delivery	Some Concerns
Arzoo 2020[156]	Bangladesh / LMIC	Urban, Tertiary	150	24-32	Hb <100g/L	Peripheral Blood Smear**	Iron Sucrose IV	Ferrous Sulphate PO 65mg TDS	8 wks.	Hb: 4 wks., 9 wks.	Some Concerns
Awomolo 2023[157]	USA / HIC	Urban, Tertiary	124	20-37	Hb <100g/L or Hct <33%	NA	Ferumoxytol IV	Ferrous Sulphate PO 65mg BD	Until Delivery	Hb: delivery	Low Risk of Bias
Bhavi 2017[158]	India / LMIC	Urban, Tertiary	112	14-34	Hb <110g/L	Ferritin <15ug/L	Iron Sucrose IV	Ferrous Fumarate PO 100mg BD	4 wks.	Hb: 4 wks., Ferritin: 4 wks.	Some Concerns
Breymann 2017[159]	Multi-country* / HIC, UMIC	Urban, Tertiary	252	16-33	***Trimester Based Hb	Ferritin <20ug/L	Ferric Carboxymaltose IV	Ferrous Sulphate PO 100mg BD	12 wks.	Hb: 3 wks.	Some Concerns
Chauhan 2023[160]	India / LMIC	Urban, Tertiary	268	18-22	Hb <90 g/L	Ferritin <30ug/L	Iron Sucrose IV	Ferrous Sulphate PO 100mg BD	4 wks.	Hb: 4 wks., 36 wks. GA, Ferritin: 4 wks., 36 wks. GA.	Some Concerns

Chavan 2021[161]	India / LMIC	Urban, Tertiary	150	12-26	Hb <100g/L	Peripheral Blood Smear**	Ferrous Sulphate PO 120mg OD	Iron Polymaltose Complex PO 100mg OD, Ferrous Fumarate PO 100mg OD	90 days	Hb: 4 wks., 8 wks., 12 wks.	Some Concerns
Chawla 2022[162]	India / LMIC	Urban, Tertiary	335	18-34	Hb <110g/L	Ferritin, Red Cell Indices	Ferric Carboxymaltose IV	Ferrous Sulphate PO 60mg BD	Until Delivery	Hb: 6 wks. (proportion not anaemic)****	High Risk of Bias
Dalal 2018[163]	India / LMIC	Urban, Tertiary	75	26-34	Hb <110g/L	Ferritin, Red Cell Indices	Ferrous Sulphate PO 100mg BD/TDS	Iron Sucrose IV	Until Delivery	Hb: 1 wk., 4 wks., Ferritin: 1 wk., 4 wks., Reticulocytes: 1 wk., 4 wks.	Some Concerns
Daniilidis 2020[164]	Greece / HIC	Urban, Tertiary	63	>12	***Trimester Based Hb	Peripheral Blood Smear**	Iron Protein Acetyl Aspartilate PO 40mg BD	Ferrous Sulphate / Fumarate / Gluconate PO 80mg OD	4 wks.	Hb: 4 wks.	Some Concerns
Das 2025[165]	India / LMIC	Urban, Tertiary	100	16-34	Hb <100g/L	Peripheral Blood Smear**	Ferrous Sulphate PO 100mg OD	Carbonyl Iron PO 100mg OD, Iron Sorbitol IM, Iron Sucrose IV	4 wks.	Hb: 4 wks.	Some Concerns
Derman 2025[127]	India / LMIC	Urban; Rural, Primary; Community	4355	14-17	Hb <100g/L	Ferritin <30ug/L	Ferrous Sulphate PO 60mg BD	Ferric Derisomaltose IV, Ferric Carboxymaltose IV	14-17 weeks minimum	Hb: 30 – 34 wks. GA (proportion not anaemic)****	Low Risk
Gupta 2014[166]	India / LMIC	Urban, Tertiary	100	24-34	Hb <90g/L	Ferritin <15ug/L	Ferrous Sulphate PO 60mg TDS	Iron Sucrose IV	4 wks.	Hb: 4 wks. Ferritin: 4 wks.	Some Concerns
Kamdi 2015[167]	India / LMIC	Urban, Tertiary	50	13-27	Hb <100g/L	Ferritin <12ug/L	Ferrous Asparto Glycinate PO 100mg OD	Ferrous Ascorbate PO 100mg OD	28 days	Hb: 2 wks., 4 wks. Ferritin: 2 wks., 4 wks.	High Risk of Bias

Kazi 2022[168]	Pakistan LMIC	Urban, Tertiary	75	10-28	Hb <100g/L	Peripheral Blood Smear**	Ferrous Sulphate PO 60mg BD	Iron Polymaltose Complex PO 100mg OD, Ferrous Ascorbate PO 100mg OD	90 days	Hb: 4 wks., 8 wks., 12 wks., Ferritin: 12 wks., Reticulocytes: 1 wk.	High Risk of Bias
Lagana 2018[169]	Italy / HIC	Urban, Tertiary	50	13-27	Hb < 105g/L	Ferritin <15ug/L	Micronised Dispersible Ferric Pyrophosphate PO 30mg OD	Ferrous Gluconate PO 80mg OD	30 days	Hb: 2 wks., 4 wks. Ferritin: 2 wks., 4 wks.	Some Concerns
Lam 2024[170]	USA / HIC	Urban, Tertiary	88	12-26	Hb <110g/L	Ferritin <25ug/L	Ferrous Sulphate PO 65mg OD	Ferrous Sulphate PO 130mg OD Alternate Days	6 wks.	Hb: 6 wks.	Some Concerns
Murugesan 2023[171]	India / LMIC	Urban, Tertiary	84	>14	Hb <110g/L	Peripheral Blood Smear**	Ferrous Sulphate PO 60mg BD	Ferrous Ascorbate PO 60mg BD, Ferrous Fumarate PO 60mg BD, Iron Sucrose IV	60 days	Hb: 8 wks.	High Risk of Bias
Nappi 2009[172]	Italy / HIC	Urban, Tertiary	97	12-36	Hb <110g/L	Ferritin <12ug/L	Ferrous Sulphate PO 100mg OD	Bovine Lactoferrin PO 100mg BD	30 days	Hb: 4 wks.	Some Concerns
Ortiz 2011[173]	Colombia and Argentina / UMIC	Urban, Tertiary	80	18-26	Hb < 105g/L	Ferritin <15ug/L	Iron Polymaltose Complex PO 100mg BD	Ferrous Sulphate PO 100mg BD	90 days	Hb: 8 wks., 12 wks.	Some Concerns
Pasrischa 2023[174]	Malawi / LIC	Rural, Primary & Community	589	27-35	Hb <110g/L	Ferritin <15ug/L	Ferric Carboxymaltose IV	Ferrous Sulphate PO 60mg BD	90 days	Hb: 36 wks. GA or delivery (proportion not anaemic)****	Low Risk of Bias
Ruangvutilert 2017[175]	Thailand / UMIC	Urban, Tertiary	80	33	Hb <100g/L or Hct <33%	Ferritin <15ug/L	Iron Sucrose IV	Ferrous Fumarate PO 200mg OD	Until Delivery	Ferritin: 36 wks. GA	Some Concerns

Saha 2007[176]	India / LMIC	Urban, Tertiary	100	14-27	Hb <90g/L	Ferritin <12ug/L	Ferrous Sulphate PO 120mg OD	Iron Polymaltose Complex PO 120mg OD	8 wks.	Hb: 8 wks. (proportion not anaemic)****	Some Concerns
Sarkate 2007[177]	India / LMIC	Urban, Tertiary	37	12-26	Hb <100g/L	Ferritin <12ug/L	Sodium Feredetate PO 66mg OD	Sodium Feredate PO 66mg BD, Ferrous Fumarate PO 100mg BD	75 days	Hb: 10 wks.	High Risk of Bias
Shim 2018[178]	Korea / HIC	Urban, Tertiary	90	16-33	***Trimester Based Hb	Ferritin <20ug/L	Ferric Carboxymaltose IV	Ferrous Sulphate PO 100mg BD	12 wks.	Hb: 3 wks.	Some Concerns
Simmons 1993[179]	Jamaica / UMIC	Urban, Community	248	14-22	Hb <110g/L	NA	Ferrous Sulphate PO 100mg BD	Placebo. Gastric Delivery Capsule PO 50mg OD	12 wks.	Hb: 6 wks., 12 wks.	High Risk of Bias
Singh 1998[180]	Singapore / HIC	Urban, Tertiary	100	20-28	Hb <90 g/L	Ferritin <20ug/L	Iron Dextrin IV	Ferrous Fumarate PO 200mg TDS	Until Delivery	Hb: 36 wks., delivery	Some Concerns
Tigga 2020[181]	India / LMIC	Rural, Tertiary	100	18-28	Hb <110g/L	Peripheral Blood Smear**	Ferrous Sulphate PO 60mg BD	Iron Sucrose IV	8 wks.	Hb: 4wks., 8 wks.	Some Concerns
Vernekar 2023[182]	India / LMIC	Urban, Tertiary	100	16-36	Hb <110g/L	Ferritin <50ug/L	Ferrous Sulphate PO 120mg OD	Iron Sucrose IV	3 months	Hb: 3 wks., 12 wks.	Some Concerns

*29 sites across seven countries (Australia, Russia, South Korea, Singapore, Sweden, Switzerland, and Turkey). †Microcytic hypochromic peripheral blood smear. ‡Trimester-specific haemoglobin thresholds: <110 g/L in the first trimester and <105 g/L in the second and third trimesters. §Primary outcome was the proportion of participants no longer anaemic, defined by haemoglobin concentration. GA = gestational age; incl. = inclusion; wks = weeks; ID = iron deficiency; Hb = haemoglobin; HIC = high-income country; LIC = low-income country; LMIC = lower-middle-income country; UMIC = upper-middle-income country; NA = not applicable.

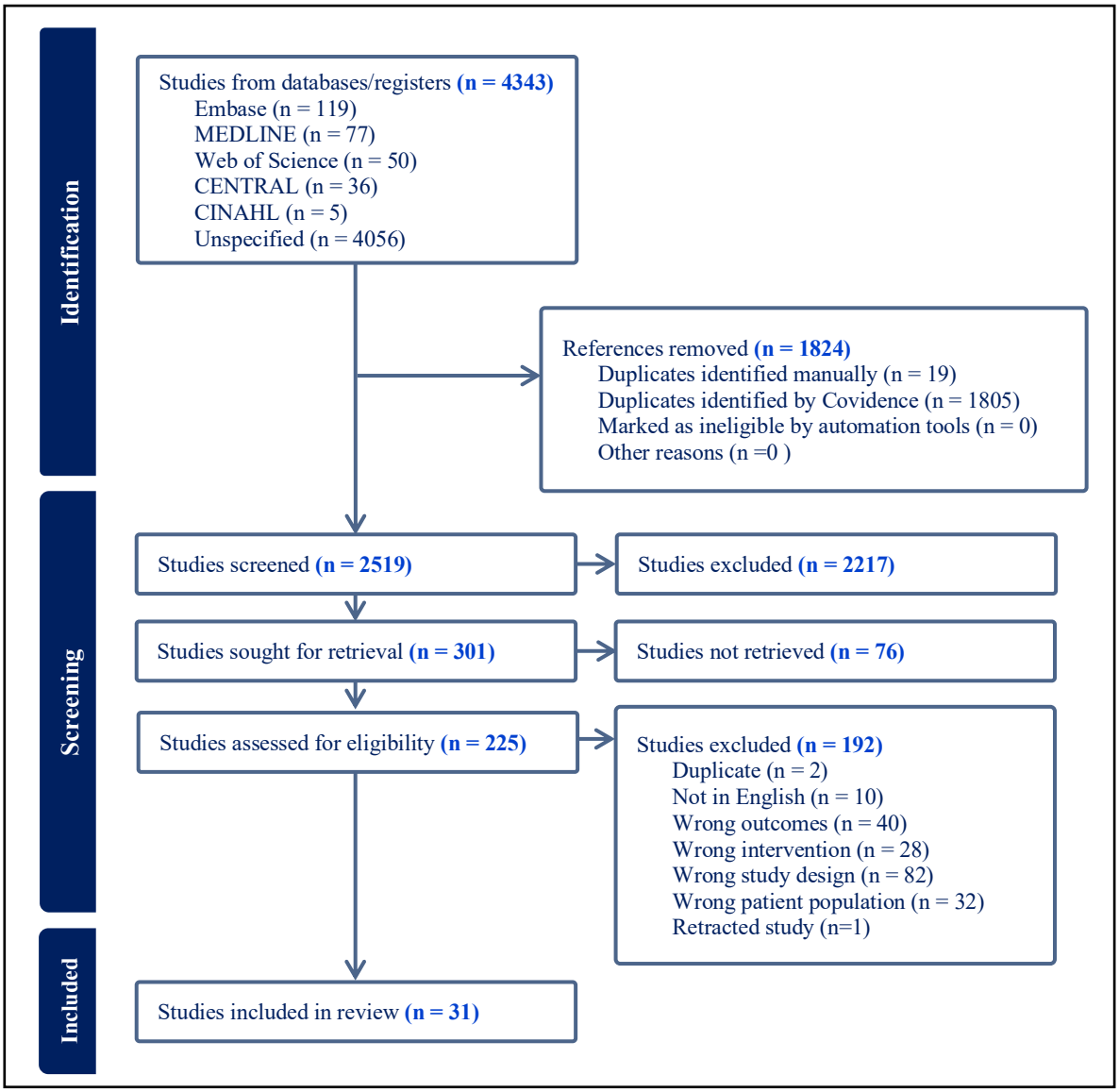


Figure 3.1 PRISMA flow diagram
Identification, screening, and inclusion of studies in the systematic review, following PRISMA 2020. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

3.3.2 Characteristics of Included Studies

The included trials were published between 1993 and 2025, with a median publication year of 2018 (IQR 2014–2022), reflecting increasing research activity over the past decade. The 31 trials were conducted across 20 countries (Figure 3.2). India contributed the most trials (n = 15), while the remaining studies were distributed across Europe, North America, Asia, Africa, and South America. By income level, the dataset comprised 8 high-income country (HIC) trials, 4 upper-middle-income country (UMIC) trials, 18 lower-middle-income country (LMIC) trials, and 1 low-income country (LIC) trial. Most studies were conducted in urban tertiary-care hospital settings. Only 3 were conducted in rural settings and 3 in primary or community-based care.

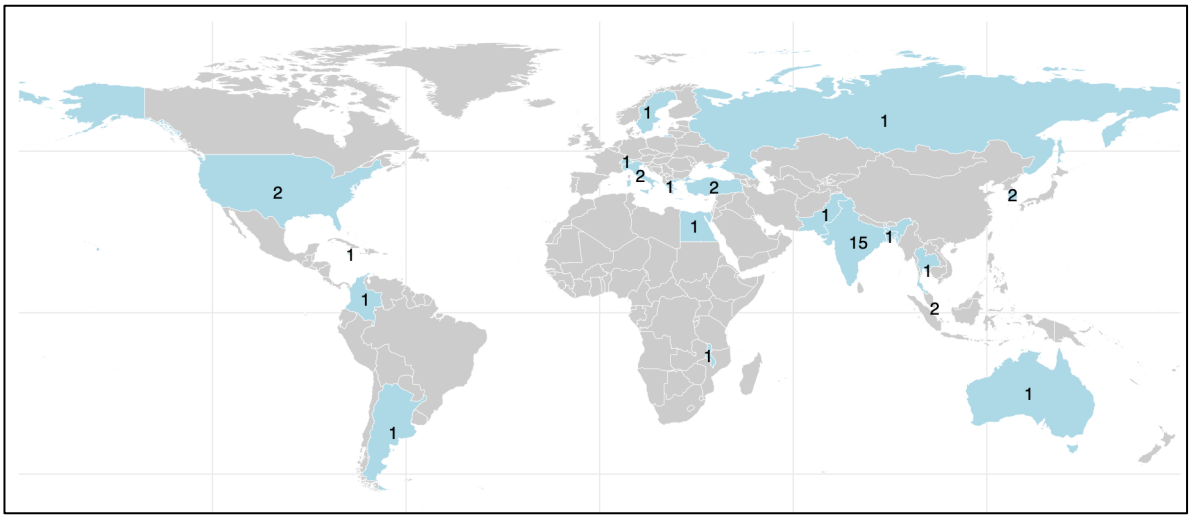


Figure 3.2 Countries included in the review
Some studies were conducted across more than one country.

Participants and Gestational Age

Sample sizes ranged from 37 to 4355 participants, with a median of 100. Gestational age inclusion criteria varied substantially, with lower limits ranging from 10 to 33 weeks' gestation (median 16) and upper limits ranging from 17 to 37 weeks' gestation (median 28). Most studies therefore recruited women in the early-to-mid second trimester and followed them through the late second or early third trimester, although a small number focused specifically on anaemia in later pregnancy.

Definitions of Anaemia and Iron Deficiency

All studies required anaemia as part of the inclusion criteria, operationalised using laboratory haemoglobin thresholds reported by the study authors. The haemoglobin thresholds ranged from 90 g/L to 110 g/L, with lower thresholds more frequently used in LMIC settings. Some studies used trimester-specific haemoglobin cut-offs or incorporated haematocrit into eligibility criteria. In addition, 24 trials required evidence of iron deficiency; ferritin-based definitions were used in 21 of these trials (range: <12–50 µg/L; median: 15 µg/L), while several LMIC studies relied on red cell indices or microcytic–hypochromic peripheral blood smear findings to indicate iron deficiency. Similar approaches were also used in studies conducted in Bangladesh and Pakistan. No trial enrolled women with IDWA.

Interventions and Comparators

Across studies, oral iron was evaluated either as the primary intervention or as a comparator. Seventeen trials compared oral with IV iron, eight compared different oral formulations, and the remainder included multi-arm designs involving both oral and parenteral therapies, including one placebo-controlled trial.

The most used oral preparation was ferrous sulphate. Other oral formulations included ferrous fumarate, ferrous ascorbate, iron polymaltose complex, carbonyl iron, sodium feredetate, and micronised ferric pyrophosphate. IV regimens most frequently included iron sucrose and ferric carboxymaltose. Ferric carboxymaltose was more commonly used in trials conducted in high-income settings, whereas iron sucrose predominated in LMIC and UMIC trials, particularly those conducted in India.

3.3.3 Biomarkers Used to Measure Response

Biomarker use across studies is summarised in Table 3.3. All 31 trials measured haemoglobin, which was the primary outcome in 30 studies. Ferritin was the second most frequently measured biomarker, reported in 25 of 31 trials (80.6%), but was designated the primary outcome in only one study.

Red cell indices, including MCV, MCH, and mean corpuscular haemoglobin concentration (MCHC), were reported in approximately one-third of studies. Serum iron was measured in 12 trials and haematocrit in 8. Other markers of iron status or erythropoietic activity, including reticulocyte count, TSAT, and sTfR, were used less frequently, appearing in fewer than one-quarter of studies.

Given the influence of inflammation on ferritin concentrations, concurrent measurement of inflammatory markers may assist interpretation of iron status biomarkers. However, assessment of inflammation was uncommon. Of the 25 trials measuring ferritin, only one (Ruangvutilert et al. 2017)[175] reported concurrent measurement of C-reactive protein (CRP), and no other inflammatory markers were reported across the included studies.

Table 3.3 Summary of biomarkers used across studies

Biomarker	Studies (n)	% Studies
Hb	31	100
Ferritin	25	80.6
Serum Iron	12	38.7
MCV	12	38.7
MCHC	10	32.3
MCH	10	32.3
TSAT	8	25.8
Hct	8	25.8
TIBC	5	16.1
Reticulocytes	6	19.4
RBC Count	3	9.7
sTfR	2	6.5
Free Erythrocyte Porphyrin	1	3.2
Transferrin	1	3.2
Zinc Porphyrin	1	3.2
Hepcidin	1	3.2
RDW	1	3.2

Hb = haemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; TSAT = transferrin saturation; TIBC = total iron-binding capacity; sTfR = soluble transferrin receptor; RDW = red cell distribution width.

Biomarkers were rarely used in isolation. Only three trials measured haemoglobin alone, and a further five relied solely on haemoglobin and ferritin. Most studies employed composite biomarker panels combining haemoglobin and ferritin with two or more additional markers.

Temporal trends in biomarker use are shown in Figure 3.3. While the use of haemoglobin and ferritin remained consistent over time, more recent studies demonstrated broader biomarker panels, including increased use of reticulocytes and emerging biomarkers such as hepcidin and sTfR.

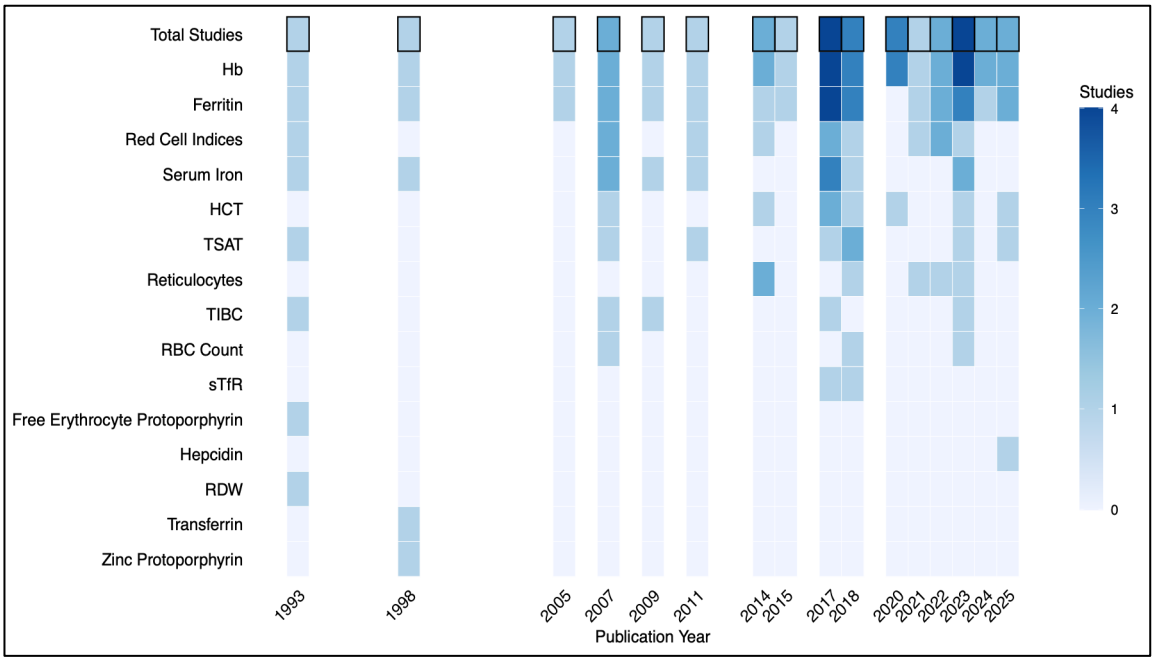


Figure 3.3 Biomarker use by publication year of study
Values indicate the number of included studies using each biomarker, by year of publication. Studies reporting multiple biomarkers contribute to more than one cell, and darker shading indicates more frequent use. Hb = haemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; TSAT = transferrin saturation; TIBC = total iron-binding capacity; sTfR = soluble transferrin receptor; RDW = red cell distribution width.

3.3.4 Timing of Response Assessment

The number of biomarker assessment time points ranged from 1 to 5 per study, with a mean of 2.5. The most common time point for assessing the response to oral iron was 4 weeks after treatment initiation, used in 20 of 31 trials. Later assessments at 8 weeks and 12 weeks were each reported in 9 and 10 trials, respectively, typically in studies with longer-duration oral iron regimens. Early assessments at 1–2 weeks were less common. Assessments at 1 week were almost exclusively performed to measure reticulocyte response, with haemoglobin also reported in one study. At 2 weeks, seven trials assessed haemoglobin alongside ferritin, reticulocyte count, or other red cell indices. Four studies assessed response at fixed gestational-age time points or at delivery, rather than at fixed intervals following treatment initiation. Patterns of biomarker use by timepoint are summarised in Figure 3.4. Haemoglobin and ferritin were most measured at 4, 8, and 12 weeks, whereas reticulocyte assessments were typically performed at 1 week. In most studies, the choice of assessment timing was not explicitly justified in relation to pregnancy-specific physiology.

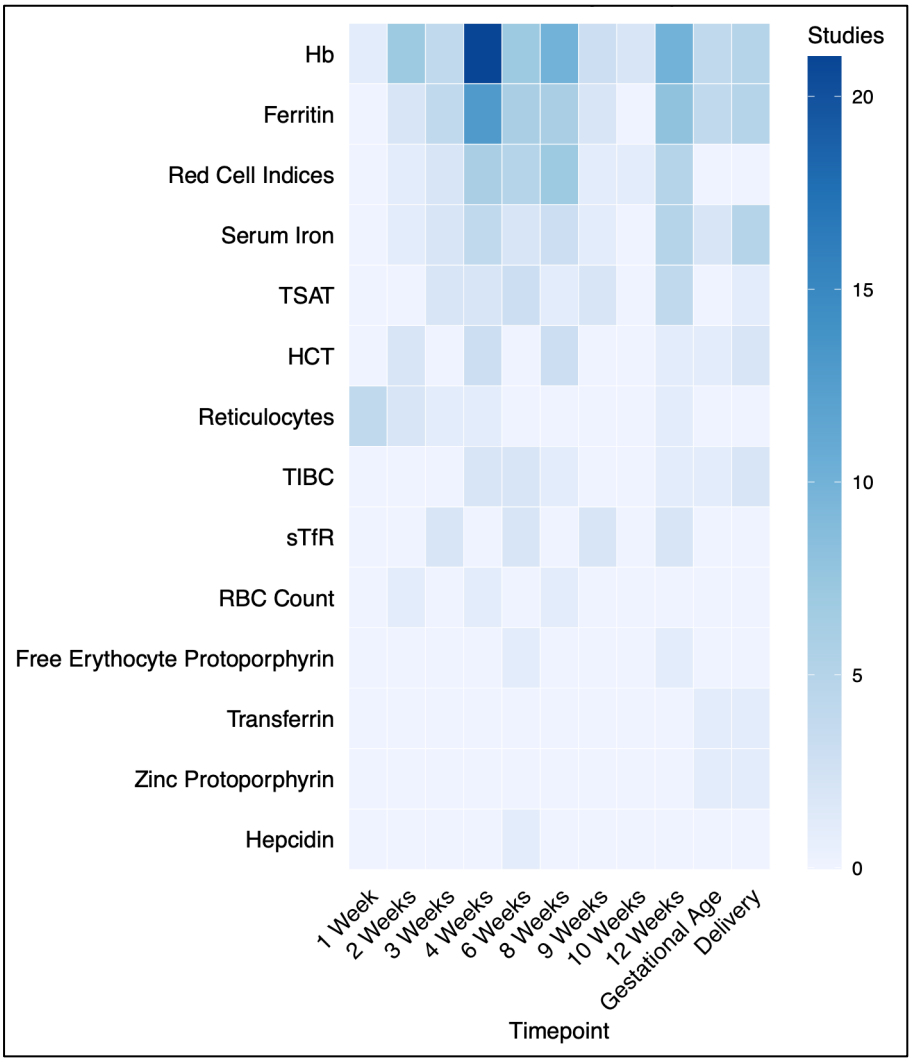


Figure 3.4 Biomarker use by assessment timepoint
Timepoints are reported as weeks following treatment initiation unless otherwise specified. "GA" indicates assessment at a fixed gestational age and "D" indicates assessment at delivery. GA = gestational age.

3.3.5 Variation by Healthcare Context

Biomarker Selection

Patterns of biomarker selection varied by healthcare context (Figure 3.5). HIC studies generally employed the broadest biomarker panels. Among the eight HIC trials, seven used multi-biomarker panels comprising four or more biomarkers, whereas one measured haemoglobin alone. Ferritin and serum iron were included in all seven multi-biomarker HIC trials, and hepcidin and sTfR were measured exclusively in HIC studies.

UMIC studies also used multi-biomarker panels, although these were typically less extensive than those used in HIC settings. In contrast, LMIC studies, particularly those conducted in India, more

commonly used narrower biomarker panels. These studies were less likely to include ferritin and more likely to rely on red cell indices and reticulocyte counts. Reticulocyte measurement was confined to LMIC studies.

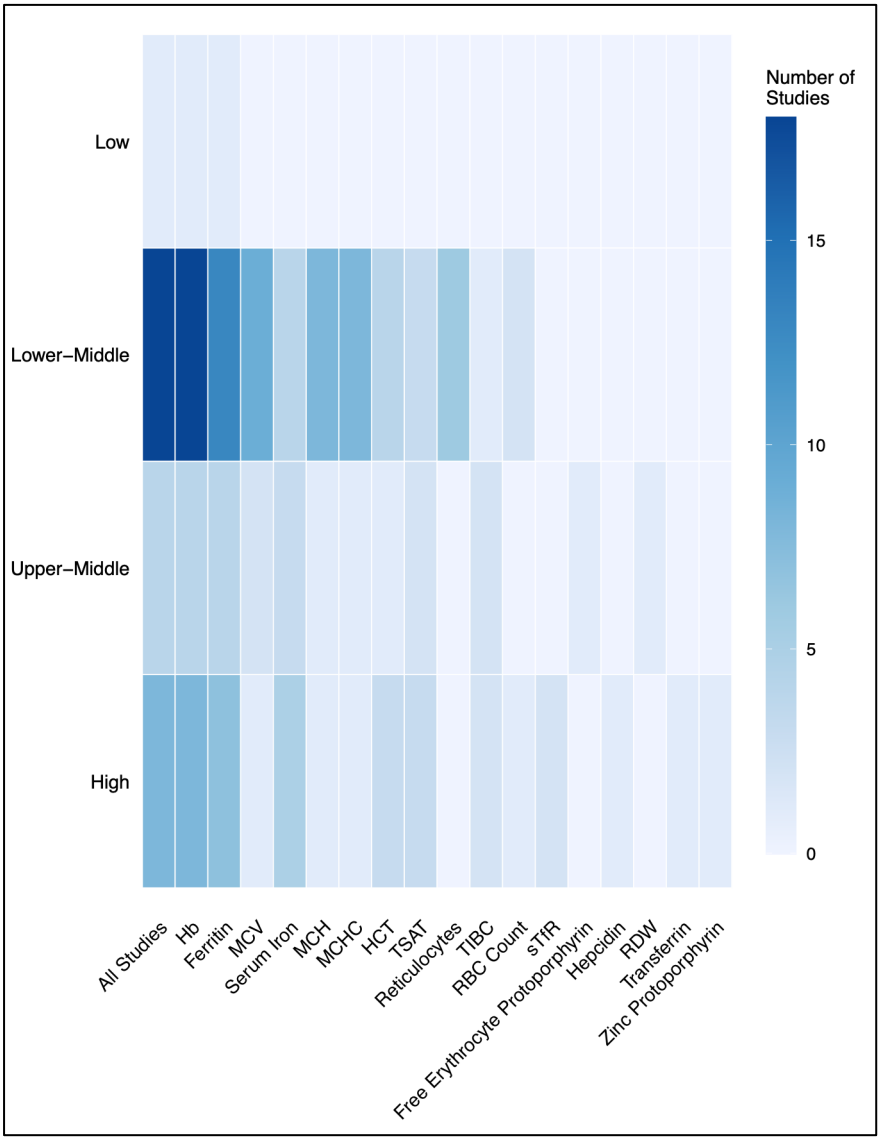


Figure 3.5 Biomarker use by country income level
Values indicate the number of included studies using each biomarker, by country income category. Studies reporting multiple biomarkers contribute to more than one cell. Hb = haemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; TSAT = transferrin saturation; TIBC = total iron-binding capacity; sTfR = soluble transferrin receptor; RDW = red cell distribution width.

Timing of Assessment

Timing of response assessment also varied across income settings. Table 3.4 summarises the distribution of assessment timepoints by income category. LMIC studies most assessed response

at 4 weeks, with early assessments at 1–2 weeks also primarily observed in these settings, usually linked to reticulocyte measurement.

In contrast, HIC studies showed greater variation in timing, with primary assessments more often occurring at 6 weeks, late gestation, or delivery. UMIC studies commonly included intermediate and later follow-up time points, such as 12-week assessments. The single LIC study measured biomarkers only in late pregnancy or at delivery.

Table 3.4 Choice of timepoint to measure response, by income level

Timepoint (wks.)	All Studies (n=31)	HIC (n=8)	UMIC (n=5)	LMIC (n=18)	LIC (n=1)
1	**4**	0	0	4	0
2	**7**	1	1	5	0
3	**4**	1	1	2	0
4	**20**	3	2	15	0
6	**7**	2	2	3	0
8	**9**	0	1	8	0
9	**3**	1	1	1	0
10	**2**	0	0	2	0
12	**10**	1	3	6	0
GA	**4**	1	1	1	1
D	**5**	2	2	1	1

Timepoints are reported as weeks following treatment initiation unless otherwise specified. “GA” indicates assessment at a fixed gestational age and “D” indicates assessment at delivery. GA = gestational age.

3.3.6 Sensitivity Analysis

India accounted for nearly half of all included trials (15/31). Compared with studies conducted elsewhere, Indian trials more frequently relied on erythropoietic and red cell–based measures and less commonly incorporated biomarkers of iron storage and transport, such as ferritin and TSAT. Indian studies also tended to assess response at multiple time points, most commonly at 4, 8, and 12 weeks following treatment initiation. In contrast, studies conducted outside India, spanning HIC, UMIC, and LIC settings, more often included ferritin, TSAT, and other markers of iron metabolism, but generally used fewer assessment time points.

Despite these differences in monitoring approaches, the overall findings were unchanged when Indian studies were examined separately. Haemoglobin remained the only biomarker measured in all studies, ferritin remained the second most used biomarker, and 4 weeks remained the most frequently selected assessment time point. Full results are provided in Appendix 3, Supplementary Table S3.3 and Supplementary Table S3.4.

3.3.7 Risk of Bias

Risk of bias was assessed using the Cochrane ROB-2 tool to provide a structured summary of methodological quality across the included studies. Four trials were judged to be at low risk of bias, 21 had some concerns, and 6 at high risk of bias. A visual summary is provided in Figure 3.6.

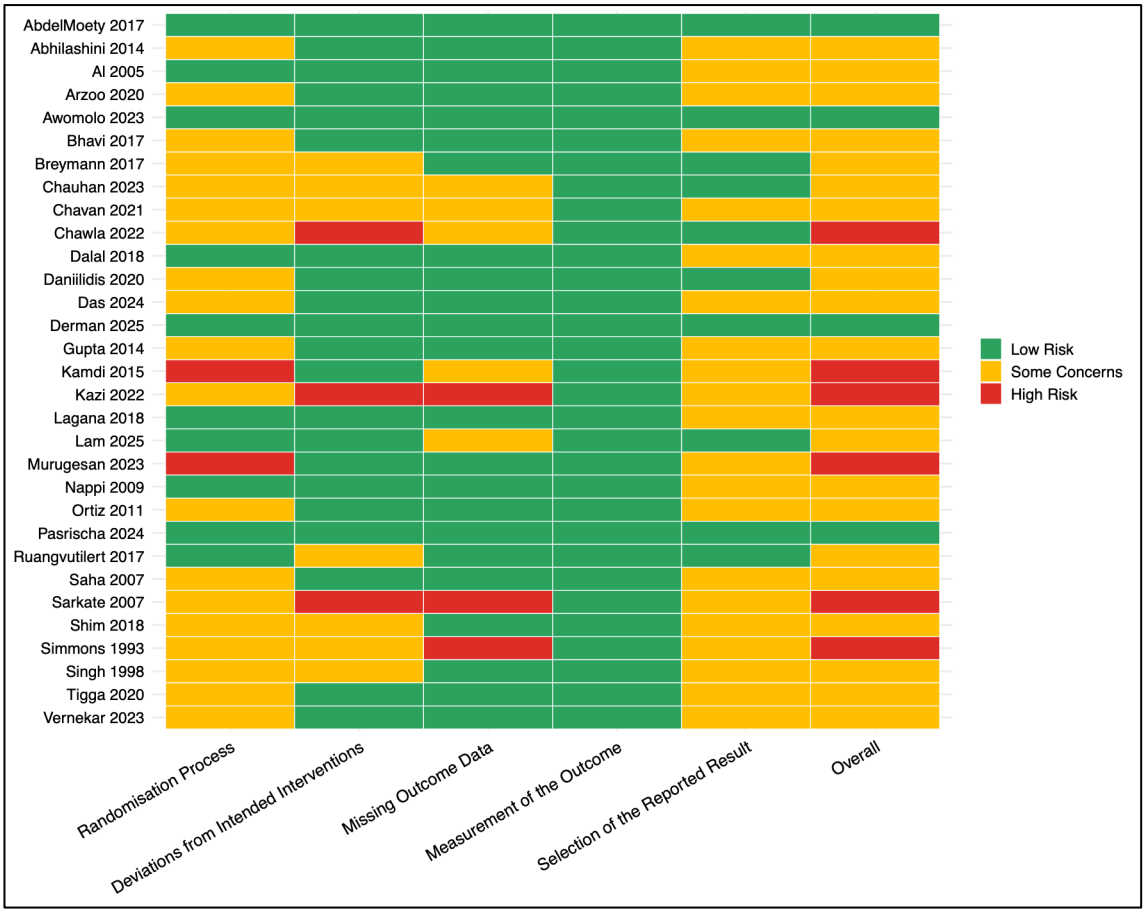


Figure 3.6 Risk of bias summary
Risk of bias across the five domains assessed using ROB-2, summarised for the included studies. Each domain is rated as low (green), some concerns (yellow), or high (red) risk of bias.

