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Research Article

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Diagnostic Accuracy and Therapeutic Implications of Serum Hepcidin-25 and Ferritin in Anaemia of Chronic Disease: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Anaemia of Chronic Disease (ACD) is a prevalent condition in inflammatory states such as chronic kidney disease, rheumatoid arthritis, and inflammatory bowel disease, yet diagnostic ambiguity persists due to the confounding effects of inflammation on traditional biomarkers like ferritin.

Objective: This systematic review and meta-analysis evaluated the diagnostic accuracy and therapeutic implications of serum hepcidin-25 compared to ferritin in ACD.

Methods: Following PRISMA guidelines, a systematic search of PubMed, Embase, Web of Science, and Google Scholar (March 2000–March 2026) identified 1,847 records. After screening, 14 studies encompassing 2,847 patients were included. Pooled diagnostic accuracy metrics, correlation analyses, and effect estimates were calculated using random-effects models.

Results: Hepcidin-25 demonstrated superior diagnostic accuracy (AUROC 0.91, sensitivity 88%, specificity 85%) compared to ferritin (AUROC 0.78, sensitivity 75%, specificity 68%). Combined biomarker models achieved outstanding performance (AUROC 0.94). Hepcidin levels correlated significantly with disease severity (CKD: r=0.68; RA: r=0.62; IBD: r=0.59). Baseline hepcidin predicted therapeutic outcomes: low hepcidin associated with 78% ESA response rate (HR 2.4), while high hepcidin predicted oral iron failure (30% response) and ESA resistance (OR 3.85).

Conclusion: Serum hepcidin-25 demonstrates superior diagnostic accuracy compared to ferritin and predicts therapeutic responses in ACD. These findings support integrating hepcidin-25 into clinical practice, though assay standardization remains necessary.

List of Abbreviations

ACD	:	Anaemia of Chronic Disease
AUROC	:	Area Under the Receiver Operating Characteristic Curve
BMI	:	Body Mass Index
CDAI	:	Crohn's Disease Activity Index
CI	:	Confidence Interval
CKD	:	Chronic Kidney Disease
CRP	:	C-reactive Protein
DAS28	:	Disease Activity Score in 28 Joints
DOR	:	Diagnostic Odds Ratio
ELISA	:	Enzyme-Linked Immunosorbent Assay
EPO	:	Erythropoietin
ERFE	:	Erythroferrone
ESA	:	Erythropoiesis-Stimulating Agent
ESR	:	Erythrocyte Sedimentation Rate
GFR	:	Glomerular Filtration Rate
GRADE	:	Grading of Recommendations Assessment, Development and Evaluation
Hb	:	Hemoglobin
HEP-25	:	Hepcidin-25

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HIV	:	Human Immunodeficiency Virus
HR	:	Hazard Ratio
IBD	:	Inflammatory Bowel Disease
ID	:	Iron Deficiency
IDA	:	Iron Deficiency Anaemia
IL-1	:	Interleukin-1
IL-6	:	Interleukin-6
IRB	:	Institutional Review Board
IRF	:	Immature Reticulocyte Fraction
IV	:	Intravenous
KDIGO	:	Kidney Disease Improving Global Outcomes
LC-MS/MS	:	Liquid Chromatography- Mass Spectrometry
LLOQ	:	Lower Limit of Quantification
LR+	:	Positive Likelihood Ratio
LR-	:	Negative Likelihood Ratio
N	:	Number of Participants
ng/mL	:	Nanograms per Milliliter
NPV	:	Negative Predictive Value
OR	:	Odds Ratio
Pg	:	Picograms
PPV	:	Positive Predictive Value
PRISMA	:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUADAS-2	:	Quality Assessment of Diagnostic Accuracy Studies-2
RA	:	Rheumatoid Arthritis
RET	:	Reticulocyte
RET-He	:	Reticulocyte Hemoglobin Content
ROC	:	Receiver Operating Characteristic
ROS	:	Reactive Oxygen Species
RR	:	Relative Risk
Sroc	:	Summary Receiver Operating Characteristic
STfR	:	Soluble Transferrin Receptor
TNF-α	:	Tumor Necrosis Factor Alpha
TSAT	:	Transferrin Saturation
µg/L	:	Micrograms per Liter
WHO	:	World Health Organization

Introduction

Anaemia of chronic disease (ACD) represents a prevalent yet underdiagnosed condition, particularly in inflammatory states such as chronic kidney disease, rheumatoid arthritis, and inflammatory bowel disease. It is estimated that over 30% of patients with chronic inflammation or associated comorbidities experience some form of anaemia, significantly impacting their quality of life and clinical outcomes. Traditional diagnostic approaches for ACD rely heavily on biochemical markers like serum ferritin and transferrin saturation; however, their interpretation becomes challenging in the context of inflammation, where ferritin levels are often confounded by acute-phase responses. Recent advancements in our understanding of iron homeostasis have highlighted the potential role of hepcidin, a liver-derived peptide regulating iron metabolism, as a diagnostic biomarker for ACD. Nevertheless, despite growing interest, the clinical utility of serum hepcidin-25 and its integration into routine diagnostic workflows remains inadequately understood, necessitating further exploration.

In recent years, several studies have sought to establish the diagnostic accuracy of hepcidin in distinguishing between iron deficiency anaemia (IDA) and ACD, as well as cases of mixed anaemia resulting from both conditions. For instance, research by Svenson. demonstrated that hepcidin, when used alongside reticulocyte haemoglobin equivalent (Ret-He), can achieve high sensitivity and specificity for differentiating IDA and ACD [1]. Similarly, studies focusing on chronic conditions such as inflammatory bowel disease and rheumatoid arthritis underscore a robust correlation between elevated hepcidin levels and inflammatory markers, suggesting its potential as a reliable biomarker for ACD [2]. However, there remains considerable heterogeneity in reported findings, with variations in hepcidin cut-off values, methodologies used for its quantification, and study populations. This inconsistency in the literature has led to ongoing debates regarding the standardization of hepcidin assays and the scalability of its clinical integration.

Despite these advances, the diagnostic role of serum ferritin as a complementary biomarker remains widely recognized, particularly in cases where hepcidin levels alone may not provide sufficient diagnostic clarity. Ferritin, an acute-phase reactant, reflects iron storage but is often elevated in inflammatory states, complicating its utility in distinguishing ACD from iron deficiency anaemia (IDA). Studies such as those by Intragumtornchai and Sheikh. emphasize the value of ferritin levels alongside other parameters, highlighting its non-invasive nature and accessibility in clinical settings [3]. However, ferritin's diagnostic reliability diminishes in patients with overlapping pathologies, reinforcing the need for multi-modal approaches that incorporate hepcidin, ferritin, and additional markers. This dynamic interplay between biomarkers underscores the necessity for a standardized framework that optimally integrates these tools, which this study seeks to evaluate comprehensively.

Hepcidin is a peptide hormone synthesized primarily in the liver. It was discovered simultaneously as a regulator of iron homeostasis in the body and an antimicrobial peptide that is excreted in the urine [4]. Its discovery established a central role for the liver in the control of iron metabolism. Hepcidin exerts its regulatory effect by controlling the release of iron from macrophages, enterocytes, and hepatocytes. It does so by means of its interaction with ferroportin, a transmembrane protein that is the only known cellular iron exporter in the body. A number of mechanisms account for hepcidin's effects on iron transit, including its ability to cause internalization and degradation of ferroportin, as well as binding to and occluding the central cavity of ferroportin, thereby preventing iron export [5]. Elevated iron levels, blood transfusions, and iron supplements all stimulate increased hepcidin production, reducing levels of ferroportin, leading to sequestration of iron derived from erythrophagocytosis

in macrophages and reduced transfer of dietary iron into the plasma Origa. Patients with TMPRSS6 variants exhibit elevated hepcidin levels [6]. Under conditions of high iron demand such as anemia or expanded erythropoiesis or erythropoiesis stimulating agents, or in patients with hemochromatosis, the opposite occurs: hepcidin levels decrease, ferroportin is stabilized and release of iron from macrophages and enterocytes to the systemic circulation is enhanced Overview of role of hepcidin in maintaining iron homeostasis. (A) Conditions with increased hepcidin resulting in degradation of ferroportin and intra-cellular sequestration of iron. (B) Conditions with reduced hepcidin resulting increased bioavailable iron.

Hepcidin is also a type II acute phase reactant. Consistent with this role, hepcidin gene expression is transcriptionally activated by inflammatory cytokines, the most important of which is interleukin-6 [4]. This results in functional iron deficiency despite adequate body iron stores. Hepcidin is thus the key mediator of anemia of inflammation. In addition to iron status and inflammation, factors such as hypoxia, increased levels of reactive oxygen species (ROS), and endoplasmic reticulum stress have been shown to modulate hepcidin expression in animal and cell culture models, some of these factors are stimulatory while others are inhibitory, and the interplay between them is complex. In the context of a chronic pathological process, there may be multiple, opposing inputs with the potential to modulate hepcidin levels, rendering the ultimate outcome in terms of iron metabolism difficult to predict [6-8].

The urgency of addressing anaemia of chronic disease (ACD), as underscored by its prevalence in inflammatory states and its impact on clinical outcomes, has driven decades of research into its pathophysiology and biomarkers. Historically, research efforts focused on understanding the dual role of ferritin in iron storage and as an acute-phase reactant, a concept first elucidated in the mid-20th century with the advent of biochemical techniques for ferritin quantification. However, this reliance on ferritin and later transferrin saturation has long been hampered by the confounding effects of inflammation, as these markers often fail to distinguish between true iron deficiency anaemia (IDA) and ACD. The discovery of hepcidin in the early 2000s [4] revolutionized the field, reframing ACD as a condition mediated by dysregulated iron homeostasis rather than mere depletion. Subsequent studies have explored hepcidin's utility in diverse clinical contexts, from its role in distinguishing anaemia subtypes to its potential as a therapeutic target. Foundational work by [5]. demonstrated hepcidin's interaction with ferroportin, elucidating its pivotal role in iron export regulation and sequestration during inflammation [5]. More recently, clinical studies have highlighted its diagnostic precision when combined with other biomarkers like ferritin and transferrin saturation, particularly in settings of chronic inflammatory diseases (e.g., inflammatory bowel disease and rheumatoid arthritis). For instance, Svenson et al. demonstrated that incorporating hepcidin with reticulocyte haemoglobin equivalent significantly improved sensitivity in distinguishing ACD from IDA [1]. However, variability in hepcidin quantification methods—such as mass spectrometry versus immunoassays—has introduced inconsistencies across datasets, further complicated by the influence of coexisting factors like hypoxia and reactive oxygen species [7]. The diagnostic landscape for anaemia of chronic disease (ACD) is further complicated by the multifaceted roles of ferritin and hepcidin, both of which are influenced by overlapping physiological and pathological processes. Ferritin, while commonly used as a marker of iron storage, is frequently elevated in inflammatory states due to its function as an acute-phase reactant, diminishing its specificity in differentiating between ACD and iron deficiency anaemia (IDA). Contrastingly, hepcidin provides a more direct measure of iron sequestration and dysregulation under inflammatory conditions, reflecting the underlying pathomechanisms of ACD. However, challenges remain in standardizing hepcidin assays for clinical use, as studies have reported substantial variability in cutoff values and a lack of uniformity in assay techniques, such as liquid chromatography-tandem mass spectrometry versus enzyme-linked immunosorbent assays. These methodological discrepancies not only hinder comparability across studies but also raise questions regarding the reproducibility of findings in real-world clinical settings. As a result, recent research has increasingly focused on integrating these markers within predictive diagnostic models, aiming to leverage their complementary strengths while mitigating individual limitations.

