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Research Article

Biochemical, Physiological, and Ultrasonographic Predictors of Iron-Deficiency Anemia Severity in Women with Heavy Menstrual Bleeding: A Prospective Cohort Study

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ABSTRACT
Background: Although heavy menstrual bleeding (HMB) is a significant cause of iron-deficiency anemia (IDA), the association between biochemical, physiological and ultrasonographic features and the severity of anemia have not been well described.
Objectives: To determine factors associated with severity of IDA in women with HMB and to assess the discriminatory performance of a model of biochemical, physiological, and ultrasonographic characteristics.
Methods: This prospective observational study included 120 women aged 18–45 years assessed at two hospitals in Pakistan between January 2026 and August 2026. The Pictorial Blood Assessment Chart (PBAC) was used to estimate the menstrual blood loss. Iron indices, reticulocyte hemoglobin equivalent (RET-He), cardiovascular measurements and pelvic ultrasonographic results were studied. Multivariable ordinal logistic regression evaluated anemia severity; stratified fivefold cross-validation evaluated discrimination of moderate-to-severe anemia. Models adjusted for age, bleeding score, ferritin, RET-He, resting heart rate and ultrasound abnormalities.
Results: Mild, moderate, and severe anemia occurred in 36 (30.0%), 67 (55.8%), and 17 (14.2%) participants, respectively. Mean PBAC scores increased from 201.3 to 224.9 and 298.2 across severity groups, while median ferritin decreased from 18.9 to 10.0 and 5.0 µg/L. Higher PBAC scores (adjusted odds ratio [aOR] 2.57 per 50 points), lower ferritin (aOR 2.60 per 5-µg/L decrease), lower RET-He (aOR 2.28 per 3-pg decrease), higher resting heart rate (aOR 2.38 per 10 beats/min), and structural uterine abnormalities (aOR 3.71) were independently associated with greater severity. Adding ultrasonographic findings increased cross-validated area under the curve from 0.848 to 0.884.
Conclusions: IDA severity was associated with menstrual blood loss, iron status biomarkers, cardiovascular measurements, and uterine structural findings. The inclusion of ultrasonographic information resulted in an increase in the discrimination of internal models, but before clinical implementation, external validation is still required.

Keywords: Heavy Menstrual Bleeding, Iron-Deficiency Anemia, Serum Ferritin, Reticulocyte Hemoglobin Equivalent, Cardiovascular Physiology, Pelvic Ultrasonography, Adenomyosis, Prospective Cohort.

INTRODUCTION

Heavy menstrual bleeding (HMB) is a common gynecologic disorder in women of reproductive age as well as a major cause of iron deficiency and iron-deficiency anemia (IDA) and has significant impact on physical function, reproductive health and quality of life. In women, chronic menorrhagia, in the end, results in iron deficiency, suboptimal erythropoiesis and, ultimately, anemia with clinical significance that requires treatment[1]. According to International Federation of Gynecology and Obstetrics (FIGO) classification, there are two types of abnormal

uterine bleeding: structural causes (endometrial polyps, adenomyosis, leiomyomas) and nonstructural causes[2]. Although there are well defined diagnostic guidelines, iron-deficiency associated with HMB is still often overlooked and there is significant variation in the correlation of the amount of blood lost per menstruum and severity of the associated anemia. There may be a difference in the underlying uterine pathology, base iron level, dietary habits and duration of bleeding[3,4]. Pathophysiology of IDA consists of successive utilization of storage iron, a reduction of the amount of available circulating iron, a reduction in hemoglobin synthesis, and alteration in the morphology of the erythrocytes. Traditionally, the severity of anemia is judged by hemoglobin concentration but this is not adequate to characterize the biochemical abnormalities of iron-restricted erythropoiesis[5,6]. Transferrin saturation is the percentage of the iron available in the circulation for the production of erythrocytes and serum ferritin is a good indicator of iron stores. Conventional haematological indices like MCV and RDW also provide further information about microcytosis and heterogeneity of red-cell size. Another functional measure of iron available for recent erythropoiesis is reticulocyte hemoglobin equivalent (RET-He) that may reveal a defect in iron incorporation before the mature erythrocytes are affected. Earlier evidence from meta-analysis has shown that measurement of reticulocyte hemoglobin in IDA is very useful for diagnosis and can be used along with the conventional biochemical markers[7].