Trials judged to be at low risk of bias generally reported clear methods for randomisation, allocation concealment, and outcome assessment. These studies universally measured haemoglobin, and most assessed the response at a single later time point, typically at 12 weeks, late gestation, or delivery. Ferritin was measured in a minority of low-risk studies. Studies judged to be at high risk of bias were more likely to have limited reporting of randomisation procedures, open-label designs, or concerns regarding incomplete outcome reporting. They also more frequently incorporated multiple biomarkers, including ferritin and red cell indices, and assessed treatment response at several time points throughout the intervention period. Across all studies, outcome measurement was consistently judged to be at low risk of bias, reflecting the use of objective, laboratory-based biomarkers.

3.4 Discussion

3.4.1 Summary of Key Findings

This systematic review examined how response to oral iron therapy during pregnancy is assessed in clinical research. Three key findings emerged. First, although haemoglobin was measured in all included studies, it was rarely used in isolation. Most trials incorporated ferritin and, in many cases, additional biomarkers of iron status or erythropoietic activity. Second, there was substantial variation in the timing of response assessment, with no consistent approach across studies and little evidence that assessment time points were anchored to the physiological changes of pregnancy. Third, monitoring practices varied across healthcare contexts, with broader biomarker panels more commonly used in higher-income settings and more limited approaches observed in lower-resource contexts.

3.4.2 Interpretation of Findings

This review identified substantial heterogeneity in how response to oral iron therapy is assessed during pregnancy. Variation was observed in the biomarkers selected, the timing of assessment, and the definitions used to characterise treatment response. Similar variation was identified in clinical guideline recommendations in Chapter 2, suggesting that uncertainty about how treatment

response should be assessed is present not only in guidelines but also in the underlying research evidence base. The following sections explore each of these factors in further detail.

3.4.2.1 Biomarker Selection and Interpretation

Although haemoglobin was measured in all included studies, it was rarely used in isolation. Most trials incorporated ferritin and, in many cases, additional biomarkers of iron status or erythropoietic activity. This finding is notable because current clinical guideline recommendations for monitoring response to oral iron therapy rely predominantly on haemoglobin.[53]

The broader biomarker panels observed in clinical research may reflect recognition of haemoglobin's limitations as a sole marker of treatment response during pregnancy. Nevertheless, the continued prominence of haemoglobin is unsurprising given its central role in clinical practice and its established association with adverse maternal and perinatal outcomes.[28,29,183] Its interpretation during pregnancy, however, is complicated by the substantial physiological adaptations that occur throughout gestation. Plasma volume expansion, changes in red cell mass, and alterations in iron metabolism all influence haemoglobin concentration throughout pregnancy.[86] Consequently, observed changes in haemoglobin may reflect both treatment response and normal physiological adaptation.

Ferritin was the second most commonly measured biomarker and was included in most studies. Ferritin may identify replenishment of iron stores before this is reflected in haemoglobin. However, interpreting ferritin during pregnancy presents several challenges. Diagnostic ferritin thresholds were applied inconsistently across studies, complicating comparisons between populations and limiting interpretation of treatment response. Ferritin is also an acute-phase reactant and may be elevated in the presence of inflammation independent of iron status,[89] yet concurrent measurement of inflammatory markers was almost absent across the included studies, with only one trial reporting CRP. Furthermore, ferritin concentrations decline physiologically across pregnancy, even among iron-replete women, reflecting increasing maternal iron requirements, plasma volume expansion, and placental iron transfer.[12,69,184] Together, these factors

complicate the interpretation of ferritin trajectories over time and raise questions regarding the application of static thresholds throughout gestation. Emerging literature exploring trimester-specific ferritin thresholds reflects increasing recognition that physiologically informed approaches may be required to interpret iron status during pregnancy.[69]

Other biomarkers of iron transport and utilisation, including TSAT, total iron-binding capacity, and sTfR, were used less frequently. Although these markers may provide complementary information regarding iron availability and utilisation, their physiological trajectories, clinically meaningful thresholds, and relationships with maternal and neonatal outcomes during pregnancy remain incompletely characterised.[93,94]

The variation observed in biomarker selection, therefore, appears to reflect ongoing uncertainty regarding which aspects of iron physiology should be prioritised when assessing response to oral iron therapy during pregnancy. Different biomarkers provide information about different dimensions of iron biology, including erythropoiesis, iron stores, and iron availability, yet their relative importance for defining treatment response remains unclear. Although broader biomarker panels suggest recognition that haemoglobin alone may be insufficient, the clinical significance of many alternative biomarkers remains uncertain. In many studies, biomarkers function primarily as surrogate outcomes, with limited evidence linking biomarker changes to clinically meaningful maternal or neonatal outcomes. Consequently, differences in biomarker response may not necessarily translate into differences in clinical benefit.

3.4.2.2 Timing of Response Assessment

Across trials, the timing of response assessment broadly reflected the expected kinetics of individual biomarkers in non-pregnant populations. Reticulocyte counts were typically measured first, consistent with their role as markers of the early erythropoietic response,[185] whereas haemoglobin and ferritin were generally assessed later. Haemoglobin was most commonly measured at 4 weeks, consistent with several international guideline recommendations[51,133,136,186] and the expected timing of haemoglobin recovery following iron supplementation in non-

pregnant populations. However, most studies used fixed intervals following treatment initiation, with relatively few incorporating gestational age into assessment schedules.

While these intervals align with expected biomarker responses to iron supplementation in non-pregnant populations, they do not account for the substantial physiological changes that occur across gestation. The interpretation of biomarker change during pregnancy, therefore, differs from that in non-pregnant adults. Previous studies have suggested that crude changes in haemoglobin may misrepresent treatment response during pregnancy, with gestational age-adjusted haemoglobin Z-scores providing a more physiologically informed assessment by expressing haemoglobin relative to gestational age-specific expected values.[187] Subsequent work has shown that absolute rises in haemoglobin may reflect movement along the haemodilution curve rather than true treatment effects, with Z-score adjustment often reducing the apparent response.[188–190] Other studies, including Mumtaz et al. (2000), have similarly shown that accounting for gestational physiology can alter the interpretation of iron-therapy outcomes.[191]

Despite this, gestational age was rarely incorporated into response assessment within the studies included in this review. Instead, treatment response was almost universally assessed using changes in biomarker concentrations measured at fixed intervals following treatment initiation. As a result, observed changes may partly reflect normal physiological trajectories rather than the biological effects of supplementation alone.

3.4.2.3 Healthcare Context and Variation in Response Assessment

The healthcare context appeared to influence both biomarker selection and the timing of response assessment. Studies conducted in lower-resource settings more commonly relied on haemoglobin and red cell indices, whereas studies conducted in higher-income settings more frequently incorporated ferritin and other biomarkers of iron metabolism. These differences likely reflect variation in laboratory infrastructure, assay availability, and the feasibility of repeated follow-up.[7,192,193] Evidence suggests that red cell indices have limited diagnostic accuracy compared with serum ferritin for detecting iron deficiency and iron deficiency anaemia during pregnancy,[194] yet they remain attractive in settings where access to biochemical testing is limited.

Importantly, the healthcare context may shape not only how treatment response is monitored in clinical practice but also how the effectiveness of oral iron therapy is evaluated in research. Haemoglobin and red cell indices can be measured relatively inexpensively and are widely available, whereas ferritin, sTfR, and other biomarkers of iron metabolism require more specialised laboratory testing. Consequently, studies conducted in different settings may prioritise different biomarkers and capture different dimensions of treatment response, even when evaluating similar interventions. This may contribute to heterogeneity across the literature and complicate the comparison of findings between settings.

3.4.3 Strengths and Limitations

This review is the first to systematically examine how haematological response to oral iron therapy is assessed in pregnancy, including both biomarker selection and timing of assessment. A comprehensive search strategy was applied across multiple databases in accordance with PRISMA 2020 guidance, and the review was conducted using a prospectively registered protocol. Screening, data extraction, and risk-of-bias assessment were performed independently by two reviewers using standardised methods. Restricting inclusion to RCTs strengthened internal validity by ensuring that biomarker selection reflected prespecified outcome assessment rather than incidental measurement within observational studies. In addition, stratification of findings by country, World Bank income level, and healthcare setting enabled exploration of contextual variation in monitoring practices.

Several limitations should be considered. First, this review was descriptive by design and did not evaluate the diagnostic accuracy, clinical utility, or comparative performance of individual biomarkers. Consequently, the findings describe how treatment response is assessed but cannot determine which biomarkers or approaches are most informative or clinically meaningful.

Second, the composition of the evidence base may have influenced the findings. Although restricting inclusion to RCTs strengthened internal validity, the exclusion of observational cohort studies may have narrowed the range of monitoring approaches identified. Cohort studies may capture biomarker selection and follow-up schedules that more closely reflect routine clinical

practice, and their exclusion may therefore limit the generalisability of the findings to real-world care. In addition, restricting inclusion to trials reporting biochemical primary outcomes may have favoured studies conducted in lower- and middle-income countries, where biomarker-based endpoints were more commonly incorporated, whereas studies in higher-income settings more frequently prioritised clinical primary outcomes. This may have contributed to the predominance of studies from LMICs, particularly India. The review was also limited by substantial heterogeneity in biomarker selection, definitions of response, timing of assessment, and gestational age at enrolment, which precluded meta-analysis and limited comparability across studies. While this heterogeneity represents a central finding of the review, it also constrained the ability to draw quantitative conclusions.

Third, the interpretation of contextual variation should be undertaken cautiously. The healthcare context was assessed using broad classifications, such as the World Bank income group, which may not fully capture variation in healthcare systems, laboratory access, or clinical practice. Furthermore, monitoring strategies employed within clinical trials may differ from those used in routine care, where biomarker availability, follow-up, and resource constraints may influence monitoring practices.

Finally, the Cochrane ROB-2 tool provided a structured assessment of methodological quality. However, as ROB-2 was developed to assess the risk of bias in estimates of intervention effect rather than to evaluate descriptive questions, it was less well suited to evaluating the rationale, completeness, or appropriateness of biomarker selection and assessment timing. As a result, risk-of-bias findings should be interpreted as contextual information regarding overall study quality rather than as a direct assessment of the monitoring approaches themselves.

3.4.4 Implications for Research, Practice and Policy

The findings of this review suggest that assessment of treatment response during pregnancy remains conceptually and operationally inconsistent. Different studies used different biomarkers, measured response at different time points, and applied different definitions of response. Consequently, women receiving similar treatment may be classified differently depending on

which biomarkers are measured and how the response is defined. This variability may contribute to the heterogeneity in monitoring recommendations identified in Chapter 2 and highlights the limited evidence available to support a single approach to response assessment during pregnancy.

These uncertainties have potential implications for clinical decision-making. Identification of treatment failure is frequently used to guide escalation from oral to intravenous iron therapy. However, the findings of this review suggest little consensus on which biomarkers to use to assess response, when to measure response, or what degree of change constitutes a clinically meaningful change. Emerging evidence also suggests that apparent non-response may reflect multifactorial anaemia rather than iron deficiency alone, further complicating the interpretation of treatment response.[195]

More broadly, the findings suggest that the challenge may be less about measuring additional biomarkers and more about understanding what biomarker changes represent during pregnancy. Interpretation of treatment response requires consideration of both the effects of supplementation and the physiological adaptations of gestation. Approaches that account for gestational age and expected physiological change may therefore provide a more meaningful assessment of response than absolute changes in biomarker concentrations alone.

For researchers, these findings highlight the need for more physiologically informed frameworks for assessing treatment response during pregnancy. Future studies should improve understanding of biomarker trajectories across gestation, establish pregnancy-specific approaches to response assessment, and evaluate how biomarker changes relate to clinically meaningful maternal and neonatal outcomes. The heterogeneity identified in this review also highlights the potential value of core outcome set initiatives, such as the Core Outcome Measures in Effectiveness Trials (COMET) Initiative, in improving consistency and comparability across studies.[196] However, standardisation should not necessarily require adopting a single biomarker or a single response definition. Rather, future approaches may need to recognise that different biomarkers capture different dimensions of iron physiology and may be informative for different clinical and research purposes.

3.5 Conclusion

This systematic review examined how the haematological response to oral iron therapy during pregnancy is assessed in clinical research. Although haemoglobin was measured in all included studies, most incorporated additional biomarkers of iron status or erythropoietic activity, and substantial variation was observed in both biomarker selection and assessment timing. Ferritin was the second-most-used biomarker, while reticulocyte indices, sTfR, TSAT, and other measures were used less frequently. Response was most assessed at four weeks following treatment initiation, although monitoring approaches varied considerably across studies and healthcare settings.

These findings suggest that there is no consistent approach to assessing response to oral iron therapy during pregnancy within the current research literature. The heterogeneity observed in biomarker selection, assessment timing, and response definitions mirrors the variation identified in clinical guideline recommendations in Chapter 2 and highlights ongoing uncertainty about how treatment response should be defined and interpreted during pregnancy. Importantly, the findings of this review are descriptive and do not establish that any one biomarker or monitoring strategy is superior. In part, this may reflect a more fundamental uncertainty regarding how commonly used iron biomarkers behave during pregnancy and what constitutes a meaningful treatment response.

This review raises an important question: how do commonly used iron biomarkers behave during pregnancy under oral iron supplementation, and how should observed changes be interpreted in the context of normal physiological adaptation? Chapter 4 addresses this question through a longitudinal analysis of iron biomarkers within a randomised trial of oral iron supplementation during pregnancy.

Chapter 4 - **Impact of Iron Supplementation Frequency on Iron Biomarkers During Pregnancy:** A Laboratory Sub-Analysis of the PANDA Randomised Controlled Trial

Publication Status

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Statement of Contribution

I conceived the sub-analysis study with N.R., S.J.S., and D.C., and developed the sub-analysis protocol. I catalogued all frozen samples with N.R. I performed ELISAs under the supervision of H.D. and A.A., and developed the statistical analysis plan and performed all statistical analyses. I wrote the first draft of the manuscript and was responsible for final data analysis and data integrity. R.M. performed the biochemistry assays for CRP, transferrin, and serum iron. C.H., T.H., and C.B. assisted with data management and transfer. S.J.S. and D.C. conceived the main PANDA trial.

4.1 Introduction

This chapter presents a secondary analysis of the PANDA randomised trial, examining the effects of different oral iron supplementation regimens on iron status biomarkers during pregnancy.

Chapters 2 and 3 demonstrated substantial variation in recommendations for iron supplementation and in approaches to assessing treatment response during pregnancy. However, interpretation of biomarker changes in response to supplementation is complicated by the physiological adaptations of pregnancy, which independently influence many commonly used markers of iron status.

Using longitudinal data from the PANDA trial, this chapter examines trajectories of ferritin, TSAT, hepcidin, sTfR, and related biomarkers among non-anaemic, pregnant women receiving daily, alternate-day, or three-times-weekly oral iron supplementation. By evaluating biomarker behaviour within a controlled trial setting, this analysis provides insight into the extent to which observed changes reflect supplementation effects versus the underlying physiological changes of pregnancy.

4.1.1 Iron Requirements and Biomarker Assessment in Pregnancy

Iron requirements increase substantially during pregnancy to support maternal erythropoiesis, placental development, and foetal growth.[12,197] Demand rises most rapidly during the second and third trimesters, coinciding with the period when iron deficiency and anaemia are most commonly diagnosed.[24] In the UK, anaemia affects approximately 23–30% of pregnancies and increases across gestation, rising from 2–5% at booking to 15–20% by the third trimester in unselected maternity populations.[24,198,199]

Although a range of biomarkers are used to assess iron status during pregnancy, their interpretation is complicated by the physiological adaptations of gestation, including plasma volume expansion, altered erythropoiesis, and changes in iron regulation, as discussed in Chapter 1. Ferritin and TSAT generally decline across pregnancy and may also be influenced by inflammation.[54,56] sTfR, which reflects cellular iron demand, is less sensitive to inflammation but

may increase as erythropoietic activity rises during gestation.[93,94] Composite measures such as the ferritin index (sTfR/$\log_{10}$ ferritin) have been proposed as markers of functional iron deficiency, although their role in pregnancy remains incompletely understood.[81]

Hepcidin, the principal regulator of iron homeostasis, is of particular interest because it regulates intestinal iron absorption and the mobilisation of iron from stores. During pregnancy, hepcidin concentrations are progressively suppressed to facilitate increased maternal iron absorption and placental iron transfer.[12,90,200] Although hepcidin has emerged as a promising biomarker of iron regulation, pregnancy-specific reference ranges remain limited, and its clinical role has yet to be established. Furthermore, sTfR and hepcidin assays lack standardisation across platforms.

4.1.2 The Role of Prophylactic Oral Iron

Prophylactic oral iron supplementation is widely used to prevent iron deficiency during pregnancy, although recommendations vary across healthcare settings. In populations with a high burden of anaemia, including many LMICs, routine supplementation is recommended for all pregnant women.[133] In contrast, high-income settings such as the UK generally favour targeted supplementation due to uncertainty about the balance between preventing iron deficiency and avoiding unnecessary treatment in iron-replete women.[51,197] As demonstrated in Chapter 2, this uncertainty is reflected in variation in recommendations for prophylactic iron use and oral iron dosing regimens.

There is also limited consensus regarding the optimal supplementation strategy. In non-pregnant populations, alternate-day dosing has been proposed to reduce gastrointestinal side effects and improve iron absorption. This approach is based on the physiological response of hepcidin, which rises transiently following oral iron administration and reduces subsequent intestinal iron absorption.[123,201,202] Allowing hepcidin concentrations to return to baseline between doses, therefore, improves fractional iron absorption. Whether this physiological rationale applies during pregnancy remains uncertain, as hepcidin concentrations are progressively suppressed throughout gestation to support maternal iron absorption and placental iron transfer.[12,90,200] Consequently, it

is unclear whether the advantages reported with alternate-day dosing in non-pregnant populations are maintained in pregnant women.

The PANDA dose-finding trial,[203] a multicentre randomised trial in non-anaemic pregnant women, compared three prophylactic regimens: 200 mg ferrous sulphate administered daily, on alternate days, or three times weekly. While the primary trial evaluated adherence, side effects, and haemoglobin maintenance, this secondary analysis examines longitudinal changes in iron biomarkers to explore how different supplementation regimens influence biomarker trajectories during pregnancy.

4.1.3 Rationale, Aim and Research Questions

Chapters 2 and 3 demonstrated substantial variation in recommendations for oral iron supplementation and in approaches to assessing iron status during pregnancy. However, relatively little is known about how commonly used iron biomarkers behave across pregnancy under different prophylactic oral iron supplementation regimens. Understanding these biomarker trajectories is important for interpreting changes in iron status during pregnancy and for informing approaches to monitoring and supplementation.

The PANDA trial provided an opportunity to examine longitudinal changes in iron biomarkers among women receiving daily, alternate-day, or three-times-weekly oral iron supplementation. By evaluating biomarkers that reflect different aspects of iron metabolism and erythropoiesis, this chapter explores how biomarker trajectories vary according to supplementation frequency and how these changes should be interpreted in the context of normal pregnancy physiology.

Aim

To evaluate the effect of different prophylactic oral iron dosing regimens on key iron biomarkers during pregnancy.

Research Questions

1. Do daily, alternate-day, and three-times-weekly oral iron supplementation regimens produce different patterns of change in key iron biomarkers across pregnancy?

2. What do longitudinal changes in iron biomarkers suggest about the influence of supplementation regimen and physiological adaptation during pregnancy?

4.2 Methods

4.2.1 Study Design & Collaboration

This chapter reports a secondary analysis of Workstream 2 of the PANDA Programme, a three-arm, open-label, multicentre, dose-finding randomised trial evaluating prophylactic oral iron supplementation during pregnancy.[203] Participants were randomised in a 1:1:1 ratio to receive 200 mg ferrous sulphate daily, on alternate days, or three times weekly. A total of 300 non-anaemic pregnant women were recruited before 14 weeks' gestation and followed until delivery. Venous blood samples were collected at randomisation (<14 weeks' gestation) and follow-up (28 weeks' gestation ±2 weeks) (see Figure 4.1).

The present analysis examined longitudinal changes in iron status biomarkers across the three supplementation regimens. The analysis was conducted in collaboration with the PANDA trial team. The parent trial was funded by the NIHR Blood and Transplant Research Unit, which provided access to biological samples and trial data.

Serum samples were stored at the Nuffield Orthopaedic Centre and the Women's Centre at the John Radcliffe Hospital. Laboratory analyses were performed at the Weatherall Institute of Molecular Medicine (WIMM) and the John Radcliffe Hospital Biochemistry Laboratory.

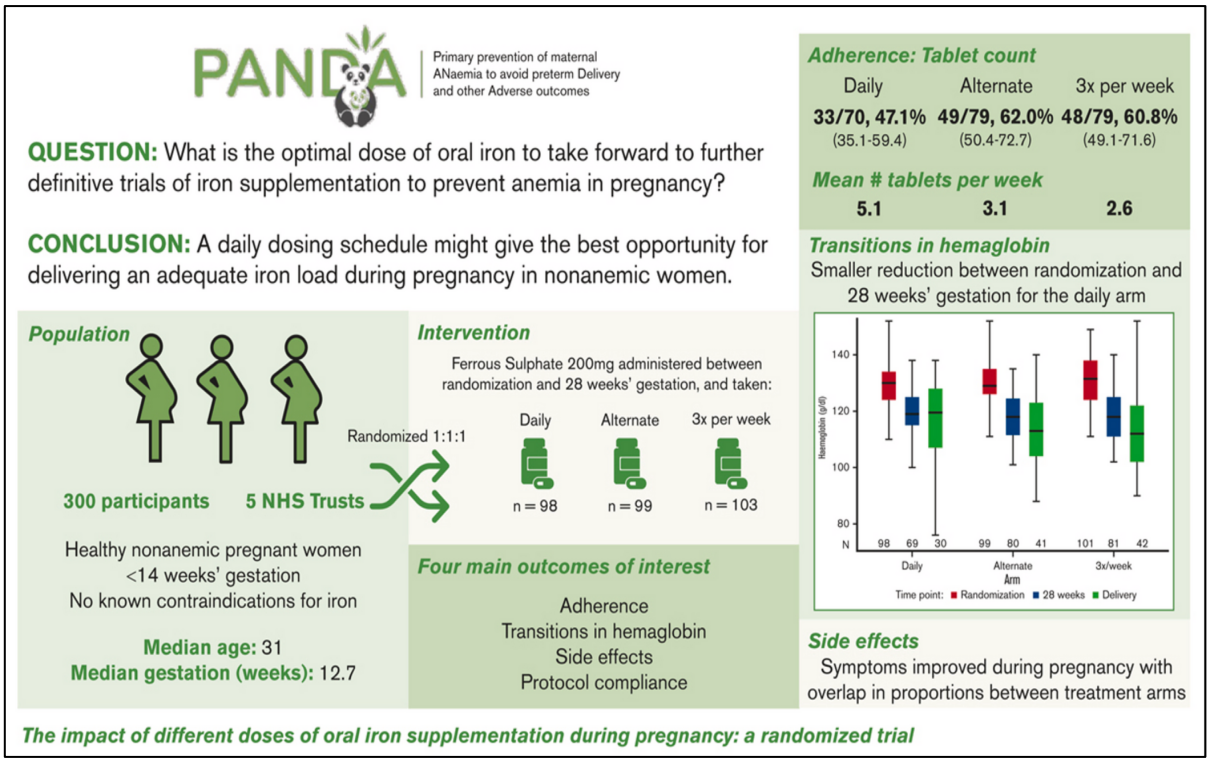


Figure 4.1 PANDA dose-finding trial visual abstract
Author-provided version of the visual abstract accompanying Stanworth et al. (2024),[203] reproduced with the authors' permission. The original publication is licensed under the Creative Commons Attribution–NonCommercial–NoDerivatives 4.0 International licence (CC BY-NC-ND 4.0). PANDA = Primary prevention of maternal Anaemia to avoid preterm Delivery and other Adverse outcomes.

4.2.2 Study Population and Sample Availability

Of the 300 participants in the original trial, 135 were randomly selected across all participating sites and treatment groups to have their serum samples stored for future analysis. The remaining participants did not have samples stored due to feasibility and cost constraints rather than clinical or demographic factors.

A total of 311 serum samples from 135 participants were stored at −80°C at the Nuffield Orthopaedic Centre. These included paired baseline and follow-up samples, as well as a smaller number of delivery samples (n=42).

Samples were assigned unique identifiers and recorded with corresponding participant ID, timepoint, and storage location to ensure traceability throughout transfer and analysis. Samples were catalogued at the Nuffield Orthopaedic Centre, and a Material Transfer Agreement (MTA) was completed to enable transfer of samples to the WIMM for laboratory analysis. A Data Transfer Agreement was also completed to access baseline participant characteristics and

routinely collected laboratory data, including haemoglobin, mean cell volume (MCV), MCH, and ferritin, from the parent trial dataset.

4.2.3 Laboratory Methods

Following approval of the MTA, all serum samples were transferred on dry ice to the WIMM for storage and analysis. Laboratory procedures were conducted under supervision in accordance with established research laboratory protocols.

4.2.3.1 Assayed Biomarkers

Haemoglobin, MCV, MCH, and ferritin were measured locally at participating NHS Trust sites using site-specific analysers as part of routine antenatal care and were available from the parent trial. In addition to these biomarkers, further measurements were performed on all samples included in this sub-analysis:

- **Hepcidin-25:** measured using a competitive enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmbH, Marburg, Germany; EIA-5782),
- **sTfR:** measured using a sandwich ELISA (DRG Instruments GmbH, Marburg, Germany; EIA-5925),
- **Serum iron, transferrin, and CRP:** measured using automated assays on an Abbott Architect C16000 analyser,
- **TSAT:** calculated from serum iron and transferrin concentrations using standard methods,
- **Ferritin index:** calculated as sTfR divided by $\log_{10}$-transformed ferritin (sTfR / $\log_{10}$ ferritin).

Biomarker selection was guided by their clinical relevance and their complementary roles in iron physiology. Ferritin and TSAT were included as measures of iron stores and circulating iron availability, respectively. Hepcidin was included as a central regulator of iron absorption and mobilisation, with particular relevance to alternate-day dosing mechanisms.[201] sTfR was selected as a marker of cellular iron demand and erythropoietic activity, with lower sensitivity to inflammation than ferritin. The ferritin index was included as a composite marker integrating

cellular iron demand and iron stores. CRP was included as a marker of inflammation to support interpretation of biomarker results.

4.2.3.2 ELISAs

Hepcidin and sTfR concentrations were measured using ELISAs following manufacturer protocols. sTfR was measured by sandwich ELISA, and hepcidin by competitive ELISA. Samples were thawed, vortexed, and centrifuged before analysis. Plate layouts were predefined to ensure that all samples from the same participant (baseline, follow-up, and delivery) were analysed on the same plate, minimising inter-assay variability. Each 96-well plate included six standards and two quality-control samples (run in duplicate), alongside up to 40 participant samples, also run in duplicate (see Figure 4.2) All assays were completed within five weeks to reduce batch effects, including temperature fluctuations and reagent variability. ELISA Procedure (sTfR Sandwich ELISA)

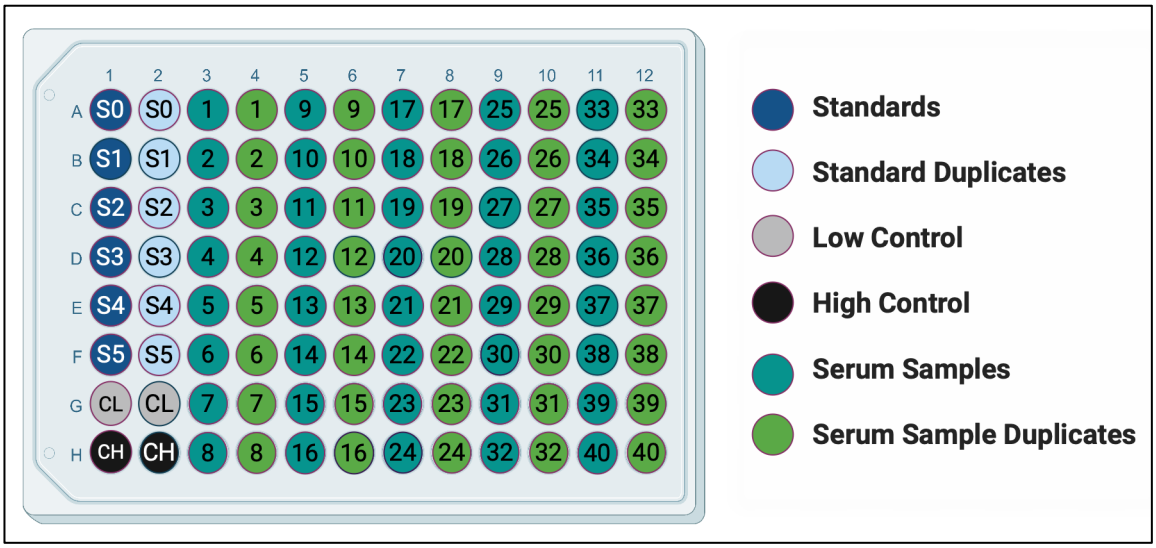


Figure 4.2 ELISA plate layout
Created in BioRender (Haynes, 2026; https://BioRender.com/1x6y6ef). ELISA = enzyme-linked immunosorbent assay.

A standardised workflow was followed for all sandwich ELISAs (see Figure 4.3). Serum samples were diluted according to the manufacturer's instructions (10 µL serum in 490 µL diluent) and analysed alongside standards and quality-control samples. Samples, standards, and controls were added to pre-coated ELISA plates and incubated at room temperature with shaking. Plates were washed according to the manufacturer's protocol, incubated with an enzyme-conjugated detection

antibody, developed with a substrate solution, and read at 450 nm with a spectrophotometer. Concentrations were calculated using four-parameter logistic (4-PL) standard curves, with dilution factors applied to derive final serum concentrations.

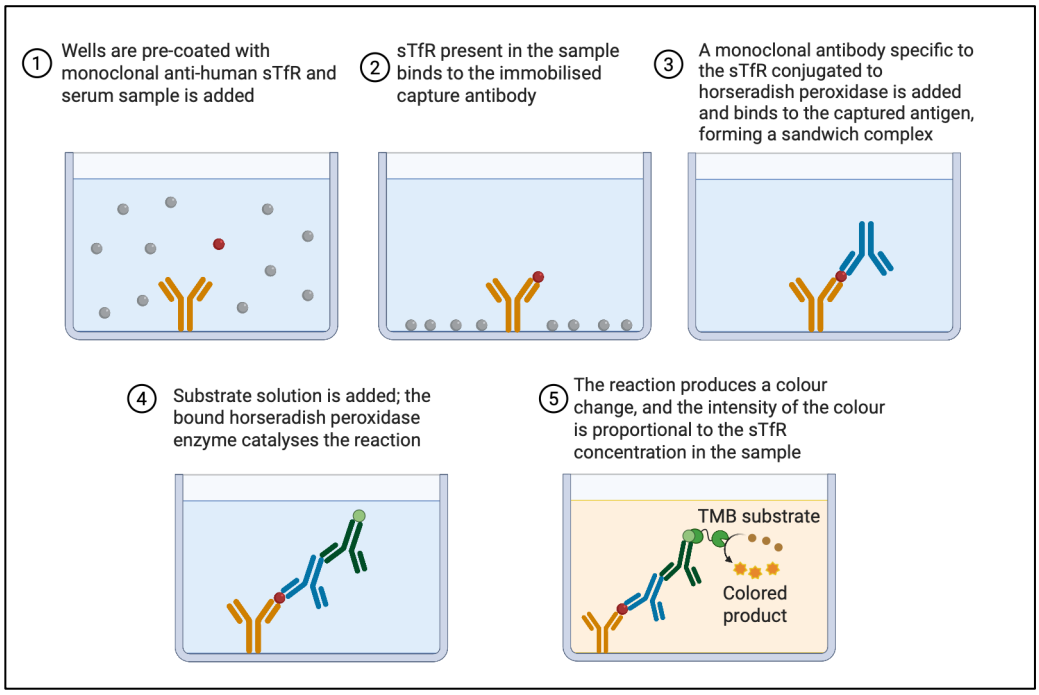


Figure 4.3 Sandwich ELISA process
Created in BioRender (Haynes, 2026; https://BioRender.com/x62qhnn). ELISA = enzyme-linked immunosorbent assay.

Competitive ELISA (Hepcidin)

A competitive ELISA format was used to measure hepcidin (Figure 4.4). In this assay, serum hepcidin and enzyme-labelled hepcidin competed simultaneously for antibody-binding sites during a single incubation step prior to washing. Following substrate development, optical density was measured at 450 nm. In contrast to the sandwich ELISA, signal intensity in the competitive assay was inversely proportional to analyte concentration.

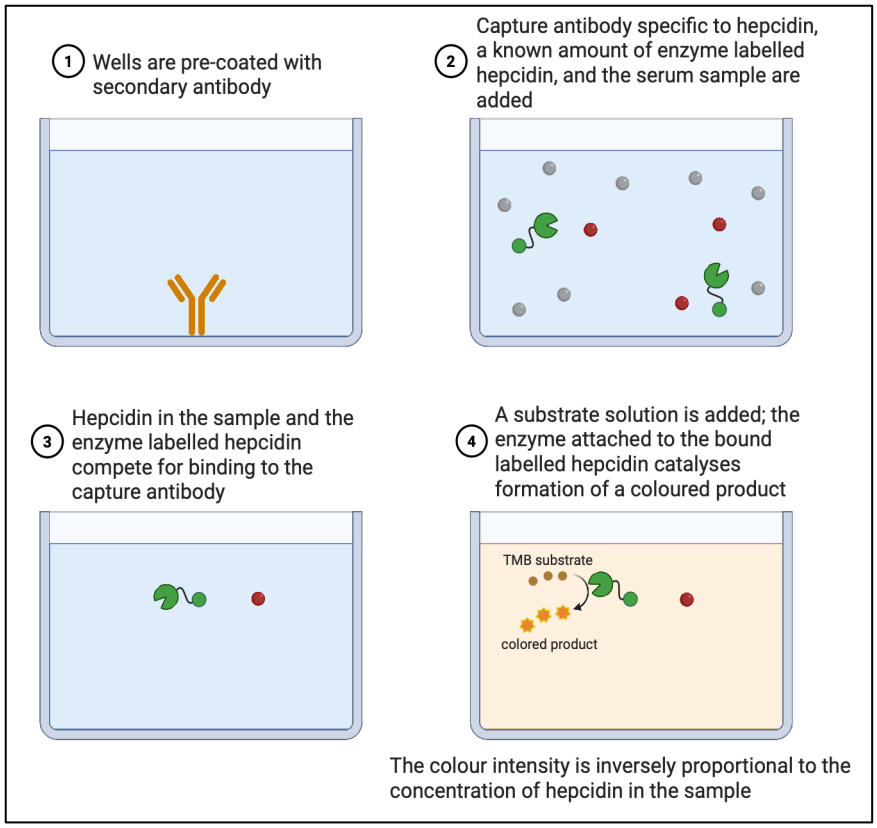


Figure 4.4 Hepcidin competitive ELISA process
Created in BioRender (Haynes, 2026; https://BioRender.com/4qd8390). ELISA = enzyme-linked immunosorbent assay.

