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Underdiagnosis of iron deficiency anaemia in HIV-infected individuals: a pilot study using soluble transferrin receptors and intensive bone marrow iron stores to improve the diagnosis

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ABSTRACT

Aim We compared soluble transferrin receptors (sTfR), serum ferritin, mean cell volume (MCV) of red cells and the sTfR-ferritin index with the intensive method bone marrow trephine (BMT) iron stores in the diagnosis of iron deficiency anaemia (IDA) in Human Immunodeficiency Virus (HIV)-positive hospitalised participants.

Methods In this cross-sectional study, we recruited hospitalised HIV-positive and coronavirus of 2019 (COVID-19)-negative adults with anaemia who required a bone marrow examination as part of their diagnostic workup. We measured the full blood count, ferritin, sTfR and assessed iron using the intensive method in Haemotoxylin and Eosin (H&E)-stained BMT core biopsies of consenting participants.

Results Of the 60 enrolled participants, 57 were evaluable. Thirteen (22.80%) had IDA on H&E BMT iron stores assessment, and 44 (77.19%) had anaemia of chronic diseases (ACD). The sTfR and the sTfR-ferritin index had sensitivities of 61.54% and 53.85%, respectively, for IDA diagnosis. The sensitivity and specificity of ferritin was 7.69% and 92.31%, respectively. The sTfR and sTfR-ferritin index's diagnostic specificity was relatively low at 46.15% and 38.46%, respectively.

Conclusion In this pilot study in HIV-positive participants, the prevalence of iron deficiency using the BMT assessment was low. Both the sTfR and the sTfR-ferritin index had a better quantitative correlation to bone marrow iron stores when compared with the MCV and ferritin and, may be more accurate surrogate markers of IDA.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ South Africa has a high burden of HIV and anaemia in HIV-infected individuals is a very common finding. IDA and of ACD may copresent and are initially indistinguishable in patients with chronic infections like HIV. The current routine best test in our setting to differentiate between IDA and ACD is ferritin. Ferritin however has a very low sensitivity compared against the gold-standard test to diagnose IDA, the bone marrow iron stores.

WHAT THIS STUDY ADDS

⇒ We explored one of the newer markers to differentiate IDA and ACD, the sTfR. We also assesed bone marrow iron stores using the intensive method. The sTfR and sTfR-ferritin index have a stronger quantitative correlation with bone marrow iron stores and, therefore, better surrogate markers of IDA when compared with the MCV and ferritin in HIV-positive individuals.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Differentiating between the two forms of anaemia is crucial for an appropriate management as the aetiology and treatment are different. Institutions can consider making these newer markers of IDA as standard of practice.

INTRODUCTION

Iron deficiency anaemia (IDA) remains the leading cause of anaemia globally and in developing countries in particular.[1] South Africa has a high burden of Human Immunodeficiency Virus (HIV) infection, with HIV ranking fifth among the leading causes of death in 2016.[2] The prevalence of anaemia in HIV-infected individuals is as high as 95%, with a multifactorial aetiology including anaemia of chronic disease (ACD); drug-induced anaemia; malignancies and nutritional deficiencies.[3] The ACD, also known as functional IDA, is characterised by elevated hepcidin levels in both the acute and chronic phases of HIV infection, which results in iron-restricted erythropoiesis.[4]

Concurrent presentation of IDA and ACD in HIV-positive individuals is not unusual. However, the prevalence and pathophysiology of this simultaneous presentation is currently poorly understood and documented.[3] Measuring the plasma ferritin concentration is still currently the accepted best non-invasive test for iron deficiency.[5] The World Health Organisation (WHO) defines ferritin of <15 µg/L as diagnostic of IDA in the appropriate clinical setting, whereas the American Society of Haematology recommends a cut-off of 30 µg/L to improve sensitivity.[5 6] However, ferritin, an acute

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phase reactant, is elevated in acute and chronic HIV infection and may be misleading if elevated. In addition, transferrin and transferrin saturation correlates poorly with bone marrow iron stores in individuals with chronic conditions.[5] Furthermore, both IDA and ACD can manifest as microcytosis making the mean cell volume (MCV) unhelpful in distinguishing between these two conditions.[3]

The gold standard to diagnose IDA is an assessment of bone marrow iron stores.[7] However, marrow sampling and examination is invasive; expensive and labour intensive.[8] Traditionally, bone marrow iron stores are quantified following visualisation with Perl's stain on bone marrow aspirate (BMA).[7] The Perl's stain iron grading assessment is subjective in this setting, with significant interobserver variability. The poor predictive value of absent BMA iron stores to diagnose IDA and often poor quality of BMA for iron assessment has highlighted the need for better methods to assess bone marrow iron stores [9].