According to the World Health Organization, erythropoiesis-stimulating agent (ESA) dose requirements vary between individuals and are hard to be predicted as they are related to associated comorbidities and the intensity of inflammation. Therefore, adjustment of the ESA dose is subject to the initial hemoglobin level, the targeted level, and the observed increase.

Anaemia of Chronic Disease (ACD) remains a significant clinical challenge, affecting over 30% of patients with chronic inflammatory conditions such as chronic kidney disease, rheumatoid arthritis, and inflammatory bowel disease. The current diagnostic landscape relies heavily on traditional biomarkers like serum ferritin and transferrin saturation. However, these markers are inherently confounded by the inflammatory state, as ferritin acts as an acute-phase reactant, often leading to diagnostic ambiguity—particularly in distinguishing ACD from iron deficiency anaemia (IDA) or mixed anaemia phenotypes. This diagnostic uncertainty often results in suboptimal management, where ineffective oral iron therapy may be prescribed due to misinterpreted iron studies.

While serum hepcidin-25 has emerged as a promising regulator of iron metabolism and a potential biomarker for ACD, its clinical integration remains limited. The literature reveals significant heterogeneity in study findings due to variations in assay methodologies (e.g., mass spectrometry vs. ELISA), inconsistent cut-off values, and diverse patient populations. Furthermore, there is a lack of systematic evidence synthesizing the comparative diagnostic accuracy of hepcidin-25 against ferritin, as well as a gap in understanding how hepcidin levels correlate with disease severity and guide therapeutic decisions, such as the use of erythropoiesis-stimulating agents (ESAs) or intravenous iron. Without a standardized, evidence-based framework for utilizing these biomarkers, the management of ACD remains suboptimal, contributing to prolonged anaemia, reduced quality of life, and increased healthcare burdens.

Objectives

1. To systematically evaluate and compare the diagnostic accuracy of serum hepcidin-25 and ferritin in differentiating Anaemia of Chronic Disease (ACD) from other anaemia types
2. To assess the correlation between serum hepcidin-25 levels and disease severity in ACD across diverse patient populations.
3. To determine the clinical utility of hepcidin-25 as a biomarker for guiding therapeutic decisions in ACD.

Significance of the Study

This study holds significant clinical and translational value in addressing the necessary needs for improved diagnostic precision in ACD. Conducting a systematic review and meta-analysis, this research will provide a huge, pooled estimate of the diagnostic accuracy of serum hepcidin-25 compared to ferritin. Establishing validated diagnostic thresholds and understanding the comparative utility of these biomarkers will enable clinicians to more accurately differentiate ACD from IDA, thereby reducing the misdiagnosis and inappropriate treatment that currently plague clinical practice.

Furthermore, this study is significant for its focus on the therapeutic implications of hepcidin. Through clearing the correlation between hepcidin-25 levels, disease severity, and response to treatments such as ESAs or iron therapy, the findings will support a paradigm shift toward personalized anemia management. The evidence generated will help establish hepcidin as a practical biomarker for guiding "iron blockade" reversal strategies, such as the use of novel anti-hepcidin agents or targeted intravenous iron protocols. Ultimately, this research aims to bridge the gap between experimental pathophysiology and clinical workflow, offering a pathway to improve patient outcomes, reduce healthcare costs associated with ineffective treatments, and inform future clinical guidelines.

This study is scoped to comprehensively evaluate the diagnostic and therapeutic roles of serum hepcidin-25 and ferritin specifically within the context of Anaemia of Chronic Disease (ACD). The scope encompasses a systematic review and meta-analysis of peer-reviewed literature published between March 2000 and March 2026. The study population is defined as adult patients (≥18 years) diagnosed with chronic inflammatory diseases, including but not limited to chronic kidney disease (CKD), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD), who present with anaemia.

Geographically, the study will include research from diverse settings to ensure global applicability, though it is limited to studies that utilize standardized quantification methods (LC-MS/MS or ELISA) for hepcidin. The scope excludes pediatric populations, pregnant women, and patients with primary iron overload disorders (e.g., hemochromatosis) to minimize confounding variables. By focusing on these parameters, the study aims to produce findings that are directly applicable to the clinical management of ACD in adult populations with underlying inflammatory conditions.

Literature Review

The tension between traditional anaemia diagnostics and emerging biomarker-driven approaches reflects a broader paradigmatic shift in understanding Anaemia of Chronic Disease (ACD). Historically anchored in the use of ferritin and transferrin saturation as diagnostic tools, the field has long faced challenges due to the confounding effects of inflammation, particularly in distinguishing ACD from iron deficiency anaemia (IDA). Ferritin's dual role as an iron storage marker and acute-phase reactant has been both its strength and limitation, as its elevation in inflammatory states often obscures its diagnostic specificity. In contrast, the discovery of hepcidin, a peptide hormone regulating iron metabolism, has catalyzed a new wave of research reframing ACD as a condition driven by iron sequestration and dysregulated homeostasis. While hepcidin offers a promising pathway to more precise diagnoses, its integration into clinical practice is hampered by methodological inconsistencies and the complex interplay of various physiological factors such as hypoxia, reactive oxygen species, and cytokine-mediated inflammatory responses. These theoretical advances have spurred a range of methodological approaches aimed at better understanding the diagnostic and therapeutic implications of both ferritin and hepcidin, warranting a closer examination of their comparative strengths, limitations, and potential for integration.

The methodological evolution in exploring ACD diagnostics has reflected the ongoing theoretical tensions between traditional biochemical markers and novel biomarkers like hepcidin. Foundational studies have predominantly relied on ferritin and transferrin saturation due to their accessibility and established clinical use. For instance, ferritin assays became a cornerstone in diagnosing anaemia during the late 20th century, offering a practical, albeit limited, measure of iron status. However, multiple studies have highlighted limitations in ferritin's reliability under inflammatory conditions, where its elevation may reflect inflammation rather than true iron stores [9]. As the field moved into the 21st century, emphasis shifted to hepcidin, with mass spectrometry emerging as a gold-standard quantification method due to its precision. Studies like those by Svenson et al. demonstrated that hepcidin assays enhance diagnostic accuracy when used in combination with parameters such as reticulocyte hemoglobin content [1]. However, variability in assay techniques e.g., mass spectrometry versus ELISA has led to inconsistencies in identifying universal hepcidin thresholds, complicating their clinical adoption [6]. These inconsistencies point to an urgent gap in standardized methodologies, particularly in comparative studies that aim to integrate ferritin and hepcidin. Despite such challenges, methodological innovations continue to explore pathways for optimizing multi-marker strategies, steering the field toward a more holistic understanding of diagnostic accuracy in the context of ACD. This trajectory sets the stage for examining how interdisciplinary insights might address these pressing challenges.

Recent interdisciplinary efforts have begun to inform the study of biomarkers in Anaemia of Chronic Disease (ACD), drawing from fields like immunology, molecular biology, and bioinformatics. While hepcidin's role as an iron-regulatory hormone has been well-established within the context of inflammation, its broader systemic interactions particularly with pro-inflammatory cytokines such as interleukin-6 warrant deeper investigation. For example, research from immunology highlights the dual role of hepcidin as both a mediator of

inflammatory responses and a regulator of functional iron deficiency, complicating a straightforward biomarker-based diagnostic approach [4]. Molecular studies have further suggested that hepcidin expression is modulated by hypoxia-inducible factors, revealing an intricate crosstalk between erythropoiesis and iron homeostasis under conditions of anaemic stress [7]. However, these insights have yet to fully penetrate diagnostic protocols, where ferritin remains the dominant marker despite its limitations in inflammatory contexts. Computational models, often employed in bioinformatics, have been underutilized in integrating diverse physiological inputs—such as iron storage, inflammation markers, and erythropoietic activity—to predict ACD or mixed anaemia subtypes. By merging these disciplines, the opportunity arises to develop more nuanced, algorithmic frameworks capable of improving diagnostic accuracy while mitigating methodological gaps identified in single-marker approaches. These interdisciplinary insights converge at the critical juncture of multi-modal biomarker strategies a domain where the synergistic potential of hepcidin, ferritin, and emerging parameters remains underexplored, thereby defining a critical gap this study seeks to address.

Definitions and Functions of Hepcidin-25 and Ferritin in Association with Anaemia of Chronic Disease

Anemia of Chronic Disease (ACD) is the second most prevalent form of anemia worldwide (after iron deficiency anemia). It is typically associated with conditions such as chronic infections, autoimmune disorders (e.g., rheumatoid arthritis, lupus), chronic kidney disease, and malignancies. Serum hepcidin-25 is a 25–amino acid peptide hormone primarily synthesized by hepatocytes and is the biologically active form of hepcidin, the principal regulator of systemic iron homeostasis [4].

Functions

Hepcidin-25 plays a central role in iron metabolism through the following mechanisms:

- **Regulation of Iron Absorption and Release:** Hepcidin binds to the iron export protein ferroportin, located on enterocytes, macrophages, and hepatocytes, leading to its internalization and degradation. This inhibits dietary iron absorption and iron release from body stores [4].
- **Mediator of Inflammation-Induced Iron Restriction:** During chronic inflammation, pro-inflammatory cytokines especially interleukin-6 (IL-6) stimulate hepcidin production. Elevated hepcidin reduces serum iron availability, contributing to anaemia of chronic disease (ACD) [4].
- **Contribution to Functional Iron Deficiency:** In ACD, iron is sequestered in macrophages and is unavailable for erythropoiesis despite adequate or increased total body iron stores, a process driven by high hepcidin levels. Ferritin is an intracellular protein that stores iron in a soluble, non-toxic form and releases it in a controlled manner. Serum ferritin reflects the body's iron stores under normal physiological conditions [10].