4.2.3.3 Quality Control and Data Capture

Raw absorbance data were exported from the plate reader and processed in Microsoft Excel. For each assay, the following were recorded:

- Raw optical density (OD) values
- Standard curve fit (four-parameter logistic model)
- Calculated sample concentrations
- Mean replicate values
- Coefficient of variation (%CV)

All samples, standards, and controls were analysed in duplicate, and mean values were used for analysis. Assay validity was assessed using standard and control performance, and plates outside acceptable ranges were repeated. Samples with CV >10% between duplicates were re-assayed until acceptable.

As sTfR concentrations were measured in diluted samples, a dilution factor of 50 was applied to derive final serum concentrations. Biomarker data were subsequently merged with participant metadata, including study ID, treatment group, and timepoint, to create the final analytical dataset.

4.2.3.4 Biochemistry Laboratory Assays

Serum iron, transferrin, and CRP were analysed at the John Radcliffe Hospital Biochemistry Laboratory using standardised clinical protocols on an Abbott Architect C16000 analyser**.**

Samples were processed in batches to minimise batch effects, with samples from the same participant analysed together where possible.

TSAT was calculated from serum iron and transferrin concentrations using the formula:

$$\text{TSAT (\%)} = \frac{\text{Serum Iron (µmol/L)} \times 100}{\text{Transferrin (g/L)} \times 25.1}$$

In line with laboratory recommendations, samples with evidence of haemolysis (haemolysis index >0.25; n=7) were excluded from TSAT analysis due to the risk of artificially elevated serum iron concentrations. These samples were not re-analysed due to insufficient remaining sample volume.

4.2.4 Data Management and Statistical Analysis

All data were recorded, cleaned, and anonymised in Microsoft Excel, with each participant assigned a unique trial ID. Treatment groups were blinded during analysis. Statistical significance was set at $p < 0.05$. All analyses, figures, and visualisations were conducted using GraphPad Prism (Version 10.6.1) and RStudio (Version 2025.09.2+418).

4.2.4.1 Descriptive Analysis

Baseline characteristics of the sub-sample were summarised descriptively and compared with the full PANDA cohort to assess representativeness.[203]

Adherence to oral iron supplementation was assessed using tablet counts at the follow-up visit, as described in the parent PANDA trial.[203] Participants returned supplement bottles, and remaining tablets were counted. The mean number of tablets taken per week was calculated by dividing the

total number of tablets consumed by the treatment duration. Adherence was defined as returned tablets within ±15% of the expected number. For this sub-analysis, adherence was summarised by treatment group as the mean number of tablets taken per week (SD) and the mean proportion of expected doses taken (%).

Anaemia was defined using trimester-specific haemoglobin thresholds (Hb <110 g/L at baseline and <105 g/L at follow-up), and iron deficiency was defined as ferritin <30 µg/L, consistent with UK guidelines.[51] The incidence of anaemia at follow-up and the prevalence of iron deficiency at baseline and follow-up were calculated for each treatment group.

Agreement between ferritin- and TSAT-based classifications of iron deficiency (ferritin <30 µg/L; TSAT <20%) was assessed by cross-tabulation at baseline and follow-up. Participants were classified into four groups (both normal, ferritin-only deficiency, TSAT-only deficiency, both deficient), and concordance and discordance were summarised as proportions. Differences in CRP concentrations across ferritin–TSAT classification groups were assessed using the Kruskal–Wallis test.

4.2.4.2 Data Preparation and Transformation

Continuous variables were assessed for normality using statistical tests and visual inspection of Q–Q plots (see Figure 4.5). Variables with right-skewed distributions (ferritin, hepcidin, sTfR, TSAT, and CRP) were $\log_{10}$-transformed prior to analysis. CRP values reported as <1.00 mg/L were recoded to 0.99 mg/L to permit log transformation. For descriptive reporting, summary statistics were presented on the original scale as mean ± standard deviation (SD) for normally distributed variables and median (interquartile range, IQR) for skewed variables. Change scores (Δ=follow-up−baseline) were calculated (after transformation where required) and summarised descriptively.

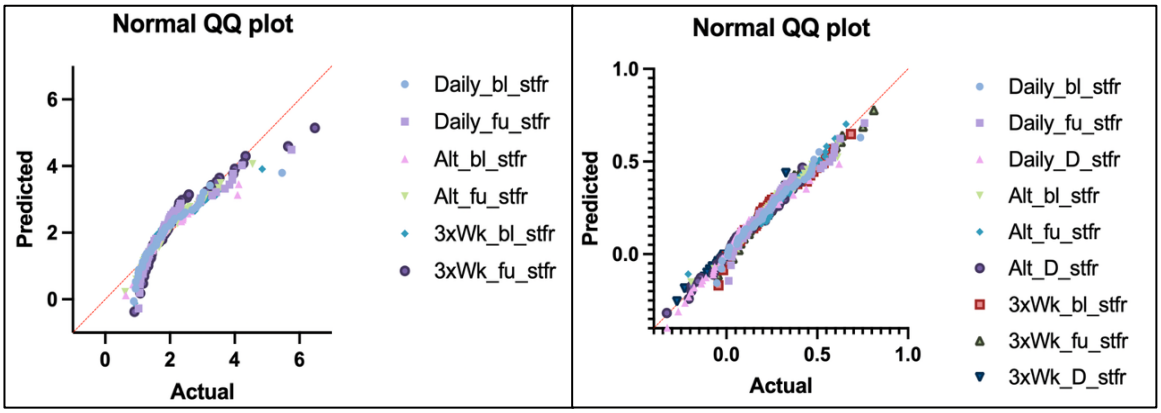


Figure 4.5 Q–Q plots for raw and log_{10}-transformed sTfR data
Quantile–quantile (Q–Q) plots for the raw sTfR data (left) and the log_{10}-transformed sTfR data (right). sTfR = soluble transferrin receptor.

4.2.4.3 Within- and Between-Group Comparisons

Within-group changes in biomarker concentrations were assessed using paired t-tests. Between-group comparisons at baseline, follow-up, and for change scores were examined using one-way Analysis of variance (ANOVA) or Welch's ANOVA where variances were unequal. All tests were two-tailed.

4.2.4.4 Linear Mixed-Effects Models

To account for repeated measures, adjust for inflammation, and evaluate group–time interactions, linear mixed-effects models (LMMs) were fitted in R using restricted maximum likelihood estimation (REML). As each participant contributed biomarker measurements at two timepoints, observations were not independent; inclusion of a participant-level random intercept allowed within-individual correlation to be appropriately modelled.[204] CRP was included as a covariate to account for potential inflammation-related effects on iron biomarkers, particularly ferritin. The dataset was restructured into long format, with each row representing a single biomarker measurement per participant per timepoint.

Fixed effects included treatment group (daily, alternate-day, three-times-weekly), timepoint (baseline, follow-up), the group × time interaction, and log_{10}(CRP) as a continuous covariate. Participant ID was included as a random intercept to account for within-individual correlation. The full model specification is provided in Appendix 4: Supplementary Table S4.1.

Models were fitted using the *lmer()* function in the lme4 package. P-values were estimated using Satterthwaite's approximation (lmerTest). Estimated marginal means and contrasts (emmeans) were used to assess (i) within-group changes, (ii) between-group differences in change and (iii) between-group differences at follow-up, with Tukey correction for multiple comparisons.

Model assumptions (normality, homoscedasticity, and multicollinearity) were assessed using residual diagnostics, Shapiro–Wilk tests, and variance inflation factors (VIF) <5. The coefficient for $\log_{10}$(CRP) was used to assess independent associations between biomarkers and inflammation.

4.2.4.5 Assessment of CRP–Biomarker Associations

To explore the potential influence of inflammation on iron biomarker interpretation beyond model-based adjustment, the association between CRP and iron biomarkers (ferritin, TSAT, and sTfR) was assessed using $\log_{10}$-transformed values. Spearman correlation coefficients were calculated at baseline and follow-up.

4.2.4.6 Handling of Missing Data

Across all variables, missing data were minimal (<10%) and were assumed to be missing completely at random. Analyses were therefore conducted using a complete-case approach, with participants missing biomarker data at either time point excluded from analyses of the relevant variable. Under the assumption of missing completely at random, this approach was considered unlikely to introduce substantial bias.[205]

4.2.4.7 Sample Size and Power Considerations

The PANDA parent trial was not powered to detect small between-group differences in iron biomarkers examined in this sub-analysis. A priori calculations, based on a small, expected effect size (Cohen's f=0.10), indicated that approximately 967 participants would have been required to detect such differences (α=0.05, three groups, 80% power), substantially exceeding the available sample size.

Post hoc power calculations using observed effect sizes confirmed limited power for between-group comparisons (Cohen's f = 0.05–0.13; power ~7–12%). In contrast, within-group changes from baseline to follow-up showed moderate to large effect sizes (Cohen's d=0.31–0.66), corresponding to high power (83% to >99%).

These findings indicate that, although the study was sufficiently powered to detect biomarker changes across pregnancy, it was underpowered to detect small differences between supplementation regimens. A summary of effect sizes and achieved power is provided in Appendix 4: Supplementary Table S4.2.

4.2.5 Ethical Considerations

The PANDA trial and this secondary laboratory analysis were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Ethical approval for the parent trial was granted by the NHS Research Ethics Committee (REC 21/WA/0247), and regulatory approval was provided by the Medicines and Healthcare products Regulatory Agency (Clinical Trial Authorisation 25224/0006/001-0001). The original trial consent included permission for participants' data and samples to be used in future ethically approved research. However, an ethics amendment was required to permit the transfer of stored blood samples from participating NHS Trusts to Oxford for laboratory analysis, and it was approved before the transfer of samples. All participants had provided informed written consent.

4.3 Results

4.3.1 Baseline Characteristics

A total of 135 participants had paired baseline and follow-up samples available for analysis. There were no statistically significant differences in the baseline characteristics between treatment groups (Table 4.1). Baseline characteristics across treatment groups within the sub-analysis were also representative of the full PANDA trial cohort. Approximately 19% of participants (n = 26) were taking iron-containing supplements at the time of randomisation, with a broadly similar

distribution across treatment groups (22.2% daily, 17.0% alternate-day, 18.6% three-times weekly), as shown in Table 4.1.

Table 4.1 Baseline characteristics of participants, by supplementation regimen

	Daily (n=45)	Alternate (n=47)	Three Times Weekly (n=43)	Total (N=135)	P value*
Age (y) (median + IQR)	32 (29-32)	31 (28-35)	31 (28-34)	31 (28 – 34)	0.9945
Socioeconomic Status (quintile)					0.8985
1 (most deprived)	8 (17.8)	8 (17)	6 (14.0)	22 (16.3)	
2	3 (6.7)	8 (17)	8 (18.6)	19 (14.1)	
3	5 (11.1)	4 (8.5)	4 (9.3)	13 (9.6)	
4	4 (8.9)	3 (6.4)	4 (8.4)	11 (8.1)	
5 (least deprived)	3 (6.7)	5 (10.6)	4 (2.3)	12 (8.9)	
Gestation (wks.)	12.7 (11.9-13.1)	12.7 (12.2 – 13.1)	12.7 (11.9 -13.0)	12.7 (12.0 – 13.1)	0.7418
Ethnicity					0.3463
White	37 (82.2)	42 (89.4)	38 (88.4)	117 (86.7)	
Mixed	0 (0)	0 (0)	1 (2.3)	1 (0.7)	
Asian or Asian British	7 (15.6)	4 (8.5)	3 (7.0)	14 (10.4)	
Black, African, Caribbean, Black British	1 (2.2)	1 (2.1)	1 (2.3)	3 (2.2)	
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoker	5 (11.1)	2 (4.3)	2 (4.7)	9 (6.7)	0.4651
Height (cm)	164 (160-168)	165 (162-169)	165 (161-171)	165 (160 – 170)	0.7322
Weight (kg)	69 (61-82)	76 (64-96)	75 (62-84)	71 (62 – 88)	0.5423
BMI (kg/m2)	26 (23-30)	27 (23-35)	28 (21-31)	27 (23 – 32)	0.6592
Parity	1 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)	0.6358
Baseline Hb (g/L)	130 (123-134)	129 (126-137)	131 (123-137)	130 (125 – 136)	0.6480
Iron-containing supplements being taken at baseline	10 (22.2)	8 (17.0)	8 (18.6)	26 (19.3)	0.8252
Known haemoglobinopathy trait	1 (2.2)	1 (2.1)	2 (4.7)	4 (3.0)	0.6907
Previous Anaemia					0.3958
Outside of Pregnancy	7 (15.6)	4 (8.5)	6 (14.0)	17 (12.6)	
During Pregnancy	3 (6.7)	6 (12.8)	6 (14.0)	15 (11.1)	
Both	0	1 (2.1)	0 (0.0)	1 (0.7)	
Comorbidity†	6 (13.3)	8 (17.0)	11 (25.6)	25 (18.5)	0.3405
At least 1 previous termination	3 (6.7)	5 (10.6)	1 (2.3)	9 (6.7)	0.3257
At least 1 previous miscarriage or still birth	10 (22.2)	14 (29.8)	14 (32.6)	38 (28.1)	0.5343
At least 1 previous PPH (>1L)	3 (6.7)	5 (10.6)	7 (16.3)	15 (11.1)	0.3554

Data are n (%) for categorical variables and median (IQR) for continuous variables. *Between-group comparisons used the Kruskal–Wallis test (continuous) and Fisher's exact test (categorical). †Comorbidities include hypertension, cardiac disease, thyroid disorders, renal disease, pre-existing diabetes, clotting disorders, and anticoagulant therapy. The Index of Multiple Deprivation (IMD, 2019) was derived from participants' postcodes; data were unavailable for 58 participants who did not consent, and two participants had missing baseline haemoglobin. BMI = body mass index; IQR = interquartile range; IMD = Index of Multiple Deprivation.

4.3.2 Tablet Count Adherence

Tablet count adherence data were available for 125/135 participants (10 missing: 6 three-times-weekly, 2 alternate-day, 2 daily). The mean number of tablets taken per week was 5.23 (SD 1.66) in the daily arm, 3.20 (SD 0.98) in the alternate-day arm, and 2.94 (SD 0.90) in the three-times-weekly arm (Appendix 4: Supplementary Table S4.3), corresponding to a mean of 74.8%, 91.3%, and 98.1% of expected weekly doses taken, respectively. The proportion of participants classified as adherent (within ±15% of expected weekly dose) was 46.5%, 56.8%, and 62.2% in the daily, alternate-day, and three-times-weekly groups, respectively, consistent with adherence patterns reported in the full trial cohort.[203] Despite lower adherence in the daily arm, participants in this group received substantially more iron overall, with a mean of 1,046 mg of ferrous sulphate per week, compared with 640 mg and 588 mg per week in the alternate-day and three-times-weekly arms, respectively.

4.3.3 Incidence of Iron Deficiency and Anaemia at Follow-Up

The proportion of participants meeting biochemical criteria for iron deficiency increased substantially from baseline to follow-up across all treatment groups. Using the current UK ferritin threshold (<30 μg/L), 69–72% of women in each regimen were classified as iron-deficient at 26–30 weeks' gestation, compared with 19–31% at randomisation (Appendix 4: Supplementary Table S4.4). Similar patterns were observed when iron deficiency was defined using TSAT <20%. When a ferritin threshold of <50 μg/L was applied, an alternative cut-off proposed in the emerging literature,[69] the proportion classified as iron deficient at follow-up was 92.9%, 86.0% and 90.5% in the daily, alternate-day and three-times-weekly groups, respectively (Appendix 4: Supplementary Table S4.4). The overall agreement between ferritin- and TSAT-based classifications was 72.6% at baseline, declining to 65.2% at follow-up, indicating increasing discordance between biomarkers as pregnancy progressed (Appendix 4: Supplementary Table S4.5). Discordance between ferritin and TSAT classifications was associated with differences in inflammatory status. Participants with low TSAT but ferritin ≥30 μg/L had significantly higher

baseline CRP concentrations than other groups ($p = 0.003$). This pattern was not observed at follow-up, where CRP concentrations were lower overall and more similar across groups.

Despite these high rates of biochemical iron deficiency, haemoglobin concentrations remained within the normal range for most participants at follow-up, and the incidence of new-onset anaemia (<105 g/L) remained below 5% in all groups.

4.3.4 Maternal Iron Status Biomarkers

Table 4.2 and Figure 4.6 summarise the unadjusted biomarker concentrations at baseline and follow-up. Across all treatment groups, haemoglobin declined significantly ($p < 0.0001$) from randomisation to follow-up, reflecting expected haemodilution, but not necessarily attributable to haemodilution alone. Ferritin concentrations also fell by approximately 50% in each arm, with a statistically significant ($p < 0.0001$) change seen in all groups, indicating declining iron stores across pregnancy. TSAT showed a similar pattern, decreasing markedly between baseline and follow-up in all three regimens ($p < 0.001$), reflecting both reduced circulating iron and increased transferrin concentrations. Hepcidin concentrations were markedly reduced at follow-up in all groups, with all reductions statistically significant ($p < 0.0001$), consistent with previously reported patterns of hepcidin dynamics during pregnancy.[206]

sTfR concentrations increased significantly over the study period in the alternate-day and three-times-weekly groups ($p < 0.01$), indicating increasing cellular iron demand. The smaller sTfR rise in the daily arm did not reach statistical significance ($p = 0.1223$).

The ferritin index (sTfR/[$\log_{10}$ ferritin]) increased significantly from baseline to follow-up in all three treatment groups (all $p < 0.0001$), reflecting the combined effect of rising cellular iron demand (sTfR) and declining iron stores (ferritin) across gestation. The magnitude of the increase was greatest in the alternate-day group and smallest in the daily group.

No statistically significant differences between treatment groups were detected at baseline or follow-up for any biomarker examined. Similarly, when comparing the magnitude of change

between groups, no significant differences were observed in unadjusted analyses. All three regimens demonstrated broadly similar biomarker trajectories, and the direction of change in each biomarker was consistent across groups.

CRP concentrations were generally low across all treatment groups at both time points. Mean CRP concentrations did not differ significantly between groups at baseline (Welch's ANOVA, $p = 0.2121$) or at follow-up ($p = 0.2568$). Although a higher proportion of participants in the alternate-day group had CRP concentrations >5 mg/L at baseline (Appendix 4: Supplementary Table S4.6), the distribution of CRP categories (≤5 mg/L vs >5 mg/L) did not differ significantly between treatment groups at baseline (Fisher's exact test, $p = 0.2431$) or at follow-up ($p = 0.9218$).

Table 4.2 Maternal iron biomarkers by supplementation group, at baseline and follow-up

Biomarker	Time	Daily (n=45)	Alternate Day (n=47)	Three-Times-Weekly (n=43)	p-value[1]
Hb (g/L)	BL	129.2 ± 8.3	131.4 ± 8.1	130.1 ± 8.9	0.4645
	FU	118.7 ± 8.9 (n=42)	121.5 ± 7.8	118.1 ± 9.8 (n=42)	0.1488
	Δ (p^2)	−10.5 ± 8.0 (<0.0001)	−9.9 ± 8.0 (<0.0001)	−11.8 ± 8.2 (<0.0001)	0.5405
MCV (fL)	BL	88.4 ± 5.2	88.5 ± 4.2	88.8 ± 4.9	0.9026
	FU	91.8 ± 5.7	91.7 ± 5.3	90.6 ± 4.5 (n=42)	0.5045
	Δ (p^2)	+3.38 ± 3.2 (<0.0001)	+3.19 ± 4.3 (<0.0001)	+1.71 ± 4.6 (0.0108)	0.1002
MCH (pg)	BL	29.8 ± 1.8	29.9 ± 1.6	29.6 ± 1.8	0.6601
	FU	30.9 ± 2.0	30.8 ± 1.9	30.3 ± 1.9 (n=42)	0.2750
	Δ (p^2)	+1.11 ± 1.2 (<0.0001)	+0.89 ± 1.1 (<0.0001)	+0.69 ± 1.2 (0.0008)	0.2574
Ferritin (µg/L)	BL	50.0 (26.0–79.5)	44.0 (29.8–72.5) (n=46)	47.0 (30.8–77.3) (n=42)	0.5250
	FU	23.0 (15.8–32.3) (n=42)	22.0 (15.0–32.0) (n=43)	21.5 (11.8–31.0) (n=42)	0.9046
	Δ (p^2)	−18.5 (−39.0 to −5.75) (<0.0001)	−23.5 (−47.5 to −12.8) (<0.0001)	−29.0 (−56.0 to −14.5) (<0.0001)	0.0995
Serum Iron (µmol/L)	BL	18.5 (14.7–23.7) (n=43)	18.1 (13.6–23.4) (n=45)	20.2 (12.8–25.2)	0.8583
	FU	15.3 (12.1–21.4) (n=44)	15.5 (12.0–20.5) (n=45)	14.9 (9.6–22.0)	0.6162
	Δ (p^2)	−1.19 (−6.57 to 1.32) (0.1447)	−1.01 (−6.83 to 3.00) (0.2210)	−1.42 (−12.9 to 5.82) (0.1053)	0.7792
Transferrin (g/L)	BL	2.97 ± 0.53 (n=43)	2.92 ± 0.43 (n=45)	2.93 ± 0.49	0.9090
	FU	3.56 ± 0.60 (n=44)	3.62 ± 0.50 (n=45)	3.73 ± 0.80	0.5573
	Δ (p^2)	+0.61 ± 0.48 (<0.0001)	+0.72 ± 0.45 (<0.0001)	+0.80 ± 0.65 (<0.0001)	0.2948
TSAT (%)	BL	26.1 (18.7–32.5) (n=43)	24.7 (18.3–33.9) (n=45)	28.5 (17.9–36.8)	0.9282
	FU	18.4 (14.2–26.4) (n=44)	17.7 (12.6–23.6) (n=45)	16.3 (10.3–24.7)	0.5118
	Δ (p^2)	−6.78 (−11.9 to 0.72) (0.0002)	−5.80 (−13.1 to −0.72) (0.0001)	−5.69 (−21.0 to 0.44) (0.0004)	0.6152
sTfR (mg/L)	BL	1.71 (1.25–2.23)	1.63 (1.29–2.03)	1.60 (1.22–2.15)	0.8672
	FU	1.81 (1.35–2.32)	2.00 (1.66–2.53)	2.07 (1.45–3.13)	0.4665
	Δ (p^2)	+0.13 (−0.27 to 0.56) (0.1223)	+0.19 (−0.14 to 0.89) (0.0041)	+0.38 (−0.07 to 0.89) (0.0003)	0.4625
Ferritin Index[3]	BL	−1.39 ± 0.39	−1.46 ± 0.43 (n=42)	−1.45 ± 0.32 (n=46)	0.6481
	FU	−1.08 ± 0.34 (n=42)	−1.00 ± 0.45 (n=42)	−1.06 ± 0.39 (n=43)	0.6876
	Δ (p^2)	+0.29 ± 0.43 (<0.0001)	+0.47 ± 0.33 (<0.0001)	+0.44 ± 0.35 (<0.0001)	0.0660
Hepcidin (ng/mL)	BL	6.66 (3.40–13.10)	9.97 (4.29–20.98)	9.39 (4.89–17.37)	0.3201
	FU	2.96 (1.71–4.34)	1.97 (1.21–4.26)	2.38 (1.02–5.65)	0.3184
	Δ (p^2)	−3.89 (−11.1 to 0.09) (<0.0001)	−7.73 (−18.8 to −2.22) (<0.0001)	−5.13 (−11.8 to −1.39) (<0.0001)	0.0810
CRP (mg/L)	BL	3.10 (1.84–7.21)	5.07 (2.21–9.00)	3.60 (1.90–9.11)	0.2121
	FU	3.34 (1.64–6.96)	4.06 (2.31–6.95)	4.20 (2.31–9.40)	0.2568
	Δ (p^2)	−0.07 (−0.92 to 0.88) (0.8516)	−0.54 (−1.92 to 0.32) (0.1117)	+0.11 (−2.90 to 1.21) (0.5814)	0.2877

Values are mean ± SD or median (IQR), as appropriate. Δ = follow-up − baseline. [1]Between-group comparisons by one-way ANOVA or Kruskal–Wallis test. [2]Within-group comparisons by paired t-test or Wilcoxon signed-rank test. [3]Ferritin index = sTfR/$\log_{10}$(ferritin). Ferritin, hepcidin, sTfR, CRP, and TSAT were log-transformed prior to analysis. Missing data (<10%) were handled by complete-case analysis; sample sizes are shown only where they differed from group totals. Significance: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. ANOVA = analysis of variance; BL = baseline; CRP = C-reactive protein; FU = follow-up; Hb = haemoglobin; IQR = interquartile range; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; SD = standard deviation; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

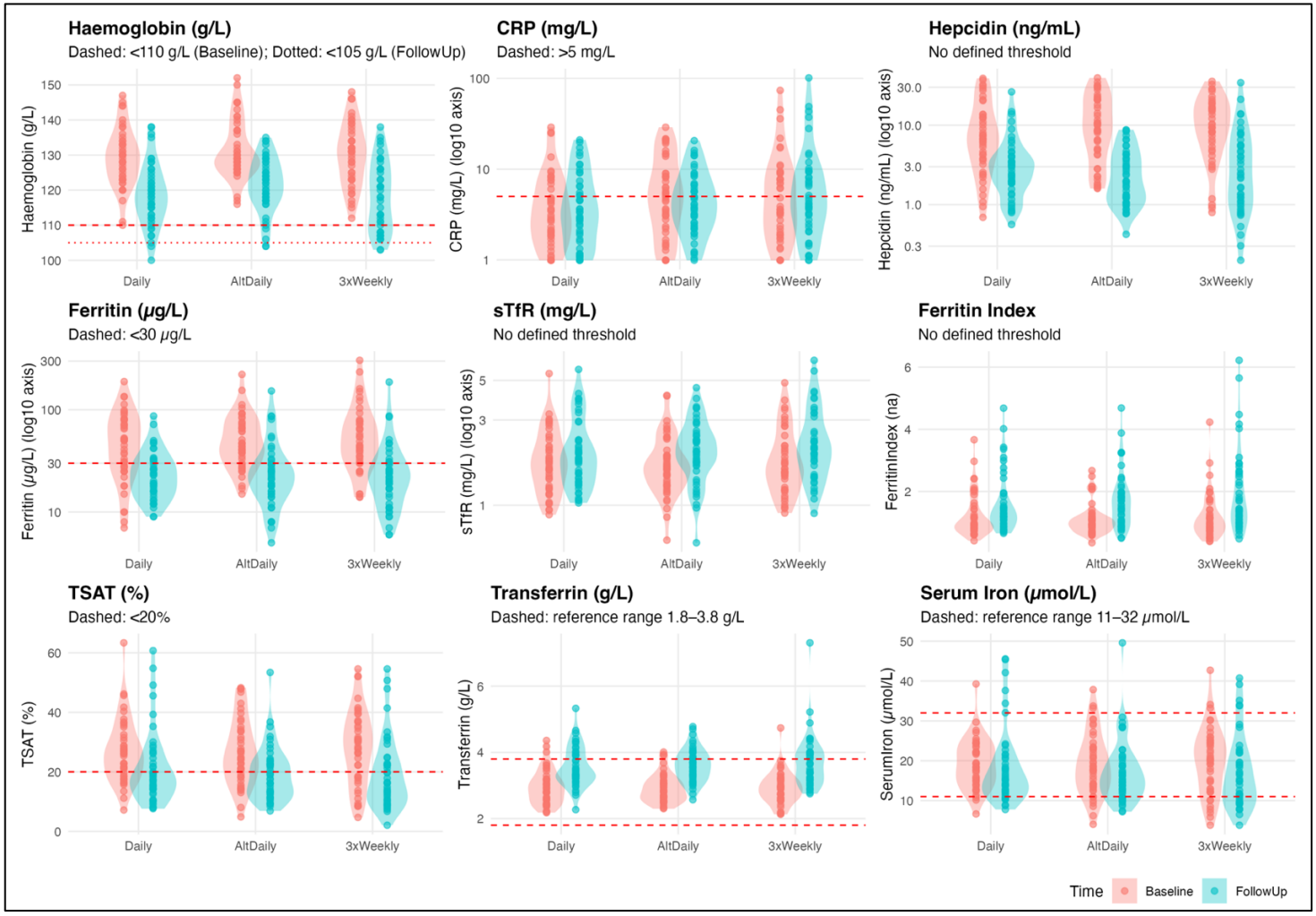


Figure 4.6 Distributions of biomarkers by supplementation regimen and timepoint
Violin plots show the distribution of values, with individual participant measurements overlaid. Ferritin, sTfR, CRP, and hepcidin are shown on a $\log_{10}$ scale. Dashed horizontal lines indicate diagnostic or reference thresholds where applicable (haemoglobin <110 g/L at baseline and <105 g/L at follow-up; ferritin <30 µg/L; TSAT <20%; CRP >5 mg/L; transferrin 1.8–3.8 g/L; serum iron 11–32 µmol/L). No established diagnostic threshold is shown for the ferritin index or hepcidin. CRP = C-reactive protein; Hb = haemoglobin; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

4.3.5 Inflammation Adjustment with Linear Mixed-Effects Models

To account for repeated measures within participants and the potential influence of inflammation, linear mixed-effects models adjusting for $\log_{10}$(CRP) were fitted for each biomarker. Adjusted baseline and follow-up estimates, along with within-group contrasts, are presented in Table 4.3.

Table 4.3 Adjusted baseline and follow-up means for iron biomarkers, with absolute and percentage within-group change

Biomarker	Treatment Arm	Baseline (Adjusted Mean [95% CI])	Follow Up (Adjusted Mean [95% CI])	Absolute Change	Percent Change (%)	p (Within-Group)
Ferritin (µg/L)	Daily	42.05 (34.38–51.42)	23.19 (18.88–28.49)	-18.86	-44.8	<0.001***
	Alternate Day	48.69 (39.94–59.35)	22.02 (17.99–26.96)	-26.67	-54.8	<0.001***
	Three-Times-Weekly	51.09 (41.55–62.83)	21.36 (17.37–26.27)	-29.73	-58.2	<0.001***
Serum Iron (\mol/L)	Daily	17.8 (15.5–20.0)	15.8 (14.1–18.2)	−2.0	−11.2	0.210
	Alternate Day	17.8 (15.8–20.0)	15.8 (13.8–17.8)	−2.0	−11.2	0.096
	Three-Times-Weekly	18.2 (15.8–20.4)	15.5 (13.5–17.4)	−2.7	−14.8	0.047*
Transferrin (g/L)	Daily	2.97 (2.80–3.14)	3.58 (3.41–3.74)	+0.61	+20.5	<0.001***
	Alternate Day	2.90 (2.74–3.07)	3.62 (3.46–3.79)	+0.72	+24.8	<0.001***
	Three-Times-Weekly	2.92 (2.75–3.09)	3.72 (3.55–3.88)	+0.80	+27.4	<0.001***
TSAT (%)	Daily	23.85 (20.74–27.44)	17.98 (15.65–20.65)	-5.87	-24.6	0.001**
	Alternate Day	24.65 (21.5–28.26)	17.41 (15.19–19.95)	-7.25	-29.4	<0.001***
	Three-Times-Weekly	24.92 (21.66–28.66)	16.93 (14.71–19.48)	-7.99	-32.1	<0.001***
sTfR (mg/L)	Daily	1.74 (1.54–1.96)	1.93 (1.71–2.18)	0.19	11	0.084
	Alternate Day	1.66 (1.48–1.87)	1.99 (1.77–2.23)	0.33	19.6	0.003**
	Three-Times-Weekly	1.73 (1.53–1.96)	2.13 (1.88–2.41)	0.4	23	<0.001***
Ferritin Index³	Daily	1.25 (0.99–1.51)	1.64 (1.37–1.91)	+0.39	+31.4	0.006**
	Alternate Day	1.09 (0.83 – 1.35)	1.73 (1.46–1.99)	+0.64	+58.5	<0.001***
	Three-Times-Weekly	1.20 (0.93–1.47)	1.97 (1.71–2.24)	+0.78	+64.6	<0.001***
Hepcidin (ng/mL)	Daily	6.54 (4.92–8.7)	2.81 (2.11–3.73)	-3.73	-57.1	<0.001***
	Alternate Day	8.79 (6.65–11.61)	2.18 (1.65–2.87)	-6.61	-75.2	<0.001***
	Three-Times-Weekly	8.66 (6.47–11.58)	2.49 (1.86–3.33)	-6.17	-71.3	<0.001***

Values are adjusted means (95% CI) from CRP-adjusted linear mixed-effects models, back-transformed to original units. sTfR, hepcidin, ferritin, TSAT, and serum iron were modelled on the $\log_{10}$ scale and back-transformed for presentation; transferrin, MCV, MCH, and the ferritin index were modelled on their original scales. Absolute and percentage changes reflect adjusted within-group differences (follow-up minus baseline); percentage change is not reported for the ferritin index. p-values correspond to within-group contrasts estimated using Satterthwaite's approximation. Significance: ns ≥ 0.05; *< 0.05; **< 0.01; ***< 0.001. CI = confidence interval; CRP = C-reactive protein; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

In adjusted analyses, ferritin, TSAT, and hepcidin decreased significantly ($p < 0.01$), and ferritin index increased significantly ($p < 0.01$), from baseline to follow-up across all three dosing regimens. The adjusted direction and magnitude of change were consistent with unadjusted findings. sTfR increased significantly in the alternate-day ($p = 0.003$) and three-times-weekly ($p < 0.001$) groups in the CRP-adjusted models, whereas the change in the daily group did not reach statistical significance ($p = 0.084$), mirroring the pattern observed in the unadjusted analyses Figure 4.7).

Between-group comparisons of adjusted change values (Group × Time interaction) are summarised in Table 4.4. After adjusting for inflammation and repeated measures, biomarker trajectories were largely similar across the three dosing regimens. Ferritin, TSAT, and sTfR showed no significant differences in the change between groups, indicating that dosing frequency did not materially alter the overall direction or extent of biomarker change. There was only one significant between-group difference observed; the alternate-day group exhibited a greater decline in hepcidin from baseline to follow-up than the daily group ($p = 0.027$). Other between-group differences were small and not statistically significant, potentially reflecting limited power to detect modest dose-related effects, despite directionally consistent trends favouring daily dosing across biomarkers.