The intensive bone marrow iron store assessment protocol developed by Phiri and colleagues is superior to Perl's stain method.[9] Unlike Perl's stain method, which evaluates iron in haemosiderin of marrow fragments only, the intensive method evaluates iron in marrow fragments, macrophages and erythroblasts. This intense iron evaluation becomes critical in settings where chronic inflammatory diseases resulting in ACD or functional iron deficiency are common.[9] The bone marrow trephine (BMT) iron staining was not only demonstrated to be comparable to BMA Perl staining for iron,[10] it was also shown to be superior to Perl's stain for visualising and assessing bone marrow iron stores in a study by Stuart-Smith *et al* of 155 bone marrow samples that had 61% of marrow samples having absent iron on BMA but positive on BMT.[11]

The soluble transferrin receptors (sTfRs) are the truncated forms of the transferrin receptors without their transmembrane and cytoplasmic domains and circulate bound to transferrin.[12] The cells with the most significant number of transferrin receptors are the bone marrow nucleated red blood cells.[12] The circulating sTfR concentration was shown to be proportional to the cellular expression of the membrane-associated transferrin receptor and correlated with increased cellular iron needs (ie, IDA) and increased cellular proliferation (ie, haemorrhage, hypersplenism, haematinics and haemolysis). The sTfR level is elevated in IDA but is unaffected by inflammation and, therefore, has the potential to better differentiate IDA from ACD.[13] Furthermore, the use of sTfR/log ferritin index is a reasonable estimate of body iron as sTfR reflects the degree of availability of iron for cells and ferritin reflects storage iron compartment.[13]

To our knowledge, there are currently no studies comparing BMT iron stores and sTfR in HIV-positive individuals. This study evaluated the diagnostic usefulness of sTfR, ferritin, MCV and sTfR-ferritin index compared with Haematoxylin and Eosin (H&E) BMT intensive iron stores assessment to diagnose IDA in HIV-infected individuals.

PARTICIPANTS AND METHODS

Study design and setting

This is a pilot cross-sectional study, recruiting patients prospectively and was conducted at the Klerksdorp/Tshepong Hospital Complex in South Africa from October 2020 to May 2021. All study participants provided written informed consent before study-related activities and the study was conducted in compliance with the Declaration of Helsinki principles.

Study population

Consenting consecutive adult (≥18 years) male and female participants admitted to the Internal Medicine wards were enrolled in the study if they met study eligibility criteria. The key inclusion criteria were the presence of anaemia (haemoglobin of <130 g/L in men and <120 g/L in women) together with a requirement for bone marrow examination as decided on by the treating doctor, a signed informed consent, a positive HIV test and a negative coronovirus of 2019 (COVID-19) polymerase chain reaction (PCR) test. Participants <18 years of age, those with known haematologic malignancies, patients that had already received blood transfusions during the admission, patients who were on iron supplementation and those with anaemia due to blood loss were excluded.

Definitions

We used the WHO definition of anaemia.[13 14] In addition, a diagnosis of IDA was made if the ferritin was <30 µg/L as defined by the American Society of Haematology.[5 6] Adjunct diagnostic criteria for IDA were a microcytosis with an MCV <80 fL, the sTfR of >55.0 nmol/L (as per the manufacturer's instructions (Roche Diagnostics, Indianapolis, USA)) and sTfR-ferritin index of>1.4.[13 15]

Routine blood analyses

The hospital diagnostic laboratory processed the full blood count (FBC), ferritin, CD4 count HIV viral load bloods collected as part of the participants' routine diagnostic workup. The FBC analyses were done on the Siemens Advia 2120i (Erlangen, Germany). The ferritin was analysed on the Roche Cobas 6000 (Indianapolis, USA). The CD4 count was run on the Beckman Coulter Aquios CL analyser (Indianapolis, USA), and the HIV viral load was analysed with the Abbott Molecular Alinity analyser (Illinois, USA).

Soluble transferrin receptor analyses

Five millilitres of serum were taken for sTfR analysis. The sample was centrifuged at 3500 revolutions per minute (rpm) for 5 min, aliquoted into cryovials and stored in a −80°C freezer. The sTfR level was measured on the Cobas c501 clinical analyser as per the manufacturer's instructions (Roche Diagnostics, Indianapolis, USA). This validated test method used 110 µL of serum and had a coefficient of variation of 1.6%–4.0% with an accuracy of 93%–98% low-quality and high-quality control samples run.