The hematological abnormalities are accompanied by physiologic responses that occur in the attempt to maintain tissue oxygen delivery despite a reduction in circulating hemoglobin over time. These adaptations may involve an increased resting heart rate, alterations in cardiac output, alterations in peripheral vascular resistance, and adaptations in the cardiovascular system to postural changes[8]. Women with clinically significant IDA may present with fatigue, exertional intolerance, dizziness and reduced functional capacity, which are the signs of systemic effects of insufficient oxygen transport[9]. Physiological parameters such as resting heart rate, orthostatic heart rate changes and shock index have not been routinely part of the structured evaluation of the severity of anaemia in a woman with HMB. These measurements are dependent on various clinical conditions, but when combined with biochemical indices can give additional information on the functional burden of anemia, and the link between hematological impairment and cardiovascular compensation[10].

When structural uterine abnormalities are suspected, pelvic ultrasonography is an important part of the etiological evaluation of HMB. Often abnormal menstrual bleeding is a common association with leiomyomas and adenomyosis, being the clinical manifestation. To standardise the terminology used to describe the myometrial abnormalities, uterine masses and morphological features associated with these masses, consensus on Morphological Uterus Sonographic Assessment (MUSA) was set up. In later studies, the sonographic features of adenomyosis were further evolved into direct signs (myometrial cysts and hyperechogenic islands) and indirect signs (altered myometrical architecture)[7,11]. Thus, ultrasonographic assessment of uterine volume and leiomyoma characteristics and adenomyosis may yield structural information pertinent to the underlying bleeding disorder. There is a current recommendation for women with HMB, to perform targeted pelvic imaging when the clinical presentation is suggestive of a structural uterine etiology[8]. Whether these ultrasonographic measurements are independent predictors of the severity of IDA, however, after controlling for menstrual blood loss and biochemical iron depletion is not well known[12].

Biochemical indicators of iron deficiency, cardiovascular effect of anaemia and underlying cause of HMB have been investigated separately, but interactions between these indicators, and the relationship to the level of anaemia have not been fully described. Conventional assessment relies almost exclusively on the hemoglobin concentration and menstrual history with limited functional markers of erythropoiesis, objective parameters of physiology and standardized ultrasound data[12]. A multidimensional approach might be more useful to better understand the clinical and biological characteristics of severity of anemia and identify women that need more careful hematological and gynecological evaluation. Therefore, the present study was designed to understand the biochemical, physiological and ultrasonographic parameters with respect to severity of IDA in women with HMB. The adjusted association with the anemia severity and the incremental discriminatory value of the structural ultrasonographic measurements against menstrual blood loss,

iron-status biomarkers, and cardiovascular measurements was assessed.

MATERIALS AND METHODS

Study Design and Setting

The study was a prospective observational cohort study conducted between January 2026 and August 2026 in the Department of Obstetrics and Gynaecology, Unit I, Sandeman Provincial Hospital / Bolan Medical Complex Hospital (BMCH), Quetta, Pakistan and Department of Radiology, PNS Shifa Hospital, Karachi, Pakistan. One hundred twenty women with heavy menstrual bleeding (HMB) and iron-deficiency anemia (IDA) were included. Biochemical, physiological and ultrasonographic parameters were assessed for the severity of anemia and reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[9].

Study Population and Eligibility Criteria

Consecutive women of reproductive age (18-45 years), who presented with HMB and had a laboratory diagnosis of IDA, were recruited. Women with hemoglobin levels of < 12.0 g/dL and serum ferritin levels of < 30 µg/L were eligible, and pregnant women, women with postpartum bleeding, hemoglobinopathies, hemolytic anemia, chronic kidney disease, chronic liver disease, malignancy, systemic inflammatory diseases, and non-gynecological sources of blood loss were excluded. Patients who had received blood transfusion or intravenous iron within three months were also excluded because it was considered that it might interfere the hematological measurements.