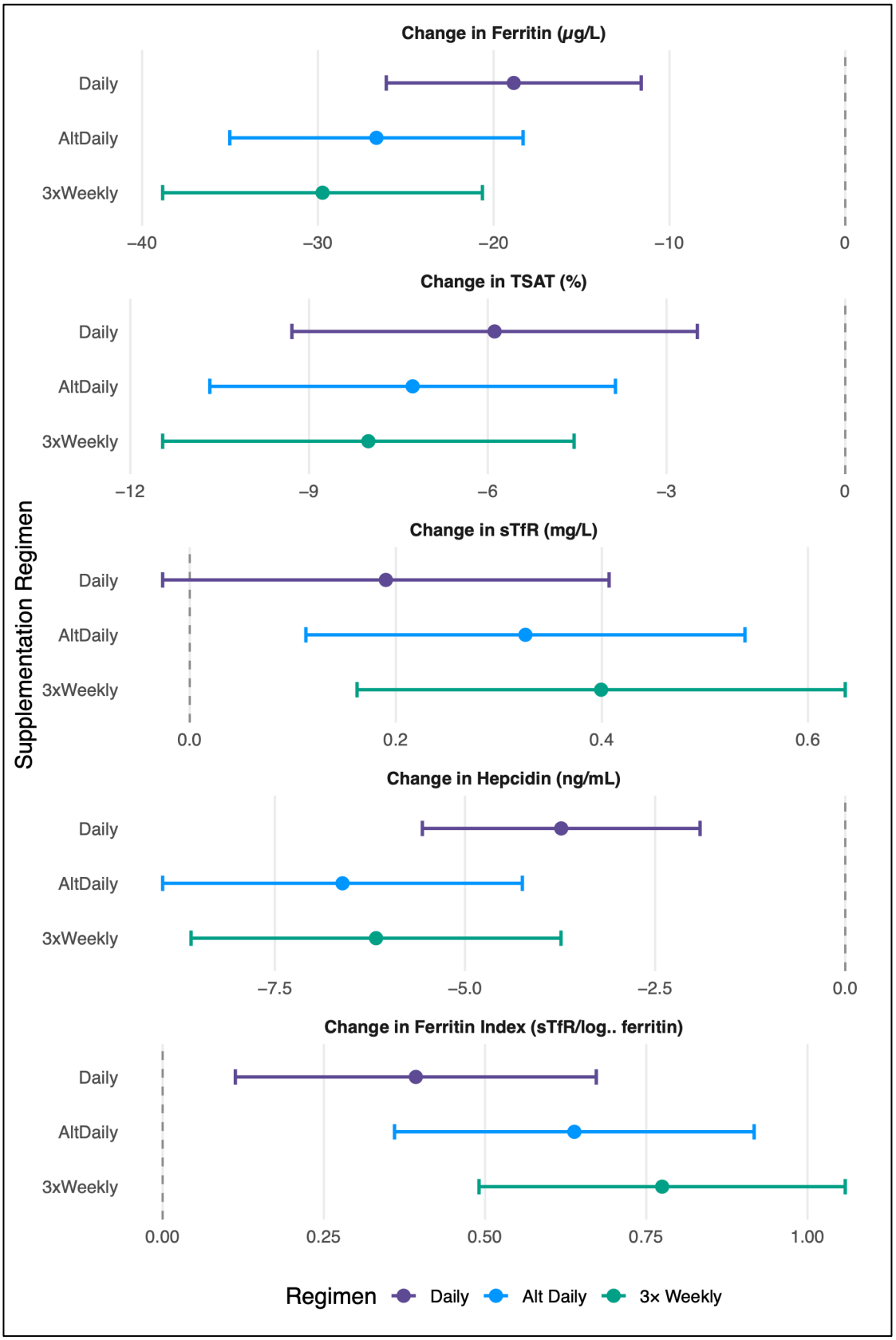


Figure 4.7 Adjusted within-group changes in iron biomarkers, by supplementation regimen
Adjusted within-group changes from baseline to follow-up in ferritin, TSAT, sTfR, hepcidin, and the ferritin index, by oral iron supplementation regimen. Ferritin, TSAT, sTfR, and hepcidin were modelled on the $\log_{10}$ scale and back-transformed for presentation; the ferritin index was analysed on its original scale. Points show adjusted mean changes with 95% confidence intervals, and the dashed line indicates no change. CI = confidence interval; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

Table 4.4 Between-group differences in adjusted within-group changes in biomarkers

Biomarker	Comparison	Effect (95% CI)	P value
Ferritin	Three-Times-Weekly vs Alternate Day	−0.034 [−0.158, 0.090]	0.587
	Three-Times-Weekly vs Daily	−0.120 [−0.245, 0.004]	0.057
	Alternate Day vs Daily	−0.086 [−0.209, 0.037]	0.169
Serum Iron	Three-Times-Weekly vs Alternate Day	−0.012 [−0.104, 0.081]	0.802
	Three-Times-Weekly vs Daily	−0.025 [−0.118, 0.068]	0.597
	Alternate Day vs Daily	−0.013 [−0.105, 0.079]	0.778
Transferrin	Three-Times-Weekly vs Alternate Day	0.076 [−0.156, 0.307]	0.518
	Three-Times-Weekly vs Daily	0.189 [−0.043, 0.422]	0.110
	Alternate Day vs Daily	0.113 [−0.118, 0.345]	0.334
TSAT	Three-Times-Weekly vs Alternate Day	−0.017 [−0.114, 0.081]	0.735
	Three-Times-Weekly vs Daily	−0.045 [−0.143, 0.053]	0.364
	Alternate Day vs Daily	−0.028 [−0.126, 0.069]	0.565
sTfR	Three-Times-Weekly vs Alternate Day	0.012 [−0.061, 0.085]	0.740
	Three-Times-Weekly vs Daily	0.045 [−0.028, 0.118]	0.228
	Alternate Day vs Daily	0.033 [−0.039, 0.105]	0.370
Ferritin Index	Three-Times-Weekly vs Alternate Day	-0.14 [-0.53, 0.25]	0.500
	Three-Times-Weekly vs Daily	-0.38 [-0.78, 0.02]	0.060
	Alternate Day vs Daily	-0.25 [-0.64, 0.14]	0.136
Hepcidin	Three-Times-Weekly vs Alternate Day	0.065 [−0.150, 0.279]	0.552
	Three-Times-Weekly vs Daily	−0.174 [−0.390, 0.042]	0.114
	Alternate Day vs Daily	−0.239 [−0.450, −0.027]	**0.027***

Between-group contrasts represent differences in adjusted within-group change (follow-up − baseline) between dosing regimens. Models included treatment group, time, the group × time interaction, and $\log_{10}$(CRP) as fixed effects, with a random intercept for participant. Estimates are on the $\log_{10}$-transformed scale for ferritin, serum iron, TSAT, sTfR, and hepcidin, and on the original scale for transferrin and the ferritin index. CI = confidence interval; CRP = C-reactive protein; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

CRP was included in all models to assess the independent contribution of inflammation to biomarker behaviour, and the estimated effect of $\log_{10}$(CRP) on each biomarker was calculated (Appendix 4: Supplementary Table S4.7). Adjustment for CRP did not materially alter biomarker trajectories across treatment groups, and the absence of a statistically significant rise in sTfR in the daily dosing group persisted after adjustment for CRP, indicating that differences in inflammatory status were unlikely to explain between-group differences. Although the alternate-day group showed a greater decline in hepcidin than the daily group in adjusted analyses, CRP was not independently associated with hepcidin concentrations in the mixed models, suggesting that this difference reflects physiological or dosing-related factors rather than baseline inflammation. CRP was not independently associated with ferritin, hepcidin, ferritin index, or sTfR in the mixed models. TSAT showed a modest negative association with CRP.

Model diagnostics are summarised in Appendix 4: Supplementary Table S4.8 and indicate no major violations of model assumptions.

4.3.6 CRP–Biomarker Associations

Correlations between CRP and iron biomarkers are presented in Appendix 4 (Supplementary Table S4.9 and Supplementary Figure S4.11). These analyses provide an unadjusted assessment of the relationships between inflammation and iron biomarkers and complement the findings from the linear mixed-effects models. There was no evidence of a meaningful association between CRP and ferritin at baseline ($\rho = -0.038$, $p = 0.66$) or follow-up ($\rho = -0.133$, $p = 0.136$). Similarly, no significant association was observed between CRP and sTfR at baseline ($\rho = 0.129$, $p = 0.136$) or follow-up ($\rho = 0.047$, $p = 0.588$). By contrast, TSAT showed a moderate negative correlation with CRP at baseline ($\rho = -0.526$, $p < 0.001$) and a weaker but still significant negative correlation at follow-up ($\rho = -0.331$, $p < 0.001$).

4.4 Discussion

4.4.1 Summary of Key Findings

This chapter examined the effect of daily, alternate-day, and three-times-weekly prophylactic oral iron supplementation on iron biomarker trajectories during pregnancy using data from the PANDA randomised trial. Four main findings emerged. First, ferritin, TSAT, and hepcidin declined significantly from randomisation to follow-up across all supplementation regimens, suggesting that pregnancy-related changes in iron metabolism occurred irrespective of dosing frequency. Second, sTfR increased significantly in the alternate-day and three-times-weekly groups but not in the daily group, although differences between regimens were generally modest. Third, the observed biomarker trajectories remained largely unchanged after adjustment for inflammation, indicating that inflammation was not the primary driver of the patterns observed. Fourth, despite ongoing supplementation, most participants met biochemical criteria for iron deficiency at follow-up regardless of regimen, while haemoglobin concentrations remained within the normal range and incident anaemia remained uncommon.

Collectively, these findings suggest that physiological changes occurring during pregnancy substantially influence iron biomarker trajectories. Although modest differences were observed between supplementation regimens, none of the evaluated supplementation strategies prevented the pregnancy-associated decline in iron status.

4.4.2 Biomarker Interpretation in Pregnancy

The observed biomarker trajectories broadly align with other longitudinal studies describing declining ferritin, hepcidin, and TSAT across gestation, alongside a progressive rise in sTfR, reflecting increased maternal–foetal iron demand, plasma volume expansion, and coordinated iron regulation.[12,89,90,207] Consistent with these findings, Bar et al. analysed longitudinal laboratory data from more than 300,000 pregnancies and demonstrated coordinated changes across haematological, inflammatory, metabolic, and biochemical biomarkers throughout gestation, highlighting the extensive physiological adaptations that occur during pregnancy.[208] However, the biological mechanisms underlying these trajectories remain incompletely understood, complicating the interpretation of changes in iron biomarkers during pregnancy.

Ferritin declined in all arms, reflecting haemodilution, mobilisation of stores, and increased iron use,[12,69,184] with possible contribution from pregnancy-related immunomodulation.[209] TSAT fell consistently, in line with rising transferrin and redistribution of iron towards maternal and foetal needs.[12] Hepcidin fell markedly in all groups, consistent with the profound suppression that occurs in healthy pregnancy to facilitate iron absorption and placental transfer.[12,90,197] While the alternate-day group demonstrated a slightly greater decline in hepcidin, differences between regimens were small relative to the overall pregnancy-driven suppression.

The sTfR findings align with previous studies showing a progressive rise across gestation,[210–212] reflecting increased cellular iron demand and erythropoietic activity.[207,213] Importantly, the rise in the daily group did not reach statistical significance ($p = 0.084$), suggesting that cellular iron supply may have been more adequately maintained. These findings are consistent with the possibility that daily dosing may better meet the increasing erythropoietic demands of pregnancy, although this exploratory sub-analysis was underpowered for between-group comparisons and

should therefore be interpreted with caution. The ferritin index (sTfR/$\log_{10}$ferritin), which integrates cellular iron demand with iron stores,[81] increased in all groups but showed the smallest increase in the daily arm, providing further, complementary support for this interpretation.

Despite prophylactic supplementation, most participants met biochemical criteria for iron deficiency at follow-up. In the daily group, this occurred without a significant rise in sTfR, suggesting erythropoietic iron demands may have remained adequately met despite declining ferritin and TSAT. This discordance highlights the challenge of interpreting iron status biomarkers in late pregnancy, where it is difficult to determine which biochemical thresholds reflect true functional iron deficiency. Current cut-offs are largely derived from non-pregnant populations, and longitudinal studies consistently demonstrate a physiological decline in ferritin across gestation.[12,69,89,184,209] Recent analyses by Mei et al.[69] and Addo et al.[68] have proposed physiologically derived ferritin thresholds of approximately 25 µg/L in the first trimester and 20 µg/L in later pregnancy, while Fan et al.[67] highlighted the limitations of applying fixed diagnostic cut-offs across gestation. Together, these studies support the need for physiologically informed, trimester-specific thresholds to better distinguish normal gestational adaptation from clinically meaningful iron deficiency.

CRP had little independent association with most biomarkers, likely reflecting the cohort's low overall inflammatory burden and the dominant physiological suppression of ferritin and hepcidin across pregnancy.[12,89,90,197] The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project demonstrated that inflammation can substantially influence ferritin concentrations but has a more limited effect on sTfR.[76,207,213] Consistent with these findings, little evidence of an association between sTfR and CRP was observed, and adjustment for CRP did not explain between-group differences in hepcidin or sTfR trajectories. TSAT retained its expected negative association with CRP, consistent with inflammation-mediated reductions in circulating serum iron.[197] Overall, these findings suggest that inflammation was not a major driver of biomarker variability in this cohort and that the challenges

in interpretation primarily reflect physiological changes across gestation rather than inflammatory effects.

4.4.3 Strengths and Limitations

Strengths of this analysis include the randomised design of the parent trial, comparability of baseline characteristics across treatment groups, and inclusion of a comprehensive biomarker panel spanning iron stores, circulating availability, cellular demand, and regulatory control. Laboratory assays were conducted with strong internal quality control, and paired samples from the same participant were measured on the same plate to minimise inter-assay variability. The use of linear mixed-effects models allowed appropriate handling of repeated measures and adjustment for inflammation while preserving within-participant correlations.

However, several limitations should be considered. This sub-analysis was exploratory and hypothesis-generating, and the sample size was determined by feasibility and cost constraints rather than formal power calculations. Post hoc analyses confirmed limited power to detect small between-group differences; therefore, findings regarding dosing regimens should be interpreted with caution.

The parent PANDA trial also did not include an unsupplemented or placebo control arm. Consequently, although the observed biomarker trajectories were consistent with established physiological changes across pregnancy, it is not possible to determine how these trajectories might have differed in the absence of iron supplementation. The analysis therefore allows comparison of relative differences between supplementation regimens but cannot quantify the extent to which supplementation modified or attenuated pregnancy-related changes in iron biomarkers.

The cohort was predominantly White British (86.7%), non-anaemic at enrolment, and managed within a structured trial setting with active adherence support. These factors may limit generalisability to more diverse populations, to routine clinical settings where adherence may be lower, and to anaemic women, in whom effects on iron status may be more pronounced.

Several methodological factors may have contributed to variability in biomarker measurements. Ferritin was measured locally at participating NHS sites using site-specific analysers, and inter-site variability cannot be excluded. In addition, iron biomarkers are influenced by recent oral iron intake and diurnal variation in iron metabolism.[123] The timing of blood sampling relative to iron dosing was not standardised, which may have contributed to variability, particularly for serum iron and TSAT.

Although adjustment for inflammation was performed, the analysis was limited by the availability of CRP alone. The BRINDA approach was originally developed using both CRP and α1-acid glycoprotein (AGP) to capture different phases of the inflammatory response. AGP was not available in this study, and therefore, residual confounding by inflammation may have persisted despite adjustment.[214]

Multiple statistical comparisons increase the risk of Type I error, and isolated findings, such as the greater reduction in hepcidin in the alternate-day group, should therefore be interpreted cautiously. The absence of linked maternal and neonatal outcome data limits the interpretation of the clinical significance of observed biomarker changes. Finally, the exclusion of anaemic women results in a relatively homogeneous cohort that aids interpretation but limits applicability to populations at the highest risk of iron deficiency and anaemia globally.

4.4.4 Clinical and Research Implications

The parent PANDA trial demonstrated broadly similar haemoglobin outcomes across all three regimens, with daily dosing achieving the most consistent maintenance of haemoglobin and comparable adherence and side-effect profiles.[203] Adherence in this sub-cohort was consistent with that reported in the parent trial, although these rates were achieved within a clinical trial setting and may not reflect adherence in routine practice. The low incidence of anaemia across all groups (~4–5%) suggests that prophylactic supplementation was effective in maintaining haemoglobin concentrations during pregnancy. However, substantial changes were observed in

ferritin, TSAT, hepcidin, sTfR, and ferritin index despite relatively stable haemoglobin concentrations. These findings suggest that haemoglobin alone may provide an incomplete picture of iron status during pregnancy and support the use of additional biomarkers when evaluating the physiological effects of supplementation.

This chapter provides exploratory mechanistic evidence consistent with a possible directional advantage of daily dosing beyond haemoglobin. The absence of a significant sTfR rise and the smaller ferritin index increase in the daily arm both suggest that daily dosing may better sustain erythropoietic iron supply across pregnancy. This contrasts with the findings of a recent systematic review[100] showing similar efficacy between alternate-day and daily iron dosing; however, that review focused on the treatment of established anaemia, with haemoglobin as the primary outcome, whereas this sub-analysis addressed prophylactic dosing in non-anaemic women. While these results favour daily prophylactic dosing, larger, adequately powered, outcome-focused studies are needed to support the rationale for the main PANDA trial, a large pragmatic study that has begun enrolling 11,020 women to compare once-daily oral iron with placebo (ISRCTN16425597).

The choice of biomarker thresholds used to define iron deficiency has important implications for clinical decision-making during pregnancy. Ferritin <30 µg/L is increasingly used to justify treatment escalation in pregnancy, including decisions around IV iron.[89] Although an early study suggested that sTfR outperforms ferritin in detecting iron deficiency,[215] its diagnostic utility remains unclear, particularly because proposed diagnostic thresholds are assay-dependent and vary widely across contexts.[148,216,217] If the decline in ferritin partly reflects physiological adaptation rather than a complete functional deficiency in late pregnancy, there is a risk of over-treatment. At the same time, a recent randomised trial using ferritin <30 µg/L as an eligibility criterion for IV iron in non-anaemic women reported a significant reduction in foetal growth restriction; however, this was not a primary outcome and requires confirmation in larger trials.[128] This finding nonetheless highlights that the clinical consequences of iron deficiency in pregnancy extend beyond haemoglobin. Ultimately, these findings highlight the need for pregnancy-specific,

functionally validated thresholds that can meaningfully distinguish physiological adaptation from true deficiency and appropriately guide treatment decisions.

These findings also help contextualise the variation identified in Chapters 2 and 3. While earlier chapters demonstrated heterogeneity in clinical recommendations, biomarker selection, and definitions of response, the present analysis suggests that some of this uncertainty may reflect the challenges of interpreting iron biomarkers in the physiological environment of pregnancy. This highlights the importance of considering gestational physiology when assessing iron status and developing pregnancy-specific approaches to biomarker interpretation.

4.5 Conclusion

In summary, this chapter examined the effect of the frequency of prophylactic oral iron supplementation on iron biomarker trajectories during pregnancy. Ferritin, TSAT, and hepcidin declined across all supplementation regimens, while most participants met biochemical criteria for iron deficiency at follow-up despite preserved haemoglobin concentrations and low rates of incident anaemia. Although the absence of a significant rise in sTfR in the daily supplementation group may suggest better support for increasing erythropoietic iron demands, this exploratory sub-analysis was underpowered for between-group comparisons and larger studies are required to confirm these findings.

These findings highlight the challenges of interpreting iron biomarkers during pregnancy. Biomarker trajectories were influenced not only by the supplementation regimen but also by the physiological adaptations of gestation, making it difficult to distinguish normal pregnancy-related change from clinically meaningful iron deficiency. The findings therefore support the need for pregnancy-specific approaches to biomarker interpretation and for thresholds that account for the physiological context in which measurements are obtained.

The next chapter shifts beyond pregnancy to examine iron deficiency and anaemia in a postpartum population with a higher burden of disease and multiple potential nutritional contributors to anaemia. This provides an opportunity to assess how iron biomarkers perform in a different

maternal health setting, where the physiological adaptations of pregnancy are no longer the predominant influence on biomarker interpretation and the causes of anaemia may be more readily distinguished.

Chapter 5 - **Prevalence and Aetiology of Postpartum Anaemia in Rural India:** A Nested Sub-study of the SMARThealth Pregnancy Trial

Publication Status

A version of this chapter is currently being prepared for publication as a peer-reviewed journal article.

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Statement of Contribution

This chapter reports a nested observational sub-study within the SMARTHealth Pregnancy 2 trial. I designed the sub-study, including the research questions, biomarker selection, analytical approach, and ethics amendment. Data collection was undertaken by the SHP2 trial team in India (SRT, DP, AG, JH). Venous sampling and laboratory analyses were conducted by accredited laboratories and All India Institute of Medical Sciences (AIIMS), New Delhi (TA, AR). Data management was conducted by The George Institute for Global Health, India (SRT, DP, AG). I conducted all statistical analyses, interpreted the findings, and wrote this chapter, with input from supervisors and collaborators.

5.1 Introduction

This chapter examines the assessment of anaemia and iron status among women at 12 months postpartum in rural India. Chapters 2–4 examined the use of iron status biomarkers across clinical guidelines, pregnancy research, and a controlled antenatal supplementation trial. These studies identified uncertainty regarding how to assess iron deficiency and treatment response and demonstrated that biomarker interpretation may vary with the biomarker measured, the threshold applied, and the physiological context in which assessment occurs.

This chapter extends these questions beyond pregnancy. It shifts from antenatal populations in the UK to community-based postpartum care in rural India, where anaemia remains highly prevalent and may reflect multiple overlapping nutritional deficiencies, inflammation, and differences in diagnostic infrastructure. This setting provides an opportunity to examine the assessment of iron deficiency and anaemia in a population representative of settings where the global burden of anaemia is greatest.

This chapter presents a nested sub-study of the SMARThealth Pregnancy 2 (SHP2) trial conducted in Siddipet, a rural district in Telangana, India. Using a panel of iron and nutritional biomarkers together with paired capillary and venous haemoglobin measurements, the study first examines whether the prevalence of postpartum anaemia differed between women enrolled in the intervention and control arms of the trial. It then investigates the prevalence and likely causes of postpartum anaemia, the agreement between capillary point-of-care and laboratory-based venous haemoglobin measurements, and the agreement among biomarkers used to classify iron deficiency.

5.1.1 Postpartum Anaemia in India

Postpartum anaemia is a major global maternal health problem, with the highest burden occurring in LMICs, where nutritional deficiencies, limited antenatal care access, postpartum blood loss, and infection contribute substantially to risk.[4,193,218,219] Although definitions vary according to the postpartum period assessed, lower haemoglobin thresholds are commonly used during the

immediate postpartum and early postnatal periods, typically within the first 24–48 hours and the first weeks after delivery. Thresholds of <100 g/L or <110 g/L are commonly used during these earlier postpartum stages in some definitions, whereas later postpartum assessment often returns to the non-pregnant haemoglobin threshold of <120 g/L.[49] Postpartum anaemia is also increasingly recognised as a preconception health issue. For many women, particularly in settings with short inter-pregnancy intervals, the postpartum period represents the nutritional starting point for future pregnancies. Persistent anaemia and iron deficiency may therefore influence not only maternal recovery after childbirth but also nutritional status entering subsequent pregnancies, contributing to intergenerational cycles of poor maternal and child health.[220]

India bears one of the largest global burdens of anaemia, with over half of women of reproductive age affected, and prevalence is particularly high in rural and socioeconomically disadvantaged populations.[219,221] This scale of burden was also reflected in the findings of Chapter 3, which identified India as the most frequently represented LMIC setting across oral iron supplementation trials in pregnancy, emphasising both the public health importance of the problem and the extent to which evidence generation in this area has been concentrated in the Indian context.

This high prevalence likely reflects a combination of biological, nutritional, and structural factors. Repeated pregnancies with short interpregnancy intervals may prevent replenishment of maternal iron stores, while dietary patterns with limited iron bioavailability may further contribute to nutritional vulnerability.[193,219] Socioeconomic constraints, food insecurity, and inequities in access to nutrition and healthcare may additionally influence maternal nutritional status and recovery following pregnancy.[222,223]

Iron deficiency has traditionally been considered the predominant cause of anaemia in India.[219,224] Despite multiple national anaemia control and iron supplementation initiatives, including the Anaemia Mukt Bharat strategy launched in 2018, anaemia prevalence among Indian women has remained persistently high.[98,219,221] India's National Family Health Survey (NFHS) data demonstrate little improvement over time, with anaemia affecting 53% of women aged 15–49

years in NFHS-4 (2015–16) and 57% in NFHS-5 (2019–21).[225,226] Anaemia among pregnant women similarly increased from 50.4% to 52.2% over the same period. Despite successive national anaemia control initiatives, these findings suggest limited progress in reducing the burden of anaemia among Indian women.

5.1.2 Healthcare Context and Anaemia Screening in Rural India

India has a mixed and highly decentralised healthcare system in which responsibility for governance, financing, and service delivery is shared across central and state governments, with substantial involvement of the private sector. Public healthcare is intended to be universally available and is primarily organised and delivered at the state level, resulting in considerable variation in access, infrastructure, and service quality across the country. The central Ministry of Health and Family Welfare provides overall policy direction and funds national health programmes, while state governments are responsible for implementation and day-to-day delivery.[227,228]

The public sector is structured as a tiered referral system. In rural areas, care begins at sub-centres and primary health centres (PHCs), with referral upward to community health centres, district hospitals, and tertiary facilities. Under Indian public health standards, PHCs serve as the first point of contact with a medical officer for rural populations and are typically responsible for a catchment population of approximately 20,000–30,000 people, depending on geography. PHCs provide preventive, promotive, and basic curative services, including antenatal care, anaemia screening, immunisation programmes, management of common illnesses, and referral to higher levels of care when required. More recently, many PHCs have been strengthened under the Ayushman Bharat programme through the establishment of Health and Wellness Centres, which aim to expand access to comprehensive primary healthcare, including maternal health services, non-communicable disease screening, essential diagnostics, and health promotion activities.[229,230]

At the community level, frontline workers, including Accredited Social Health Activists (ASHAs), Auxiliary Nurse Midwives, and Anganwadi workers, play a central role in maternal

and child health screening and referral. In practice, however, shortages of staff, diagnostics, medicines, and infrastructure, particularly in rural and underserved areas, limit the public system's capacity to deliver timely and comprehensive care.[231]

For maternal health specifically, access to laboratory-based diagnostics, specialist referral, and postpartum follow-up may vary substantially by setting. In many rural settings, anaemia screening relies on point-of-care capillary haemoglobin devices due to their portability, low cost, and feasibility for community-based use.[232] These devices are widely used in antenatal and postpartum screening programmes and have been used in the NFHSs and primary care initiatives. Several studies have demonstrated that digital haemoglobinometers provide acceptable sensitivity and specificity for detecting anaemia at a population level, supporting their use in large-scale screening and public health programmes.[233,234]

However, even modest systematic differences between capillary and venous haemoglobin measurements can meaningfully alter estimates of anaemia prevalence in high-burden populations.[235,236] Capillary measurements may additionally be influenced by sampling technique, peripheral perfusion, environmental conditions, and device calibration, potentially contributing to variation in classification.[237] While point-of-care devices are pragmatic and scalable, they provide limited information on iron status or alternative causes of anaemia and may therefore be insufficient to distinguish between different forms of nutritional or inflammatory anaemia when used in isolation.

5.1.3 Multifactorial Anaemia and Iron Biomarker Interpretation

Although iron deficiency is widely recognised as a major contributor to maternal anaemia, accumulating evidence from India suggests that postpartum anaemia is frequently multifactorial. In many settings, anaemia reflects overlapping contributions from iron, vitamin B12, and folate deficiencies, chronic inflammation, infection, and inherited haemoglobin disorders.[195,238–240] As a result, assuming that anaemia primarily reflects iron deficiency may oversimplify its underlying

causes and lead to misclassification of women who would benefit from alternative investigations or interventions.

In a large cohort study of postnatal women in India, Nair et al. reported that 61% of women remained anaemic despite widespread iron supplementation, while only approximately one-third of anaemic women had low ferritin concentrations.[195] The study also demonstrated poor concordance between haemoglobin and ferritin during pregnancy and the postpartum period, suggesting that iron deficiency alone did not account for most anaemia cases and that inflammation may substantially influence the assessment of iron deficiency.[195] Regional studies from Telangana and Haryana, the states in which SHP2 was conducted, have similarly reported high prevalences of vitamin B12 and folate deficiency among women of reproductive age.[239,241] Additionally, inherited haemoglobin disorders, including the sickle cell trait and β-thalassaemia, are recognised contributors to the anaemia burden in India, although their relative contribution compared with nutritional deficiencies remains unclear in postpartum populations.[238,242,243]

However, despite recognition that multiple nutritional deficiencies frequently coexist, interventions targeting multiple micronutrients have not consistently demonstrated greater reductions in anaemia than iron–folic acid supplementation alone.[97,244] Careful characterisation of the causes of anaemia, therefore, remains important for informing future intervention strategies.

In populations where multiple nutritional deficiencies, inflammation, and other causes of anaemia coexist, interpretation of iron status biomarkers may be particularly challenging. As discussed in Chapters 1 and 4, ferritin and sTfR reflect different aspects of iron metabolism and erythropoiesis. Ferritin provides an estimate of iron stores but is influenced by inflammation as an acute-phase reactant, while sTfR may better reflect iron-restricted erythropoiesis and is generally considered less sensitive to inflammatory processes. However, the interpretation of sTfR is complicated by assay variability, altered erythropoietic activity, and the lack of universally accepted thresholds, particularly in maternal populations.[80]

Against this background, important uncertainties remain regarding the causes of postpartum anaemia and the interpretation of iron status biomarkers in rural Indian populations. In particular, it remains unclear how different biomarkers and measurement approaches influence the classification of iron deficiency and anaemia in settings where multiple nutritional deficiencies and inflammatory processes may coexist.

5.1.4 The SMARThealth Pregnancy 2 Trial

To address these questions, this chapter uses data from the SHP2 trial, a type 2 implementation-effectiveness cluster-RCT conducted across 60 rural and semi-rural villages in the states of Telangana and Haryana in India. The trial evaluated a community health worker-led digital intervention aimed at improving identification, referral and management of high-risk conditions during and after pregnancy.[245,246] Randomisation occurred at the level of the PHC, with pregnant women enrolled into pre-randomised intervention or control clusters. This secondary analysis presents data from the Telangana site due to ethics approval delays at the Haryana site.

The primary effectiveness outcome of the parent trial was anaemia prevalence at 12 months postpartum, assessed using capillary point-of-care haemoglobin measured by ASHAs to reflect routine community-based practice. In the Telangana site, no difference in anaemia prevalence at 12 months postpartum was observed between the intervention and control groups. However, a high prevalence of postpartum anaemia was observed in both arms, raising questions regarding the underlying causes of anaemia in this population and the implications of relying on capillary point-of-care testing for anaemia classification.

This nested sub-study was conducted on a subset of women from the Telangana site of the SHP2 cohort, using venous haemoglobin and a panel of iron and nutritional biomarkers, including ferritin, sTfR, folate, vitamin B12, CRP, and hepcidin. This enabled more detailed characterisation of postpartum anaemia and its potential causes, while also allowing evaluation of agreement between different biomarkers and haemoglobin measurement approaches.

5.1.5 Rationale, Aim, and Research Questions

The 12-month postpartum time point extends beyond the period typically captured by routine postnatal follow-up while remaining relevant to maternal iron recovery and future reproductive health. Examining anaemia at this stage provides an opportunity to investigate the persistence and potential causes of postpartum anaemia, and to evaluate how different biomarkers and haemoglobin measurement approaches influence the classification of iron deficiency and anaemia in this setting.

Aim

To investigate the prevalence and aetiology of postpartum anaemia and evaluate the influence of biomarker selection and measurement approach on the classification of anaemia and iron deficiency amongst women in rural India.

Research Questions

1. Are there differences in postpartum anaemia prevalence between women enrolled in the intervention and control arms of the SHP2 trial?

2. What is the prevalence and likely aetiology of postpartum anaemia at 12 months, including the contribution of iron deficiency, folate deficiency, vitamin B12 deficiency, and inflammation in this population?

3. How closely do capillary point-of-care haemoglobin measurements captured under real-world field conditions agree with laboratory-based venous haemoglobin, and what are the implications for anaemia classification?

4. To what extent do ferritin- and sTfR-based definitions of iron deficiency agree, and how does inflammation influence this agreement in an Indian population?

5.2 Methods

5.2.1 Study Design and Setting

This chapter reports a prospectively designed nested observational sub-study within the SHP2 trial as previously described. Randomisation occurred at the level of the PHC prior to participant recruitment, with pregnant women subsequently enrolled into pre-randomised intervention or control clusters. Detailed methods of the parent trial have been published previously.[245,246]

The SHP2 trial was implemented across rural and semi-rural PHC catchment areas in the states of Telangana and Haryana. PHCs were selected based on feasibility and existing collaborations, with two villages randomly selected within each PHC catchment. The anaemia sub-study was conducted in Siddipet district, Telangana. Although originally planned to recruit across both trial states, delays in ethics approval in Haryana restricted implementation to Telangana, which contributed 16 PHCs and 32 villages. The consequences of this for sample size and statistical power are described in Section 5.2.8.

The anaemia sub-study was designed to incorporate venous blood sampling and biochemical analysis into the 12-month postpartum follow-up. Field implementation and coordination were undertaken by the George Institute India trial team. Venous sampling and initial haematology testing were conducted by local, accredited laboratories, with biochemical assays performed at the All-India Institute of Medical Sciences (AIIMS), New Delhi.

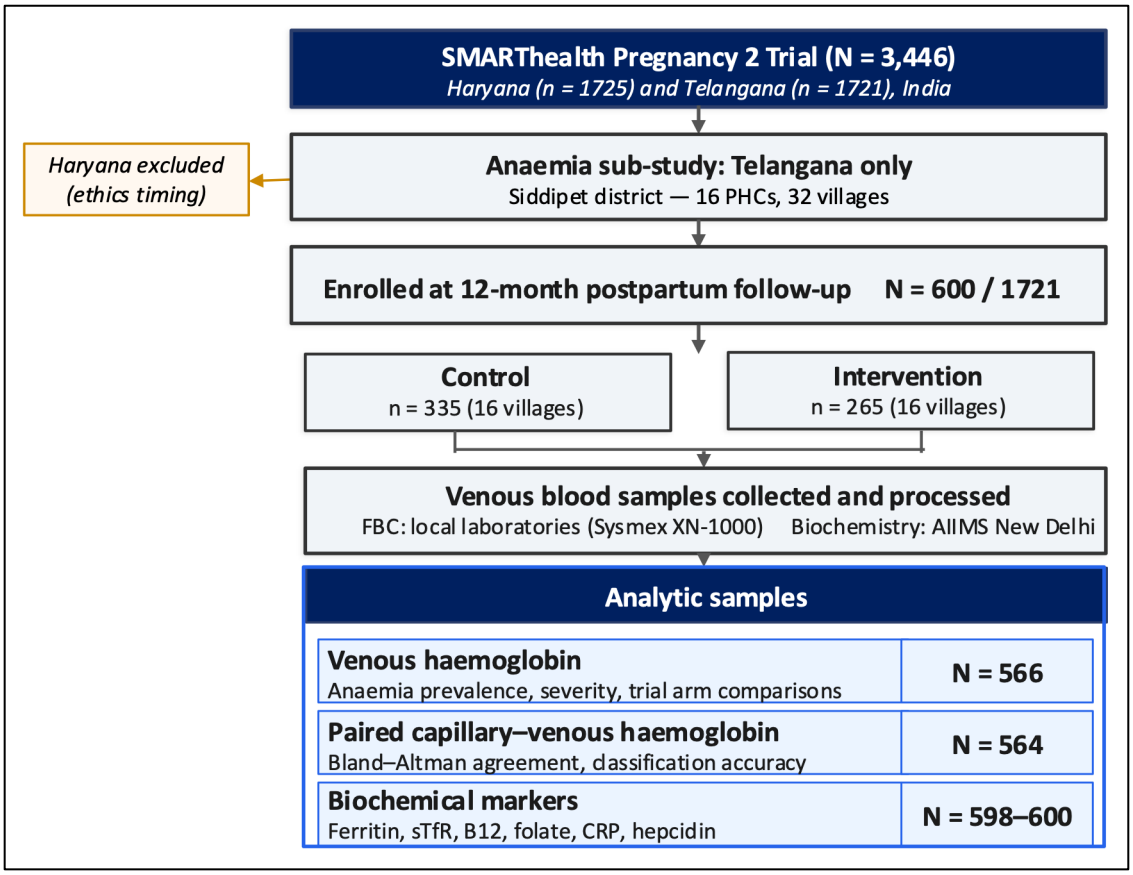


Figure 5.1 Flow chart of study design
Venous haemoglobin was missing for 34 women (control 31, intervention 3), clustered in three control villages; full blood count (FBC) was missing for 38 women; biochemical markers had ≤2 missing values each; and CRP was complete. Denominators are reported throughout the results. FBC = full blood count; Hb = haemoglobin; CRP = C-reactive protein.

5.2.2 Participants and Recruitment

All women who completed the scheduled 12-month postpartum follow-up at the Telangana SHP2 trial sites from April 2025 onwards were eligible for inclusion in the anaemia sub-study. Women were excluded only if they declined or withdrew consent for venous blood collection.