Bone marrow analyses

All participants had standard BMT core biopsy sampling using a 12G needle under local anaesthesia. The trephine specimens were paraffin wax-embedded overnight and sectioned into 3 µm slices before being stained with H&E and cover slipped for microscopic examination. BMT core biopsies were quality assured by two pathologists, and those with at least three marrow spaces were assessed for iron. The trephine cores were examined at 20× magnification in 10 high power fields. All fields, including marrow fragments, macrophages and erythroblasts, were examined for the presence of iron using the intensive histological iron grading method shown in table 1.[9]

Statistical analyses

The results of these analyses were collated in a REDCap database together with the participant anonymised demographic information.

Table 1 Evaluation of iron status using the intensive histological grading method

Iron in fragment*	Iron in macrophage†	Iron in erythroblast‡	Iron status diagnosis
Present	Present	Present	Normal (No iron deficiency)
Present	Absent	Present	
Present	Present	Absent	Functional iron deficiency (ACD)
Present	Absent	Absent	
Absent	Present	Present	Iron stores deficiency (IDA)
Absent	Absent	Present	
Absent	Present	Absent	Functional and iron stores deficiency
Absent	Absent	Absent	

*Positive fragment iron: fragment grade ≥2.
†Positive macrophage iron: iron present in a reticular cell.
‡Positive erythroblast iron: iron present in >30% of erythroblasts.
ACD, anaemia of chronic disease; IDA, iron deficiency anaemia.

Frequencies and percentages were calculated for categorical variables stratified by BMT iron status (IDA vs ACD). For continuous measures, medians (IQRs) and means (SD) were determined by BMT iron status. For statistical comparison by BMT iron status, χ^2 or Fisher's exact test were used for categorical variables; whereas Kruskal Wallis and Student's t test were used to compare the medians and means.

To assess the accuracy of tests used for BMT iron status, receiver operating characteristics (ROC) curves were determined using logistic regression. The tests that were assessed using the ROC curves were Ferritin, MCV, sTfR (nmol/L) and sTfR-ferritin index. Sensitivity, specificity, positive and negative predictive values were assessed to determine the validity of the test based on the BMT iron status as the gold standard. Statistical analysis was conducted in SAS Enterprise guide V.7.15 (SAS Institute, Cary, North Carolina) using SAS/STAT procedures PROC FREQ, PROC MEANS, PROC TTEST, PROC NPAR1WAY and PROC LOGISTIC.

RESULTS

A total of 60 participants met the inclusion criteria and were enrolled in the study. Three participants were excluded due to incomplete data (see figure 1). Of the remaining 57, 13 (22.81%) had IDA, and 44 (77.19%) had ACD based on intensive method BMT iron store assessment. The demographic and laboratory characteristics of the study population are shown in table 2. More than half the participants were women (54.39%, n=31), of which the majority (54%) had ACD. The study population median age was 38 years (range 18–70 years). Most participants (75%) had severe anaemia, and the median haemoglobin across the study population was 640 g/L. The anaemia was normochromic in half the study population (52%), with only 9 (15%) of participants presenting with the microcytic anaemia. Only two participants had ferritin below 30 µg/L, one was iron

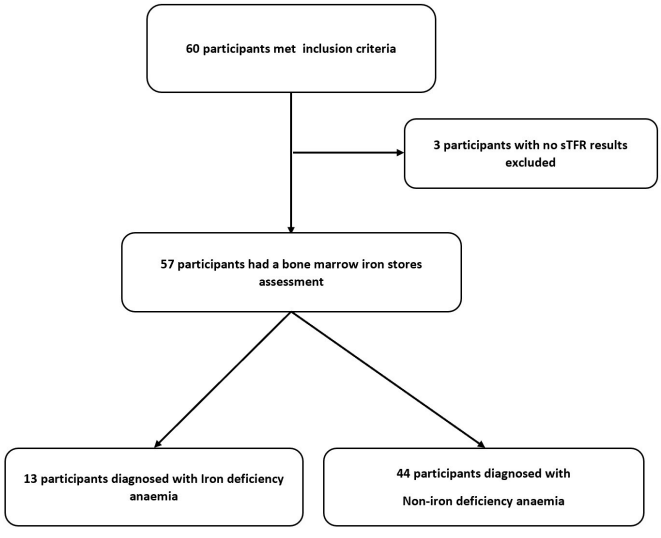

Figure 1 Disposition of study participants. sTfR, soluble transferrin receptors.