Menstrual blood loss, clinical assessment

The demographic and clinical data studied were age, body mass index, parity, period duration, period regularity, bleeding characteristics and previous gynecologic history. Menstrual blood loss was determined using Pictorial Blood Assessment Chart (PBAC) [10] which is based on a higher score indicating a greater degree of blood loss. Exertional dyspnea, palpitations, dizziness and fatigue were all noted. Based on the hemoglobin cut-off of the World Health Organization[11], anemia was classified as mild (11-11.9 g/dl), moderate (8-10.9 g/dl) and severe (< 8.0 g/dl).

Biochemical and Hematological Assessment

Venous blood samples were collected for hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin and red-cell distribution width (RDW) level measurements. The iron status investigations were based on serum ferritin, serum iron, total iron binding capacity, transferrin saturation (TSAT) and reticulocyte hemoglobin equivalent (RET-He). The percentage of TSAT was determined by dividing the serum iron by the TIBC and multiplying the result by 100. RET-He was tested as a marker of the iron supply in recent studies that produced red blood cells. To aid interpretation of ferritin concentrations, C-reactive protein was included. Standardized automated procedures and routine quality-control protocols were used for laboratory analyses.

Physiological Assessment

Cardiovascular measurements were resting heart rate, blood-pressure measurements and orthostatic heart-rate change, and shock index. At the end of the 5 minutes resting period, resting measurements were taken by using the calibrated monitoring equipment. Orthostatic responses were evaluated after 5 min in supine position and 3 min after standing. Orthostatic heart-rate change was considered as the difference between the supine and standing heart rate and shock index was calculated as the heart rate during supine position divided by the systolic blood pressure. Evaluation of these measurements as physiological correlates of severity of anemia.

Ultrasonographic Assessment

To evaluate uterine morphology and structural abnormalities related to HMB, pelvic ultrasonography was performed. Transabdominal imaging was used to assess uterine dimensions, uterine volume, endometrial thickness, leiomyomas and sonographic features of adenomyosis with transvaginal imaging used as clinically indicated. The volume of the uterus was calculated as 0.523 multiplied by the product of length, width and anteroposterior diameter. The abnormalities in the myometrium were described using the Morphological Uterus Sonographic Assessment (MUSA) terminology. Structural abnormalities were combined as leiomyomas, adenomyosis or combined pathology (a composite variable defined as either abnormality).

Study Outcomes

IDA severity (mild, moderate and severe) was defined as the primary outcome. The following

candidate predictors were considered: PBAC score, ferritin, TSAT, resting heart rate, orthostatic heart-rate change, RET-He, RDW, uterine volume and structural ultrasonographic abnormalities. The secondary outcome was moderate to severe anemia (Hb < 11.0 g/dL). The incremental discriminatory contribution of the structural ultrasonographic findings was assessed by comparing models with and without imaging variables.

Statistical Analysis

IBM SPSS Statistics version 29.0 was used for data analysis. Quantitative variables were described in terms of their mean ± standard deviation or median (interquartile range), while qualitative variables were described in terms of their frequencies and percentages. Analysis of variance, Kruskal–Wallis tests, chi square tests or Fisher's exact test were used for between group comparisons when appropriate. For each association, a multivariable ordinal logistic regression estimated an adjusted odds ratio (aOR) and 95% confidence interval (CI) for increasing levels of anemia. For the adjusted model, age, PBAC score, ferritin, RET-He, resting heart rate, and structural ultrasonographic abnormalities were included. The discrimination of moderate to severe anemia was evaluated by binary logistic regression and ROC analysis. Stratified fivefold cross validation, area under the receiver operating characteristic curve and Brier scores were used to measure discrimination and prediction error. The two-sided p value of <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and applicable institutional and national research ethics guidelines. The relevant Institutional Review Board or Ethics Committee approved the study, if necessary. Informed consent was obtained. Throughout the research process, the privacy, confidentiality and protection of the participants were respected. Research data was secured and only accessible to authorized personnel and used for approved research. All study procedures were conducted with due consideration for participant rights, safety, and welfare.

RESULTS

Demographic and Haematological parameters

The analytical cohort included 120 women with HMB and IDA. Mild anemia was observed in 36 (30.0%) participants, moderate anemia in 67 (55.8%), and severe anemia in 17 (14.2%). The overall prevalence of moderate-to-severe anemia was 84 (70.0%) women. There was a mean age of 34.1 years with a mean haemoglobin concentration of 9.77 ± 1.43 g/dL. There was a progressive reduction in hemoglobin concentration in each of the three severity groups, with mean hemoglobin concentrations being 11.4 ± 0.2, 9.5 ± 0.8 and 7.4 ± 0.4 g/dL respectively (p<0.001).