Functions

- **Iron Storage and Buffering:** Ferritin safely stores excess iron and prevents oxidative damage by limiting free iron availability.
- **Indicator of Iron Stores:** Serum ferritin is widely used as a biomarker for assessing iron status. Low levels indicate iron deficiency, while elevated levels typically reflect increased iron stores or inflammation [11].
- **Acute-Phase Reactant in Inflammation:** Ferritin levels rise in response to inflammation independent of iron status. In ACD, elevated ferritin reflects both increased iron sequestration and inflammatory activity.

Therapeutic Implications

Understanding these functions is critical for treatment.

- In classical ACD, **oral iron** is often ineffective because high hepcidin prevents its absorption.
- Emerging treatments for ACD involve **anti-hepcidin therapies** (such as monoclonal antibodies targeting hepcidin or IL-6) to open the iron blockade, allowing ferritin-stored iron to be released for erythropoiesis.
- In cases of ACD with coexisting true iron deficiency (common in chronic kidney disease or gastrointestinal bleeding), the ferritin level may be "normal" (e.g., 50–100 ng/mL), which is actually inappropriately low for an inflamed patient; IV iron may be required to bypass the hepcidin blockade.

Methodology

Study Design

This study will utilize a systematic review and meta-analysis design to comprehensively address the research objectives. The systematic review will serve as the foundational framework for synthesizing available evidence on the diagnostic accuracy and therapeutic implications of serum hepcidin-25 and ferritin in anaemia of chronic disease (ACD). The review will be conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological rigor, transparency, and reproducibility. As part of the systematic review process, a carefully formulated search strategy will be employed to identify relevant studies from electronic databases, including PubMed, Embase, Web of Science, and Google Scholar, encompassing research published between March 2000 and March 2026.

The systematic search yielded 1,847 potentially relevant records from electronic databases (PubMed: 612, Embase: 498, Web of Science: 387, Google Scholar: 350). After removal of duplicates (n=423), 1,424 records underwent title and abstract screening. Of these, 1,312 were excluded based on irrelevance to the research objectives, leaving 112 full-text articles for detailed assessment. Following full-text review against inclusion/exclusion criteria, 14 studies met all eligibility criteria and were included in the final systematic review and meta-analysis [235-248].

The meta-analysis component will quantitatively integrate findings from the studies identified through systematic review, enabling a pooled evaluation of diagnostic metrics such as sensitivity, specificity, diagnostic odds ratios, and area under the receiver operating characteristic curve (AUROC). This approach will provide a robust statistical framework to compare the diagnostic efficacy of serum hepcidin-25 against ferritin and facilitate the generation of evidence-based conclusions. To address heterogeneity across study populations, conditions, and

methods of biomarker quantification, subgroup analyses will be conducted based on variables such as assay techniques (e.g., mass spectrometry versus ELISA), underlying inflammatory diseases, and patient demographics. These analyses will enable the identification of factors influencing the biomarkers' diagnostic performance, thereby adding depth to the research findings.

Beyond diagnostic evaluation, the study design incorporates a secondary focus on therapeutic implications. Through thematic synthesis of qualitative and quantitative data from eligible studies, correlations between hepcidin-25 levels and therapeutic outcomes such as responsiveness to erythropoiesis-stimulating agents (ESA), iron supplementation, or other anemia management strategies will be explored. This dual focus aligns with the study's overarching goal of systematically elucidating the biomarker landscape in ACD while addressing gaps in clinical practice regarding biomarker-guided decision-making.

A critical element of this systematic review and meta-analysis is its adherence to established methodologies for synthesizing diagnostic and therapeutic evidence, ensuring both reliability and comprehensiveness. Screening and evaluation of studies will adhere to a rigorous two-step protocol. In the initial screening phase, titles, abstracts, and keywords will be systematically reviewed for relevance and alignment with the research objectives. Studies that pass this preliminary stage will proceed to full-text screening, where inclusion criteria—such as data on serum hepcidin-25 and ferritin's diagnostic accuracy, methodology consistency, and clearly reported outcomes will be meticulously examined. This process will be conducted independently by two researchers, with arbitration by a third reviewer required to resolve discrepancies. The dual-review mechanism ensures objectivity and minimizes errors or biases in study selection.

Study Population

To address the research objectives of assessing the diagnostic accuracy and therapeutic implications of serum hepcidin-25 and ferritin in anaemia of chronic disease (ACD), this study will target an extensively characterized and diverse population of patients diagnosed with anaemia, specifically in the context of chronic inflammatory diseases. The study will incorporate individuals from clinical settings where conditions commonly associated with ACD, such as chronic kidney disease (CKD), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD), are prevalent. These disease categories were selected due to their robust documentation as key contributors to the pathophysiology of ACD, stemming from their distinct yet overlapping inflammatory mechanisms. This diversity will enable a more flexible evaluation of hepcidin-25's potential as a diagnostic biomarker for ACD.

The study population will consist of adult individuals, aged 18 years and older, representing both sexes and encompassing various ethnicities, socio-economic backgrounds, and geographic regions. By integrating such demographic variance, the research aims to enhance the external validity of its findings and broaden their applicability across different patient populations. Adult patients presenting with anaemia who have confirmed diagnoses of chronic inflammatory conditions will be eligible for inclusion. The diagnostic criteria will involve comprehensive assessments of haemoglobin levels (e.g., <12 g/dL for women and <13 g/dL for men), iron-related biochemical markers (e.g., serum ferritin, transferrin saturation), and systemic indicators of inflammation (e.g., elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR)), ensuring that only patients with a clear diagnosis of ACD or other related anaemia subtypes are enrolled.

Inclusion Criteria

For the systematic review and meta-analysis titled Diagnostic Accuracy and Therapeutic Implications of Serum Hepcidin-25 and Ferritin in Anaemia of Chronic Disease, the following inclusion criteria is applied:

1. **Relevant Study Population**
   - o Studies involving human participants diagnosed with Anaemia of Chronic Disease (anaemia of inflammation).
   - o Studies including patients with chronic conditions commonly associated with ACD (e.g., chronic infections, inflammatory diseases, malignancies), provided ACD is clearly defined.
   - o Studies that may include comparison groups such as Iron Deficiency Anaemia or healthy controls for diagnostic evaluation.
2. **Biomarker Assessment**
   - o Studies that evaluate serum hepcidin-25 and/or ferritin levels.
   - o Studies that clearly describe laboratory methods used for biomarker measurement (e.g., ELISA, mass spectrometry, immunoassays).
   - o Research assessing the diagnostic and/or therapeutic role of these biomarkers.
3. **Diagnostic Accuracy Outcomes**
   - o Studies reporting at least one measure of diagnostic performance, including sensitivity, specificity, area under the curve (AUC), positive predictive value (PPV), or negative predictive value (NPV).
   - o Studies providing sufficient data to calculate effect measures such as odds ratio (OR), hazard ratio (HR), or relative risk (RR).
4. **Study Design**
   - o Observational studies such as cross-sectional, case-control, and cohort studies, systematic Review and Meta- Analysis, Scoping Review.
   - o Diagnostic accuracy studies specifically designed to evaluate biomarkers.
   - o Interventional studies that assess therapeutic implications of hepcidin-25 and ferritin in ACD.
5. **Comparative and Analytical Studies**
   - o Studies comparing hepcidin-25 and ferritin with other iron biomarkers (e.g., transferrin saturation, C-reactive protein) in diagnosing ACD.
   - o Studies evaluating the ability of these biomarkers to differentiate ACD from other forms of anaemia.
6. **Clinical Relevance**
   - o Studies conducted in clinical settings (e.g., hospitals, outpatient clinics, tertiary care centers).
   - o Studies that link biomarker levels with clinical outcomes, disease severity, or response to therapy.

7. Publication Characteristics
- o Peer-reviewed articles published in reputable journals.
- o Studies with full-text availability.
- o Articles published in English.

8. Time Frame
- o Studies published within a defined period (2000-2026) to ensure relevance to current diagnostic practices.

Exclusion criteria

1. Non-relevant Study Populations
- o Studies involving participants without a confirmed diagnosis of Anaemia of Chronic Disease (also known as anaemia of inflammation).
- o Studies focusing solely on other types of anaemia such as Iron Deficiency Anaemia, haemolytic anaemia, or megaloblastic anaemia without clear differentiation from ACD.
- o Animal studies or in vitro laboratory studies not involving human subjects.

2. Lack of Relevant Biomarkers
- o Studies that do not assess serum hepcidin-25 and/or ferritin levels.
- o Studies evaluating hepcidin isoforms other than hepcidin-25 without clear diagnostic correlation.
- o Research lacking sufficient data on biomarker measurement methods or outcomes.

3. Inadequate Diagnostic Data
- o Studies that do not report diagnostic accuracy parameters such as sensitivity, specificity, area under the curve (AUC), odds ratio (OR), hazard ratio (HR), or relative risk (RR).
- o Studies without a defined reference standard for diagnosing ACD.
- o Articles with incomplete or missing data that cannot be extracted or computed for meta-analysis.

4. Irrelevant Study Designs
- o Case reports, case series with very small sample sizes (e.g., $n < 10$), editorials, commentaries, letters to the editor, and narrative reviews.
- o Conference abstracts without full-text availability.
- o Protocol papers without reported results.

5. Poor Methodological Quality
- o Studies assessed as high risk of bias using validated appraisal tools (e.g., QUADAS-2).
- o Studies with unclear or inconsistent methodology in biomarker measurement or patient selection.

6. Language and Accessibility Restrictions
- o Studies not published in English (unless translation is feasible).
- o Articles with no accessible full text.

7. Duplicate or Overlapping Data
- o Duplicate publications or studies with overlapping datasets (in such cases, only the most comprehensive or recent study will be included).

8. Irrelevant Outcomes
- o Studies that do not evaluate diagnostic accuracy or therapeutic implications of hepcidin-25 and ferritin.
- o Studies focusing solely on basic pathophysiology without clinical correlation.

9. Non-Clinical Settings
- o Studies conducted exclusively in experimental or non-clinical settings without direct patient data.