Table 2 Demographic and laboratory characteristics of study participants

Variable	Overall (n=57)	IDA (n=13)	ACD (n=44)	P value
Sex				
Female (%)	31/57 (54.39)	7/13 (53.85)	24/44 (54.55)	0.9999
Male (%)	26/57 (45.61)	6/13 (46.15)	20/44 (45.45)	
Age				
18–35 (%)	20/57 (35.09)	5/13 (38.46)	15/44 (34.09)	0.7538
36–70 (%)	37/57 (64.91)	8/13 (61.54)	29/44 (65.91)	
Median (IQR)	38.0 (32.0–52)	39.0 (35.0–48)	38.0 (31.0–54)	0.7826
Mean (SD)	41.46 (12.69)	40.31 (9.28)	41.80 (13.60)	0.7138
Min, Max	(18–70)	(29–60)	(18–70)	
Anaemia severity (Hb) g/L				
Mild (>110) (%)	1/57 (1.75)	1/13 (7.69)	0/44 (0.00)	0.1763
Moderate (80–110) (%)	13/57 (22.81)	3/13 (23.08)	10/44 (22.73)	
Severe (<80) (%)	43/57 (75.44)	9/13 (69.23)	34/44 (77.27)	
Median (IQR)	64 (53–77)	61 (49–88)	64.5 (53.5–76)	0.9848
Mean (SD)	64.2 (24.3)	65.2 (29.4)	63.9 (22.9)	0.8626
Min, Max	(10–120)	(20–120)	(10–110)	
Mean cell volume fL				
Microcytic (<80) (%)	9/57 (15.79)	2/13 (15.38)	7/44 (15.91)	0.1240
Normocytic (80–100) (%)	30/57 (52.63)	4/13 (30.77)	26/44 (59.09)	
Macrocytic (>100) (%)	18/57 (31.58)	7/13 (53.85)	11/44 (25.00)	
Median (IQR)	92.0 (84.8–105)	100 (88.9–107)	92.0 (84.5–101)	0.2868
Mean (SD)	93.71 (13.64)	97.46 (16.31)	92.60 (12.75)	0.2628
Min, Max	(69–127)	(69–127)	(72–124)	
Ferritin ug/L				
<30 (%)	2/57 (3.51)	1/13 (7.69)	1/44 (2.27)	0.4073
>30 (%)	55/57 (96.49)	12/13 (92.31)	43/44 (97.73)	
Median (IQR)	972 (237–2629)	723 (154–1423)	1149 (298–2665)	0.5683
Mean (SD)	5551 (16 673)	11 351 (28 198)	3838 (11 267)	0.3648
Min, Max	(4–100 000)	(4–100 000)	(12–73 000)	
sTfR nmol/L				
<55 (%)	34/57 (59.65)	6/13 (46.15)	28/44 (63.64)	0.3389
>55 (%)	23/57 (40.35)	7/13 (53.85)	16/44 (36.36)	
Median (IQR)	49.9 (28.4–72.7)	58.1 (44.3–105)	48.6 (28.2–68)	0.2309
Mean (SD)	77.16 (98.34)	97.60 (93.06)	71.12 (100.1)	0.3985
Min, Max	(6–615)	(22–300)	(6–615)	
sTfR-Ferritin index				
<1.4 (%)	31/57 (54.39)	5/13 (38.46)	26/44 (59.09)	0.2198
>1.4 (%)	26/57 (45.61)	8/13 (61.54)	18/44 (40.91)	
Median (IQR)	1.37 (0.69–2.41)	1.97 (0.99–4.76)	1.28 (0.69–2.07)	0.1862
Mean (SD)	3.51 (7.57)	5.65 (11.26)	2.87 (6.13)	0.4074
Min, Max	(0–42)	(0–42)	(0–33)	

fl, femtolitre; Hb, haemoglobin; IDA, iron deficiency anaemia; IQR, interquartile range; Max, maximum; Min, minimum; N; sTfR, soluble transferrin receptor.