Participants were approached at the scheduled follow-up visit by trained study staff. Eligible participants received a written information sheet and a verbal explanation in the local language. Written informed consent was obtained prior to venepuncture, with witnessed consent procedures used where required to accommodate participants with low literacy.

5.2.3 Specimen Collection and Laboratory Procedures

5.2.3.1 Sample Collection and Transport

At the 12-month postpartum follow-up, participants underwent routine trial assessments including capillary haemoglobin measurement using the TrueHb haemoglobinometer (Wrig Nanosystems, India), performed by ASHAs during home visits. Women consenting to the sub-study additionally provided a venous blood sample, collected on the same day by trained laboratory technicians either at home or at local sub-centres. Samples were immediately placed in insulated containers with ice packs and transported at 2–4°C to accredited laboratories located approximately 30–40 km from the collection sites. FBC analysis was performed on fresh whole blood using automated haematology analysers. Following FBC analysis, samples were centrifuged and serum aliquots stored at −20°C before transport on dry ice to AIIMS, New Delhi. Biochemical assays were performed at AIIMS, and the remaining samples were stored at −80°C in accordance with local protocols.

5.2.3.2 Laboratory Analysis

Biomarker selection was informed by findings from earlier chapters of this thesis and existing literature on anaemia among women of reproductive age in India. The panel was designed to characterise haematological status, iron deficiency, non-iron nutritional causes, and inflammation. FBC parameters included haemoglobin, erythrocyte indices (MCV, MCH, MCHC), RDW, reticulocyte count, white blood cell count, and platelet count. Iron status was assessed using ferritin, and sTfR with the ferritin index (sTfR/$\log_{10}$ ferritin) calculated as a composite marker. Vitamin B12 and folate were measured to assess non-iron nutritional causes. Inflammation was assessed using CRP, and hepcidin was measured as a marker of iron regulation.

FBC testing was performed using a Sysmex XN-1000 analyser. Biochemical assays were conducted at AIIMS using the Roche cobas 8000 platform (Elecsys immunoassays) for ferritin, vitamin B12, folate, and high-sensitivity CRP. sTfR was measured using the Beckman Coulter Access assay, with values converted from nmol/L to mg/L (conversion factor 0.0738). Hepcidin was measured using a commercial ELISA (Invitrogen EEL073).

Quality control included manufacturer-provided internal controls and external quality assurance through Bio-Rad programmes; assay characteristics are summarised in Appendix 5: Supplementary Table S5.1.

Interleukin-6 and haemoglobinopathy screening were planned but not completed due to logistical and budgetary constraints. The Mentzer index (MCV/RBC) was calculated as a screening indicator of possible thalassaemia trait, recognising that this does not replace formal haemoglobin electrophoresis.[247]

5.2.4 Data Management and Confidentiality

All samples were labelled using unique study identification numbers, with no personal identifiers. Laboratory staff were blinded to trial arm allocation. Laboratory and trial datasets were linked using secure identifier matching, and all data were de-identified prior to analysis. All data were stored in encrypted, password-protected databases with access restricted to authorised personnel. Data management was conducted by The George Institute for Global Health, India, with data retained in India in accordance with local data governance requirements. In accordance with Indian law, all samples were analysed in India.

5.2.5 Definitions

5.2.5.1 Anaemia and Severity

Anaemia was defined as venous haemoglobin <120 g/L, with venous haemoglobin treated as the reference standard. Severity was classified according to WHO thresholds for non-pregnant women as mild (110–119 g/L), moderate (80–109 g/L), and severe (<80 g/L).[49]

5.2.5.2 Full Blood Count Characterisation

FBC indices were used descriptively to characterise red cell morphology. Anaemia was classified by MCV as microcytic (<80 fL), normocytic (80–100 fL), or macrocytic (>100 fL). Hypochromia was defined as MCH <27 pg, and anisocytosis as RDW-CV >14.5%. The Mentzer index (MCV/RBC) was calculated as a screening indicator, with values ≥13 suggestive of iron deficiency and <13 suggestive of thalassaemia trait.[247]

5.2.5.3 Iron Status

Iron deficiency was defined primarily as ferritin <30 µg/L, with the lower threshold of <15 µg/L examined in sensitivity analyses. sTfR (≥1.55 mg/L; ≥21 nmol/L for the Beckman Coulter Access assay) was used as a complementary marker of functional iron deficiency. Iron deficiency anaemia (IDA) was defined as ferritin <30 µg/L in the presence of venous-defined anaemia. The ferritin index [sTfR (mg/L) / log10 ferritin (µg/L)] was calculated as a composite marker of iron-restricted erythropoiesis, with a threshold of ≥1.03 based on manufacturer recommendations for the Beckman Coulter Access assay; as ferritin was measured on the Roche Elecsys platform, this threshold was interpreted as an approximate indicator.

5.2.5.4 Other Nutritional and Inflammatory Markers

Vitamin B12 status was categorised as deficient (<200 pg/mL), borderline (200–300 pg/mL), or adequate (>300 pg/mL).[248] Folate status was categorised as deficient (<2 ng/mL), borderline (2–4 ng/mL), or adequate (>4 ng/mL).[249] Anaemia was classified as multifactorial, where two or more nutritional deficiencies were present. Inflammation was defined as CRP >5 mg/L.[214] Participants with elevated CRP were retained in all analyses, and iron deficiency was defined by ferritin <30 µg/L irrespective of CRP status; the influence of inflammation on ferritin-based classification was examined separately in a sensitivity analysis (Section 5.2.7.6). Hepcidin was analysed as a continuous variable because clinically validated thresholds have not been established.

5.2.6 Obstetric and Delivery Outcome Data Collection

Obstetric and delivery outcomes were collected as part of the SHP2 trial through structured interviews administered by trained study staff at the 3- and 12-month postpartum follow-up visits, using standardised case report forms covering the period from delivery through the first postpartum year. Variables included mode of delivery (vaginal, instrumental, or caesarean section), birth weight, preterm delivery (<37 weeks), live birth status, perinatal loss (stillbirth or neonatal death), infant feeding practices, and timing of breastfeeding initiation. Maternal and neonatal hospitalisations, length of stay, and major maternal (e.g. pre-eclampsia, postpartum

haemorrhage, infection, thromboembolism) and neonatal complications (e.g. respiratory distress, hypoglycaemia, jaundice, special care admission) were also recorded. All obstetric and delivery outcomes were self-reported and were not independently verified against medical records.

5.2.7 Statistical Analysis

All analyses were conducted in RStudio (version 2025.09.2+418). Data cleaning included standardising variable names, assessing missingness, checking for implausible values, and harmonising units across datasets. Values reported with inequality symbols (e.g. "<100") were recorded to the nearest detectable value; ferritin values reported in ng/mL were treated as equivalent to µg/L; and CRP values were converted to mg/L where required. Missingness was assessed for all key variables before analysis. Several biomarkers were non-normally distributed and are therefore summarised as medians with interquartile ranges; skewed variables were log-transformed where required for regression analyses. Each analysis was restricted to participants with complete data for the variables it required, and denominators are reported explicitly throughout.

Analyses followed the four research questions. After descriptive summaries of the cohort, the intervention and control arms were compared; the prevalence and aetiology of postpartum anaemia were then characterised in the combined cohort, followed by an assessment of agreement between capillary and venous haemoglobin and between ferritin- and sTfR-based definitions of iron deficiency. Analyses of the combined cohort are presented by anaemia status where relevant.

5.2.7.1 Descriptive Analyses

Participant characteristics and biomarker values were summarised overall and by trial arm. Categorical variables are presented as counts and percentages, and continuous variables as medians with interquartile ranges, given the non-normal distribution of several biomarkers. Birth and delivery outcomes were summarised descriptively. Anaemia prevalence and severity at 12 months postpartum were calculated using venous haemoglobin. Nutritional deficiencies, elevated CRP, and hepcidin concentrations were summarised overall, by trial arm, and among women with

venous-defined anaemia. Baseline characteristics are presented descriptively by trial arm, without significance testing. As allocation was randomised, any baseline differences are assumed to have arisen by chance, while variables considered prognostically important were accounted for through pre-specified adjustment in the trial-arm models.

5.2.7.2 Trial Arm Comparisons

Differences between the intervention and control arms in anaemia prevalence and related haematological and biochemical outcomes were evaluated using chi-squared or Fisher's exact tests for categorical outcomes and Mann–Whitney U tests for continuous outcomes. Mixed-effects regression models were then used to examine associations between trial arm and anaemia-related outcomes at 12 months postpartum: binary outcomes (venous-defined anaemia and IDA, as defined in Section 5.2.5) using mixed-effects logistic regression, and continuous outcomes (venous haemoglobin) using linear mixed-effects models, with trial arm as a fixed effect.

All models were adjusted a priori for baseline point-of-care haemoglobin, maternal age, parity (nulliparous versus multiparous), body mass index (BMI), gestational age at enrolment, and delivery mode. These were selected for their established associations with maternal anaemia and iron status and included as prognostic factors to improve precision and account for chance imbalances arising from cluster randomisation across a limited number of PHCs. Continuous covariates were standardised as z-scores. For ferritin-based outcomes, log10-transformed CRP was additionally included, as inflammation influences ferritin concentrations. As randomisation in the parent trial occurred at the PHC level, all models included a random intercept for PHC to account for clustering at the level of intervention allocation. Models were fitted using the lme4 package, with results reported as adjusted odds ratios (aORs), mean differences, or geometric mean ratios with 95% confidence intervals. Convergence and distributional assumptions were checked, with no substantive violations identified.

5.2.7.3 Prevalence and Aetiology of Postpartum Anaemia

The prevalence and likely aetiology of postpartum anaemia were characterised primarily descriptively. Among women with venous-defined anaemia, the proportion attributable to each

contributing factor (iron deficiency, folate deficiency, vitamin B12 deficiency, and inflammation, as defined in Section 5.2.5) was calculated, and women with two or more nutritional deficiencies were classified as having multifactorial anaemia. The deficiency profile of anaemic women was compared with that of non-anaemic women to identify which deficiencies were most strongly associated with anaemia in this multifactorial setting, using chi-squared or Fisher's exact tests for categorical variables and Mann–Whitney U tests for continuous variables. As a sensitivity analysis, ferritin was adjusted for inflammation, as described in Section 5.2.7.6, and the resulting prevalence of iron deficiency was compared with the primary ferritin-based definition.

5.2.7.4 Agreement Between Capillary and Venous Haemoglobin

Agreement between capillary point-of-care and venous haemoglobin was assessed among participants with both measurements available. Continuous agreement was evaluated using Bland–Altman analysis, with mean bias and 95% limits of agreement (±1.96 SD of the mean difference); proportional bias was assessed by regressing the difference between measurements on their mean. Classification agreement at the anaemia threshold (Section 5.2.5) was evaluated using overall agreement, Cohen's kappa, sensitivity, and specificity, with venous haemoglobin as the reference standard.

5.2.7.5 Iron Biomarker Comparison and Agreement

Agreement between the iron deficiency definitions in Section 5.2.5 was evaluated by calculating prevalence for each biomarker individually and in combination, using marker-specific denominators where missingness varied across assays. Concordance between ferritin- and sTfR-based classifications was assessed using percentage agreement and Cohen's kappa, for the full cohort and stratified by inflammatory status (CRP ≤5 versus >5 mg/L) and anaemia status, to examine the influence of inflammation and anaemia on biomarker agreement. Associations between CRP and iron biomarkers were assessed using regression of log-transformed ferritin, sTfR, and hepcidin. Iron status among anaemic women was visualised using a scatter plot of ferritin against sTfR with clinical thresholds overlaid.

5.2.7.6 Inflammation Adjustment of Ferritin (BRINDA Sensitivity Analysis)

To assess the influence of inflammation on the ferritin-based classification of iron deficiency, ferritin levels were adjusted using a regression-based correction informed by the BRINDA approach.[214] Given AGP was unavailable, the adjustment was based on CRP alone. A cohort-specific regression coefficient was derived from the association between log-transformed ferritin and CRP, and ferritin was corrected to a reference CRP concentration of 0.5 mg/L. Adjusted ferritin values were then used in sensitivity analyses: the prevalence of iron deficiency based on adjusted ferritin was compared with the primary ferritin-based definition, and the number of participants reclassified from iron-replete to iron-deficient after adjustment was examined.

5.2.7.7 Missing Data

Missingness was assessed for all key variables before analysis. Analyses were restricted to participants with non-missing values for the variables required for each analysis, and denominators were explicitly reported throughout.

5.2.8 Sample Size and Power

The anaemia sub-study was originally designed to compare anaemia prevalence between the intervention and control arms of the SHP2 trial. A target of 900 women (approximately 450 per state) was calculated to provide 80% power to detect a 10-percentage-point absolute difference in anaemia prevalence between arms at a two-sided α of 0.05, allowing for 15% missing samples.

As described in Section 5.2.1, delays in ethics approval in Haryana restricted recruitment to Telangana, yielding a final sample of 600 participants, with venous haemoglobin available for 566. At the achieved sample size, the study had approximately 67% power to detect the pre-specified 10-percentage-point difference. No meaningful clustering of anaemia prevalence was observed at the PHC level (ICC ≈ 0), and adjustment for clustering did not materially alter this estimate. The study was therefore underpowered relative to its original design for detecting between-arm differences, and trial-arm comparisons were interpreted with caution. The

descriptive, biomarker, and agreement analyses, however, were pre-specified components of the study protocol and were independent of the between-arm comparison.

5.2.9 Ethical Considerations

This sub-study was conducted under ethics approvals granted to the parent SHP2 trial by the George Institute for Global Health India Institutional Ethics Committee (Project No. 22/2021) and the University of Oxford Tropical Research Ethics Committee (Ref 27–21). An amendment covering additional venous blood sampling was approved on 29 August 2024.

5.3 Results

5.3.1 Study Cohort and Data Completeness

A total of 600 women were included in the sub-study (control n = 335; intervention n = 265), recruited from 32 villages nested within 16 PHCs. Mean village size was 20.9 (SD 9.7) in control villages and 16.6 (SD 7.8) in intervention villages. Overall, missingness was low (<10% for all variables). Venous haemoglobin and FBC measurements were missing for 34 participants (5.7%), predominantly in the control arm (31/335, 9.3%) versus the intervention arm (3/265, 1.1%); missingness in biochemical markers was <5% for all markers, and CRP data were complete. The missing venous samples were attributable to sampling failures clustered in three control-arm villages and appeared to be related to field sampling procedures rather than participant characteristics.

Baseline characteristics are presented in Table 5.1. Median gestational age at enrolment was 15 weeks (IQR 13–18). Participants were young (mean age 24.5 years), universally married, and almost all reported a non-vegetarian diet (98.5%). Characteristics were broadly similar between arms, with two exceptions relevant to later analyses: women in the intervention arm were less likely to be multiparous (47.5% vs 60.6%) and had slightly higher baseline point-of-care haemoglobin (median 105 vs 101 g/L) than women in the control arm.

Table 5.1 Baseline characteristics of sub-study participants, overall and by trial arm

Characteristic	Overall N = 600	Control n = 335	Intervention n = 265
Age (years)			
Mean (SD)	24.5 (3.8)	24.5 (3.9)	24.6 (3.6)
Median (Q1, Q3)	24 (22, 27)	24 (22, 27)	24 (22, 27)
Anthropometry			
Height, cm — Mean (SD)	152.7 (4.6)	152.5 (4.9)	152.9 (4.2)
Weight, kg — Mean (SD)	49.7 (9.6)	49.1 (10.2)	50.4 (8.8)
BMI, kg/m² — Mean (SD)	21.3 (4.0)	21.1 (4.2)	21.5 (3.6)
BMI — Median (Q1, Q3)	20.8 (18.5, 23.3)	20.0 (18.2, 23.4)	21.1 (18.8, 23.3)
Sociodemographic			
Religion (Hindu)	570 (95.0%)	322 (96.1%)	248 (93.6%)
Married	600 (100%)	335 (100%)	265 (100%)
Diet (Non-vegetarian/Pescatarian)	591 (98.5%)	330 (98.5%)	261 (98.5%)
Education (Undergraduate or above)	362 (60.3%)	202 (60.3%)	160 (60.4%)
Education (Up to secondary school)	228 (38.0%)	128 (38.2%)	100 (37.7%)
Education (Illiterate)	10 (1.7%)	5 (1.5%)	5 (1.9%)
Lifestyle			
Smoking (Yes)	0 (0%)	0 (0%)	0 (0%)
Alcohol (Yes)	18 (3.0%)	18 (5.4%)	0 (0%)
Employment and income			
Self-employed (full or part time)	64 (10.7%)	42 (12.5%)	22 (8.3%)
Husband employed full time	542 (90.3%)	310 (92.5%)	232 (87.5%)
Household income, INR — Median (Q1, Q3)	15,000 (15,000, 20,000)	15,000 (14,000, 20,000)	15,000 (15,000, 20,000)
Household income N non-missing	507	245	262
Obstetric history			
Gestational age at enrolment, weeks — Median (Q1, Q3)	15 (13, 18)	15 (13, 18)	15 (14, 18)
Nulliparous	271 (45.2%)	132 (39.4%)	139 (52.5%)
Multiparous (≥1 previous pregnancy)	329 (54.8%)	203 (60.6%)	126 (47.5%)
Number of live births — Median (Q1, Q3)*	1 (1, 1)	1 (1, 1)	1 (1, 1)
High-risk condition in previous pregnancy†	225 / 329 (68.4%)	136 / 203 (67.0%)	89 / 126 (70.6%)
High-risk condition identified at baseline	103 (17.2%)	71 (21.2%)	32 (12.1%)
Clinical and medical history			
Past medical history‡	58 (9.7%)	38 (11.3%)	20 (7.5%)
Family history of CVD/diabetes/hypertension/stroke	187 (31.2%)	124 (37.0%)	63 (23.8%)
Taking regular medications	181 (30.2%)	175 (52.2%)	6 (2.3%)
Haemoglobin			
Haemoglobin (POC capillary), g/L — Median (Q1, Q3)	103.0 (94.0, 110.0)	101.0 (89.0, 110.0)	105.0 (98.0, 112.0)

*Among multiparous women only (n = 329). †Any high-risk condition recorded in obstetric history. ‡Past medical history was defined as self-reported history of any of: cardiac disease, hypertension, respiratory disease, gastrointestinal disease, diabetes, thyroid disease, polycystic ovarian syndrome, kidney disease, tuberculosis, malaria, HIV, syphilis, epilepsy, or cancer. BMI = body mass index; POC = point-of-care; CVD = cardiovascular disease.

5.3.2 Obstetric and Delivery Characteristics

Obstetric and delivery outcomes are summarised in Table 5.2. Most women were delivered by caesarean section (408/600, 68.1%), in similar proportions across arms. Median birth weight was 2.8 kg (IQR 2.5–3.0), and low birth weight (<2.5 kg) occurred in 45 infants (7.5%). Postpartum complications were uncommon (34/600, 5.7%), most often sepsis or infection (2.3%) and pre-eclampsia/eclampsia (1.3%); postpartum haemorrhage occurred in 6 women (1.0%) and transfusion in 2 (0.3%). These rates should be read with caution: outcomes were self-reported by participants and could not be verified against medical records; women delivered in both public and private facilities.

Table 5.2 Obstetric and delivery outcomes, by trial arm

Variable	Overall (N=600)	Control (n=335)	Intervention (n=264)
Mode of Delivery			
Normal vaginal	179 (29.9%)	99 (29.6%)	80 (30.3%)
Instrumental	12 (2.0%)	12 (3.6%)	0 (0%)
Caesarean section	408 (68.1%)	224 (66.9%)	184 (69.7%)
Neonatal Outcomes			
Birth Weight (Median [IQR], kg)	2.8 [2.5, 3.0]	2.7 [2.5, 3.0]	3.0 [2.6, 3.0]
Low birthweight (<2.5 kg)	45 (7.5%)	32 (9.6%)	13 (4.9%)
Perinatal loss	1 (0.2%)	0 (0%)	1 (0.4%)
Maternal Outcomes			
Any postpartum complication	34 (5.7%)	29 (8.7%)	5 (1.9%)
Sepsis/infection	14 (2.34%)	14 (4.2%)	0 (0%)
Preeclampsia/eclampsia	8 (1.34%)	8 (2.4%)	0 (0%)
PPH	6 (1.0%)	6 (1.8%)	0 (0%)
Blood transfusion	2 (0.33%)	2 (0.6%)	0 (0%)
Other complications	11 (1.84%)	7 (2.1%)	4 (1.5%)
DVT	1 (0.17%)	0 (0%)	1 (0.4%)
Vaginal/perineal infection	1 (0.17%)	1 (0.3%)	0 (0%)
Hysterectomy	1 (0.17%)	1 (0.3%)	0 (0%)

PPH = postpartum haemorrhage; DVT = deep vein thrombosis.

5.3.3 Comparison of Intervention and Control Arms

Biomarkers at 12 months postpartum are summarised by arm in Table 5.3. Anaemia prevalence was similar in the control and intervention arms (39.1% vs 40.1%; p = 0.821), median venous haemoglobin was identical in both groups (122.0 g/L), and no significant differences were observed across the principal iron, inflammatory, or nutritional biomarkers. Although baseline

differences were present between trial arms, biomarker distributions at 12 months postpartum were broadly similar.

Table 5.3 Haematological and biochemical biomarkers at 12 months postpartum, by trial arm

Variable	Control (n=335)	Intervention (n=265)	p-value
Anaemia and Haemoglobin			
Venous-defined anaemia (<120 g/L)	119/304 (39.1%)	105/262 (40.1%)	0.821
Venous Hb (g/L)	122.0 [115.0, 129.0]	122.0 [115.0, 129.0]	0.873
POC Hb (g/L)	103.0 [92.0, 112.0]	103.0 [95.2, 110.0]	0.482
Iron Status Biomarkers			
Ferritin (µg/L)	34.0 [19.1, 55.4]	31.8 [17.3, 51.4]	0.211
Ferritin <30 µg/L (deficiency)	139/334 (41.6%)	121/265 (45.7%)	0.337
sTfR (mg/L)	1.8 [1.4, 2.4]	1.8 [1.4, 2.4]	0.947
sTfR ≥1.55 mg/L (deficiency)	221/335 (66.0%)	175/265 (66.0%)	0.999
Ferritin Index	1.3 [0.9, 1.7]	1.2 [0.9, 1.8]	0.776
Ferritin Index ≥1.03 (deficiency)	210/333 (63.1%)	168/265 (63.4%)	0.950
Hepcidin (ng/mL)	3.9 [2.0, 5.7]	3.9 [1.9, 5.3]	0.738
Inflammation and Nutrition			
CRP (mg/L)	1.1 [0.4, 3.0]	1.1 [0.5, 3.0]	0.808
Elevated CRP (>5 mg/L)	43/335 (12.8%)	33/265 (12.5%)	0.903
Vitamin B12 (pg/mL)	334.5 [262.0, 451.8]	358.0 [261.0, 497.0]	0.149
Vitamin B12 deficiency	37/334 (11.1%)	23/265 (8.7%)	0.337
Folate (ng/mL)	2.5 [1.9, 3.8]	2.8 [2.0, 4.1]	0.071
Folate deficiency (<2 ng/mL)	101/334 (30.2%)	63/265 (23.8%)	0.086

Values are median [IQR] or n/N (%). Continuous variables were compared using Mann–Whitney U tests and categorical variables using chi-squared or Fisher's exact tests, as appropriate. IQR = interquartile range.

Adjusted mixed-effects models are shown in Table 5.4. There was no evidence of a difference between trial arms in the odds of venous-defined anaemia (aOR 1.47, 95% CI 0.97–2.22; p = 0.070) or venous haemoglobin concentration (mean difference −2.36 g/L, 95% CI −5.25 to 0.54). However, IDA (ferritin <30 µg/L in the presence of anaemia) was more common in the intervention arm after adjustment for prespecified covariates (aOR 1.94, 95% CI 1.19–3.16; p = 0.007). Lower baseline haemoglobin and multiparity were independently associated with higher odds of IDA in the adjusted model. Notably, women in the intervention arm had higher baseline haemoglobin concentrations and were less likely to be multiparous at enrolment. No clear association was observed between CRP and IDA after adjustment.

Table 5.4 Adjusted mixed-effects regression models for anaemia and iron deficiency anaemia at 12 months postpartum

Predictor	Venous Anaemia aOR (95% CI)	p-value	IDA aOR (95% CI)	p-value
Intervention vs Control	1.47 (0.97–2.22)	0.070	1.94 (1.19–3.16)	0.007
Baseline POC Hb (per SD)	0.63 (0.52–0.78)	<0.001	0.60 (0.47–0.75)	<0.001
Maternal age (per SD)	0.90 (0.74–1.10)	0.306	0.91 (0.73–1.15)	0.450
Multiparous vs nulliparous	1.36 (0.91–2.05)	0.135	1.77 (1.10–2.83)	0.019
BMI (per SD)	0.92 (0.75–1.13)	0.404	1.00 (0.77–1.29)	0.999
Gestational age (per SD)	1.05 (0.86–1.27)	0.638	1.16 (0.93–1.44)	0.194
Caesarean vs vaginal/instrumental birth	0.92 (0.62–1.39)	0.704	1.05 (0.66–1.68)	0.831
Log_{10}(CRP)	—	—	0.73 (0.47–1.15)	0.173

Models include a random intercept for primary health centre (PHC) to account for cluster randomisation, and were adjusted for baseline point-of-care haemoglobin, maternal age, parity, body mass index, gestational age at enrolment, and delivery mode. Continuous covariates were standardised as z-scores. The iron deficiency anaemia model additionally adjusted for $\log_{10}$(CRP). Results are adjusted odds ratios (aOR) with 95% confidence intervals. PHC = primary health centre; CRP = C-reactive protein; aOR = adjusted odds ratio.

As anaemia prevalence, haemoglobin concentrations, and most biomarker distributions were broadly similar between trial arms, subsequent analyses were conducted in the combined cohort and, where relevant, stratified by anaemia status.

5.3.4 Prevalence and Aetiology of Postpartum Anaemia

Using venous haemoglobin (<120 g/L), 224 of 566 women (39.6%) were anaemic at 12 months postpartum. Anaemia was predominantly mild (140/224, 62.5%) or moderate (83/224, 37.1%), with one woman classified as severe. By morphology, anaemia was most often normocytic (146/221, 66.1%), then macrocytic (45/221, 20.4%) and microcytic (30/221, 13.6%); hypochromia was present in 61.5%, and anisocytosis in 70.6% (Appendix 5: Supplementary Table S5.2), and the Mentzer index suggested possible thalassaemia trait in three women. Postpartum anaemia was therefore heterogeneous in morphology rather than the microcytic phenotype classically associated with iron deficiency.

Biomarkers by anaemia status are shown in Table 5.5. Iron status biomarkers differed markedly: ferritin and hepcidin were lower, and sTfR and the ferritin index higher, in anaemic than non-anaemic women (all $p < 0.001$). The red cell indices also differed (lower haematocrit, RBC, MCV, MCH, MCHC; higher RDW-CV; all $p < 0.001$). In contrast, vitamin B12 ($p = 0.334$), folate ($p =$

0.575), and CRP (p = 0.113) did not differ significantly between anaemic and non-anaemic women, nor did RDW-SD, platelets, or the Mentzer index.

Table 5.5 Haematological and biochemical biomarkers at 12 months postpartum, by anaemia status

Biomarker	Anaemic (n=224)	Non-anaemic (n=342)	p-value
Haemoglobin Measurements			
Venous Hb, g/L	112.0 [105.0, 117.0]	128.0 [124.0, 133.0]	<0.001
POC Hb, g/L	100.0 [89.0, 108.0]	106.0 [98.0, 113.0]	<0.001
Iron Status Biomarkers			
Ferritin, µg/L	21.7 [11.9, 39.8]	37.5 [25.9, 59.1]	<0.001
sTfR, mg/L	2.1 [1.6, 3.0]	1.7 [1.3, 2.2]	<0.001
Ferritin Index	1.6 [1.1, 2.6]	1.1 [0.8, 1.5]	<0.001
Hepcidin, ng/mL	2.9 [1.5, 5.0]	4.2 [2.8, 5.7]	<0.001
Inflammation and Nutrition Biomarkers			
CRP, mg/L	1.0 [0.4, 2.5]	1.2 [0.5, 3.2]	0.113
Vitamin B12, pg/mL	340.5 [244.8, 496.5]	347.0 [269.0, 468.0]	0.334
Folate, ng/mL	2.7 [2.0, 4.0]	2.6 [2.0, 4.0]	0.575
Full Blood Count Parameters			
Haematocrit, %	39.4 [36.9, 41.8]	44.4 [42.4, 46.5]	<0.001
RBC, $\times 10^6$/µL	4.3 [4.0, 4.6]	4.6 [4.4, 4.8]	<0.001
MCV, fL	92.0 [85.5, 98.6]	96.7 [91.8, 101.3]	<0.001
MCH, pg	25.8 [23.8, 28.0]	27.9 [26.8, 29.2]	<0.001
MCHC, g/dL	28.2 [27.0, 29.8]	28.9 [27.8, 30.2]	<0.001
RDW-CV, %	15.5 [14.3, 17.0]	14.5 [13.8, 15.5]	<0.001
RDW-SD, fL	50.0 [46.5, 55.8]	50.9 [47.2, 55.5]	0.314
WBC, $\times 10^3$/µL	7.0 [5.8, 8.7]	7.5 [6.4, 9.0]	0.016
Platelets, $\times 10^3$/µL	296.0 [251.0, 340.0]	307.0 [262.0, 348.0]	0.167
Mentzer index	21.4 [18.8, 23.9]	20.9 [19.3, 22.9]	0.317

Values are median [IQR]. P-values derived from Mann-Whitney U tests.

Nutritional deficiencies were common (Table 5.6). Iron deficiency was substantially more frequent among anaemic women, regardless of definition: by the primary ferritin threshold (<30 µg/L), 60.3% of anaemic versus 34.0% of non-anaemic women were iron deficient, with similar patterns using CRP-adjusted ferritin, sTfR, and the ferritin index. Vitamin B12 deficiency was also more common in anaemic women (14.3% vs 7.6%; p = 0.016), whereas folate deficiency and elevated CRP were similarly distributed. Overall, 99 of 599 women (16.5%) had two or more concurrent nutritional deficiencies, more common among anaemic women (22.8% vs 12.6%; p = 0.002). Postpartum anaemia in this cohort was thus frequently multifactorial, with iron deficiency the leading but not the sole contributor.

Table 5.6 Iron deficiency, nutritional deficiency, and inflammation classifications at 12 months postpartum, by anaemia status

Variable	Anaemic (n=224)	Non-anaemic (n=342)	p-value
Iron Deficiency			
Ferritin <15 µg/L	78/224 (34.8%)	35/341 (10.3%)	<0.001
Ferritin <30 µg/L	135/224 (60.3%)	116/341 (34.0%)	<0.001
Ferritin <30 µg/L (CRP-adjusted, BRINDA sensitivity analysis)	156/224 (69.6%)	149/341 (43.7%)	<0.001
sTfR ≥1.55 mg/L	171/224 (76.3%)	210/342 (61.4%)	<0.001
Ferritin Index ≥1.03	173/224 (77.2%)	191/341 (56.0%)	<0.001
Other			
Vitamin B12 Deficient (<200 pg/mL)	32/224 (14.3%)	26/341 (7.6%)	0.016
Folate Deficient (<2 ng/mL)	56/224 (25.0%)	94/341 (27.6%)	0.563
Multiple nutritional deficiencies*	51/224 (22.8%)	43/341 (12.6%)	0.002
Elevated CRP (>5 mg/L)	26/224 (11.6%)	45/342 (13.2%)	0.678

Multiple nutritional deficiency was defined as two or more concurrent deficiencies among ferritin <30 µg/L, vitamin B12 <200 pg/mL, and folate <2 ng/mL.

5.3.5 Agreement Between Capillary and Venous Haemoglobin

Among 564 women with paired measurements, capillary point-of-care haemoglobin was substantially lower than venous haemoglobin (mean bias −17.3 g/L; 95% limits of agreement −48.7 to 14.1 g/L; Figure 5.2), with proportional bias indicating that disagreement widened across the haemoglobin range ($\beta = 0.29$, $p < 0.001$). This translated into a large difference in classification: venous haemoglobin classified 39.6% of women as anaemic, whereas capillary testing classified 87.2%. Agreement at the <120 g/L threshold was poor (overall agreement 47.5%; sensitivity 93.3%, specificity 17.6%; Figure 5.3; Appendix 5: Supplementary Table S5.3), with 281 women (49.8%) false-positive and 15 (2.7%) false-negative by point-of-care testing. Capillary point-of-care haemoglobin, therefore, substantially overestimated the prevalence of anaemia and agreed poorly with venous classification in this population.

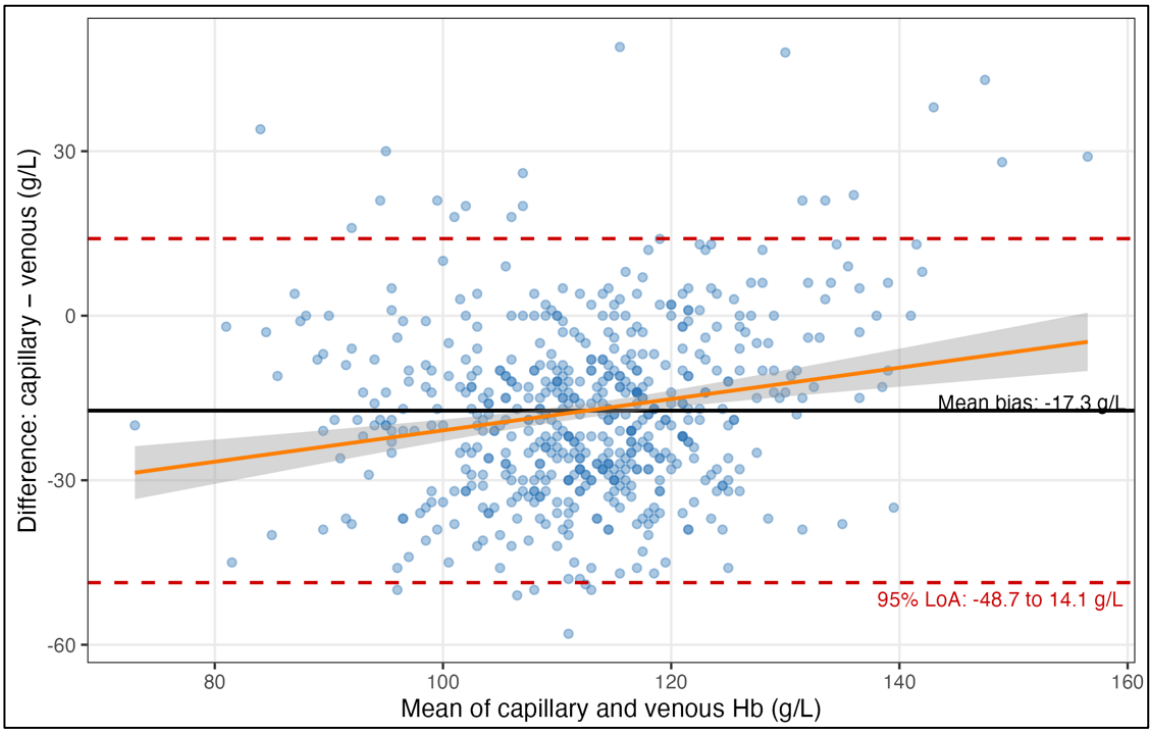


Figure 5.2 Bland–Altman plot comparing capillary point-of-care and venous haemoglobin
n = 564. The solid line indicates the mean bias and the dashed red lines the 95% limits of agreement (LoA); the orange line is the regression fit, indicating proportional bias. LoA = limits of agreement; Hb = haemoglobin.