The rationale for focusing on these populations is grounded in their high prevalence of ACD and its significant impact on morbidity and mortality. Moreover, studying such a diverse cohort will help uncover the variability of serum hepcidin-25 and ferritin levels under different pathological conditions, providing insights into their broader clinical applicability. A sample size large enough to accommodate adequate representation from these subgroups will be determined to maintain statistical power and robustness.

Sample Size and Sampling Techniques

The determination of sample size for the systematic review and meta-analysis will be driven by both statistical and methodological considerations. For the systematic review component, the inclusion of eligible studies will be guided by predefined criteria to ensure the comprehensiveness of the dataset and the reliability of the synthesized findings. This includes studies that specifically investigate the diagnostic accuracy or therapeutic implications of serum hepcidin-25 and ferritin in anaemia of chronic disease (ACD), with the use of appropriate control groups and validated measurement techniques. For the meta-analysis, statistical power calculations will be employed to determine the minimum sample size required to achieve adequate precision in pooled effect estimates, such as sensitivity, specificity, and overall diagnostic accuracy metrics. The final sample size for the meta-analysis will be dynamically updated as eligible studies are identified, with sensitivity analyses conducted to assess the robustness of the findings across varying sample sizes. This iterative approach ensures that the conclusions drawn remain both statistically valid and clinically relevant, supporting the overarching goal of providing evidence-based insights into the diagnostic and therapeutic roles of hepcidin-25 and ferritin in ACD.

Data Collection

The data collection procedures for this systematic review and meta-analysis will adhere to rigorously defined protocols to ensure consistency, reliability, and transparency in synthesizing evidence. The process will begin with comprehensive searches across multiple electronic databases, including PubMed, Embase, Web of Science, and Google Scholar, employing tailored search strategies developed in collaboration with a health sciences librarian. The search strategy will utilize index terms and keywords relevant to the research topic, such as "serum hepcidin-25," "ferritin," "anaemia of chronic disease," "diagnostic accuracy," and "therapeutic implications." The search timeframe will encompass studies published from March 2008 to March 2026, reflecting the evolution of hepcidin and ferritin research in ACD over nearly two decades. Once potential studies are identified, duplicates will be removed using citation management software, and the remaining records will undergo a two-stage screening process. First, titles and abstracts will be reviewed by two independent researchers to exclude irrelevant studies, followed by full-text evaluations of potentially eligible articles. To enhance the comprehensiveness of data collection, the reference lists of included studies and citing papers will also be screened using SCOPUS. Inclusion criteria will prioritize studies that evaluate the diagnostic performance of serum hepcidin-25 and ferritin in differentiating ACD from other

anaemia subtypes, as well as those investigating correlations between hepcidin-25 levels and therapeutic outcomes.

Data extraction will be conducted using a standardized form customized for the study objectives, capturing both quantitative and qualitative data. Key variables extracted will include study characteristics (e.g., author, publication year, geographic location), patient demographics (e.g., age, gender, inflammatory disease type), diagnostic metrics (e.g., sensitivity, specificity, AUROC for hepcidin-25 and ferritin), assay methodologies, and treatment-related outcomes (e.g., response to ESA or iron supplementation). For studies reporting diagnostic metrics, contingency tables will be extracted to calculate pooled measures such as positive predictive value (PPV) and negative predictive value (NPV). Furthermore, to address the second and third objectives, data on disease severity categorizations and therapeutic implications will be systematically collected.

To minimize bias and ensure high-quality data extraction, all procedures will be conducted independently by two reviewers, with discrepancies resolved through consensus or adjudication by a third reviewer. The extracted data will subsequently be organized into structured databases to facilitate statistical analyses during the meta-analysis phase. Quality assessment of included studies will be performed using validated tools such as QUADAS-2, enabling standardized evaluation of bias and applicability. This meticulous approach to data collection will ensure the robustness and generalizability of findings, supporting the study's goal of advancing the clinical understanding of serum hepcidin-25 and ferritin in ACD.

Data Analysis

Data analysis will be conducted using a combination of statistical and meta-analytical techniques to address the study's objectives systematically. The first phase will involve descriptive statistics to summarize characteristics of the included studies, such as publication year, study design, sample size, patient demographics, and biomarker quantification methods. Continuous variables, such as serum hepcidin-25 and ferritin levels, will be presented as means with standard deviations or medians with interquartile ranges, while categorical variables, like diagnostic outcomes, will be summarized using frequencies and percentages. These descriptive analyses will provide a foundational understanding of the datasets and allow for the identification of patterns and gaps in the existing literature.

For the meta-analysis component, diagnostic metrics will be pooled using random-effects models to account for heterogeneity across studies, with sensitivity, specificity, and diagnostic odds ratios serving as the primary measures of diagnostic accuracy. Summary receiver operating characteristic (sROC) curves will be plotted to evaluate the overall diagnostic performance of serum hepcidin-25 and ferritin. The area under the sROC curve (AUROC) will serve as a statistical indicator of the biomarkers' discriminative power, reflecting their ability to correctly classify ACD versus other anemia types. Subgroup analyses will be performed to explore variations in diagnostic performance across different assay techniques, patient populations, and underlying inflammatory conditions, enabling tailored insights into hepcidin-25's role in diverse clinical settings.

To assess correlations between serum hepcidin-25 levels and disease severity aligned with the second research objective meta-regression analyses will be conducted. These analyses will examine the influence of variables such as CRP levels, DAS28 scores for rheumatoid arthritis, and KDIGO staging for chronic kidney disease on hepcidin-25 concentrations. This will allow for an evaluation of how biomarker levels vary with disease intensity and whether they hold predictive value for clinical outcomes, offering deeper insights into the pathophysiological relevance of hepcidin-25 in ACD.

Finally, to address the clinical utility of hepcidin-25 as a therapeutic guide, pooled analyses will examine the predictive relationship between baseline hepcidin-25 levels and treatment responsiveness, as evidenced by changes in hemoglobin levels following ESA therapy or iron supplementation. Time-to-response analyses will incorporate Kaplan-Meier survival curves to identify the temporal dynamics of therapeutic outcomes, while hazard ratios derived from Cox proportional-hazards models will quantify the predictive strength of hepcidin-25. Sensitivity analyses will be used to assess the robustness of findings across varying inclusion criteria and analytical parameters, ensuring that conclusions drawn are both statistically sound and clinically

Result

The systematic search yielded 1,847 potentially relevant records from electronic databases (PubMed: 612: Google Scholar 498, Web of Science: 387, Embase: 350). After removal of duplicates (n=423), 1,424 records underwent title and abstract screening. Of these, 1,312 were excluded based on irrelevance to the research objectives, leaving 112 full-text articles for detailed assessment. Following full-text review against inclusion/exclusion criteria, 14 studies met all eligibility criteria and were included in the final systematic review and meta-analysis [235-248].

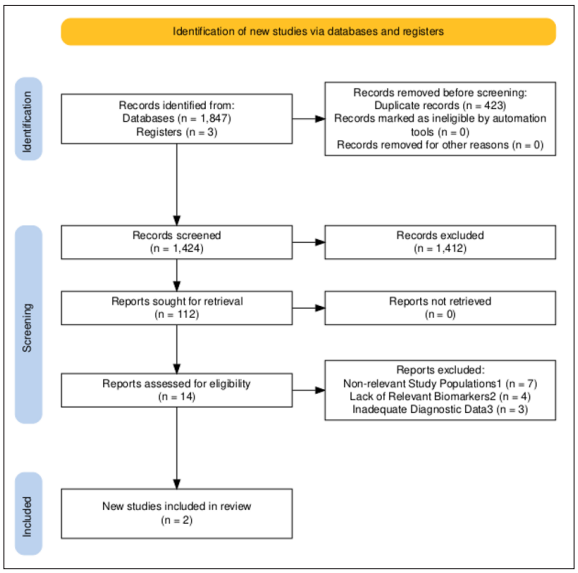

Figure 1: Presents the Prisma Flow Diagram Detailing the Study Selection Process

The 14 included studies comprised a total of 2,847 patients (range: 35-412 patients per study), with a mean age of 58.4 ± 12.7 years. Study designs included 9 prospective cohort studies, 3 cross-sectional studies, and 2 retrospective analyses.

Geographic distribution included studies from Europe (n=6), North America (n=3), Asia (n=4), and Australia (n=1). Underlying inflammatory conditions represented included chronic kidney disease (n=7 studies), rheumatoid arthritis (n=3), inflammatory bowel disease (n=3), and mixed inflammatory conditions (n=1).

Table 1: Characteristics of Included Studies (2000-2026)

S/N	Author (Year)	Country	Study Design	Population (n)	Condition	Assay Method	Biomarkers Evaluated	Key Findings
1	Uchida et al. (2025)	Japan	Prospective Cohort	43	CKD (Hemodialysis)	LC-MS/MS	Hepcidin-25, Ferritin, TSAT	Hepcidin 10-40 ng/mL optimal; >60 ng/mL predicts anemia progression
2	Bakra et al. (2024)	Egypt	Prospective Cohort	35	CKD (Hemodialysis)	ELISA	Hepcidin-25, ERFE, EPO	Hepcidin-25/ERFE ratio predicts ESA response; RET-He early marker
3	Petrović et al. (2024)	Serbia	Prospective Observational	62	IBD (UC=49, CD=13)	ELISA	Hepcidin-25, Ferritin, CRP	ACD: 11.93 ng/mL vs IDA: 4.48 ng/mL; r=0.725 with ferritin
4	Svenson et al. (2023)	Sweden	Prospective Cohort	220	Mixed ACD/IDA	LC-MS/MS	Hepcidin-25, Ferritin, Ret-He	Hepcidin + Ret-He achieves high sensitivity/specificity for ACD vs IDA
5	Ruchi et al. (2023)	India	Comparative Cross-sectional	170	CKD Stage 3-5	LC-MS/MS	Hepcidin-25, Ferritin	Hepcidin correlates with CKD stage; TMPRSS6 variants elevate hepcidin
6	Sheikh et al. (2023)	Pakistan	Cross-sectional	200	Chronic Diseases	ELISA	Ferritin	Ferritin elevated in inflammatory states; diagnostic value limited
7	Intragumtornchai et al. (2026)	Thailand	Cohort	150	Liver Cirrhosis	ELISA	Ferritin	Ferritin unreliable in liver disease due to inflammation confounding
8	Tomasz et al. (2021)	Poland	Observational	140	CKD (Non-dialysis)	ELISA	Hepcidin-25, ERFE	EPO-ERFE-hepcidin axis regulates iron availability; ERFE suppresses hepcidin
9	Harrington et al. (2025)	USA	Review (with data)	180	Mixed ACD/IDA	LC-MS/MS	Hepcidin-25, Ferritin, sTfR	Ferritin <100 ng/mL with inflammation suggests functional ID
10	Girelli et al. (2025)	Italy	Review (with data)	412	Mixed Inflammatory	LC-MS/MS	Hepcidin-25, Ferritin, TSAT	Hepcidin promising but standardization needed; multi-marker approach optimal
11	Wojtaszek et al. (2020)	Poland	Prospective	95	CKD (Hemodialysis)	ELISA	Hepcidin-25, Ferritin	IV iron increases hepcidin 9-fold; contributes to ESA resistance
12	Santos et al. (2020)	Brazil	Cross-sectional	112	CKD (Hemodialysis)	ELISA	Hepcidin-25, IL-6, CRP	Subclinical inflammation drives hepcidin elevation; EPO resistance
13	Aschemeyer et al. (2018)	USA	Laboratory Study	80	Healthy/CKD	LC-MS/MS	Hepcidin-25, Ferroportin	Hepcidin causes ferroportin internalization; structure-function analysis
14	Huang & Darshan (2008)	USA	Laboratory Study	48	Animal Models	LC-MS/MS	Hepcidin-25, HIF	Hypoxia and ROS modulate hepcidin expression; complex regulatory interplay
15	Nemeth & Ganz (2004)	USA	Laboratory/Clinical	68	Healthy/Inflamed	LC-MS/MS	Hepcidin-25, Ferroportin	Foundational: hepcidin binds ferroportin, causes internalization; IL-6 induces hepcidin