Table 3 Analytic performance of ferritin, MCV, sTfR and sTfR-ferritin index in the diagnosis of IDA

Parameter	Sensitivity	Specificity	PPV	NPV
Ferritin	1/13 (7.69)	12/13 (92.31)	1/2 (50.00)	43/55 (78.18)
MCV	2/13 (15.38)	11/13 (84.62)	2/9 (22.22)	37/48 (77.08)
sTfR	7/13 (53.85)	6/13 (46.15)	7/23 (30.43)	28/34 (82.35)
sTfR-ferritin index	8/13 (61.54)	5/13 (38.46)	8/26 (30.77)	26/31 (83.87)

MCV, mean cell volume; NPV, negative predictive value; PPV, positive predictive value; sTfR, soluble transferrin receptor.

deficient, and the second was not iron deficient. The median ferritin for the population was 972 µg/L. Using the sTFR cut-off of 55 nmol/L as recommended by the reagent suppliers, 34 (60%) had a low sTfR level, of which six were iron deficient by BMT examination. Using an sTfR-Ferritin index cut-off of 1.4, most participants (54%, n=31) were below the 1.4, and the median sTfR-Ferritin index for the population was 1.34. The analytical performance of the MCV, ferritin, sTFR and sTfR-ferritin index in the diagnosis of iron deficiency is shown in table 3. The sensitivities were 54% and 62% for sTfR and sTfR-ferritin index respectively, compared with sensitivities of 8% and 15% for ferritin and MCV, respectively. The negative predictive value was high across all four adjunct iron parameters. Both ferritin and MCV had high specificities (92% and 84%, respectively).

The area under the curve (AUC) of ROC curves was highest for sTfR-ferritin index, followed by sTfR (0.62 vs 0.61, respectively). Ferritin had the lowest area of 0.45 (see table 4).

DISCUSSION

Anaemia is a common clinical presentation, with IDA and ACD being the most common entities in clinical practice.[16] However, the pathogenesis, aetiology and therapeutic interventions of IDA and ACD are different, so it is crucial to differentiate between the two.[6] Measurement of ferritin and MCV is often used in diagnosing IDA. Still, these are less useful in chronic infections such as HIV because ferritin is an acute-phase reactant and MCV is low or normal in both IDA and ACD. On the other hand, the sTfR is elevated in IDA and is unaffected by chronic inflammation or infection such as HIV and, therefore, has the potential to differentiate IDA from ACD better.[13] In this pilot study, we evaluated the diagnostic usefulness of sTfR, ferritin, MCV and sTfR-ferritin index compared with BMT-intensive iron store (the gold standard) assessment to differentiate IDA and ACD in HIV-infected individuals.

In our cohort of 57 participants, 22% had IDA, and 77% had ACD based on BMT histological iron store assessment. While IDA is the most common anaemia in general,[17] it was less common in this cohort for several reasons. First, the participants had anaemia in the setting of HIV, which is usually associated with multifactorial aetiology, with ACD being the most common.[16] Second, participants included in this study were hospitalised, and, therefore, those with IDA responding to iron supplementation were excluded from the study. Third, the most typical reason for iron deficiency in our setting is blood loss secondary to gastrointestinal or gynaecologic blood loss. Nowadays, endoscopic examination and gynaecologic procedures are all performed as outpatient procedures and would not be reflected in this cohort. We excluded the outpatient participants because most of these patients do not require a bone marrow examination as part of their hospital diagnostic workup. Our findings that IDA is less common than ACD concur with other studies with similar settings.[16] In the Cape Town study on patients with HIV and TB, the mean prevalence of IDA was <3%, ranging from 0% to 32%. Our IDA prevalence of 22% concurred with these published results.[16]

In our cohort, only two participants had ferritin <30 µg/L, one had IDA, and the second had ACD (table 2). These results are not surprising because, in HIV infection, we expect the ferritin to increase as it is an acute-phase reactant.[18] In our population, the mean ferritin was 5551 µg/L (range 4 000–100 000 µg/L), emphasising that it should not be used in the diagnostic workup of patients suspected of having IDA if they have concurrent chronic infection. Hyperferritenaemia is well described in chronic infection, including HIV and COVID-19 infection.[19 20] Hyperferritenaemia, however, lacks specificity as a surrogate marker of infection.[19 20]

As shown in table 2, there were nine participants in our cohort with an MCV <80 fl. Most participants in the study had a normal MCV (n=30, 52%) or high MCV (n=18, 31%). Some of our participants were on antiretroviral treatment that can elevate the MCV, so a normal or high MCV is not unexpected. The net effect is to make the diagnosis of IDA less obvious when looking at an MCV in isolation.[16 21] The HIV-associated macrocytosis may reflect many aetiological factors, including nutritional deficiency (B12 or folate deficiency); liver toxicity or bone marrow toxicity.[21 22] The significance of a normal MCV, which occurred in a third of participants, is that it does not help in differentiating IDA and ACD.