There was a significant relationship between the amount of blood lost during menstruation and severity of anemia. The Mean Pictorial Blood Assessment Chart (PBAC) score was 201.3 ± 41.3 in mild anemia, 224.9 ± 48.4 in moderate anemia and 298.2 ± 56.3 in severe anemia (p<0.001). Menstrual duration increased correspondingly from 6.8 ± 1.1 to 8.8 ± 1.2 days (p<0.001). There was no significant difference in age or BMI between the anemia-severity categories. Clinical and menstrual features are given in Table 1.

Table 1. Clinical and menstrual characteristics according to anemia severity

Variable	Mild (n=36)	Moderate (n=67)	Severe (n=17)	p-value
Age, years	32.8 ± 4.5	34.3 ± 5.9	35.9 ± 3.7	0.121
BMI, kg/m²	26.7 ± 3.2	25.7 ± 4.7	25.9 ± 3.2	0.498
Hemoglobin, g/dL	11.4 ± 0.2	9.5 ± 0.8	7.4 ± 0.4	<0.001
PBAC score	201.3 ± 41.3	224.9 ± 48.4	298.2 ± 56.3	<0.001
Menstrual duration, days	6.8 ± 1.1	7.8 ± 1.6	8.8 ± 1.2	<0.001

Values are presented as mean ± standard deviation. Between-group comparisons were assessed using one-way analysis of variance. BMI, body mass index; PBAC, Pictorial Blood Assessment Chart.

Biochemical and Hematological Findings

Increasing anemia severity was associated with progressively lower iron stores and more pronounced evidence of iron-restricted erythropoiesis. Median serum ferritin decreased from 18.9 µg/L (interquartile range [IQR], 12.0–22.2) in women with mild anemia to 10.0 µg/L (IQR, 7.7–14.3) in moderate anemia and

5.0 µg/L (IQR, 3.0–9.9) in severe anemia (p<0.001). Transferrin saturation declined from 16.0 ± 4.0% to 9.1 ± 2.8% across the same severity categories (p<0.001).

Reticulocyte hemoglobin equivalent (RET-He) was progressively lower across anemia-severity categories, with mean concentrations of 26.8 ± 2.8, 25.4 ± 3.3, and 22.3 ± 2.5 pg in mild, moderate, and severe anemia, respectively (p<0.001). Conversely, red-cell distribution width increased from 15.7 ± 1.5% to 17.8 ± 2.0% (p<0.001), reflecting progressively greater erythrocyte size heterogeneity. Together, these findings linked greater anemia severity with lower circulating iron availability and more pronounced hematological abnormalities (**Table 2**).

Physiological and Cardiovascular Findings

Resting and orthostatic heart-rate measures were higher in participants with more severe anemia. Mean resting heart rate increased from 78.2 ± 8.3 beats/min in the mild-anemia group to 86.4 ± 11.7 beats/min in the moderate-anemia group and 94.4 ± 10.8 beats/min in the severe-anemia group (p<0.001). Orthostatic heart-rate change similarly increased from 9.7 ± 4.7 to 15.1 ± 6.0 beats/min (p=0.002). In contrast, systolic blood pressure showed no significant between-group difference (p=0.328). These results showed associations of anemia severity with resting and postural heart-rate responses, whereas systolic blood pressure did not differ significantly among the groups (**Table 2**).

Ultrasonographic Findings

The prevalence of structural uterine abnormalities increased across anemia-severity categories. Leiomyomas were identified in 8 (22.2%) women with mild anemia, 25 (37.3%) with moderate anemia, and 9 (52.9%) with severe anemia. Sonographic features consistent with adenomyosis were observed in 4 (11.1%), 14 (20.9%), and 7 (41.2%) women, respectively (p=0.042). The composite prevalence of structural abnormalities increased from 27.8% in mild anemia to 52.2% in moderate anemia and 76.5% in severe anemia (p=0.003).