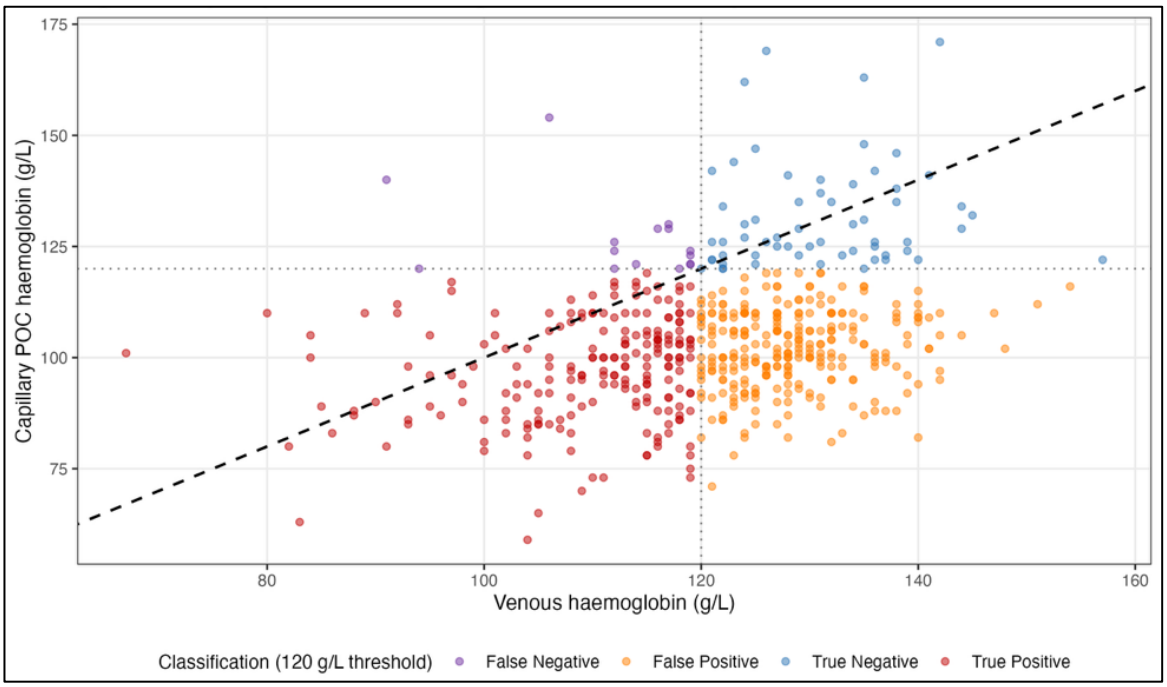


Figure 5.3 Agreement between capillary and venous haemoglobin classification
The dashed line indicates perfect agreement and the grey dotted line the 120 g/L anaemia threshold. Hb = haemoglobin.

5.3.6 Iron Deficiency Classification and Biomarker Agreement

Estimated iron deficiency prevalence varied markedly by definition and was consistently higher among anaemic women (Figure 5.4). At the primary ferritin threshold (<30 µg/L), 60.3% of

anaemic versus 34.0% of non-anaemic women were iron deficient; after BRINDA correction, this rose to 69.6% and 43.7%, respectively. sTfR and the ferritin index again yielded higher estimates (76.3% and 77.2% of anaemic women; 61.4% and 56.0% of non-anaemic women), and across all definitions, 88.4% of anaemic and 72.2% of non-anaemic women were classified as iron deficient. Biochemical evidence of iron deficiency was common even among non-anaemic women, with more than half classified as iron-deficient by sTfR or the ferritin index.

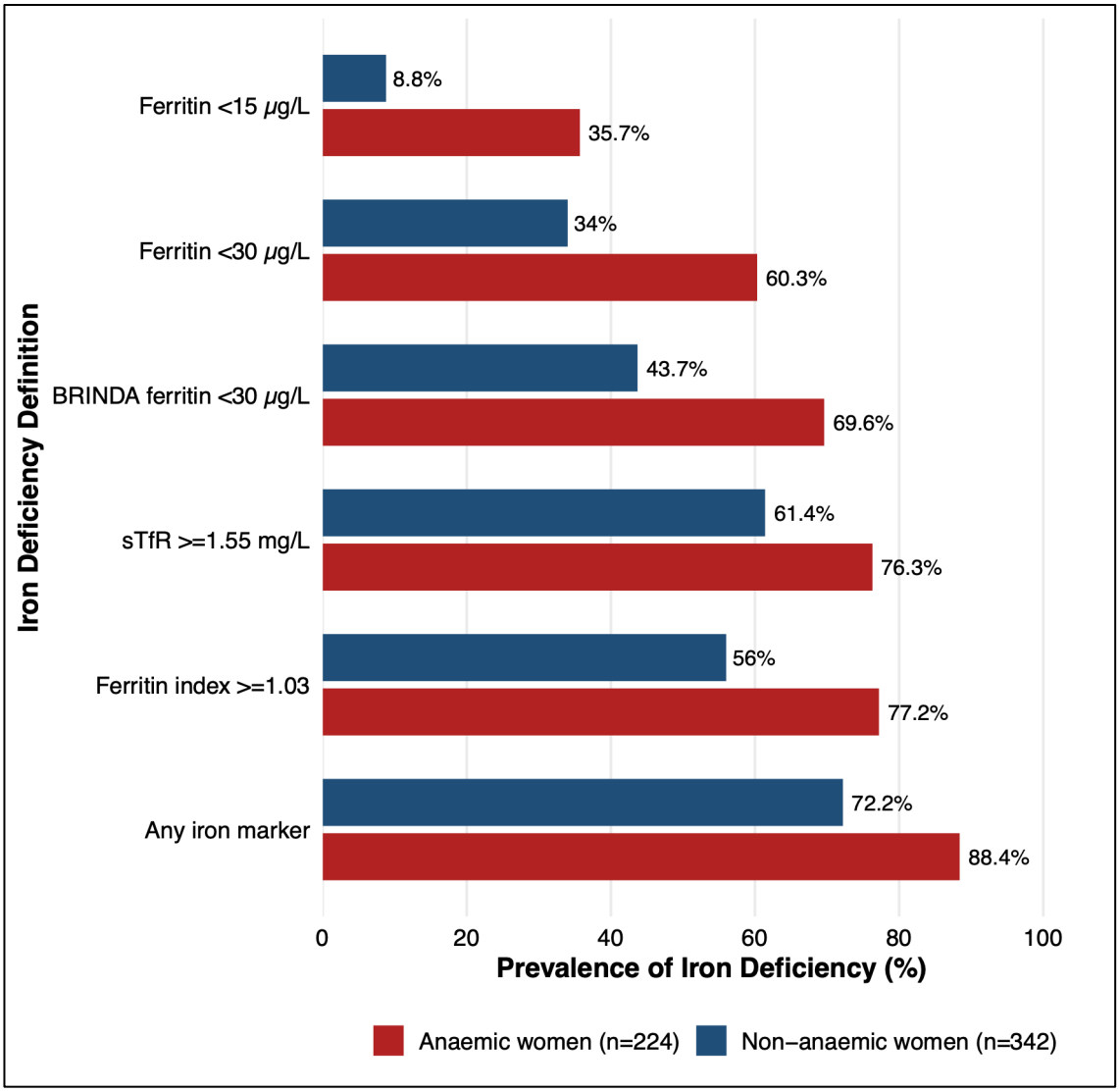


Figure 5.4 Iron deficiency prevalence, by anaemia status and biomarker definition
Prevalence of iron deficiency among women with and without venous-defined anaemia, stratified by biomarker definition (anaemic n = 224; non-anaemic n = 342). Biomarker-specific denominators vary slightly because of missing laboratory data. Each bar is an independent classification of the same women under a different biomarker definition; the categories are not mutually exclusive, and a woman may be classified as iron-deficient under more than one definition. BRINDA = Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia; sTfR = soluble transferrin receptor.

The proportion of anaemic women with at least one identifiable nutritional deficiency varied according to the iron deficiency definition applied: among the 224 anaemic women, 166 (74.1%) had at least one identifiable nutritional deficiency using ferritin <30 µg/L with B12 or folate deficiency, rising to 207 (92.4%) when any iron biomarker definition was used, reducing anaemia without an identifiable nutritional cause from 25.9% to 7.6%.

Agreement between definitions varied (Appendix 5: Supplementary Table S5.4): ferritin (<30 µg/L) versus sTfR (≥1.55 mg/L) was moderate (67.9%), driven mainly by women ferritin-replete but sTfR-deficient; ferritin versus the ferritin index was 73.2%; and sTfR versus the ferritin index was high (94.6%). Visualisation of ferritin against sTfR among anaemic women (Figure 5.5) showed considerable spread across thresholds, with many discordant cases, particularly normal ferritin with elevated sTfR. Ferritin- and sTfR-based approaches, therefore, did not identify the same women as iron-deficient.

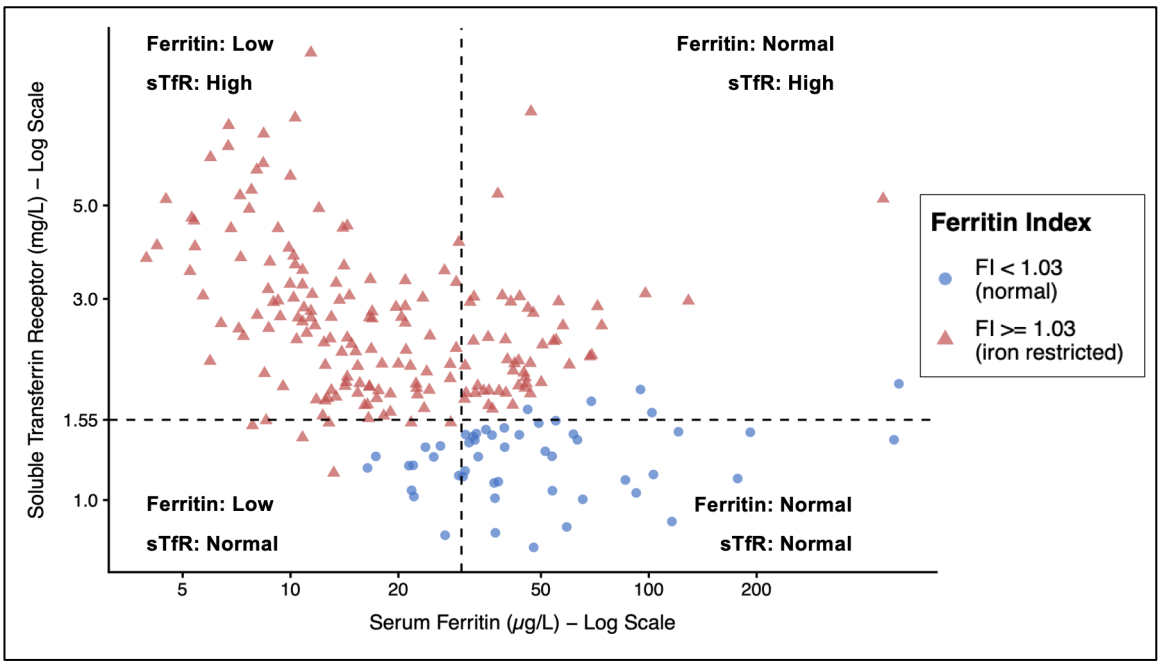


Figure 5.5 Iron status in anaemic women: ferritin versus soluble transferrin receptor
Relationship between serum ferritin and soluble transferrin receptor (sTfR) among women with venous-defined anaemia. Axes are log-transformed, with clinical thresholds indicated (ferritin 30 µg/L; sTfR 1.55 mg/L), and points are coloured by ferritin index classification. The distribution shows heterogeneity in iron status and strong concordance between sTfR and the ferritin index. sTfR = soluble transferrin receptor.

5.3.7 Influence of Inflammation on Iron Biomarker Interpretation

Inflammation was modest overall (76/600, 12.7% with CRP >5 mg/L) but influenced both biomarker agreement and ferritin-based estimates. Agreement between ferritin- and sTfR-based classification was lower among women with elevated CRP than without (50.0% vs 70.2%), and CRP was positively associated with ferritin and hepcidin but not sTfR (Figure 5.6; Appendix 5: Supplementary Table S5.5). BRINDA adjustment of ferritin increased iron deficiency prevalence from 43.4% to 52.8% overall and from 60.3% to 69.6% among anaemic women; 70 women were reclassified from replete to deficient, of whom 46 (65.7%) were also sTfR-deficient. Inflammation, therefore, contributed to discordance between definitions and materially affected ferritin-based estimates in this cohort.

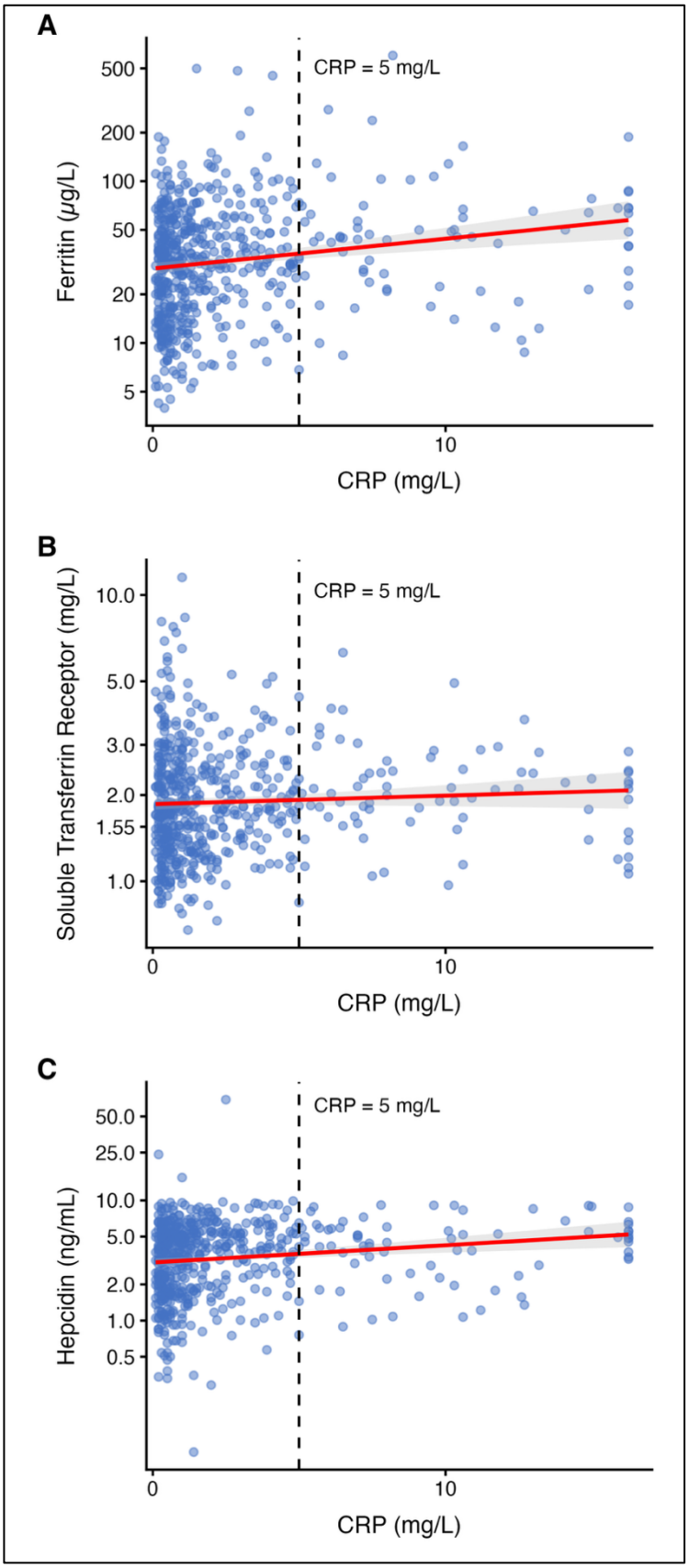


Figure 5.6 Associations between CRP and iron biomarkers
(a) CRP versus ferritin, (b) CRP versus sTfR, and (c) CRP versus hepcidin. The y-axis is $\log_{10}$ and regression was fitted on the log scale (n = 571; CRP truncated at the 98th percentile, 16.3 mg/L). CRP = C-reactive protein; sTfR = soluble transferrin receptor.

5.4 Discussion

5.4.1 Summary of Key Findings

In this nested sub-study within the SHP2 trial, the intervention was not associated with a lower prevalence of anaemia at 12 months postpartum in the Telangana cohort. Beyond the trial comparison, four key findings emerged. First, anaemia remained common at 12 months postpartum, affecting approximately two in five women, as measured by venous haemoglobin. Second, anaemia was frequently multifactorial, with iron deficiency often occurring alongside folate and vitamin B12 deficiency and, to a lesser extent, inflammation. Third, agreement between capillary point-of-care testing and venous haemoglobin was poor. Capillary testing demonstrated high sensitivity but very low specificity, resulting in substantial overestimation of the prevalence of anaemia. Finally, estimates of iron deficiency varied considerably according to the biomarker used. Ferritin, sTfR, and the ferritin index showed only modest agreement and frequently identified different women as iron-deficient, highlighting the challenges of interpreting iron-status biomarkers in this population.

5.4.2 Interpretation of Findings

Ultimately, these findings show that postpartum anaemia here was common, multifactorial, and strongly shaped by the methods used to assess both anaemia and iron deficiency. The following sections consider each in turn, in relation to the literature and to maternal health programmes in low- and middle-income settings.

5.4.2.1 Prevalence of Postpartum Anaemia

No clear difference in overall anaemia prevalence was observed between the intervention and control arms at 12 months postpartum. This finding may reflect the context in which the intervention was delivered. Rather than replacing routine antenatal care, the intervention was implemented within an existing health system where women in both arms continued to receive standard anaemia screening and management. Subsequent linkage to government antenatal records indicated that women frequently underwent venous haemoglobin testing and received

routine anaemia care throughout pregnancy, which may have reduced the potential for the intervention to produce an additional effect on postpartum haemoglobin outcomes.

Although adjusted analyses suggested higher odds of IDA in the intervention arm, this finding should be interpreted cautiously. The sub-study was underpowered for between-group comparisons and was not designed primarily to evaluate intervention effectiveness. Additionally, baseline differences in parity and haemoglobin between the groups may have contributed to the observed association. The absence of a corresponding difference in overall anaemia prevalence further suggests that this finding should not necessarily be interpreted as evidence of a detrimental effect of the intervention. Rather, it may reflect residual confounding, unmeasured differences between groups, or chance variation within a relatively small exploratory sub-study.

Regardless of allocation, anaemia remained common, affecting roughly two in five women at 12 months postpartum. This is consistent with evidence that the prevalence of anaemia in India has remained persistently high despite widespread antenatal iron–folic acid supplementation and substantial investment in maternal anaemia control.[219,250] For context, NFHS-5 estimated anaemia at 57.8% among non-pregnant women, 57.6% among all women aged 15–49, and 53.2% among pregnant women in Telangana,[251] although those estimates used capillary measurement and are not directly comparable. Persistence despite routine antenatal screening, iron–folic acid supplementation, and, for the intervention arm, additional SHP2 support suggests current approaches may be insufficient to address all contributors. Plausible factors include poor adherence, discontinuation after delivery, dietary inhibitors of iron absorption, the ongoing nutritional demands of the postpartum period, and broader social determinants.[252,253] Unfortunately, adherence data were not collected, so its contribution here cannot be quantified.

Consistent with a cumulative burden across pregnancies, both multiparity and lower baseline haemoglobin were associated with higher odds of anaemia and IDA, pointing to the value of considering maternal iron status across the reproductive life course rather than within single pregnancies.[15,220,254]

5.4.2.2 Multifactorial Anaemia and Nutritional Deficiencies

Iron deficiency appeared to be the principal contributor to postpartum anaemia in this cohort. It was the most prevalent micronutrient deficiency and the most common abnormality identified among anaemic women. Consistent with this, iron biomarkers (ferritin, sTfR, and the ferritin index) differed significantly between anaemic and non-anaemic women (all $p < 0.001$), whereas vitamin B12 and folate concentrations did not ($p = 0.334$ and $p = 0.575$, respectively). These findings suggest that iron status was more strongly associated with anaemia than other nutritional biomarkers measured in this study.

However, iron deficiency alone did not fully explain the burden of anaemia. It frequently coexisted with folate and vitamin B12 deficiencies, and a proportion of anaemia remained *'unexplained'* by the measured biomarkers. This pattern is consistent with growing evidence from India that postpartum anaemia often reflects multiple overlapping nutritional and non-nutritional factors rather than a single deficiency state.[239,241] Folate deficiency was common across both anaemic and non-anaemic women, suggesting broader nutritional vulnerability within the population. This finding aligns with systematic reviews and regional studies from Telangana that have reported overlapping micronutrient deficiencies among women of reproductive age.[240,241] Vitamin B12 deficiency was less prevalent in this cohort (~10%) than reported in some national estimates (~53%),[240] which may reflect the predominantly non-vegetarian diet of this study population.

The predominance of normocytic anaemia further supports a multifactorial aetiology. While iron deficiency is classically associated with microcytosis and folate or vitamin B12 deficiency with macrocytosis, the predominance of normocytic indices observed here suggests that many women may have had overlapping deficiencies, early iron deficiency, inflammation or other contributing factors that did not produce a characteristic red cell phenotype.[73,255] Inflammation, although a recognised contributor to anaemia through iron sequestration, appeared to play a secondary role relative to iron deficiency in this cohort.[78,256] Elevated CRP concentrations were observed in a substantial proportion of women, and inflammation clearly influenced the interpretation of some

biomarkers, particularly ferritin. However, hepcidin concentrations remained generally low, suggesting that iron deficiency rather than inflammation-driven iron restriction predominated. This is consistent with the increase in iron deficiency prevalence observed following BRINDA correction and the high prevalence of iron deficiency identified across multiple biomarkers.[256,257]

Other unmeasured causes may also have contributed to anaemia, including chronic infections such as helminthic disease and malaria,[252] additional micronutrient deficiencies such as copper deficiency,[258] and inherited haemoglobin disorders. The β-thalassaemia trait affects an estimated 3–5% of individuals in some regions of India.[238] Although only 3 of 221 anaemic women (1.4%) had a Mentzer index suggestive of thalassaemia, this index is a screening rather than a diagnostic tool and may perform less reliably in the presence of coexisting iron deficiency.[247] As confirmatory investigations such as haemoglobin electrophoresis were not performed, haemoglobinopathies could not be excluded and may have contributed to anaemia in a small proportion of women.

5.4.2.3 Point-of-Care Haemoglobin Misclassification

Agreement between capillary point-of-care and venous haemoglobin measurements was poor, and the choice of measurement method substantially altered estimates of anaemia prevalence. Anaemia affected 39.6% of women when assessed using venous haemoglobin, compared with 87.2% when assessed using capillary point-of-care testing. Although capillary testing demonstrated high sensitivity, its very low specificity resulted in substantial over-classification of anaemia, highlighting the extent to which prevalence estimates may be influenced by measurement approach in community settings.

The magnitude of disagreement observed in this study exceeds that reported in formal validation studies of the TrueHb device in India, which found sensitivities of 72–92% and specificities of 81–88%.[259,260] However, the findings are consistent with the wider literature demonstrating variable agreement between capillary and venous haemoglobin measurements across pregnant and non-pregnant populations.[233,261,262] This discrepancy may reflect the difference between

device performance under validation conditions and performance during routine implementation. Capillary sampling is inherently more susceptible to variation arising from peripheral perfusion, skin temperature, sampling technique, and tissue-fluid contamination.[237] In the present study, measurements were obtained across multiple rural communities by numerous ASHAs under routine programme conditions rather than standardised research settings. Although the interval between paired capillary and venous samples was not standardised, short-term physiological variation is unlikely to account for discrepancies of the magnitude observed.[263]

Despite poor agreement with venous haemoglobin, the high sensitivity of capillary testing may still support a role in community-based screening programmes. In settings where the priority is identifying women who may require further assessment, few cases of anaemia are likely to be missed. However, the low specificity observed in this study means that many women would be incorrectly classified as anaemic if capillary testing were used in isolation. The implications of this misclassification depend on the intended purpose of testing and the consequences of a positive result. In settings where a positive screen leads primarily to oral iron–folic acid supplementation, the harms associated with over-classification may be relatively limited. In contrast, where results inform more costly investigations or interventions, poor specificity may have greater consequences. This distinction is considered further in the implications section.

5.4.2.4 Iron Deficiency and Biomarker Choice

Estimates of iron deficiency varied substantially according to the biomarker used. Ferritin identified fewer women as iron deficient than either sTfR or the ferritin index, and agreement between biomarkers was limited. Ferritin primarily reflects iron stores, whereas sTfR reflects cellular iron demand and iron-restricted erythropoiesis.[56,80] As a result, these biomarkers capture related yet distinct aspects of iron metabolism and may identify different individuals as iron-deficient.

Agreement between ferritin and sTfR was poorer among women with elevated CRP concentrations, suggesting that inflammation may contribute to discordant classification. However, inflammation alone is unlikely to explain the observed differences. Although BRINDA

correction increased ferritin-based estimates of iron deficiency, sTfR continued to identify a substantially larger proportion of women as iron deficient. Raised sTfR is also not specific to iron deficiency, increasing with expanded erythropoiesis including haemoglobinopathies, haemolysis, and recovery from blood loss,[264] so its higher estimates warrant caution.

These differences affected not only estimates of iron deficiency prevalence but also the attribution of anaemia aetiology. Incorporating multiple biomarkers reduced the proportion of anaemia classified as unexplained from approximately one-quarter of cases when ferritin alone was considered to less than 10%. Iron deficiency was also common among women without anaemia, although prevalence estimates varied considerably according to the biomarker used. These findings demonstrate that conclusions regarding both the burden of iron deficiency and its contribution to anaemia depend, in part, on the biomarkers and diagnostic thresholds applied.

Iron deficiency could not be confirmed against an independent reference standard in the present study, making it impossible to determine which biomarker most accurately reflected iron status in this population. Nor can it be determined whether the additional women identified by sTfR or the ferritin index differ meaningfully in terms of symptoms, outcomes, or response to treatment. Consequently, although biomarker choice clearly influences estimates of iron deficiency and anaemia aetiology, whether one classification approach is more clinically informative than another cannot be determined from the present study.[265]

Finally, any potential advantages of sTfR or the ferritin index must be considered alongside practical constraints. These assays are more expensive, require laboratory infrastructure, are less accessible in lower-resource settings, and are complicated by assay variability and platform-specific thresholds.[266,267] Although these biomarkers produced different estimates of iron deficiency and anaemia aetiology, their role in routine clinical care remains uncertain and must be balanced against cost, laboratory capacity, and feasibility within community-based programmes.

5.4.3 Strengths and Limitations

The study's strengths include prospective nesting within a large cluster-randomised trial in a real-world community setting, providing evidence on anaemia detection using point-of-care devices under routine conditions; the availability of paired capillary and venous measurements, rarely assessed in large population-based studies; and a comprehensive biomarker panel (ferritin, sTfR, vitamin B12, folate, CRP) enabling characterisation of anaemia beyond haemoglobin alone, uncommon this far into the postpartum period.

Several limitations apply. The single-state setting (Telangana) limits generalisability to regions with different diets, infection burden, and healthcare access. Haemoglobinopathy screening was not performed, so inherited disorders could not be assessed directly. Inflammation was assessed using CRP alone, without AGP, which limited the ability to fully account for its effects on ferritin concentrations.[76] Baseline haemoglobin was measured by point-of-care rather than venous testing; adherence data were unavailable, limiting interpretation of treatment exposure; biomarkers were measured at a single time point, precluding longitudinal assessment; and IV iron use data were unavailable, which may affect interpretation of between-arm differences. Missing venous measurements were clustered within a small number of villages rather than occurring completely at random, representing a potential source of bias despite the low overall proportion of missing data. Obstetric outcomes were self-reported and not verified against clinical records, introducing the possibility of under-ascertainment or misclassification. In addition, although multiple iron biomarkers were assessed, no independent reference standard for iron deficiency was available, meaning the study could not determine which biomarker classification most accurately reflected iron status. Finally, the study was not powered to detect between-arm differences in anaemia or IDA, and these exploratory comparisons should therefore be interpreted with caution.

5.4.4 Clinical, Research and Policy Implications

These findings have implications for maternal healthcare, surveillance, policy, and research. First, persistent anaemia and nutritional deficiency at 12 months postpartum may affect maternal wellbeing, including fatigue, physical functioning, cognition, and quality of life.[4,35] In addition,

the later postpartum period often overlaps with the preconception interval for a subsequent pregnancy. Women who remain iron deficient or anaemic at 12 months postpartum may therefore enter a future pregnancy with depleted iron stores and reduced physiological reserve.[14,15,17] These findings support approaches to maternal nutrition and anaemia prevention that extend across the reproductive life course rather than focusing solely on individual pregnancies. They also highlight the potential value of closer follow-up for women at higher risk of persistent anaemia, including those with lower baseline haemoglobin concentrations and multiparity.

Second, the measurement findings have implications for anaemia surveillance. National surveys such as NFHS rely on capillary haemoglobin measurement, whereas many research studies and clinical services use venous haemoglobin. Differences in measurement methodology may therefore contribute to variation in reported anaemia prevalence across settings.[250,268] Although this study was not designed as a formal validation study, the poor agreement observed between capillary and venous haemoglobin under routine implementation conditions highlights the importance of measurement methodology when interpreting anaemia burden and evaluating programme impact. Concerns regarding capillary haemoglobin measurement have already had national consequences in India, with haemoglobin testing omitted from NFHS-6 and population estimates redirected towards venous-based surveys.[269] These findings do not support abandoning point-of-care testing, which remains essential for scalable community screening. Rather, they support a clearer distinction between screening and diagnosis, with point-of-care testing used to identify women requiring further assessment or low-risk treatment, and venous confirmation reserved for situations where greater diagnostic certainty is required, particularly before costlier or higher-risk interventions such as intravenous iron or transfusion outside the setting of acute bleeding.

Third, the findings raise questions about how anaemia interventions are designed and targeted. SHP2, like many maternal anaemia programmes, centred on haemoglobin-based screening and iron–folic acid supplementation. Iron deficiency remained common, underscoring the continued importance of iron–folic acid supplementation, but coexisting nutritional deficiencies and

unexplained anaemia indicate that it does not account for the entire burden. Equally, the presence of multiple deficiencies does not necessarily imply that broader supplementation strategies will reduce maternal anaemia. Although multiple micronutrient supplementation improves several birth outcomes, its effect on maternal anaemia appears limited, with recent evidence from India reaching similar conclusions.[97,244,270,271] For settings such as Telangana, the most immediate implication may therefore be improved identification and follow-up of women with persistent anaemia, alongside consideration of alternative nutritional deficiencies and further investigation where anaemia persists despite supplementation.

Finally, estimates of both iron deficiency and anaemia aetiology depended on the diagnostic approach used. Haemoglobin measurement method and biomarker selection each influenced classification, while inflammation further complicated biomarker interpretation. Yet the clinical significance of these differences remains uncertain. Without an independent reference standard for iron deficiency, it is not possible to determine which biomarker most accurately reflects iron status, nor whether one classification approach is more clinically informative than another. The strengths, limitations, and potential roles of the measurement approaches examined in this study are summarised in Table 5.7. Overall, these findings highlight the need for diagnostic strategies that balance accuracy, feasibility, and affordability, while improving understanding of why postpartum iron deficiency and anaemia persist in resource-constrained settings.

Table 5.7 Influence of different haemoglobin and iron biomarkers on the classification of anaemia and iron deficiency in maternal health

Test	Strengths	Limitations	Potential role
Capillary POC Hb	High sensitivity; scalable	Over-classifies anaemia	Screening
Venous Hb	Diagnostic reference	Infrastructure required	Confirmation and treatment decisions
Ferritin	Reflects iron stores	Affected by inflammation	First-line assessment of iron stores
sTfR	Reflects iron-restricted erythropoiesis	Non-specific; costly	Additional assessment where ferritin interpretation is uncertain
Ferritin index	Captures stores + demand	Complex; threshold dependent	Research and composite assessment

5.5 Conclusion

Postpartum anaemia, in this cohort in rural India, was common, multifactorial, and strongly influenced by the methods used to assess it. Approximately two in five women remained anaemic at 12 months postpartum. Iron deficiency was the predominant nutritional deficiency identified, but frequently coexisted with folate deficiency, vitamin B12 deficiency, and, to a lesser extent, inflammation, highlighting the overlapping contributors to anaemia in this population.

A key finding of this chapter is that estimates of both iron deficiency and anaemia aetiology depended substantially on the diagnostic approach used. Capillary point-of-care testing demonstrated poor agreement with venous haemoglobin and substantially overestimated anaemia prevalence, while ferritin, sTfR, and the ferritin index identified different women as iron deficient. These findings demonstrate that anaemia classification is influenced not only by underlying biology but also by measurement approach, biomarker selection, and healthcare context.

By examining anaemia and iron deficiency in a community-based postpartum population in a lower-resource setting, this chapter extends the findings of the preceding chapters beyond pregnancy and into the postpartum period. The findings further support the broader thesis argument that biomarkers commonly used to assess iron status do not provide equivalent information and may classify the same woman differently depending on the physiological, clinical, and measurement context in which they are applied. Chapter 6 draws together these findings with the wider evidence presented throughout the thesis and considers their implications for maternal anaemia research, clinical practice, and public health policy.

Chapter 6 - **Discussion and Conclusions:** The Limits of Iron Status Biomarkers in Maternal Health Without Context

Statement of Contribution

This chapter is my own work and has not been published elsewhere.

6.1 Chapter Overview

This thesis examined how iron status biomarkers are used and interpreted in the assessment and monitoring of iron deficiency and anaemia in maternal health. Through four complementary studies, it explored variation in clinical recommendations, approaches to assessing treatment response, biomarker behaviour across pregnancy, and the classification of iron deficiency and anaemia in a postpartum population.

Although each study addressed a distinct research question, common themes emerged. Across clinical guidelines, research studies, pregnancy cohorts, and postpartum populations, the interpretation of iron status biomarkers was shaped not only by the biomarkers themselves but also by the circumstances in which they were measured and applied.

Table 6.1 summarises the objectives, study designs, settings, and principal findings of each empirical chapter. **Error! Reference source not found.** presents the conceptual framework that emerged from the synthesis of these findings and forms the basis of the discussion that follows.

The remainder of this chapter integrates the findings of the four empirical studies, considers the strengths and limitations of the thesis, and discusses their implications for clinical practice, policy, and future research, before outlining the thesis's overall contribution.

Table 6.1 Summary of thesis objectives, study designs, settings, and principal findings

Objective	Study and methodology	Setting	Principal finding
Objective 1: To characterise variation in recommendations for the assessment, treatment and monitoring of iron deficiency and anaemia across UK maternity guidelines.	Cross-sectional analysis of local NHS maternity guidelines (Chapter 2)	UK NHS maternity services	UK maternity guidelines showed substantial variation in screening, diagnosis, treatment and monitoring, greatest in areas where the evidence base remains limited or uncertain.
Objective 2: To examine how response to oral iron therapy during pregnancy has been assessed in clinical research.	Systematic review of trials of oral iron therapy in pregnancy (Chapter 3)	International research literature	Response was assessed using heterogeneous biomarkers, reassessment intervals, and definitions of response, with limited standardisation across studies.
Objective 3: To evaluate the effect of different prophylactic oral iron dosing regimens on key iron biomarkers during pregnancy.	Sub-analysis of a randomised controlled trial (PANDA; Chapter 4)	UK antenatal cohort	Biomarker trajectories were strongly influenced by physiological adaptation during pregnancy, with only modest differences between supplementation regimens.
Objective 4: To investigate the prevalence and aetiology of postpartum anaemia and evaluate the influence of biomarker selection and measurement approach on classification of anaemia and iron deficiency.	Sub-study nested within a cluster-randomised trial (SMARThealth Pregnancy 2; Chapter 5)	Rural India, postpartum population	Postpartum anaemia was common and multifactorial, while biomarker selection and measurement approach substantially influenced classification of iron deficiency and anaemia.

PANDA = Primary prevention of maternal Anaemia to avoid preterm Delivery and other Adverse outcomes; NHS = National Health Service.