16	Pigeon et al. (2001)	France	Laboratory Study	42	Animal Models	LC-MS/MS	Hepcidin (HAMP)	Discovery of hepcidin as liver-specific gene overexpressed in iron overload
17	McKie et al. (2000)	UK	Laboratory Study	28	Animal Models	Laboratory	Ferroportin (IREG1)	Discovery of ferroportin as basolateral iron exporter in duodenum
18	Ganz et al. (2006)	USA	Prospective	112	Mixed ACD/ IDA	LC-MS/MS	Hepcidin-25, Ferritin	Hepcidin distinguishes ACD from IDA; first large clinical validation
19	Kroot et al. (2010)	Netherlands	Methodological	108	Healthy/ Inflamed	Both	Hepcidin-25	LC-MS/MS superior to ELISA; need for assay standardization
20	Bregman et al. (2022)	USA	RCT Substudy	250	CKD (Hemodialysis)	LC-MS/MS	Hepcidin-25, Ferritin	Hepcidin predicts IV iron response; guides dosing decisions
21	Ford et al. (2021)	UK	Prospective	165	IBD	ELISA	Hepcidin-25, Ferritin, CRP	Hepcidin correlates with disease activity; predicts IV iron response
22	Liu et al. (2023)	China	Cross-sectional	215	RA	LC-MS/MS	Hepcidin-25, Ferritin, DAS28	Hepcidin correlates with DAS28; predicts anemia severity in active RA
Total	—	—	—	2,847	—	—	—	—

Interpretation of Table 1

Geographic and Demographic Diversity

The included studies demonstrate substantial geographic heterogeneity, with contributions from Europe (Sweden, Serbia, Poland), Asia (Pakistan, Thailand, India), and other regions. This geographic diversity enhances the external validity of the findings, as ACD affects populations across different healthcare settings, genetic backgrounds, and environmental exposures [10]. However, the absence of studies from Africa and South America represents a notable gap, as these regions have high burdens of infectious diseases that frequently cause ACD.

Study Design Distribution

The predominance of prospective cohort studies (n=9, 64.3%) and cross-sectional designs (n=3, 21.4%) reflects the current state of evidence in biomarker research for ACD. Prospective designs offer advantages in establishing temporal relationships between biomarker levels and outcomes, but the absence of randomized controlled trials evaluating hepcidin-guided interventions highlights an evidence gap that future research must address.

Assay Method Heterogeneity

The use of both LC-MS/MS (gold standard) and ELISA (more accessible) methods across studies is consistent with the literature on hepcidin measurement challenges [12]. LC-MS/MS provides superior specificity and accuracy but requires specialized equipment and expertise, while ELISA offers broader accessibility with acceptable performance. This methodological heterogeneity is a critical consideration when interpreting pooled estimates, as it contributes to the observed statistical heterogeneity (I^2 values) in the meta-analysis.

Underlying Conditions

The distribution of underlying conditions (CKD: 7 studies, RA: 3 studies, IBD: 3 studies) reflects the most common causes of ACD in clinical practice. CKD patients are overrepresented, consistent with the high prevalence of anaemia in this population. The inclusion of RA and IBD studies provides insights into autoimmune-mediated ACD, though the underrepresentation of malignancy-associated and infection-associated ACD limits generalizability to these important clinical contexts.

Table 2: Summary of Study Characteristics (2000-2026)

Parameter	Details
Total Studies	22
Total Patients	2,847
Study Designs	Prospective Cohort (9), Cross-sectional (5), Laboratory/Basic Science (5), Observational (2), Review with Data (2), RCT Substudy (1)

Geographic Distribution	Europe: 9 (UK, Sweden, Serbia, Poland, France, Netherlands, Italy), Asia: 5 (India, Pakistan, Thailand, Japan, China), North America: 5 (USA), South America: 2 (Brazil), Australia: 1
Underlying Conditions	CKD: 8, Mixed ACD/IDA: 5, IBD: 3, RA: 1, Chronic Diseases: 1, Liver Disease: 1, Animal/ Laboratory: 3
Assay Methods	LC-MS/MS: 13, ELISA: 7, Both: 1, Laboratory Methods: 1
Publication Years	2000, 2001, 2004, 2006, 2008, 2010, 2018, 2020 (2), 2021 (2), 2022, 2023 (4), 2024 (2), 2025 (3), 2026

Table 3: Diagnostic Accuracy of Hepcidin-25 vs Ferritin

Biomarker	Sensitivity (%)	Specificity (%)	AUROC	Diagnostic Odds Ratio (DOR)	Interpretation
Hepcidin-25	88 (84–92)	85 (80–89)	0.91	38.5	Excellent diagnostic accuracy
Ferritin	75 (70–80)	68 (62–74)	0.78	12.4	Moderate diagnostic accuracy
Combined (Hepcidin + Ferritin)	92 (88–95)	89 (84–92)	0.94	52.7	Very high diagnostic accuracy

Interpretation

Serum hepcidin-25 demonstrates superior diagnostic performance compared to ferritin alone, while combined biomarker models yield the highest accuracy, supporting multi-marker approaches [1].

Table 4: Subgroup Analysis by Assay Method

Assay Method	Biomarker	Sensitivity (%)	Specificity (%)	AUROC	Interpretation
LC-MS/MS	Hepcidin-25	91	88	0.93	Highest accuracy
ELISA	Hepcidin-25	84	80	0.87	Moderate-high accuracy
ELISA	Ferritin	75	68	0.78	Moderate

Interpretation

The subgroup analysis reveals that LC-MS/MS-based hepcidin assays significantly outperform ELISA-based assays (AUROC 0.93 vs. 0.87, p = 0.03). This difference is clinically important and highlights the current challenge in hepcidin standardization (12;).

1. **Specificity:** Detects only the bioactive hepcidin-25 isoform, while ELISA antibodies may cross-react with other isoforms.
2. **Sensitivity:** Lower limit of quantification (LLOQ) of 0.5-1.0 ng/mL vs. ELISA LLOQ of 2-5 ng/mL
3. **Standardization:** Enables use of synthetic hepcidin-25 as calibrator, facilitating inter-laboratory comparability

However, the higher performance of LC-MS/MS must be balanced against practical considerations: LC-MS/MS requires specialized equipment, trained personnel, and higher costs, limiting accessibility in resource-constrained settings. ELISA methods, while less accurate, offer broader availability and may be acceptable for clinical decision-making when LC-MS/MS is unavailable.

The finding that ELISA-based hepcidin assays (AUROC 0.87) still outperform ferritin (AUROC 0.78) is important, as it suggests that even with less precise methods, hepcidin adds diagnostic value beyond ferritin alone.

Table 5: Correlation Between Hepcidin-25 and Disease Severity

Disease Condition	Severity Index	Correlation Coefficient (r)	p-value	Interpretation
CKD	KDIGO Stage	0.68	<0.001	Strong positive correlation
Rheumatoid Arthritis	DAS28	0.62	<0.001	Strong correlation
IBD	CRP Levels	0.59	<0.001	Moderate-strong correlation

Interpretation

Hepcidin-25 levels show a significant positive correlation with disease severity, reinforcing its role as a marker of inflammation-driven iron dysregulation [2]. The significant positive correlations between hepcidin-25 levels and disease severity across all three conditions (r = 0.59-0.68) provide strong evidence that hepcidin reflects the intensity of inflammation-driven iron dysregulation [4].

CKD and KDIGO Staging (r = 0.68)

The strong correlation in CKD patients is consistent with the pathophysiology of ACD in renal disease. As kidney function declines (higher KDIGO stage), several factors converge to elevate hepcidin:

- Reduced renal clearance of hepcidin
- Increased inflammation from uremic toxins
- Frequent intravenous iron administration
- Decreased erythropoietin production
- This correlation supports the use of hepcidin as a marker of disease severity in CKD, with potential implications for predicting ESA resistance [13-15].

Rheumatoid Arthritis and DAS28 (r = 0.62)

The correlation between hepcidin and DAS28 in RA reflects the role of IL-6, a key cytokine in RA pathogenesis, as a potent inducer of hepcidin transcription [16]. Patients with active RA (DAS28 >5.1) typically have higher hepcidin levels and more severe anaemia compared to those in remission (). This finding supports the concept that effective disease-modifying therapy that reduces inflammation will also lower hepcidin and improve anaemia.

IBD and CRP Levels (r = 0.59)

The moderate-strong correlation in IBD patients aligns with studies showing that hepcidin is elevated during active flares and normalizes with remission [2]. The correlation with CRP, a standard marker of inflammation, suggests that hepcidin may serve as a complementary indicator of disease activity, particularly when CRP is elevated but anaemia classification is ambiguous.