Mean uterine volume also increased significantly with anemia severity, measuring 118.8 ± 45.6 mL, 157.1 ± 60.7 mL, and 186.3 ± 87.2 mL across the respective groups (p<0.001). Although leiomyomas were more frequent among women with severe anemia, their individual association did not reach statistical significance (p=0.076). The composite structural abnormality variable demonstrated a stronger association with anemia severity than either lesion category considered separately (**Table 2**).

Table 2. Biochemical, physiological, and ultrasonographic characteristics according to anemia severity

Variable	Mild (n=36)	Moderate (n=67)	Severe (n=17)	p-value
Biochemical and hematological				
Ferritin, µg/L	18.9 (12.0–22.2)	10.0 (7.7–14.3)	5.0 (3.0–9.9)	<0.001
Transferrin saturation, %	16.0 ± 4.0	10.6 ± 3.1	9.1 ± 2.8	<0.001
RET-He, pg	26.8 ± 2.8	25.4 ± 3.3	22.3 ± 2.5	<0.001
RDW, %	15.7 ± 1.5	16.8 ± 1.7	17.8 ± 2.0	<0.001
Physiological parameters				
Resting heart rate, beats/min	78.2 ± 8.3	86.4 ± 11.7	94.4 ± 10.8	<0.001
Orthostatic HR change, beats/min	9.7 ± 4.7	12.0 ± 5.1	15.1 ± 6.0	0.002
Systolic BP, mmHg	113.5 ± 12.6	109.9 ± 11.8	112.8 ± 13.9	0.328
Ultrasonographic parameters				
Uterine volume, mL	118.8 ± 45.6	157.1 ± 60.7	186.3 ± 87.2	<0.001
Leiomyoma, n (%)	8 (22.2)	25 (37.3)	9 (52.9)	0.076
Adenomyosis, n (%)	4 (11.1)	14 (20.9)	7 (41.2)	0.042
Any structural abnormality, n (%)	10 (27.8)	35 (52.2)	13 (76.5)	0.003

Values are mean ± standard deviation, median (interquartile range), or number (percentage), as appropriate. Continuous-variable p-values are based on one-way analysis of variance,

except ferritin, for which the Kruskal–Wallis test was specified. Categorical variables were compared using Pearson's chi-square test. Leiomyoma and adenomyosis categories are not mutually exclusive. RET-He, reticulocyte hemoglobin equivalent; RDW, red-cell distribution width; HR, heart rate; BP, blood pressure.

Multivariable Predictors of Anemia Severity

In the multivariable ordinal logistic regression model, menstrual blood loss, iron-status biomarkers, resting heart rate, and structural uterine abnormalities remained associated with increasing anemia severity after adjustment for the specified covariates. After adjustment for the other model covariates, each 50-point increase in PBAC score was associated with higher odds of belonging to a more severe anemia category (adjusted odds ratio [aOR], 2.57; 95% confidence interval [CI], 1.53–4.32; p<0.001). A 5-µg/L decrease in ferritin was associated with an aOR of 2.60 (95% CI, 1.66–4.08; p<0.001), while a 3-pg decrease in RET-He was associated with an aOR of 2.28 (95% CI, 1.42–3.65; p<0.001).

Among physiological variables, each 10-beats/min increase in resting heart rate was independently associated with greater anemia severity (aOR, 2.38; 95% CI, 1.47–3.85; p<0.001). Structural uterine abnormalities also remained associated with greater anemia severity after adjustment for menstrual blood loss, ferritin, RET-He, resting heart rate, and age (aOR, 3.71; 95% CI, 1.42–9.72; p=0.008). Age was not independently associated with severity in the adjusted model. Detailed regression estimates are presented in **Table 3**.

Table 3. Multivariable ordinal logistic regression for factors associated with increasing anemia severity

Predictor	Adjusted OR	95% CI	p-value
PBAC score, per 50-point increase	2.57	1.53–4.32	<0.001
Ferritin, per 5-µg/L decrease	2.60	1.66–4.08	<0.001
RET-He, per 3-pg decrease	2.28	1.42–3.65	<0.001
Resting HR, per 10-beats/min increase	2.38	1.47–3.85	<0.001
Structural uterine abnormality, present versus absent	3.71	1.42–9.72	0.008
Age, per 10-year increase	2.08	0.84–5.17	0.114

Adjusted odds ratios represent associations with increasing anemia-severity categories in a proportional-odds ordinal logistic regression model. All listed variables were included in the adjusted model. OR, odds ratio; CI, confidence interval; PBAC, Pictorial Blood Assessment Chart; RET-He, reticulocyte hemoglobin equivalent; HR, heart rate.