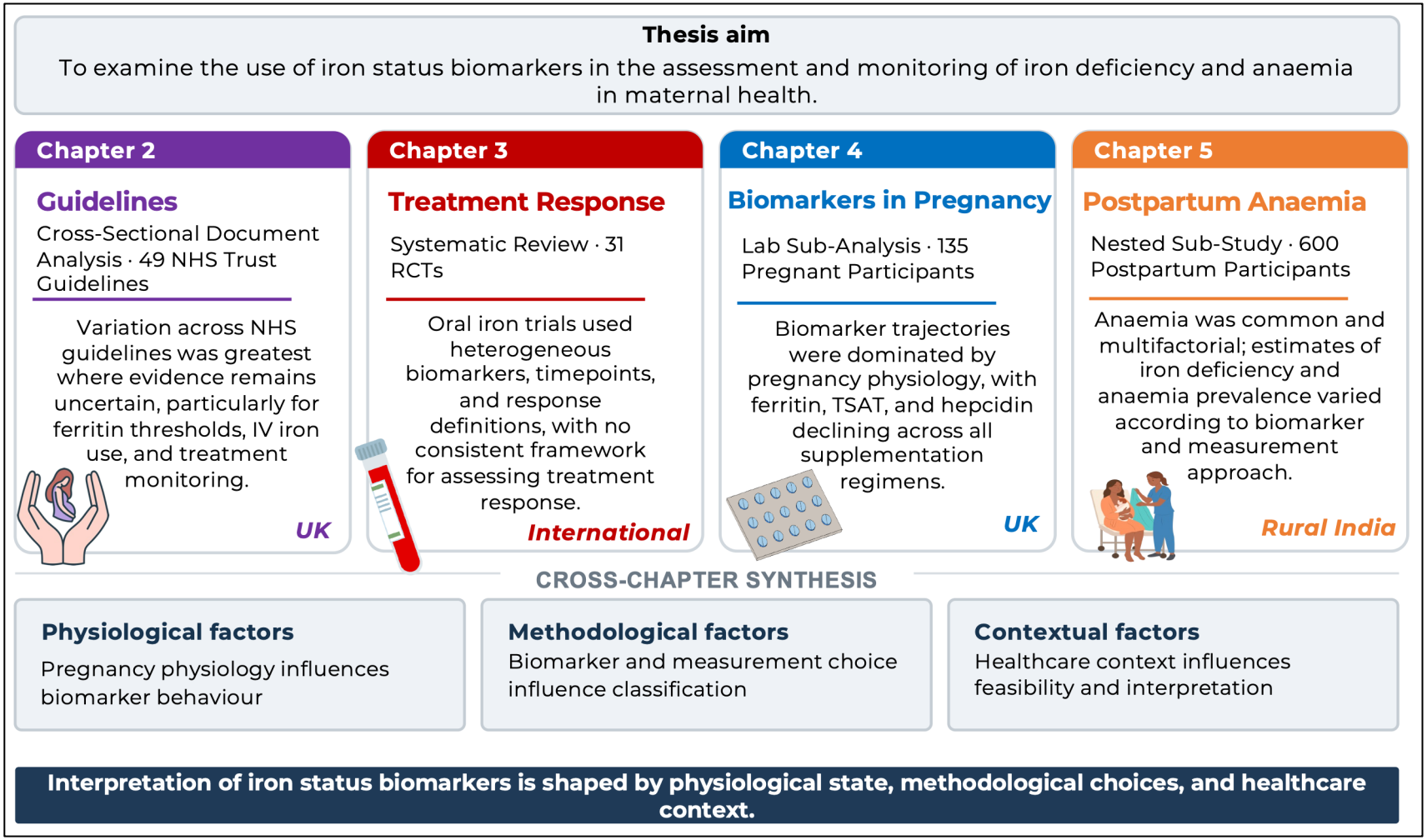


Figure 6.1 Cross-chapter synthesis of iron biomarker interpretation in maternal health
The thesis aim, the four empirical studies and their principal findings, and the three cross-cutting influences on biomarker interpretation: physiological state, methodological choices, and healthcare context.

6.2 Synthesis of Findings

The four empirical studies consistently showed that the interpretation of iron status biomarkers depended not only on the biomarkers themselves, but also on physiological state, methodological approach, and the healthcare context in which they were measured. Although explored across different populations and study designs, these three influences recur throughout the thesis and help explain the challenges of assessing iron deficiency and anaemia in maternal health. The following sections examine each study objective before drawing these observations together in the final synthesis.

6.2.1 Variation in Clinical Recommendations for Assessment and Monitoring

Chapter 2 demonstrated substantial variation in recommendations for the assessment, treatment, and monitoring of iron deficiency and anaemia across UK maternity guidelines. However, the most important observation was not simply that variation existed, but where it occurred. Recommendations regarding routine haemoglobin screening and diagnostic haemoglobin thresholds were relatively consistent and broadly aligned with the BSH guidance. In contrast, considerably greater variation was observed in ferritin screening, ferritin thresholds, monitoring strategies, definitions of treatment response, and criteria for escalation to IV iron.

This pattern is informative because it suggests that variation was not distributed randomly across clinical practice. Rather, it was concentrated in areas where uncertainty remains regarding how iron deficiency should be defined, monitored, and interpreted during pregnancy. Ferritin thresholds ranged from <15 to <50 µg/L; many guidelines specified no ferritin threshold; reassessment intervals varied substantially; and definitions of adequate response were frequently absent. Although ferritin was increasingly incorporated into antenatal screening, reflecting recognition that haemoglobin is a relatively late marker of iron deficiency,[10,19] this had not consistently translated into monitoring recommendations, with haemoglobin often remaining the sole biomarker used to assess treatment response.

Some degree of variation is expected and may reflect adaptation to local populations, service configuration, and resource availability.[106] However, the findings suggest that differences between

guidelines may reflect uncertainty within the underlying evidence base itself. This observation became an important driver of the subsequent chapters. If clinicians and guideline developers differ in how iron biomarkers should be used and interpreted, an important question is whether greater consensus exists within the research literature from which these recommendations are derived. Chapter 3 addressed this question directly by examining how response to oral iron therapy has been assessed in pregnancy research.

6.2.2 Variation in Research Approaches to Treatment Response

Chapter 3 identified substantial heterogeneity in how the response to oral iron therapy has been assessed in pregnancy research. Although haemoglobin was universally measured, it was rarely used in isolation, and considerable variation existed in the biomarkers used to evaluate treatment response, the timing of reassessment, and the criteria used to define adequate response.

The observed variation appeared to reflect both healthcare context and differing approaches to conceptualising treatment response. Studies conducted in higher-income settings were more likely to incorporate biomarkers of iron stores and iron regulation, such as ferritin and TSAT, whereas studies from lower-resource settings relied more heavily on haemoglobin and red cell indices. Resource availability, laboratory infrastructure, and assay costs likely contributed to these differences.[7,192,193] However, biomarker selection may also reflect differing priorities in treatment assessment, with some studies focusing on correction of anaemia, others on restoration of iron stores, and others on iron supply for erythropoiesis. Studies evaluating similar interventions were therefore not always assessing the same physiological outcome despite ostensibly addressing the same research question. Women receiving similar treatment may consequently have been classified differently depending on which biomarkers were measured and how the response was defined. At present, there remains limited evidence regarding which of these approaches is most clinically meaningful or most appropriate for assessing treatment response during pregnancy.

A further observation was that assessment was typically structured around fixed intervals following treatment initiation rather than the gestational stage. This approach reflects recognition that different biomarkers respond to treatment over different timeframes, with some studies

focusing on early erythropoietic responses and others on later changes in haemoglobin or iron stores. However, far less consideration was given to the influence of gestational physiology on biomarker interpretation. Biomarker values measured at the same interval after treatment initiation may therefore reflect not only treatment response but also differences in gestational timing and the physiological adaptations occurring across pregnancy. As a result, studies may have attributed changes in biomarker values to treatment effects without fully accounting for the contribution of pregnancy-related physiological change.

These findings provide important context for the variation observed in Chapter 2. The heterogeneity identified across UK maternity guidelines may reflect not simply differences in local implementation, but a similar lack of consensus within the underlying evidence base. Although biomarkers beyond haemoglobin are increasingly incorporated into both research and clinical practice, there remains limited agreement on which biomarkers to measure, when to assess them, and how to interpret their results during pregnancy. More broadly, the findings of Chapters 2 and 3 therefore suggest that uncertainty may arise not only from variation in clinical and research practice, but also from an incomplete understanding of how iron biomarkers behave during pregnancy and in response to oral iron supplementation. Chapter 4 explored this question through longitudinal assessment of iron biomarker trajectories across pregnancy.

6.2.3 Biomarker Behaviour Across Pregnancy

Chapter 4 examined the behaviour of key iron biomarkers under different prophylactic oral iron supplementation regimens during pregnancy. Although the original objective was to compare biomarker trajectories across daily, alternate-day, and three-times-weekly dosing schedules, the findings ultimately highlighted a broader challenge in interpreting iron biomarkers across pregnancy. While haemoglobin is interpreted using trimester-specific thresholds to account for haemodilution, most other iron biomarkers continue to be interpreted using fixed cut-offs despite substantial physiological changes in iron metabolism across gestation.[12,84–88] As a result, the relationship between a biomarker value and the underlying biological state it is intended to

represent may shift throughout pregnancy, raising questions about the validity of applying static thresholds within a dynamic physiological state.

This challenge was illustrated directly by the longitudinal PANDA data. Ferritin and TSAT declined across all supplementation regimens, hepcidin became markedly suppressed, and both sTfR and the ferritin index increased over time. When conventional ferritin thresholds were applied, the majority of participants were classified as iron-deficient by late pregnancy despite low rates of incident anaemia. Similar patterns have been reported elsewhere, where declining ferritin concentrations across gestation frequently coexist with relatively stable haemoglobin concentrations and low rates of clinically overt anaemia.[12,67,89] The observed pattern may reflect physiological redistribution of iron to support increasing gestational demands, clinically meaningful deficiency despite supplementation, or a combination of both. This sub-analysis and the current, evolving evidence base do not allow these possible causes to be clearly distinguished.

Several approaches have been proposed to address this uncertainty, including developing trimester-specific ferritin thresholds that may better reflect gestational physiology.[69] However, these approaches remain incompletely validated. Similarly, expanding the biomarker panel within the PANDA cohort did not resolve the uncertainty. Ferritin suggested declining iron stores, hepcidin reflected suppression of iron regulation, and the ferritin index indicated increasing iron-restricted erythropoiesis. Yet considered together, these markers still did not provide a clear means of distinguishing physiological adaptation from pathological deficiency. Consequently, the clinical significance of biomarker-defined iron deficiency during pregnancy remains unclear. If low ferritin concentrations are common despite supplementation and preserved haemoglobin levels, it is uncertain whether current thresholds identify women who would benefit from additional intervention or, at least in part, capture expected physiological adaptation.

Although expanding the biomarker panel did not resolve this uncertainty, it did provide additional insights into treatment response. Using haemoglobin alone, the three supplementation regimens appeared broadly equivalent. Alternative biomarkers suggested a more differentiated pattern, with sTfR and the ferritin index indicating relatively better preservation of erythropoietic iron supply

in the daily dosing arm. This observation highlights a broader challenge in interpreting iron biomarkers. Different biomarkers may not simply provide more information; they may provide different information. Consequently, the apparent effectiveness of an intervention may depend, at least in part, on the biomarker used to assess it. The implication is not that any one biomarker is necessarily superior, but that different biomarkers may yield different conclusions regarding treatment response because they capture distinct aspects of iron physiology.[74] Determining which of these outcomes is most clinically meaningful remains challenging, particularly given the known limitations of biomarkers such as sTfR and its absence of validated gestation-specific reference ranges.[58,93]

These findings help explain the variation observed in both Chapters 2 and 3. If currently available biomarkers cannot reliably distinguish physiological adaptation from clinically meaningful deficiency during pregnancy, variation in diagnostic thresholds, monitoring recommendations, and definitions of treatment response is expected as the evidence base evolves. The question then becomes whether this uncertainty is primarily a consequence of pregnancy physiology or whether similar difficulties persist in other maternal health settings. Chapter 5 examines this question in a contrasting postpartum population.

6.2.4 The Influence of Biomarker Selection and Measurement Approach

Chapter 5 demonstrated that uncertainty in iron status assessment is not confined to the physiology of pregnancy. Although the challenges identified in Chapter 4 arose largely from the interpretation of biomarkers within a dynamic physiological state, the postpartum findings showed that uncertainty persisted outside of pregnancy. In this setting, uncertainty arose less from gestational physiology and more from the biomarkers and measurement approaches used to define iron deficiency and anaemia.

A central finding of the SHP2 sub-study was that different biomarkers did not identify the same women as iron-deficient. Iron deficiency prevalence ranged from 43.4% with ferritin to 66.0% with sTfR and 63.2% with the ferritin index, a difference of more than 20 percentage points depending solely on the biomarker used. Agreement between ferritin- and sTfR-based

classifications was only moderate and decreased further in the presence of inflammation. These differences have practical consequences. A clinical service, surveillance programme, or research study using one biomarker will characterise the burden of iron deficiency differently from one using another. Biomarker selection is therefore not simply a methodological decision but one that determines which aspect of iron metabolism is prioritised and, consequently, how iron deficiency is characterised.

As observed in Chapter 4, incorporating additional biomarkers increased the amount of information available but did not necessarily eliminate interpretive uncertainty. In the SHP2 cohort, multi-biomarker assessment substantially reduced the proportion of anaemia classified as '*unexplained*' by nutritional causes, but at the cost of increased laboratory complexity and the need to interpret biomarkers that themselves remain imperfectly understood. Although sTfR and the ferritin index may provide additional information regarding iron-restricted erythropoiesis, particularly in the context of inflammation, uncertainty regarding assay standardisation and the absence of validated reference ranges continue to limit the routine clinical application of sTfR.[58,119]

The measurement approach influenced classification as much as biomarker selection did. In the same cohort, capillary point-of-care haemoglobin measurements systematically underestimated venous haemoglobin, resulting in substantial overestimation of the prevalence of anaemia. The difference arose not from any change in underlying iron status but from the method of measurement alone, which was dictated by the setting's available resources.

Additionally, biomarkers are inevitably influenced by inflammatory burden, comorbidity, and broader population characteristics, all of which can alter the relationship between measured values and underlying iron status.[10,54,58] Inflammation influenced biomarker interpretation within the SHP2 cohort. Agreement between ferritin- and sTfR-based classifications was lower among women with elevated CRP, and adjusting ferritin for inflammation increased the estimated prevalence of iron deficiency, suggesting that unadjusted ferritin may have underestimated deficiency in this setting. These findings highlight that biomarker interpretation may be

influenced by the inflammatory burden in the setting in which it is applied. This is one reason sTfR may offer advantages in populations with higher inflammatory burden, as it is less influenced by inflammation than ferritin, although it is itself affected by erythropoietic activity and can therefore reflect more than iron status along.[80,238,264]

Finally, interpretation was also shaped by the underlying aetiology of anaemia. Iron, folate, and vitamin B12 deficiencies frequently coexisted in the SHP2 cohort, meaning that iron biomarkers captured only one component of the underlying pathology. In such settings, an iron-centred framework provides only a partial explanation for anaemia, reinforcing the importance of considering multiple nutritional and physiological contributors when interpreting biomarker results.

The findings of Chapter 5 also suggest that iron deficiency in maternal health may be better understood as a dynamic process across the reproductive life course than as an isolated condition confined to pregnancy. Although Chapter 5 examined women at a single postpartum timepoint, biomarker values are likely to reflect the cumulative effects of pregnancy, childbirth, postpartum recovery, lactation, and, for some women, subsequent conception.[14,15,17] Biomarker interpretation should therefore consider not only current physiological status but also preceding reproductive events, extending the observations from Chapter 4 beyond pregnancy into the postpartum period.

Beyond extending the physiological observations of the preceding chapter, Chapter 5 grounds the central argument of this thesis in a population and healthcare setting where the burden of anaemia is substantial and the consequences of biomarker interpretation are likely to be greatest.[4] The findings demonstrated that postpartum anaemia remained common, that its underlying causes were frequently multifactorial, and that estimates of iron deficiency varied considerably according to the biomarker and measurement approach used. Together, these findings reinforce that uncertainty in iron status assessment arises not only from dynamic physiology but also from the methods of measurement and the context in which biomarkers are applied. These broader themes are drawn together in the following section.

6.2.5 Understanding Uncertainty in Maternal Iron Assessment

Across the four empirical chapters, three recurring influences shaped the interpretation of iron status biomarkers: the physiological state in which a biomarker was measured, the methodological approaches used to generate and interpret it, and the healthcare context in which it was applied. Despite addressing different research questions in different populations, each study demonstrated how these factors could influence the relationship between a biomarker value and the underlying biological state it was intended to represent. When not adequately considered, they contributed to uncertainty in the assessment and interpretation of iron deficiency and anaemia in maternal health populations. Each of these influences is recognised in existing literature individually; pregnancy physiology affects iron metabolism,[12] different biomarkers capture distinct dimensions of iron biology,[74] and healthcare systems differ substantially in their approaches to anaemia assessment.[7,193] However, they are rarely considered together as interacting influences on biomarker interpretation.

Figure 6.2 brings these recurring influences together into a single conceptual framework, the '*Iceberg Model*'. The biomarker value represents the observable output used in clinical and research decision-making (the tip of the iceberg), but its interpretation is shaped by underlying physiological, methodological, and contextual influences that are not always immediately apparent.

The four empirical studies illustrate how these underlying influences manifested at different levels of maternal iron assessment. Chapter 2 demonstrated that healthcare context influenced biomarker interpretation through variation in guideline recommendations, diagnostic thresholds, and monitoring strategies across UK maternity services. Chapter 3 showed that both methodological and clinical contexts influenced biomarker selection, assessment timing, and definitions of treatment response, limiting comparability between studies evaluating oral iron therapy. Chapter 4 highlighted the influence of physiological adaptation, demonstrating that biomarker trajectories across pregnancy reflected normal gestational changes in addition to those attributable to iron supplementation. Finally, Chapter 5 illustrated how physiological state, inflammation, biomarker

selection, and measurement approach together influenced estimates of both iron deficiency and anaemia within a postpartum population. Importantly, these influences rarely acted in isolation. Instead, they frequently interacted, so that the interpretation of a biomarker value reflected their combined effects rather than any single influence.

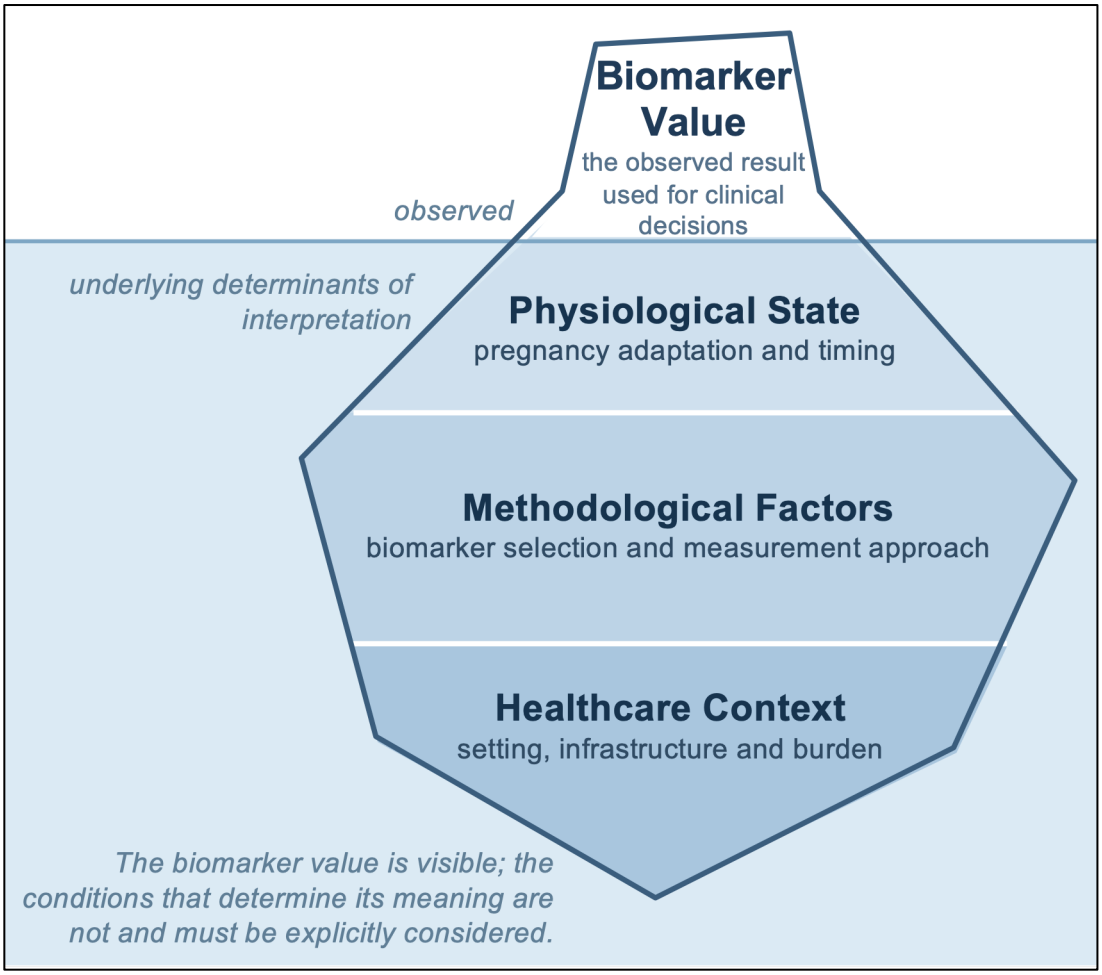


Figure 6.2 Iceberg conceptual model of iron biomarker interpretation in maternal health
The biomarker value is the observable output used in clinical and research decision-making, but its interpretation is shaped by underlying factors that are not always visible, including physiological state, methodological factors, and healthcare context.

Although these influences differed across studies, each affected how accurately biomarker measurements reflected underlying iron biology. The *'Iceberg Model'*, therefore, provides a visual representation of this synthesis, illustrating the importance of considering these interacting influences when interpreting biomarker values in maternal health.

What distinguishes this thesis is therefore not the identification of physiology, methodology, or healthcare context as individual influences on biomarker interpretation, each of which is already

recognised, but the demonstration that they recur consistently across multiple levels of enquiry. Whether examining clinical guidance, the research evidence base, a longitudinal pregnancy cohort, or a postpartum population, uncertainty arose because biomarker values could not be interpreted independently of the circumstances in which they were generated. Considered together, these findings suggest that the interpretation of iron status biomarkers in maternal health requires attention not only to the biomarker itself but also to the interactions among these underlying influences.

6.3 Strengths and Limitations of the Thesis

6.3.1 Strengths

This thesis has several important strengths that can be considered at both the level of individual study designs and at the level of the thesis as an integrated body of work.

At the level of individual studies, each empirical chapter was designed and executed to a high methodological standard appropriate to its research question. The guideline analysis employed a structured extraction framework, applied in duplicate by independent reviewers, covering a large, nationally representative sample of NHS sites. The systematic review was conducted in accordance with PRISMA 2020 guidance, with a prospectively registered protocol, duplicate screening and extraction, and risk-of-bias assessment using validated tools; it is, to date, the first systematic review to characterise both the selection and timing of biomarkers used to monitor response to oral iron therapy in pregnancy across different healthcare settings. The PANDA sub-analysis used a randomised trial as its parent study, enabling comparability of baseline characteristics across treatment groups and access to a comprehensive biomarker panel assayed under rigorous, quality-controlled laboratory conditions. Chapter 5 was prospectively nested within a large cluster-randomised trial and conducted in a real-world community setting, providing evidence of a kind seldom available in postpartum populations in lower-resource settings.

At the level of the thesis, the central strength lies in integrating these approaches into a coherent, multi-method research programme. No single study design could have addressed the central

research question in its full complexity: the systematic review alone would have identified heterogeneity in monitoring practice without clarifying its implications for biomarker interpretation, while a clinical trial sub-analysis alone would have generated new biomarker data without situating it within the broader clinical and health system contexts in which such data are used. This systems-level perspective supports a more integrated interpretation of iron deficiency and anaemia in maternal health and represents a central contribution of the thesis.

A further strength lies in the breadth of physiological and contextual insight achieved through the combined use of diverse settings and comprehensive biomarker measurement. Situating Chapters 2 and 4 within the UK and Chapter 5 within rural India generates a productive analytical contrast: the same underlying biological problem is measured differently, interpreted differently, and raises different clinical questions depending on the available resources and infrastructure. The inclusion of both pregnant and postpartum populations further allows consideration of how biomarker behaviour and interpretation vary across distinct stages of the maternal period, situating the findings within the broader continuum of iron deficiency across the reproductive years.

This contextual depth is further enhanced by the use of extended biomarker panels in Chapters 4 and 5. As demonstrated in Chapter 3, most studies rely on haemoglobin alone, or at most haemoglobin and ferritin; in contrast, this thesis incorporates sTfR, the ferritin index, hepcidin, and inflammatory markers alongside these conventional measures, enabling a more comprehensive characterisation of iron physiology than is typically available. The inclusion of hepcidin provides insight into the regulation of iron absorption under different dosing regimens in pregnancy and in the postpartum period. The availability of paired capillary and venous haemoglobin measurements in Chapter 5 offers a valuable opportunity to assess measurement agreement in a large community-based population.

The multidisciplinary and translational nature of the work represents an additional strength. The thesis draws on collaboration across haematology, obstetrics, biochemistry, primary care, global health, and spans laboratory science, clinical trials, and population-based research, enabling the

integration of mechanistic, clinical, and contextual insights across disciplinary boundaries that are often addressed in isolation.

Patient and public involvement further strengthened the thesis. Engagement with women affected by iron deficiency and anaemia, including structured workshops and education-focused dissemination activities, helped ensure that the research addressed clinically meaningful questions. Themes identified through this engagement, including inconsistency in monitoring, lack of follow-up beyond the antenatal period, and barriers to accessing reliable information, are directly reflected in the thesis's empirical focus.

6.3.2 Limitations

This thesis has several important limitations that should be considered when interpreting the findings. They can be understood across four interrelated domains: the overall design of the thesis, the design of individual studies, measurement and data constraints, and generalisability and contextual limitations.

Limitations of the Overall Thesis Design

The most fundamental structural limitation is that the thesis addresses a single overarching research question through four methodologically heterogeneous and analytically non-equivalent studies. While this diversity is a strength in breadth, it creates a corresponding limitation in analytical cohesion: there is no single framework that can formally unify the findings from each empirical chapter, and the conclusions are therefore necessarily integrative rather than definitive. Relatedly, the central argument emerged through synthesis across studies rather than through formal testing within a single design. The conceptual model presented in Section 6.2.5 was developed iteratively from the findings and should be understood as an interpretive framework rather than one that was prospectively specified or formally evaluated. While appropriate for a complex and multidimensional research question, this approach limits the extent to which causal inferences can be drawn and prevents quantification of the relative contribution of individual physiological, methodological, and contextual influences.

Limitations of Individual Study Designs

Each empirical chapter has study-specific limitations, with several cross-cutting considerations. Chapters 4 and 5 are sub-studies nested within larger trials and were not powered for the specific analyses conducted, so their findings, particularly the between-group comparisons, should be interpreted as exploratory and hypothesis-generating. The guideline analysis and systematic review are descriptive in design and do not permit causal inference; they are best understood as mapping exercises that define the scope and nature of the problem rather than evaluations of clinical effectiveness. The guideline analysis additionally reflects documented local recommendations at a single point in time, which may not fully correspond to clinical practice and may have evolved since data collection, and the systematic review may be subject to publication bias and documents heterogeneity without resolving which approaches are optimal.

Measurement and Data Constraints

There is no non-invasive reference standard for iron deficiency, and all biomarkers used in this thesis represent imperfect surrogates of underlying physiology; comparisons between biomarkers therefore reflect agreement between imperfect measures rather than validation against a definitive reference standard. Biomarker measurements were often obtained at single time points, limiting the ability to capture dynamic physiological changes, and sampling was not standardised with respect to time of day or timing since the last dose of oral iron. Inflammatory status was assessed using CRP alone, which may not fully capture inflammation-related effects on iron markers; additional markers, such as AGP or IL-6, would have enabled a more robust adjustment.[213] Further sources of variability include inter-site differences in laboratory assays, lack of standardisation in sampling conditions, and known inter-assay variability for biomarkers such as sTfR. In Chapter 5, reliance on baseline point-of-care haemoglobin introduces potential classification bias, and adherence to supplementation was not formally measured across all settings. Haemoglobinopathy screening was also not performed, limiting assessment of the contribution of inherited haematological conditions to anaemia. In addition, obstetric outcomes in Chapter 5 were self-reported and therefore susceptible to recall error and underestimation.

Generalisability and Contextual Limitations

The generalisability of findings is constrained by the populations and settings studied. The PANDA cohort consisted predominantly of non-anaemic women in a high-income setting, limiting applicability to anaemic populations and to routine clinical care. Chapter 5 was conducted in a single region of India and may not generalise to settings with different epidemiological profiles, healthcare access, or disease patterns. The guideline analysis reflects a single healthcare system and cannot be directly extrapolated to other national contexts.

6.4 Implications for Clinical Practice, Policy and Future Research

The findings of this thesis have implications for how iron status biomarkers are interpreted, applied, and studied in maternal health. Although the empirical chapters addressed distinct research questions, they identified recurring challenges related to physiological adaptation, biomarker selection, and healthcare context. As this thesis is primarily descriptive, the following sections should be interpreted as areas for further development rather than prescriptive recommendations.

6.4.1 Implications for Interpreting Biomarker Results in Clinical Practice

A central implication of this thesis is that iron status biomarkers should not be interpreted in isolation from the physiological, methodological, and clinical contexts in which they are measured. Across the empirical chapters, uncertainty arose when biomarker values were interpreted as direct proxies for iron status without sufficient consideration of factors such as gestational stage, inflammatory burden, biomarker selection, or measurement approach. The findings suggest that the clinical meaning of a biomarker result is often context-dependent and may not be fully captured by applying a single threshold in isolation.

Importantly, the findings do not support adopting a single preferred biomarker for assessing and monitoring iron deficiency in maternal health. Ferritin, sTfR, the ferritin index, hepcidin, and haemoglobin each provide information on different aspects of iron biology and are associated with distinct limitations. Expanding the biomarker panel may provide additional physiological

insight, but the findings of Chapters 4 and 5 demonstrate that this does not necessarily reduce uncertainty and may introduce further complexity in interpretation.

The principal clinical implication is therefore one of careful consideration. Biomarker results should be considered alongside the individual's physiological state, clinical presentation, and relevant contextual factors rather than being viewed as definitive indicators of iron status in isolation. While future research may help refine diagnostic thresholds, reference ranges, and monitoring strategies, the current evidence base remains insufficient to support definitive recommendations in several areas of maternal iron deficiency assessment and monitoring.

6.4.2 Implications for Policy and Public Health Practice

The findings of this thesis have implications for the incorporation of biomarker-based approaches into clinical guidelines, public health programmes, and population surveillance systems. A recurring theme across the empirical chapters was that the interpretation and application of biomarkers are shaped by the healthcare settings in which they are used. As a result, policies developed in one context may not be directly transferable to another.

Within the UK setting, findings from Chapter 2 demonstrated substantial variation in local guideline recommendations, particularly regarding ferritin thresholds, monitoring of treatment response, and criteria for escalation to IV iron. These findings suggest that improving consistency in practice may require not only continued refinement of the evidence base but also greater consensus on how existing evidence should be translated into national and, ultimately, local clinical recommendations.

In lower-resource settings, findings from Chapter 5 highlight additional challenges related to measurement methodology. Consistent with previous studies, capillary point-of-care haemoglobin measurements systematically underestimated venous haemoglobin concentrations, resulting in substantial overclassification of anaemia.[233,259,272] However, the observed high sensitivity suggests that point-of-care haemoglobin remains a useful screening tool for identifying women who may require further assessment or treatment. These findings, therefore, do not

diminish the importance of point-of-care testing, which remains essential for large-scale screening and programme delivery in many settings. Rather, they highlight the need to interpret results with an awareness of the strengths and limitations of the measurement method in real-world settings. Where feasible, investment in quality assurance processes, training, and confirmatory testing strategies may help improve measurement reliability.

The interpretation of these findings also depends on the intended purpose of measurement. A highly sensitive screening tool is well-suited to identifying women who may require further assessment or treatment, as it minimises the risk of missed cases. However, the same performance characteristics have different implications when capillary haemoglobin measurements are used to estimate the prevalence of anaemia or to evaluate programme effectiveness at the population level.[233,268,273] The low specificity observed in Chapter 5 means that many women without anaemia may also be classified as anaemic, potentially inflating estimates of disease burden. Consequently, apparent differences in anaemia prevalence or programme performance may partly reflect the characteristics of the measurement method rather than true changes in population health. This has implications for interpreting progress towards global anaemia reduction targets and for allocating healthcare resources where population estimates rely predominantly on capillary haemoglobin measurements.

Overall, the findings of this thesis suggest that policy approaches to maternal iron deficiency and anaemia should explicitly consider not only which biomarkers are used, but also the assumptions and limitations that underpin their interpretation. Greater transparency regarding these uncertainties may improve both the interpretation of evidence and the implementation of policy across diverse healthcare settings.

6.4.3 Implications for Future Research

This thesis's findings identify several priorities for future research. These priorities relate not only to the performance of individual biomarkers but also to the development of diagnostic frameworks that better account for physiological adaptation, healthcare context, and the intended purpose of testing.

There remains a need for more robust, physiologically informed approaches to defining iron deficiency during pregnancy. Current diagnostic thresholds for ferritin and other iron-status biomarkers are largely derived from observational data, expert consensus, or non-pregnant populations and often do not account for the dynamic physiological changes that occur across gestation.[64] Future research should continue to evaluate the development and validation of gestation-specific reference ranges and outcome-based diagnostic thresholds that better distinguish physiological adaptation from clinically significant deficiency. This is particularly relevant for biomarkers such as ferritin, sTfR, and hepcidin, whose interpretation remains challenging in pregnancy.

Greater standardisation is needed in the monitoring of treatment response. Chapter 3 demonstrated substantial heterogeneity in biomarker selection, assessment timing, and response definitions across clinical trials. This variation limits comparison between studies, complicates evidence synthesis, and may contribute to variation in clinical guidance. Future research should prioritise the development of standardised outcome measures and monitoring frameworks, potentially through consensus-based initiatives such as the COMET Initiative.[196]

The cost-effectiveness of alternative diagnostic and monitoring strategies also requires evaluation. Improved physiological characterisation does not necessarily translate into improved clinical outcomes or efficient use of resources. Emerging evidence from the United States suggests that ferritin-based screening using a threshold of 30 μg/L may be cost-effective compared with lower thresholds or no screening,[274] but the generalisability of these findings to other healthcare systems remains unclear. Future research should therefore evaluate not only diagnostic performance but also cost-effectiveness, acceptability, feasibility, and impact on clinically meaningful maternal and infant outcomes, with particular attention to strategies that are feasible and scalable in lower-resource settings, including approaches to adjusting for inflammation and the role of affordable multi-biomarker strategies.

More broadly, future research should ultimately move beyond evaluating biomarkers in isolation and consider how they are used in practice, since the clinical value of a biomarker depends not

only on its analytical performance but also on whether its use changes clinical decisions, improves outcomes, and remains feasible. Research that integrates biological, clinical, economic, and implementation perspectives may therefore be particularly valuable. Finally, examining iron status across the reproductive life course, through longitudinal studies spanning pregnancy, postpartum recovery, and subsequent conception, may help clarify how cumulative reproductive demands influence biomarker trajectories and the risk of iron deficiency over time.

6.4.4 Contribution of the Thesis

This thesis contributes to the maternal iron deficiency and anaemia literature in several ways.

First, it provides new empirical evidence across multiple levels of inquiry: Chapter 2 represents the first national cross-sectional analysis of NHS antenatal IDA guidelines relative to BSH recommendations; Chapter 3 is the first systematic review to characterise both the biomarkers and timing used to assess response to oral iron therapy in pregnancy; Chapter 4 contributes novel longitudinal data on the behaviour of ferritin, sTfR, hepcidin, TSAT, and the ferritin index under different oral iron regimens; and Chapter 5 extends this work to a community-based postpartum population in rural India, providing evidence on anaemia prevalence, aetiology, biomarker discordance, and capillary–venous agreement in a lower-resource setting.