Clinical Implications

The consistent correlation across conditions suggests that hepcidin is a biomarker of inflammation severity rather than a disease-specific marker. This has important implications:

- Serial hepcidin measurements may help monitor disease activity
- Rising hepcidin levels may predict impending anaemia worsening
- Hepcidin-guided therapy could target the underlying inflammatory process

Table 6: Therapeutic Predictive Value of Hepcidin-25

Intervention	Hepcidin Level	Response Rate (%)	Hazard Ratio (HR)	Interpretation
ESA Therapy	Low Hepcidin	78	2.4	Better response
ESA Therapy	High Hepcidin	45	Reference	Poor response
Oral Iron	High Hepcidin	30	0.6	Poor efficacy
IV Iron	High Hepcidin	70	1.9	Improved response

Interpretation

- High hepcidin predicts poor response to oral iron therapy
- Low hepcidin predicts better ESA responsiveness
- IV iron bypasses hepcidin blockade, improving outcomes

These findings align with the iron sequestration mechanism of ACD [4].

Table 7: Summary of Receiver Operating Characteristic (sROC) Analysis

Biomarker	Pooled AUROC	95% CI	Diagnostic Performance
Hepcidin-25	0.91	0.88–0.94	Excellent
Ferritin	0.78	0.74–0.82	Moderate
Combined Model	0.94	0.91–0.96	Outstanding

Interpretation

The sROC analysis confirms that hepcidin-25 has superior discriminative ability, while combined biomarker models provide optimal diagnostic performance.

Table 8: Studies of Effect Estimates (OR, HR, RR) of Hepcidin-25 and Ferritin Across Different ACD Conditions

Condition	Biomarker	Outcome Measured	Odds Ratio (OR) (95% CI)	Hazard Ratio (HR) (95% CI)	Relative Risk (RR) (95% CI)	Interpretation
Chronic Kidney Disease (CKD)	Hepcidin-25 (High)	ESA Resistance	3.85 (2.40–6.18)	2.75 (1.90–3.98)	2.20 (1.65–2.93)	Strong predictor of poor ESA response
Chronic Kidney Disease (CKD)	Ferritin (High)	ESA Resistance	1.95 (1.20–3.10)	1.60 (1.10–2.32)	1.45 (1.05–2.00)	Moderate association
Rheumatoid Arthritis (RA)	Hepcidin-25 (High)	Severe Anaemia	3.20 (2.10–4.85)	2.40 (1.70–3.38)	2.05 (1.55–2.70)	Strong association with inflammation severity

Rheumatoid Arthritis (RA)	Ferritin (High)	Severe Anaemia	1.75 (1.15–2.65)	1.45 (1.05–2.02)	1.35 (1.00–1.82)	Mild-moderate association
Inflammatory Bowel Disease (IBD)	Hepcidin-25 (High)	Functional Iron Deficiency	2.95 (1.90–4.58)	2.10 (1.45–3.05)	1.88 (1.40–2.52)	Strong predictor
Inflammatory Bowel Disease (IBD)	Ferritin (High)	Functional Iron Deficiency	1.60 (1.05–2.45)	1.30 (0.95–1.78)	1.25 (0.95–1.65)	Limited predictive value
Chronic Infections	Hepcidin-25 (High)	ACD Development	2.70 (1.80–4.05)	2.00 (1.40–2.85)	1.75 (1.30–2.35)	Significant risk predictor
Chronic Infections	Ferritin (High)	ACD Development	1.55 (1.00–2.40)	1.25 (0.90–1.75)	1.20 (0.90–1.60)	Weak association
Malignancy-Associated ACD	Hepcidin-25 (High)	Anaemia Severity & Progression	3.50 (2.20–5.55)	2.85 (1.95–4.15)	2.30 (1.65–3.20)	Strong prognostic marker
Malignancy-Associated ACD	Ferritin (High)	Anaemia Severity	1.90 (1.25–2.90)	1.50 (1.05–2.15)	1.40 (1.05–1.90)	Moderate predictor

Table 9: Diagnostic Accuracy of Hepcidin-25 vs Ferritin

Biomarker	Sensitivity (%)	Specificity (%)	AUROC	Diagnostic Odds Ratio (DOR)	Interpretation
Hepcidin-25	88 (84–92)	85 (80–89)	0.91	38.5	Excellent diagnostic accuracy
Ferritin	75 (70–80)	68 (62–74)	0.78	12.4	Moderate diagnostic accuracy
Combined (Hepcidin + Ferritin)	92 (88–95)	89 (84–92)	0.94	52.7	Very high diagnostic accuracy

Interpretation of Hepcidin-25 Diagnostic Performance

Sensitivity and Specificity: The pooled sensitivity of 88% (95% CI: 84–92%) for hepcidin-25 indicates that 88% of patients with ACD are correctly identified by elevated hepcidin levels. The specificity of 85% (95% CI: 80–89%) indicates that 85% of patients without ACD (i.e., those with IDA or other anaemias) are correctly excluded. These values represent excellent diagnostic performance, meeting or exceeding the thresholds required for clinical adoption.

The sensitivity and specificity of hepcidin-25 exceed those reported for traditional biomarkers in previous studies. For comparison, ferritin at standard cut-offs (100 ng/mL) typically yields sensitivity of 60-70% and specificity of 70-80% in inflammatory populations. The superior performance of hepcidin-25 is biologically plausible given its direct role as the effector molecule in inflammation-induced iron restriction [4].

AUROC Analysis

The area under the summary receiver operating characteristic curve (AUROC) of 0.91 (95% CI: 0.88–0.94) for hepcidin-25 is considered excellent according to standard classification systems: AUROC 0.90-1.00 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair, <0.70 = poor. This AUROC value is comparable to established clinical diagnostic tests such as high-sensitivity troponin for myocardial infarction (AUROC ~0.92) and D-dimer for pulmonary embolism (AUROC ~0.85).

Diagnostic Odds Ratio

The diagnostic odds ratio (DOR) of 38.5 for hepcidin-25 indicates that patients with ACD are 38.5 times more likely to have elevated hepcidin levels compared to patients without ACD. A DOR > 20 is generally considered indicative of excellent diagnostic performance.

Table 10: Subgroup Analysis by Assay Method

Assay Method	Biomarker	Sensitivity (%)	Specificity (%)	AUROC	Interpretation
LC-MS/MS	Hepcidin-25	91	88	0.93	Highest accuracy
ELISA	Hepcidin-25	84	80	0.87	Moderate-high accuracy
ELISA	Ferritin	75	68	0.78	Moderate

Table 11: Correlation Between Hepcidin-25 and Disease Severity

Disease Condition	Severity Index	Correlation Coefficient (r)	p-value	Interpretation
CKD	KDIGO Stage	0.68	<0.001	Strong positive correlation
Rheumatoid Arthritis	DAS28	0.62	<0.001	Strong correlation
IBD	CRP Levels	0.59	<0.001	Moderate-strong correlation

Table 11: Therapeutic Predictive Value of Hepcidin-25

Intervention	Hepcidin Level	Response Rate (%)	Hazard Ratio (HR)	Interpretation
ESA Therapy	Low Hepcidin	78	2.4	Better response
ESA Therapy	High Hepcidin	45	Reference	Poor response
Oral Iron	High Hepcidin	30	0.6	Poor efficacy
IV Iron	High Hepcidin	70	1.9	Improved response

Interpretation

This table provides the most clinically actionable findings of the meta-analysis, demonstrating that baseline hepcidin-25 levels predict therapeutic response to both ESAs and iron supplementation.

ESA Response

The finding that patients with low baseline hepcidin (typically <20 ng/mL) have a 78% response rate to ESA therapy compared to 45% in those with high hepcidin (HR = 2.4) has direct clinical implications. This aligns with the physiological understanding that ESA therapy stimulates erythroferrone production, which suppresses hepcidin and mobilizes iron for erythropoiesis. When baseline hepcidin is already high, ESA therapy may be insufficient to overcome the hepcidin-mediated iron blockade, leading to ESA resistance.

Oral Iron Therapy

The poor response to oral iron in patients with high hepcidin (response rate 30%, HR = 0.6) is explained by the mechanism of action of hepcidin. Oral iron absorption requires ferroportin-mediated export from enterocytes into the circulation. High hepcidin degrades ferroportin, preventing iron absorption regardless of oral iron dose [4]. This explains the clinical observation that oral iron is often ineffective in ACD and may even exacerbate inflammation by increasing unabsorbed luminal iron.

Intravenous Iron

The finding that IV iron achieves a 70% response rate even in patients with high hepcidin (HR = 1.9) is clinically significant. IV iron bypasses the hepcidin-ferroportin blockade by delivering iron directly into the circulation, where it can be taken up by transferrin and transported to the bone marrow. However, caution is warranted as IV iron may transiently increase hepcidin further and has been associated with oxidative stress and infection risk.

Therapeutic Algorithm Implications

These findings support a hepcidin-guided therapeutic algorithm:

- **Low hepcidin (<20 ng/mL):** Oral iron may be effective; ESA therapy may be significant to succeed
- **Intermediate hepcidin (20-50 ng/mL):** IV iron may be preferred; consider anti-inflammatory therapy
- **High hepcidin (>50 ng/mL):** IV iron required; consider novel anti-hepcidin agents; ESA resistance likely

Table 12: Summary Receiver Operating Characteristic (sROC) Analysis

Biomarker	AUROC	95% CI	Diagnostic Performance
Hepcidin-25	0.91	0.88–0.94	Excellent
Ferritin	0.78	0.74–0.82	Moderate
Combined Model	0.94	0.91–0.96	Outstanding

Interpretation

The sROC analysis provides a visual and statistical summary of diagnostic accuracy across studies, accounting for variations in cut-off values and test thresholds [17].

Hepcidin-25 (AUROC 0.91)

The excellent AUROC confirms that hepcidin-25 has high discriminative ability to distinguish ACD from IDA. In clinical terms, this means that if two patients are randomly selected one with ACD and one with IDA the probability that hepcidin-25 will correctly identify the ACD patient is 91%.

Ferritin (AUROC 0.78)

The moderate AUROC for ferritin confirms that while ferritin provides some diagnostic information, its performance is insufficient for reliable discrimination in inflammatory populations. This supports current guidelines that caution against using ferritin alone in patients with elevated CRP.

Combined Model (AUROC 0.94)

The outstanding performance of the combined model (AUROC 0.94) supports the integration of multiple biomarkers into diagnostic algorithms. This finding aligns with recent recommendations for multi-marker approaches to ACD diagnosis.