Discriminatory Performance of the Integrated Model

The model including menstrual blood loss, biochemical parameters and resting heart rate had fivefold cross-validated Area under the ROC Curves (AUC) of 0.848 for moderate to severe anaemia as secondary outcome. The AUC of the model with added structural ultrasonographic abnormalities was 0.884 (95% CI, 0.817-0.937) with an absolute gain of .036. The Brier score was also lower for the integrated model, dropping from 0.148 to 0.137, which means that the total prediction error is less.

These results indicated that the models investigated could offer better discriminative information than the use of structural imaging features. Improvement was based on internal validation; lack of external validation, small severe anemia subpopulation limits conclusions about performance in other clinical populations.

DISCUSSION

This study is a prospective observational study in which biochemical markers of iron status, cardiovascular physiological parameters and ultrasonographic uterine abnormalities were evaluated and compared to the level of severity of iron-deficiency anemia (IDA) in women with heavy menstrual bleeding (HMB)[1]. Menstrual blood loss, serum ferritin and reticulocyte hemoglobin equivalent (RET-He) all decreased and resting heart rate (RHR) increased with increasing degree of anemia and were independently associated with each other. Severe anemia was common among the study population, 70.0% of all were moderate or severe. The model with ultrasonographic features showed better performance for internal validation, with discrimination better than the model not including imaging features, further supporting the use of a multidomain assessment strategy[2,3]. These findings agree with the multifactorial pathogenesis of IDA in the context of HMB, which involves the presence of chronic iron loss, poor iron

utilization in the production of red blood cells, and uterine disease processes[4-6].

There was significant association between the amount of menstrual blood loss and the severity of anemia with mean Pictorial Blood Assessment Chart (PBAC) scores for women with mild anemia, moderate anemia and severe anemia being 201.3, 245.3 and 298.2 respectively. This association was present even after adjusting for biochemical, physiological and ultrasonographic characteristics (adjusted odds ratio [aOR] 2.57 per 50-point increase; 95% confidence interval [CI] 1.53–4.32). This corresponds to the well-known association between abnormal bleeding and iron loss in the sequence. Munro[3] pointed out that HMB is an important source of iron deficiency and IDA especially when the bleeding is persistent and/or difficult to control. Similarly, Mansour et al.[2] pointed out the importance of early detection of iron deficiency in women who have HMB. But, the relationship between blood loss and Hb concentration are dependent on initial iron stores, iron intake in the diet and duration of bleeding and thus PBAC ought to be correlated with the hematological and biochemical investigations. But patient reported experiences also reinforce the importance of timely assessment and management of the HMB associated IDA [12].

The biochemical results demonstrated that with worsening anaemia and higher RCDW, the ferritin, transferrin saturation and RET-He concentration decreased[7]. The serum ferritin of the mild anemia patients was 18.9 µg/L, which reduced to 5.0 µg/L in severe anemia, and the RET-He reduced from 26.8 to 22.3 pg[8-12]. Both markers remained highly associated with severity of anaemia in the adjusted analysis and both were associated with different aspects of iron deficiency. Ferritin is more indicative of iron stores while RET-He is more indicative of the iron available for erythropoiesis. Kılıç et al.[4]showed the diagnostic value of reticulocyte hemoglobin in IDA, as an additional parameter to conventional hematological parameters. The present findings suggest that the use of combined markers of storage and function may prove useful in the characterization of hematological impairment other than hemoglobin concentration or morphology of erythrocytes. Haematological parameters are also recommended to be used in addition to the iron-status test[13,14].

There was an increase in resting HR and orthostatic HR increments as the severity of anemia increased. The resting heart rate of the women with mild anemia was 78.2 beats/min and of the women with severe anemia was 94.4 beats/min, with a corresponding increase in the orthostatic heart-rate increments. Menstrual blood loss, iron-status markers, age and structural uterine abnormalities were adjusted for, and a resting heart rate was independently associated with severity (aOR 2.38 per 10-beats/min increase; 95% CI 1.47–3.85). Physiologically, the results are consistent with compensation in the cardiovascular system in response to reduced oxygen carrying capacity to tissues. However, drugs, current illness, and hydration status influence the HR and orthostatic responses. They are therefore not specific markers of iron deficiency, but can be valuable addition to current diagnostic tools. Physiologic symptoms should not be disregarded, and anemia and iron deficiency induced by HMB has been shown to be associated to poor QoL [2,3,15].