Second, the thesis contributes methodologically by integrating evidence generated through guideline analysis, systematic review, clinical trial research, and community-based epidemiology, approaches that are often undertaken in isolation, to provide a broader perspective on how biomarker evidence is generated, interpreted, and translated into practice.

Third, the thesis contributes conceptually by demonstrating that uncertainty in the assessment and interpretation of iron deficiency and anaemia arises not from individual biomarkers alone, but from the interaction between physiological state, methodological approach, and healthcare context. A key contribution of this thesis is the demonstration that these influences recur consistently across diverse maternal health contexts and study designs. By bringing these findings

within a single analytical framework, the thesis highlights the importance of considering not only the biomarker itself, but also the circumstances in which it is measured and applied.

6.5 Summary

This thesis examined how iron status biomarkers are used and interpreted in assessing and monitoring iron deficiency and anaemia in maternal health. Through four complementary empirical studies spanning guideline implementation, evidence synthesis, clinical trial research, and community-based epidemiology, it examined the challenges associated with diagnosing, monitoring, and interpreting iron deficiency and anaemia across pregnancy and the extended postpartum period.

Several key findings emerged. Variation in guideline recommendations was greatest in areas where the evidence base remained uncertain, particularly in relation to ferritin thresholds, monitoring strategies, and treatment escalation. Research practice reflected similar uncertainty, with substantial heterogeneity in biomarker selection, assessment timing, and response definitions. Longitudinal biomarker data demonstrated the difficulty of distinguishing physiological adaptation from pathological iron deficiency during pregnancy, while postpartum analyses highlighted how inflammation, multifactorial anaemia, biomarker discordance, and measurement methodology influenced estimates of both iron deficiency and anaemia.

Although these findings arose from distinct populations, study designs, and healthcare settings, they converge on a common conclusion: iron status biomarkers do not provide fixed or universal measures of iron deficiency. Their interpretation depends on the interaction between underlying physiology, the methods used to generate and interpret results, and the healthcare context in which they are applied. Consequently, apparently similar biomarker values may carry different clinical implications across populations, settings, and stages of the reproductive life course.

Rather than identifying a single optimal biomarker or diagnostic threshold, this thesis demonstrates that improving the assessment and management of iron deficiency and anaemia in maternal health requires a more nuanced approach to biomarker interpretation. Future advances

will depend not only on refining biomarkers and diagnostic thresholds, but also on recognising the physiological, methodological, and healthcare contexts that determine what biomarker results mean in practice.

6.6 Conclusion

This thesis shows that greater precision in iron biomarkers alone will not resolve the uncertainty surrounding maternal iron deficiency and anaemia. Across clinical guidance, definitions of treatment response, longitudinal measurement during pregnancy, and postpartum classification, apparently different sources of uncertainty converged on the same problem: no biomarker, threshold, or measurement approach can provide a definitive assessment independently of the circumstances in which it is generated and applied. The key new insight is that this is not a problem limited to particular biomarkers or settings, but a recurring feature of maternal iron assessment more broadly.

These findings challenge approaches that rely on single biomarkers, fixed thresholds, or uniform interpretation across maternal populations. Instead, they support an approach that considers biomarker choice, timing of measurement, analytical method, physiological state, and healthcare context when interpreting results. The broader implication is that assessment of iron status should be embedded within clinically informed, personalised care across pregnancy and the wider reproductive life course, rather than centred on biomarker values in isolation. Biomarkers remain valuable, but their greatest value lies in informing clinical judgement within the context of the individual woman, rather than replacing it.

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Appendix 1. Data and Code Availability

Statistical code and analytical workflows used in this thesis are available at:

https://github.com/sarah-haynes-wrh/haynes-dphil-2026

Due to ethical and governance restrictions, individual participant-level data from the PANDA trial and the SMARThealth Pregnancy 2 (SHP2) trial are not publicly available. Access may be considered through the relevant study governance structures.

Appendix 2. Supplementary Materials for Chapter 2

Supplementary Table S2.1 Data extraction tool, with the 2020 BSH national guideline as exemplar

A. General Information

Category	Data Extracted
Guideline Name	BSH Guidelines
Date Published	March 2020
Expiry / Review Date	Not specified

B. Screening

Haemoglobin Screening		
Timepoint	Recommended	BSH Data
Booking	Y / N	Y
28 Weeks	Y / N	Y
32–36 Weeks	Y / N	N

Serum Ferritin Screening		
Timepoint	Recommended	BSH Data
Booking	Y / N	N
28 Weeks	Y / N	N
Indication for Testing		Women at risk of iron deficiency OR haemoglobinopathy

C. Diagnostic Criteria

Diagnostic Criteria	
Parameter	BSH Threshold
1st Trimester Anaemia	Hb <110 g/L
2nd & 3rd Trimester Anaemia	Hb <105 g/L
Postpartum Anaemia	Hb <100 g/L
Iron Deficiency	Serum ferritin <30 µg/L

D. Oral Iron Treatment

Oral Iron Treatment	
Parameter	BSH Recommendation
Indication	Hb <110 g/L (1st trimester); Hb <105 g/L (2nd or 3rd trimester); At-risk women – prophylactic iron
Preparation	Ferrous fumarate or other equivalent salts
Dose	40–80 mg elemental iron daily
Duration of Treatment	3 months after Hb normalised and at least 6 weeks postpartum

E. Intravenous Iron Treatment

Intravenous Iron Treatment	
Indication	
Hb Threshold	Hb <70 g/L
Timing Threshold	Anaemic at >34 weeks gestation
Failure of Oral Iron	Intolerance or lack of response
1st Trimester Use	Not recommended
Non-iron Deficiency Anaemia	Not indicated
Preparation & Dose	
Preparation(s)	Ferric carboxymaltose (Ferinject); Ferric derisomaltose (Monoferric); Iron sucrose (Venofer). Note: BSH suggests inadequate data for cosmofer in pregnancy, though some guidelines do use it.
Dose	Weight-based: 500–1000 mg
Contraindications	
1st Trimester	Yes
Hypersensitivity	Yes
Iron Overload	Not specified
Hepatic Impairment	Yes
Active Infections	Yes

F. Monitoring Post-Treatment

Monitoring Post-Treatment	
Oral Iron Monitoring	
Test	FBC
Timing	2–3 weeks
Expected Response	Demonstrable Hb rise by 2 weeks; no numeric target defined
IV Iron Monitoring	
Test	Not specified
Timing	Not specified
Expected Response	Not specified

The data extraction tool comprised six domains (general information, screening, diagnostic criteria, oral iron treatment, intravenous iron treatment, and monitoring), each corresponding to a key area of antenatal anaemia management. The structure is shown populated with the 2020 BSH national guideline as an illustrative example. BSH = British Society for Haematology; FBC = full blood count; Hb = haemoglobin; IV = intravenous; tri = trimester; Y = yes; N = no.

Appendix 3. Supplementary Materials for Chapter 3

Supplementary Table S3.1 Search strategies

	Medline **Medline (Ovid MEDLINE® Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE®) 1946 to 14 March 2025**	
1	perinatal care/ or prenatal care/ or exp Pregnancy/	1057424
2	(pregnan* or perinatal or prenatal or antenatal).ti,ab,kf.	79414
3	1 or 2	1271293
4	exp Iron Compounds/ or exp Hematinics/	115653
5	exp Administration, Oral/	162573
6	4 and 5	1396
7	((ferrous or iron) adj4 (oral or sulphate or sulfate or gluconate or fumarate or bisglycinate or succinylate)).ti,ab,kf.	8736
8	6 or 7	9621
9	3 and 8	849
	Cochrane Central Registry of Trials	
#1	[mh "perinatal care"] or prenatal care or exp Pregnancy	7746
#2	(pregnant OR pregnancy OR perinatal OR prenatal OR antenatal):ti,ab,kw	94822
#3	#1 OR #2	95838
#4	[mh "Iron Compounds"] or [mh Hematinics]	3604
#5	[mh "Administration, Oral"]	39342
#6	#4 and #5	286
#7	(ferrous OR iron) AND (oral OR sulphate OR sulfate OR gluconate OR fumarate OR bisglycinate OR succinylate):ti,ab,kw	4009
#8	#6 OR #7	4050
#9	#3 and #8	720
	Embase 1974 to present	
1	exp antianemic agent/	270822
2	oral drug administration/	414557
3	1 and 2	5177
4	((ferrous or iron) adj4 (oral or sulphate or sulfate or gluconate or fumarate or bisglycinate or succinylate)).ti,ab,kf.	12827
5	3 or 4	17418
6	(pregnan* or perinatal or prenatal or antenatal).ti,ab,kf.	1008504
7	exp perinatal care/	78754
8	exp prenatal care/	192767
9	exp pregnancy/	822475
10	6 or 7 or 8 or 9	1329899
11	5 and 10	1476
	Web of Science:	
1	TS=(pregnan* OR perinatal OR prenatal OR antenatal)	1897305
2	TS=((ferrous OR iron) NEAR/4 (oral OR sulphate OR sulfate OR gluconate OR fumarate OR bisglycinate OR succinylate))	63107
3	#1 AND #2	1752
	CINAHL:	
1	(pregnan* OR perinatal OR prenatal OR antenatal)	324416

2	((((ferrous OR iron) AND (oral OR sulphate OR sulfate OR gluconate OR fumarate OR bisglycinate OR succinylate))))	2144
3	#1 AND #2	320

Full search strategies for the five bibliographic databases searched (MEDLINE, Cochrane CENTRAL, Embase, Web of Science, and CINAHL), showing each search line and the number of records retrieved. CENTRAL = Cochrane Central Register of Controlled Trials.

Supplementary Table S3.2 Data extraction tool, with a worked example

Covidence #		6706
Year		2017
Study ID		Breymann 2017
Title		Ferric carboxymaltose vs. oral iron in the treatment of pregnant women with iron deficiency anaemia: an international, open-label, randomized controlled trial (FER-ASAP).
Country:		Multi-country (29 sites across 7 countries: Australia, Russia, South Korea, Singapore, Sweden, Switzerland, Turkey)
Level of Income	**Word Bank Income Classification**	High Income but Upper-Middle-Income (Turkey)
Healthcare Setting	**Urban vs Rural**	Urban
	Level of Care	Tertiary Care
Sample Size		252
Population Description	**Gestational Age Inclusion Criteria**	16-33
	Anaemic	y
	Iron Deficient	y
Anaemia and ID Thresholds Used for Study Inclusion:	**Anaemia Biomarker**	Hb
	Anaemia Threshold	110 and 105 (trimester based)
	Iron Deficiency Biomarker	Ferritin
	Iron Deficiency Threshold	20
Intervention and Comparisons:	**1**	IV
	2	Oral
	3	x
	4	x
Formulation	**1**	Ferric Carboxymaltose
	2	Ferrous Sulphate
	3	x
	4	x
Dose / Frequency (elemental iron mg)	**1**	x
	2	100mg BD
	3	x
	4	x

Duration by Intervention	**1**	Within 3 Weeks
	2	12 wks.
	3	x
	4	x
Number of Participants by Intervention	**1**	126
	2	126
	3	x
	4	x
Primary Outcome	**Biomarker**	Hb
	Timepoint	3 wks.
BIOMARKERS USED TO MONITOR RESPONSE	**All Biomarkers**	Hb; Ferritin; MCH; MCHC; MCV; Serum Iron ; TSAT; sTfR
What time point were the biomarkers measured at?		3 wks., 6 wks., 9 wks., 12 wks.
Response to Oral Iron	**Response Defined?**	No
	Definition?	
Risk of Bias Assessment	**1. Bias arising from the randomisation process**	Some Concerns
	2. Bias due to deviations from intended interventions	Some Concerns
	3. Bias due to missing outcome data	Low Risk
	4. Bias in measurement of the outcome	Low Risk
	5. Bias in selection of the reported result	Low Risk
	Overall Risk of Bias Judgement	Some Concerns

The data extraction tool applied to an included randomised controlled trial as a worked example. Income classification is based on World Bank country income categories at the time of study conduct. "Y" indicates the criterion was met and "N" that it was not. Risk of bias was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool. Hb = haemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; TSAT = transferrin saturation; sTfR = soluble transferrin receptor; GA = gestational age; IV = intravenous; BD = twice daily; ID = iron deficiency.

Supplementary Table S3.3 Summary of biomarkers used across studies, with studies conducted in India shown separately

Biomarker	All Studies (n=31)	HIC (n=8)	UMIC (n=5)	LMIC (Excl. India) (n=3)	India (n=15)	LIC (n=1)
Hb	31	8	4	3	15	1
Ferritin	25	7	4	2	11	0
HCT	8	3	1	1	3	0
Hepcidin	1	1	0	0	0	0
MCH	10	1	1	2	6	0
MCV	12	1	2	2	7	0
MCHC	10	1	1	2	6	0
Serum Iron	12	5	3	1	3	0
TIBC	5	2	2	0	1	0
TSAT	8	3	2	0	3	0
RBC Count	3	1	0	0	2	0
sTfR	2	2	0	0	0	0
RDW	1	0	1	0	0	0
Free Erythrocyte Porphyrin	1	0	1	0	0	0
Reticulocytes	6	0	0	1	5	0
Transferrin	1	1	0	0	0	0
Zinc Porphyrin	1	1	0	0	0	0

Values are the number of studies reporting each biomarker. Income classification is based on World Bank country income categories at the time of study conduct, with studies conducted in India shown separately from other lower-middle-income country (LMIC) studies. Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; TSAT = transferrin saturation; TIBC = total iron-binding capacity; sTfR = soluble transferrin receptor; RDW = red cell distribution width; RBC = red blood cell; HIC = high-income country; UMIC = upper-middle-income country; LMIC = lower-middle-income country; LIC = low-income country.

Supplementary Table S3.4 Choice of timepoint to measure response, by income level, with studies conducted in India shown separately

Timepoint (wks.)	All Studies (n=31)	HIC (n=8)	UMIC (n=5)	LMIC (Excl. India) (n=3)	India (n=15)	LIC (n=1)
1	**4**	0	0	1	3	0
2	**7**	1	1	0	5	0
3	**4**	1	1	0	2	0
4	**20**	3	2	3	12	0
6	**7**	2	2	0	3	0
8	**9**	0	1	1	9	0
9	**3**	1	1	1	0	0
10	**2**	0	0	0	2	0
12	**10**	1	3	2	3	0
GA	**4**	1	1	0	1	1
D	**5**	2	2	0	1	1

Values are the number of studies assessing response at each timepoint. Timepoints are reported as weeks following treatment initiation. Income classification is based on World Bank country income categories at the time of study conduct, with studies conducted in India shown separately from other LMIC studies. GA = gestational age; D = delivery; HIC = high-income country; UMIC = upper-middle-income country; LMIC = lower-middle-income country; LIC = low-income country.

Appendix 4. Supplementary Materials for Chapter 4

Supplementary Table S4.1 Linear mixed-effects model structure, parameters, and definitions

Component	Term / Symbol	Definition / Interpretation
Outcome	$\log_{10}(\text{Biomarker}_{ij})$	Log-transformed biomarker concentration for participant *i* at timepoint *j*
Fixed effects	Group_i	Treatment group (daily, alternate-day, three-times-weekly)
	Time_j	Timepoint (baseline or follow-up)
	$\text{Group} \times \text{Time}_{ij}$	Interaction term assessing whether biomarker trajectories differ by dosing regimen
	$\log_{10}(\text{CRP}_{ij})$	Continuous covariate representing inflammation
Random effects	u_i	Participant-specific random intercept capturing individual baseline variation
Residual error	ε_{ij}	Residual error term
Indexing	i	Participant
	j	Timepoint
Coefficients	β_0	Mean baseline biomarker level in the reference group (at CRP = 1 on log scale)
	β_1	Adjusted difference between treatment groups
	β_2	Adjusted change over time within participants
	β_3	Interaction effect (difference in change between dosing regimens)
	β_4	Independent effect of CRP on biomarker concentration

Components, terms, and definitions for the linear mixed-effects models used to analyse biomarker change, with the general model form shown beneath the table. CRP = C-reactive protein.

The general model took the following form: $\log_{10}(\text{Biomarker}_{ij}) = \beta_0 + \beta_1(\text{Group}_i) + \beta_2(\text{Time}_j) + \beta_3(\text{Group} \times \text{Time}_{ij}) + \beta_4 \log_{10}(\text{CRP}_{ij}) + u_i + \epsilon_{ij}$

Supplementary Table S4.2 Retrospective power calculations for hepcidin and sTfR

Outcome	Test Type	Comparison	Effect Size (Cohen's *d* / *f*)	Sample Size (n)	Observed Power (%)
sTfR	One-way ANOVA	Between Treatment Groups: Baseline Samples	0.046 (f)	135 (3 groups)	7%
sTfR	One-way ANOVA	Between Treatment Groups: Follow-Up Samples	0.046 (f)	135	7%
sTfR	Paired t-test	Daily (BL vs FU)	0.053 (d)	45	~6%
sTfR	Paired t-test	3x Weekly (BL vs FU)	0.601 (d)	43	97%
sTfR	Paired t-test	Alternate Daily (BL vs FU)	0.166 (d)	47	~29%
Hepcidin	One-way ANOVA	Between Treatment Groups: Baseline Samples	0.134 (f)	135	~12%
Hepcidin	One-way ANOVA	Between Treatment Groups: Follow-Up Samples	0.134 (f)	135	~12%
Hepcidin	Paired t-test	Daily (BL vs FU)	0.312 (d)	45	~83%
Hepcidin	Paired t-test	3xWeekly (BL vs FU)	0.516 (d)	43	~95%
Hepcidin	Paired t-test	Alternate Daily (BL vs FU)	0.657 (d)	47	>99%

Observed (post hoc) statistical power for between-group and within-group comparisons of hepcidin and sTfR, based on the observed effect sizes and sample sizes. Effect sizes are Cohen's f for one-way ANOVA and Cohen's d for paired t-tests. ANOVA = analysis of variance; BL = baseline; FU = follow-up; sTfR = soluble transferrin receptor.

Supplementary Table S4.3 Tablet-count adherence, by treatment arm

Treatment Arm	n (data / total)	Missing n (%)	Expected tablets / week	Mean tablets / week (SD)	Mean % of expected doses taken (SD)	Adherent[1] n/N (%)
Daily	43 / 45	2 (4.4%)	7	5.23 (1.66)	74.8% (23.8%)	20/43 (46.5%)
Alternate-Day	44 / 47	3 (6.4%)	3.5	3.20 (0.98)	91.3% (27.9%)	25/44 (56.8%)
Three-Times-Weekly	37 / 43	6 (14.0%)	3	2.94 (0.90)	98.1% (30.0%)	23/37 (62.2%)
Total	124 / 135	11 (8.1%)	—	3.83 (1.61)	—	68/124 (54.8%)

Values are mean (SD) unless otherwise stated. [1]Adherence was defined as a mean weekly tablet rate within ±15% of the expected weekly dose, consistent with the parent trial definition (Stanworth et al., Blood Advances 2024). SD = standard deviation.

Supplementary Table S4.4 Prevalence of iron deficiency and anaemia, by timepoint and treatment group

	Daily (n=45)		Alternate-Daily (n=47)		Three-Times-Weekly (n=43)	
Timepoint	BL (n=45)	FU (n=42)	BL (n=46)	FU (n=43)	BL (n=42)	FU (n=42)
Iron Deficiency (Ferritin <30ug/L)	14 (31.1)	29 (69.0)	11 (23.9)	31 (72.1)	8 (19.0)	30 (71.4)
Iron Deficiency (TSAT <20%)	12 (27.9)	28 (63.6)	13 (36.2)	26 (60.5)	13 (28.9)	26 (57.8)
Iron Deficiency (Ferritin <50ug/L)	22 (48.9)	39 (92.9)	27 (58.7)	37 (86.0)	22 (52.4)	38 (90.5)
Timepoint	BL (n=45)	FU (n=45)	BL (n=47)	FU (n=47)	BL (n=43)	FU (n=42)
Anaemia (Hb <105g/L)	0 (0)	2 (4.44)	0 (0)	2 (4.26)	0 (0)	2 (4.76)

Iron deficiency was defined as serum ferritin <30 µg/L, serum ferritin <50 µg/L, or transferrin saturation (TSAT) <20%. Anaemia was defined as haemoglobin <110 g/L at baseline (9–13 weeks) and <105 g/L at follow-up (26–30 weeks). Values are n (%); denominators reflect available values at each timepoint, and missing data were handled by complete-case analysis. BL = baseline; FU = follow-up; Hb = haemoglobin; TSAT = transferrin saturation.

Supplementary Table S4.5 Agreement between ferritin and TSAT classifications

Timepoint	Ferr ≥30 & TSAT ≥20	Ferr ≥30 & TSAT <20	Ferr <30 & TSAT ≥20	Ferr <30 & TSAT <20	Discordant (%)	Agreement (%)
Baseline (n=135)	98 (72.6%)	15 (11.1%)	22 (16.3%)	17 (12.6%)	27.4%	72.6%
Follow-up (n=135)	27 (20.0%)	18 (13.3%)	29 (21.5%)	61 (45.2%)	34.8%	65.2%

Iron deficiency was defined as ferritin <30 µg/L and transferrin saturation (TSAT) <20%. Percentages are calculated within each timepoint. Discordant classification refers to participants classified as iron deficient by one marker but not the other; agreement is the proportion classified concordantly (both iron deficient or both iron replete). TSAT = transferrin saturation.

Supplementary Table S4.6 Participants in CRP categories, by treatment group and timepoint

	Daily (N=45)		Alternate-Daily (N=47)		Three-Times-Weekly (N=43)	
Timepoint	BL	FU	BL	FU	BL	FU
CRP <5mg/l (n(%))	32 (71.1)	29 (64.4)	23 (48.9)	29 (61.7)	24 (55.8)	24 (55.8)
CRP 5-10mg/l	9 (20.0)	9 (20.0)	13 (27.7)	11 (23.4)	11 (25.6)	10 (23.3)
CRP 10-20mg/l	1 (2.22)	6 (13.3)	6 (12.8)	6 (12.8)	4 (9.30)	4 (9.30)
CRP >20mg/l	3 (6.67)	1 (2.22)	5 (10.6)	1 (2.12)	4 (9.30)	5 (11.6)

Values are number (percentage) of participants within each treatment group at each timepoint. CRP categories were <5 mg/L, 5–10 mg/L, 10–20 mg/L, and >20 mg/L. Percentages use the total number of participants in each group at the corresponding timepoint; participants with missing CRP at a given timepoint are excluded. BL = baseline; CRP = C-reactive protein; FU = follow-up.

Supplementary Table S4.7 Effect of log_{10}-transformed CRP on biomarker concentrations in adjusted linear mixed-effects models

Biomarker	β (Estimate)	95% CI	*p*-value
Ferritin	–0.050	[–0.146, 0.045]	0.307
Serum Iron	-0.189	[-0.246, -0.132]	<0.001***
Transferrin	+0.162	[−0.011, 0.336]	0.068
TSAT	–0.223	[–0.286, –0.159]	<0.001***
sTfR	+0.041	[–0.014, 0.096]	0.143
Ferritin Index	+0.24	[–0.05, 0.53]	0.11
Hepcidin	–0.021	[–0.152, 0.111]	0.759

β values represent the estimated effect of log_{10}-transformed CRP on biomarker concentrations from linear mixed-effects models. sTfR, hepcidin, ferritin, TSAT, and serum iron were modelled on the log_{10} scale; transferrin and the ferritin index on their original scales. Each model included fixed effects for treatment group, time, the group × time interaction, and log_{10}(CRP), with a random intercept for participant. p-values were derived using Satterthwaite's approximation, and 95% confidence intervals are Wald intervals. Significance: ns ≥ 0.05; *< 0.05; **< 0.01; ***< 0.001. CI = confidence interval; CRP = C-reactive protein; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

Supplementary Table S4.8 Summary of model diagnostics for the CRP-adjusted linear mixed-effects models

Biomarker	Residual normality (Shapiro–Wilk / check_normality)	Error variance (check_heteroscedasticity)	Multicollinearity (max VIF; term)	Random intercept SD (PATID)	Residual SD
sTfR	**OK** (p = 0.610)	**Warn** (heteroscedasticity; p < 0.001)	4.57; Group×Time	0.128	0.123
Hepcidin	**OK** (p = 0.111)	**OK** (p = 0.999)	5.97; Group×Time	0.213	0.362
Ferritin	**OK** (p = 0.785)	**OK** (p = 0.978)	4.37; Group×Time	0.215	0.204
TSAT	**Warn** (non-normality; p < 0.001)	**Warn** (heteroscedasticity; p < 0.001)	5.28; Group×Time	0.121	0.162

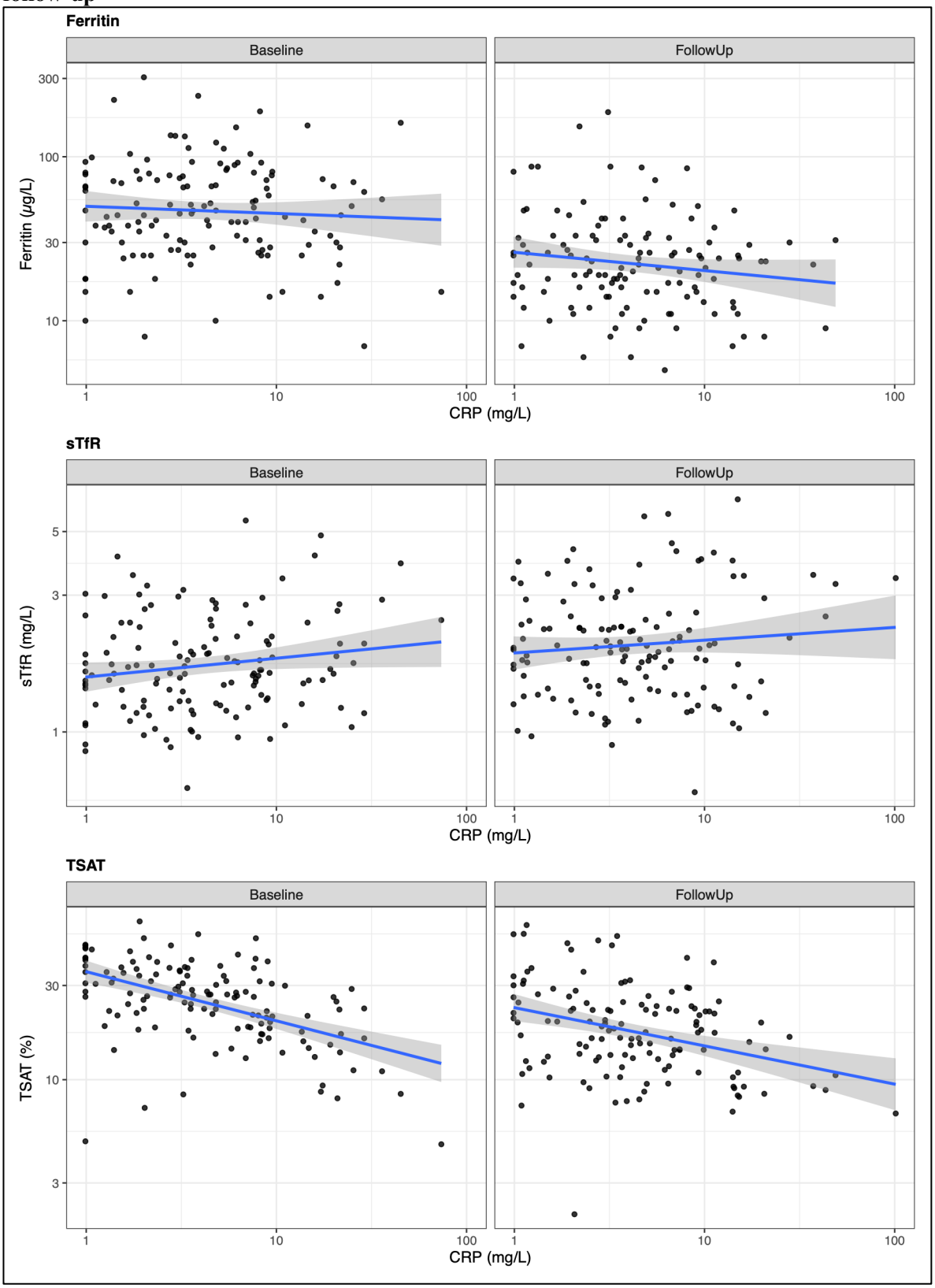
Diagnostics were computed in R using the performance package. VIF values <5 were considered acceptable; the group × time interaction showed the highest VIFs (4.37–5.97), indicating moderate but not severe multicollinearity. Where warnings arose (sTfR, TSAT), recommended mitigations include reporting with appropriate caveats and considering robust standard errors. All models were fitted by REML. CRP = C-reactive protein; REML = restricted maximum likelihood; SD = standard deviation; sTfR = soluble transferrin receptor; TSAT = transferrin saturation; VIF = variance inflation factor.

Supplementary Table S4.9 Spearman correlations between CRP and iron biomarkers, at baseline and follow-up

Biomarker	Timepoint	n	Spearman's ρ	p-value
Ferritin	Baseline	133	-0.038	0.66
Ferritin	Follow-up	127	-0.133	0.136
TSAT	Baseline	131	-0.526	<0.001
TSAT	Follow-up	132	-0.331	<0.001
sTfR	Baseline	135	0.129	0.136
sTfR	Follow-up	135	0.047	0.588

Spearman correlation coefficients were calculated using $\log_{10}$-transformed CRP and biomarker values, with p-values from Spearman's rank correlation tests. CRP = C-reactive protein; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

Supplementary Figure S4.1 Associations between CRP and iron biomarkers, at baseline and follow-up



Scatter plots show log₁₀-transformed CRP plotted against ferritin, sTfR, and TSAT at baseline and follow-up. Lines are linear fits with 95% confidence intervals. CRP = C-reactive protein; sTfR = soluble transferrin receptor; TSAT = transferrin saturation.

Appendix 5. Supplementary Materials for Chapter 5

Supplementary Table S5.1 Laboratory assay characteristics

Analyte	Platform / Kit	Method	Reporting Units	Measuring Range	LOD / LOQ	CV (%)	Notes
Full blood count (Hb, MCV, MCH, MCHC, RDW, RBC, WBC, platelets)	Sysmex XN-1000	Automated haematology analyser	Various (Hb reported in g/dL, converted to g/L)	N/A	N/A	*NA*	Performed on fresh whole blood at accredited local laboratories
Serum ferritin	Roche cobas 8000 (Elecsys Ferritin)	Electrochemiluminescence immunoassay	µg/L	0.50–2000 µg/L	LOD: 0.50 µg/L; LOQ: 2.0 µg/L	1.1–7.1	
Soluble transferrin receptor (sTfR)	Beckman Coulter Access (UniCel DXI 9000)	Immunoassay	nmol/L (converted to mg/L†)	0.05–150 nmol/L	N/R	≤8 (above 9 nmol/L) ‡	Converted using factor 0.0738 (MW monomeric non-glycosylated sTfR)
Vitamin B12	Roche cobas 8000 (Elecsys Vitamin B12 II)	Electrochemiluminescence immunoassay	pg/mL	150–2000 pg/mL	LOD: 100 pg/mL; LOQ: 150 pg/mL	2.1–7.2	
Serum folate	Roche cobas 8000 (Elecsys Folate III)	Electrochemiluminescence immunoassay	ng/mL	2.0–20.0 ng/mL	LOD: 1.2 ng/mL; LOQ: 2.0 ng/mL	1.5–4.9	
C-reactive protein (hs-CRP)	Roche cobas 8000 (CRPHS)	Immunoturbidimetric (high sensitivity)	mg/L	0.1–20 mg/L	Functional sensitivity: 0.3 mg/L	0.7–3.5	Values originally in mg/dL converted to mg/L
Hepcidin	Invitrogen EEL073 (ThermoFisher Scientific)	Solid-phase sandwich ELISA	ng/mL*	*NA*	*NA*	Intra: 4.1–5.4; Inter: 5.0–5.1	Expected range in healthy serum: 13.7–58.6 ng/mL

All biochemical assays were performed at the All India Institute of Medical Sciences (AIIMS), New Delhi, except the full blood count (performed at local accredited laboratories). *Hepcidin manufacturer's expected range for healthy serum is 13.7–58.6 ng/mL. †sTfR results in nmol/L were converted to mg/L using a factor of 0.0738. ‡sTfR total imprecision was ≤8% at concentrations above 9 nmol/L. CV = coefficient of variation; ELISA = enzyme-linked immunosorbent assay; Hb = haemoglobin; LOD = limit of detection; LOQ = limit of quantitation; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; N/A = not applicable; N/R = not reported; RBC = red blood cell; RDW = red cell distribution width; sTfR = soluble transferrin receptor; WBC = white blood cell.

Supplementary Table S5.2 Red cell morphology and haematological indices among women with venous-defined anaemia

Characteristic	n	N	%
Red cell morphology			
Normocytic	146	221	66.1
Microcytic	30	221	13.6
Macrocytic	45	221	20.4
Haematological features			
Hypochromia (MCH <27 pg)	136	221	61.5
Anisocytosis (RDW-CV >14.5%)	156	221	70.6
Mentzer index <13	3	221	1.4

Values are n/N (%), where denominators reflect participants with available full blood count indices among women with venous-defined anaemia (haemoglobin <120 g/L). Red cell morphology was classified using mean corpuscular volume (MCV); hypochromia using MCH <27 pg; anisocytosis using RDW-CV >14.5%. The Mentzer index (MCV/RBC) was used as a screening tool for possible thalassaemia. Hb = haemoglobin; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; RBC = red blood cell; RDW-CV = red cell distribution width (coefficient of variation).

Supplementary Table S5.3 Agreement between capillary point-of-care and venous haemoglobin for anaemia classification

	Venous Anaemic (<120 g/L)	Venous Not Anaemic (≥120 g/L)	Total
POC Anaemic	208 (93.3%) — TP	281 (82.4%) — FP	489
POC Not Anaemic	15 (6.7%) — FN	60 (17.6%) — TN	75
Total	**223**	**341**	**564**

Cell values are n (%) with the corresponding classification (TP, FP, FN, TN). Sensitivity 93.3% (208/223); specificity 17.6% (60/341); overall agreement 47.5% (268/564). Analyses were restricted to the 564 women with paired capillary and venous haemoglobin measurements. POC = point-of-care; TP = true positive; FP = false positive; FN = false negative; TN = true negative.

Supplementary Table S5.4 Agreement between iron deficiency biomarkers among women with venous-defined anaemia (n = 224)

Marker pair	Both positive n (%)	Marker 1 only n (%)	Marker 2 only n (%)	Neither n (%)	% agreement
Ferritin vs sTfR	117 (52.2%)	18 (8.0%)	54 (24.1%)	35 (15.6%)	67.9%
Ferritin vs Ferritin index	124 (55.4%)	11 (4.9%)	49 (21.9%)	40 (17.9%)	73.2%
sTfR vs Ferritin index	166 (74.1%)	5 (2.2%)	7 (3.1%)	46 (20.5%)	94.6%

Iron deficiency was defined as ferritin <30 µg/L; soluble transferrin receptor (sTfR) ≥1.55 mg/L (≥21 nmol/L); and the ferritin index (sTfR/$\log_{10}$ ferritin) ≥1.03. Percent agreement is the proportion of participants classified concordantly by each marker pair. sTfR = soluble transferrin receptor.

Supplementary Table S5.5 Agreement between ferritin and soluble transferrin receptor, stratified by inflammatory status, among women with venous-defined anaemia (n = 224)

CRP category	Both positive n (%)	Ferritin only n (%)	sTfR only n (%)	Neither n (%)	% agreement
>5 mg/L	10 (38.5%)	0 (0.0%)	13 (50.0%)	3 (11.5%)	50.0%
≤5 mg/L	107 (54.0%)	18 (9.1%)	41 (20.7%)	32 (16.2%)	70.2%

Iron deficiency was defined as ferritin <30 µg/L and soluble transferrin receptor (sTfR) ≥1.55 mg/L. Percent agreement is the proportion of participants classified concordantly by both biomarkers. CRP = C-reactive protein; sTfR = soluble transferrin receptor.