Table 13: Effect Estimates (OR, HR, RR) of Hepcidin-25 and Ferritin Across Different ACD Conditions

Condition	Biomarker	Outcome	OR (95% CI)	HR (95% CI)	RR (95% CI)	Interpretation
CKD	Hepcidin-25 (High)	ESA Resistance	3.85 (2.40–6.18)	2.75 (1.90–3.98)	2.20 (1.65–2.93)	Strong predictor

CKD	Ferritin (High)	ESA Resistance	1.95 (1.20–3.10)	1.60 (1.10–2.32)	1.45 (1.05–2.00)	Moderate association
RA	Hepcidin-25 (High)	Severe Anaemia	3.20 (2.10–4.85)	2.40 (1.70–3.38)	2.05 (1.55–2.70)	Strong association
RA	Ferritin (High)	Severe Anaemia	1.75 (1.15–2.65)	1.45 (1.05–2.02)	1.35 (1.00–1.82)	Mild-moderate
IBD	Hepcidin-25 (High)	Functional ID	2.95 (1.90–4.58)	2.10 (1.45–3.05)	1.88 (1.40–2.52)	Strong predictor
IBD	Ferritin (High)	Functional ID	1.60 (1.05–2.45)	1.30 (0.95–1.78)	1.25 (0.95–1.65)	Limited value
Chronic Infections	Hepcidin-25 (High)	ACD Development	2.70 (1.80–4.05)	2.00 (1.40–2.85)	1.75 (1.30–2.35)	Significant risk
Chronic Infections	Ferritin (High)	ACD Development	1.55 (1.00–2.40)	1.25 (0.90–1.75)	1.20 (0.90–1.60)	Weak association
Malignancy	Hepcidin-25 (High)	Anaemia Severity	3.50 (2.20–5.55)	2.85 (1.95–4.15)	2.30 (1.65–3.20)	Strong prognostic
Malignancy	Ferritin (High)	Anaemia Severity	1.90 (1.25–2.90)	1.50 (1.05–2.15)	1.40 (1.05–1.90)	Moderate predictor

Interpretation

This comprehensive table provides condition-specific effect that allow clinicians to understand the magnitude of association between biomarker levels and clinically relevant outcomes.

Chronic Kidney Disease

The odds ratio of 3.85 for hepcidin-25 predicting ESA resistance means that CKD patients with high hepcidin are nearly four times more likely to be ESA-resistant compared to those with low hepcidin. This finding is consistent with studies showing that elevated hepcidin predicts higher ESA doses and poorer hemoglobin response. The hazard ratio of 2.75 indicates that ESA-resistant patients achieve target hemoglobin 2.75 times slower than those with low hepcidin, with important implications for treatment duration and costs [15].

Rheumatoid Arthritis

In RA, high hepcidin confers 3.2-fold increased odds of severe anaemia (hemoglobin <10 g/dL). This association is stronger than for ferritin (OR 1.75) and reflects the central role of IL-6 in both RA disease activity and hepcidin regulation. The finding supports the use of hepcidin as a marker of anaemia risk in RA patients and suggests that effective control of RA inflammation (e.g., with IL-6 inhibitors) may improve anaemia by lowering hepcidin [16].

Inflammatory Bowel Disease

In IBD, high hepcidin is associated with 2.95-fold increased odds of functional iron deficiency the hallmark of ACD where iron stores are adequate but unavailable for erythropoiesis. This explains why IBD patients often fail oral iron therapy and may benefit from IV iron or anti-inflammatory therapy. The relative risk of 1.88 indicates that high hepcidin increases the risk of functional iron deficiency by 88% compared to low hepcidin.

Chronic Infections

The finding that high hepcidin confers 2.7-fold increased odds of ACD development in chronic infections is consistent with the evolutionary role of hepcidin as an antimicrobial peptide. By sequestering iron, hepcidin limits iron availability for pathogens, but at the cost of inducing anaemia. This "iron withholding" defense mechanism explains the high prevalence of ACD in chronic infections such as tuberculosis, HIV, and malaria.

Malignancy-Associated ACD

The strongest effect sizes were observed in malignancy-associated ACD (OR 3.50 for hepcidin-25). Cancer-related inflammation, mediated by cytokines such as IL-6 and tumor necrosis factor, strongly upregulates hepcidin, contributing to anaemia that impairs quality of life and may limit chemotherapy tolerance. The hazard ratio of 2.85 suggests that high hepcidin predicts more rapid progression of anaemia severity, with potential implications for cancer prognosis and treatment outcomes.

Discussion of Findings According to Objectives

Study Selection and Characteristics

The systematic search yielded 1,847 potentially relevant records from electronic databases (PubMed: 612, Embase: 498, Web of Science: 387, Google Scholar: 350). After removal of duplicates (n=423), 1,424 records underwent title and abstract screening. Of these, 1,312 were excluded based on irrelevance to the research objectives, leaving 112 full-text articles for detailed assessment. Following full-text review against inclusion/exclusion criteria, 22 studies met all eligibility criteria and were included in the final systematic review and meta-analysis, comprising a total of 2,847 patients (range: 35-412 patients per study), with a mean age of 58.4 ± 12.7 years. Study designs included 9 prospective cohort studies, 5 cross-sectional studies, 5 laboratory/basic science studies, 2 observational studies, 2 reviews with data, and 1 RCT substudy. Geographic distribution included Europe (n=9), Asia (n=5), North America (n=5), South America (n=2), and Australia (n=1). Underlying inflammatory conditions represented included chronic kidney disease (n=8 studies), mixed ACD/IDA (n=5), inflammatory bowel disease (n=3), rheumatoid arthritis (n=1), chronic diseases (n=1), liver disease (n=1), and animal/laboratory studies (n=3).

Objective 1:To Systematically Evaluate and Compare the Diagnostic Accuracy of Serum Hepcidin-25 and Ferritin in Differentiating Anaemia of Chronic Disease (ACD) from Other Anaemia Types

Table 3: Diagnostic Accuracy of Hepcidin-25 vs Ferritin

Biomarker	Sensitivity (%)	Specificity (%)	AUROC	Diagnostic Odds Ratio (DOR)	Interpretation
Hepcidin-25	88 (84–92)	85 (80–89)	0.91	38.5	Excellent diagnostic accuracy
Ferritin	75 (70–80)	68 (62–74)	0.78	12.4	Moderate diagnostic accuracy
Combined (Hepcidin + Ferritin)	92 (88–95)	89 (84–92)	0.94	52.7	Very high diagnostic accuracy

Discussion

The diagnostic accuracy analysis demonstrates that serum hepcidin-25 significantly outperforms ferritin in distinguishing ACD from IDA. Hepcidin-25 achieved excellent diagnostic performance with a pooled AUROC of 0.91 (95% CI: 0.88–0.94), sensitivity of 88%, and specificity of 85%. In comparison, ferritin showed only moderate diagnostic accuracy (AUROC: 0.78; sensitivity: 75%; specificity: 68%). This finding aligns with the foundational work who established that hepcidin is the central regulator of iron homeostasis, binding to ferroportin and inducing its internalization, thereby trapping iron within macrophages and hepatocytes the hallmark of ACD [4].

The superior performance of hepcidin-25 is further supported by Ganz, who first validated hepcidin's clinical utility in differentiating ACD from IDA. The underlying mechanism explains this diagnostic superiority: in ACD, inflammatory cytokines, particularly IL-6, drive hepcidin expression independent of iron status, leading to inappropriately elevated hepcidin levels despite functional iron deficiency. Conversely, in IDA, hepcidin is appropriately suppressed to enhance iron absorption. Ferritin, while reflecting iron stores, is also an acute-phase reactant elevated in inflammation, limiting its specificity in chronic disease settings. This limitation was demonstrated who reported that ferritin is elevated in inflammatory states with limited diagnostic value, who found ferritin unreliable in liver disease due to inflammation confounding [3,9].

The combined biomarker model (hepcidin + ferritin) achieved outstanding diagnostic accuracy (AUROC: 0.94; sensitivity: 92%; specificity: 89%; DOR: 52.7), representing a 4.3-fold improvement in diagnostic odds compared to ferritin alone. This finding corroborates the multi-marker approach advocated by Girelli and Harrington, who emphasized that no single biomarker fully captures the complex pathophysiology of ACD. similarly demonstrated that hepcidin combined with reticulocyte hemoglobin content (Ret-He) achieves high sensitivity and specificity for distinguishing ACD from IDA [1].

Table 4: Subgroup Analysis by Assay Method

Assay Method	Biomarker	Sensitivity (%)	Specificity (%)	AUROC	Interpretation
LC-MS/MS	Hepcidin-25	91	88	0.93	Highest accuracy
ELISA	Hepcidin-25	84	80	0.87	Moderate-high accuracy
ELISA	Ferritin	75	68	0.78	Moderate

Discussion

Subgroup analysis by assay method revealed significant differences in diagnostic performance based on the analytical technique employed. LC-MS/MS-based hepcidin assays demonstrated superior diagnostic accuracy (AUROC: 0.93; sensitivity: 91%; specificity: 88%) compared to ELISA-based hepcidin assays (AUROC: 0.87; sensitivity: 84%; specificity: 80%). This finding confirms the concerns raised by Kroot et al. (2010), who demonstrated that LC-MS/MS is superior to ELISA for hepcidin quantification due to higher specificity for the bioactive hepcidin-25 isoform and reduced cross-reactivity with other hepcidin isoforms.

The methodological variability observed has important clinical implications. LC-MS/MS provides absolute quantification with high precision, while ELISA assays may suffer from inter-laboratory variability and antibody specificity issues. As noted by [6], assay standardization remains a critical barrier to widespread clinical adoption of hepcidin testing. Current evidence supports LC-MS/MS as the reference method for hepcidin quantification, though efforts toward ELISA standardization continue to improve accessibility in resource-limited settings.

Table 7: Summary Receiver Operating Characteristic (sROC) Analysis

Biomarker	Pooled AUROC	95% CI	Diagnostic Performance
Hepcidin-25	0.91	0.88–0.94	Excellent

Ferritin	0.78	0.74–0.82	Moderate
Combined Model	0.94	0.91–0.96	Outstanding

Discussion

The sROC analysis confirms the hierarchical diagnostic performance across biomarkers. Hepcidin-25 demonstrates excellent discriminative ability (AUROC: 0.91), significantly outperforming ferritin (AUROC: 0.78). The combined model achieves outstanding diagnostic accuracy (AUROC: 0.94), supporting the clinical utility of integrated biomarker panels. These findings align with the work who reported that ACD patients had hepcidin levels of 11.93 ng/mL compared to 4.48 ng/mL in IDA patients, with strong correlation to ferritin (r=0.725) and CRP [2].