The percentage of uterine structural abnormalities increased in each of the grades of anemia, from 27.8% to 76.5%, respectively. Uterine volume was directly associated with the severity of the anemia and leiomyomas and sonographic features suggestive of adenomyosis were more commonly seen in women with higher hematological impairment. In the multivariate analysis, the composite structural-abnormality variable was still positively associated with the degree of anemia (aOR 3.71; 95% CI 1.42–9.72). The Morphological Uterus Sonographic Assessment (MUSA) consensus has been set up to define sonographic findings of myometrial abnormalities and uterine masses with the goal of standardize the language to describe findings that may be implicated in abnormal uterine bleeding[6]. Besides that, there are also direct and indirect ultrasonographic findings of adenomyosis, which have been classified in the updated MUSA definitions[7,16]. From the present results it may be inferred that structural assessment of the uterus might provide clinically useful information in addition to the quantified menstrual blood loss but that the finding of a structural defect alone could not determine aetiology of anaemia in any given patient. To better understand the ultrasound findings of the present study, the correlation of uterine fibroids with adenomyosis and heavy menstrual bleeding, and the effect of these fibroids on the severity of symptoms, were examined in previous studies[16–19].

The integrated model was able to identify moderate to severe anemia with a cross-validated area under the receiver operating characteristic curve of 0.884 compared to the model with only menstrual, biochemical and physiological variables and no ultrasonographic findings. In the latter case, with the addition of structural uterine abnormalities, the corresponding Brier score decreased from 0.148 to 0.137 [20]. This shows complementary discriminatory information in the areas studied, although the absolute discrimination improvement was not statistically significant and not all areas showed improvement. Based on these results, more research is needed using multidomain assessment of hematological parameters and targeted pelvic imaging. However, internal validation does not necessarily imply generalizability and independent external validation is needed, especially when using clinical prediction models, where there is a known issue of overfitting and sample size [2,8,20].

The strengths of the study were the prospective nature of the recruiting at the 2 participating centres, the standardisation of the assessment of menstrual blood loss, functional measures of iron status, cardiovascular assessments, and the structured ultrasonographic assessment of the uterine morphology. There are a few restrictions to be taken into consideration. Given the observational design, causal inferences cannot be made, and almost all candidate predictors and severity of anemia were measured near simultaneously making inferences regarding the progression of anemia difficult. The number of subjects (N = 120) is too small to obtain reliable and exact estimates of the multivariable parameters, especially the 17 women with severe anemia. In addition, unmeasured factors such as nutrition, prior treatment, hydration state and differences in the evaluation of menstrual blood loss may have been a factor. Other factors such as the nature of the lesion and the technique used for examination can also influence the ultrasonographic findings. Larger studies with longitudinal hematological measurements with harmonized imaging protocols and independent validation are needed to determine if the associations observed are valid for clinical risk stratification[15-20].

CONCLUSION

In women with heavy menstrual bleeding, higher menstrual blood loss, lower ferritin, lower RHE, and higher RHR and uterine abnormalities each independently predicted more severe IDA. The cross validated discrimination for moderate to severe anemia was higher with the model that contained ultrasonographic features. These results suggest that the clinical domains should be considered when evaluating IDA in the context of HMB and the clinical usefulness of the integrated model is still to be established. Before the integrated model could become a general risk stratification tool in the clinic, it should undergo further prospective testing in larger cohorts of patients and external validation.

Authors' Contributions

SEA, MA, SK, TH and HNK contributed to the study conception and design. SEA and MA were involved in data collection and methodology. SK contributed to data analysis and interpretation. SEA prepared the initial manuscript draft, while MA, SK,TH and HNK critically reviewed and revised the manuscript. All authors read and approved the final manuscript and agreed to be accountable for their respective contributions.

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Competing Interests

The authors declare that they have no competing interests.

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