Using the sTFR cut-off of 55 nmol/L, 34(60%) of our participants had a low sTfR level, of which six were iron deficient by BMT examination (table 2). The sTfR-ferritin index improved the sensitivity to 61% but resulted in a lower specificity of 38%. Our study findings in HIV-infected individuals concur with those of Koulaouzidis and colleagues, who showed that sTfR and sTfR-ferritin index have a higher sensitivity than ferritin in diagnosing IDA.[13] However, the sTfR is also elevated in conditions of increased erythroid proliferation (haemorrhage, hypersplenism, haematinics and haemolysis) and, therefore, has lower specificity than ferritin.[8]

Notwithstanding the distinct advantage of sTfR of not being affected by infection or inflammation, its use in clinical practice has been limited by its high cost and lack of standardisation of the different immunoassays.[14] In addition, the expected reference ranges are also assay dependent.[15] Therefore, the sTfR-ferritin index, which is diagnostic of IDA, is also study dependent.[15] This study used an sTfR-ferritin index of >1.4, which is an average value of the sTfR-ferritin indices in the systematic review by Anastasios and colleagues as well as used in the study by Oustamanolakis and colleagues.[13 15] Unfortunately, the Cobas c501 clinical analyser sTfR cut-off was standardised against the in-house reference material and

Table 4 AUC ROC analytic performance of ferritin, MCV, sTfR and sTfR-ferritin index in the diagnosis of IDA

Parameter	AUC
Ferritin	0.4476
MCV	0.5979
STFR	0.6101
STFR-ferritin index	0.6215

AUC, area under the curve; MCV, mean cell volume; ROC, receiver operating characteristics; sTfR, soluble transferrin receptor.

not to the 07/202 reagent, which was established as the first WHO reference reagent for sTfR in 2010.[23]

The AUC of ROC for ferritin, MCV, sTfR and sTfR-ferritin were less informative due to the small sample size but further illustrated better diagnostic ability of sTfR and sTfR-ferritin index than MCV and ferritin to diagnose IDA.

The bone marrow iron assessment remains the gold standard for iron evaluation, with the intensive method trephine core biopsy assessment favoured over marrow aspirate for several reasons. First, most aspirates are technically tricky or inadequate for accurate iron assessment. In our study, 26 (45.6%) aspirates were suboptimal. Our results are comparable to those of other studies.[5 6] Second, the Perl's stain done on marrow aspirates evaluates iron in macrophages only and not in trephine fragments and erythroid precursors, as is the case with trephine examination.[9] Third, in our experience and those of others, the frequency of suboptimal trephine core biopsy is very low compared with the frequency of aspirate sampling failure.[11] Finally, the intensive iron store assessment has been validated and found to be superior to Perl's stain in settings of chronic infections.[24 25]

Although our study looked at sTfR and sTfR-ferritin indices as better markers of IDA, there are other markers like the reticulocyte haemoglobin content (CHr) and hepcidin that can also better diagnose IDA.[26 27]

Study limitations

This study was done on hospitalised acutely ill participants expected to have higher inflammatory markers, including ferritin. The sensitivity and specificity of ferritin might not represent otherwise well clinically HIV-infected individuals. In this pilot study, the sample size is small, and, therefore, results may not be extrapolated to large participant populations. The study was conducted in a single centre in a secondary hospital and may not reflect the profiles of other tiers of healthcare delivery. The lack of standardisation in the measurement of sTfR assays may affect the reproducibility of this study results when different measurement methods are used.

The sTfR and sTfR-ferritin indices were not compared against other newer parameters like hepcidin and CHr to diagnose IDA.

CONCLUSION

In HIV-infected patients with anaemia, most have ACD with a low frequency of IDA when assessed with intensive method trephine core biopsy iron assessment. In this patient population, the sTfR and sTfR-ferritin index have a stronger quantitative correlation with bone marrow iron stores and, therefore, better surrogate markers of IDA when compared with the MCV and ferritin.

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Contributors MM conceived the project, wrote protocol, obtained approvals, collected data, analysed the data and wrote the paper. EV, NM and JM supervised the project, critically evaluated data and wrote the paper, DD and MB coordinated and analysed the trephine core biopsies iron assessments, KH and KO did the statistical analyses, RC and TS set up the sTfR assay and result analyses. MM was the guarantor for the project. All authors approved the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by University of the Witwatersrand Human Research Ethics Committee, reference number or ID M200435. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request.

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