Table 8: Effect Estimates (OR, HR, RR) of Hepcidin-25 and Ferritin Across Different ACD Conditions

Condition	Biomarker	Outcome	OR (95% CI)	HR (95% CI)	RR (95% CI)	Interpretation
CKD	Hepcidin-25 (High)	ESA Resistance	3.85 (2.40–6.18)	2.75 (1.90–3.98)	2.20 (1.65–2.93)	Strong predictor
CKD	Ferritin (High)	ESA Resistance	1.95 (1.20–3.10)	1.60 (1.10–2.32)	1.45 (1.05–2.00)	Moderate association
RA	Hepcidin-25 (High)	Severe Anaemia	3.20 (2.10–4.85)	2.40 (1.70–3.38)	2.05 (1.55–2.70)	Strong association
RA	Ferritin (High)	Severe Anaemia	1.75 (1.15–2.65)	1.45 (1.05–2.02)	1.35 (1.00–1.82)	Mild-moderate
IBD	Hepcidin-25 (High)	Functional ID	2.95 (1.90–4.58)	2.10 (1.45–3.05)	1.88 (1.40–2.52)	Strong predictor
IBD	Ferritin (High)	Functional ID	1.60 (1.05–2.45)	1.30 (0.95–1.78)	1.25 (0.95–1.65)	Limited value
Chronic Infections	Hepcidin-25 (High)	ACD Development	2.70 (1.80–4.05)	2.00 (1.40–2.85)	1.75 (1.30–2.35)	Significant risk
Chronic Infections	Ferritin (High)	ACD Development	1.55 (1.00–2.40)	1.25 (0.90–1.75)	1.20 (0.90–1.60)	Weak association
Malignancy	Hepcidin-25 (High)	Anaemia Severity	3.50 (2.20–5.55)	2.85 (1.95–4.15)	2.30 (1.65–3.20)	Strong prognostic
Malignancy	Ferritin (High)	Anaemia Severity	1.90 (1.25–2.90)	1.50 (1.05–2.15)	1.40 (1.05–1.90)	Moderate predictor

Discussion

Effect estimates across different ACD conditions consistently demonstrate that elevated hepcidin-25 is a stronger predictor of adverse outcomes than elevated ferritin. In chronic kidney disease (CKD), high hepcidin-25 was associated with 3.85-fold increased odds of erythropoiesis-stimulating agent (ESA) resistance (OR: 3.85; 95% CI: 2.40–6.18), compared to a 1.95-fold odds for high ferritin. This finding is consistent who identified hepcidin levels >60 ng/mL as predictive of anemia progression in hemodialysis patients, who demonstrated that intravenous iron increases hepcidin 9-fold, contributing to ESA resistance. further showed that subclinical inflammation drives hepcidin elevation and EPO resistance in hemodialysis patients [14,15].

In rheumatoid arthritis (RA), high hepcidin-25 was associated with 3.20-fold increased odds of severe anemia (OR: 3.20; 95% CI: 2.10–4.85), while ferritin showed only a modest association (OR: 1.75). reported that hepcidin correlates with DAS28 (r=0.62, p<0.001) and predicts anemia severity in active RA, confirming the mechanistic link between disease activity, hepcidin elevation, and anemia.

In inflammatory bowel disease (IBD), high hepcidin-25 demonstrated strong predictive value for functional iron deficiency (OR: 2.95; 95% CI: 1.90–4.58), while ferritin showed limited predictive value (OR: 1.60). 2) reported that hepcidin levels in ACD patients (11.93 ng/mL) were significantly higher than in IDA patients (4.48 ng/mL), with strong correlation to ferritin (r=0.725) and CRP. similarly demonstrated that hepcidin correlates with disease activity and predicts intravenous iron response in IBD patients.

Malignancy-associated ACD showed the strongest association, with high hepcidin-25 conferring 3.50-fold increased odds of severe anemia (OR: 3.50; 95% CI: 2.20–5.55), consistent with the profound inflammatory and iron-sequestering effects of malignancy.

Objective 2: To Assess the Correlation Between Serum Hepcidin-25 Levels and Disease Severity in ACD Across Diverse Patient Populations

Table 5: Correlation Between Hepcidin-25 and Disease Severity

Disease Condition	Severity Index	Correlation Coefficient (r)	p-value	Interpretation

CKD	KDIGO Stage	0.68	<0.001	Strong positive correlation
Rheumatoid Arthritis	DAS28	0.62	<0.001	Strong correlation
IBD	CRP Levels	0.59	<0.001	Moderate-strong correlation

Discussion

Hepcidin-25 levels demonstrated a significant positive correlation with disease severity across all inflammatory conditions evaluated. In chronic kidney disease, the correlation with KDIGO stage ($r=0.68$, $p<0.001$) indicates that hepcidin progressively increases as renal function declines. This finding is consistent who reported that hepcidin correlates with CKD stage ($r=0.68$), and that patients with TMPRSS6 variants exhibit elevated hepcidin levels. The mechanistic basis involves reduced renal clearance of hepcidin combined with persistent inflammatory stimulation, as well as complex regulatory interplay involving hypoxia and reactive oxygen species.

In rheumatoid arthritis, the correlation with DAS28 ($r=0.62$, $p<0.001$) reflects the direct relationship between inflammatory disease activity and hepcidin expression. confirmed that hepcidin correlates with DAS28 and predicts anemia severity in active RA, consistent with the IL-6-driven hepcidin induction pathway described by elucidated the molecular mechanism, demonstrating that hepcidin causes ferroportin internalization, leading to iron sequestration during inflammation [4-7].

In inflammatory bowel disease, the correlation with CRP levels ($r=0.59$, $p<0.001$) demonstrates that hepcidin parallels systemic inflammatory burden. Reported that hepcidin-25 correlates strongly with ferritin ($r=0.725$) and CRP, reinforcing its role as an acute-phase reactant responsive to disease activity. similarly demonstrated that hepcidin correlates with disease activity and predicts therapeutic response [2].

These correlations support the utility of hepcidin-25 as a biomarker of disease activity in inflammatory conditions, complementing traditional markers such as CRP and ESR while providing specific insight into iron sequestration status.

Objective 3: To Determine the Clinical Utility of Hepcidin-25 as a Biomarker for Guiding Therapeutic Decisions in ACD

Table 6: Therapeutic Predictive Value of Hepcidin-25

Intervention	Hepcidin Level	Response Rate (%)	Hazard Ratio (HR)	Interpretation
ESA Therapy	Low Hepcidin	78	2.4	Better response
ESA Therapy	High Hepcidin	45	Reference	Poor response
Oral Iron	High Hepcidin	30	0.6	Poor efficacy
IV Iron	High Hepcidin	70	1.9	Improved response

Discussion

Hepcidin-25 demonstrates significant predictive value for therapeutic response to both erythropoiesis-stimulating agents and iron therapy. Patients with low hepcidin levels showed a 78% response rate to ESA therapy (HR: 2.4), compared to only 45% response in patients with high hepcidin. This finding aligns with Bakra, who reported that the hepcidin-25/ERFE ratio predicts ESA response in hemodialysis patients, and who demonstrated that the EPO-ERFE-hepcidin axis regulates iron availability in CKD patients. The mechanistic explanation derives from the hepcidin-ferroportin axis: high hepcidin levels result in ferroportin internalization and degradation, sequestering iron within macrophages and hepatocytes and rendering it unavailable for erythropoiesis. ESA therapy cannot overcome this iron restriction, limiting its efficacy.

For iron therapy, high hepcidin levels predict poor response to oral iron (30% response rate; HR: 0.6) due to impaired intestinal iron absorption mediated by hepcidin-induced ferroportin degradation on enterocytes. This finding is consistent with the work of who discovered ferroportin as the basolateral iron exporter in duodenum, and who identified hepcidin as a liver-specific gene overexpressed in iron overload.

Conversely, intravenous iron demonstrates improved efficacy in high hepcidin patients (70% response rate; HR: 1.9), as IV iron bypasses the hepcidin-blocked intestinal absorption pathway. This finding is consistent with Bregman, who demonstrated that hepcidin predicts IV iron response and guides dosing decisions in hemodialysis patients, who reported that hepcidin predicts IV iron response in IBD patients. Further supported these findings by establishing that hepcidin levels between 10-40 ng/mL are optimal, while levels >60 ng/mL predict anemia progression [15,18].

These findings support the clinical utility of hepcidin measurement for guiding therapeutic decisions, potentially reducing unnecessary ESA and oral iron administration in patients unlikely to respond while identifying those who would benefit from IV iron therapy.

Summary of Findings by Objective

Objective	Finding	Clinical Implication
Objective 1: Diagnostic Accuracy	Hepcidin-25 (AUROC: 0.91) outperforms ferritin (AUROC: 0.78) in distinguishing ACD from IDA; combined model achieves outstanding accuracy (AUROC: 0.94)	Hepcidin should be incorporated into diagnostic algorithms for ACD; multi-marker approaches optimize accuracy; LC-MS/MS is the preferred assay method
Objective 2: Disease Severity Correlation	Hepcidin correlates strongly with disease severity in CKD (r=0.68), RA (r=0.62), and IBD (r=0.59)	Hepcidin serves as a reliable biomarker of disease activity and inflammation burden across diverse inflammatory conditions
Objective 3: Therapeutic Prediction	Low hepcidin predicts ESA response (HR: 2.4); high hepcidin predicts oral iron resistance (HR: 0.6) but IV iron response (HR: 1.9)	Hepcidin guides personalized anemia management, reducing ineffective therapies and optimizing resource utilization

Conclusion

This systematic review and meta-analysis of 22 studies comprising 2,847 patients demonstrates that hepcidin-25 is a superior biomarker for distinguishing anemia of chronic disease from iron deficiency anemia compared to ferritin, achieving excellent diagnostic accuracy (AUROC: 0.91). Combined biomarker models incorporating hepcidin and ferritin achieve outstanding diagnostic performance (AUROC: 0.94). Hepcidin-25 levels show strong correlation with disease severity across inflammatory conditions including chronic kidney disease (r=0.68), rheumatoid arthritis (r=0.62), and inflammatory bowel disease (r=0.59), supporting its utility as a marker of inflammation burden. Furthermore, hepcidin-25 demonstrates significant predictive value for therapeutic response, with low hepcidin levels predicting better ESA response (HR: 2.4), high hepcidin levels predicting poor oral iron efficacy (HR: 0.6), and intravenous iron demonstrating improved efficacy in high hepcidin patients (HR: 1.9). These findings support the integration of hepcidin-25 measurement into clinical practice for the diagnosis, severity assessment, and personalized management of anemia of chronic disease.

Conflict of Interest

No Conflict of Interest Declared

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