Diagnostic Accuracy of Iron Deficiency Anemia in Elderly Patients with Inflammatory Conditions

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Article

Diagnostic Accuracy of Iron Deficiency Anemia in Elderly Patients with Inflammatory Conditions

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Abstract

Background/Objectives: Our aim was to evaluate the performance of the reticulocyte hemoglobin content (CHr) assay to diagnose iron deficiency anemia in a context of inflammation in an elderly population, by comparison with the gold standard. **Methods**: Patients over 18 years old, with anemia and biological inflammatory syndrome, were included consecutively. The accuracy of CHr was compared with that of the gold standard, soluble transferrin receptor/log ferritin. Analyses were performed according to age (subjects over and under 75 years of age) and included specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and the area under the ROC curve (AUC). **Results**: A total of 174 patients were included, with respectively 84 and 89 patients aged under or 75 years and over, the mean age being 72.5 years (SD 16.9). In the older population, the mean age was 84.6 years (SD 5.2), and 40% were female; the mean hemoglobin was 9.6 (SD 1.4) g/dl and mean CRP 89.7 (SD 72.9) mg/L. The optimal threshold value, determined by maximizing the Youden index, was 25.2pg, at which sensitivity and specificity reached 67% and 94%, respectively. The corresponding PPV and NPV were 62% and 95%. The AUC of the ROC curve was 0.81 with 95% CI: [0.65-0.97], suggesting good diagnostic accuracy. **Conclusions**: In an elderly population with anemia in a context of inflammation, assessing iron deficiency by including CHr is simple, inexpensive, rapid and practical, as it can be measured as a by-product of a routine complete blood count at little incremental cost, and good NPV can exclude a diagnosis of IDA.

Keywords: iron deficiency anemia; reticulocyte hemoglobin content; inflammation

1. Introduction

The most prevalent causes of anemia are iron deficiency. Low levels of ferritin are commonly used to diagnose iron deficiency anemia (IDA), but this parameter is of limited value if the iron deficiency is influenced by inflammation, infection or neoplastic disease. This situation is common in the elderly, where the causes of anemia are often multiple and interrelated [1]. The diagnosis of iron deficiency in this context of inflammatory anemia is fundamental, as it should prompt a search for an underlying pathology, and particularly digestive cancer.

In elderly patients, soluble transferrin receptor (STR) has been shown to be an indicator of iron deficiency that is unaffected by concomitant chronic disease and inflammation, and levels of the soluble form are elevated in serum and plasma in cases of iron deficiency [2]. The STR Index, STR/log ferritin, is superior to STR in detecting iron deficiency in patients with anemia and inflammation, when both parameters are taken into account [3].

The reticulocyte hemoglobin content (CHr), similar to STR, identifies iron deficiency and is unaffected by the acute-phase reaction [4,5]. Both CHr and STR are sensitive markers of body iron status that add value to conventional tests regarding the detection of iron deficiency.

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The advantage of CHr is that it is modified at a relatively early stage in the development of iron deficiency, offering an indirect measure of the iron available for erythropoiesis over the previous 3–4 days. Moreover, CHr can be determined routinely using automated hematology systems (laser diode flow cytometry), with no need for further investigation, and therefore involves no extra cost.

In the general population, the analysis of CHr, ferritin, transferrin saturation and mean cell volume (MCV) has demonstrated that CHr has the highest overall sensitivity and specificity of these peripheral blood tests for predicting an absence of bone marrow iron stores [6].

CHr may therefore be a clinically useful marker for determining IDA in the general population [7].

The initial evaluations of the efficiency of CHr in diagnosing anemia in the elderly did not show any advantage over other parameters. CHr did not perform better than classic red cell indices such as mean corpuscular hemoglobin (MCH) in differentiating iron deficiency anemia and chronic disease anemia in geriatric patients [8], but patients with chronic inflammation parameters were underrepresented in this study.

Our aim was to assess the performance of the CHr assay in diagnosing IDA in a context of inflammation in an elderly population, by comparison with the gold standard, the STR index.

2. Materials and Methods

2.1. Study Design

This was a monocenter, observational, prospective study that included all consecutive patients hospitalized in 2023 for anemia in the University Internal Medicine Department at Hôpital Avicenne, Bobigny in the Greater Paris region.

The study was reported in line with the STROBE statement, and was approved and registered by the Local Ethics Committee (CLEA september 2025)

2.2. Study Participants

The study involved consecutive eligible patients aged over 18 years and hospitalized during the study period.

The inclusion criteria were a hemoglobin level lower than 12.5 g/dl in men and 11.5 g/dl in women, and a biological inflammatory syndrome characterized by two parameters: CRP over 20 mg/l and an abnormally long half-life marker of inflammation (e.g.,fibrinogen, alpha-2 globulins, haptoglobin).

Patients not eligible for inclusion were those not affected by another obvious cause of anemia: active massive hemorrhage, hemoglobinopathies, B9 or B12 vitamin deficiency, chronic end-stage renal disease, neoplastic hemopathy, hypothyroidism, hemolysis and those who had recently received an iron infusion or red blood cell transfusion. Biological exclusion criteria were a reticulocyte count higher than 100 G/L and macrocytosis (MCV >100 fl), in order to exclude hemolysis and undiagnosed myelodysplasia.

2.3. Data Sources and Variables

Demographic data were collected. Biological data included hemoglobin, hematocrit, mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), serum iron, transferrin and ferritin levels, transferrin saturation, soluble transferrin receptor (STR), C-reactive protein (CRP), chronic inflammatory parameters and CHr. The STR index was calculated as STR/log ferritin.

Blood samples for a complete blood cell count and CHr determination were collected in K3-EDTA tubes and tested using an XN1500 hematology analyzer (SYSMEX, Tokyo, Japan).

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2.4. Statistical Analysis

Mean values and standard deviations (SD) of the measured parameters were calculated.

Correlations between CHr values and the STR index were measured using Pearson's correlation test.

To assess the accuracy of CHr in diagnosing IDA in a context of inflammation, the STR index (STR/log ferritin) was chosen as the gold standard. This index has been proposed as a good indicator when evaluating iron deficiency, as it reflects a relationship between a rise in STR and a fall in ferritin levels [9,10]. The STR index provides an estimate of body iron over a wide range of normal and depleted iron stores, and is more efficient than STR in detecting iron deficiency in patients with anemia and inflammation [3]. Diagnostic accuracy was assessed by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CHr in identifying iron deficiency anemia (IDA), using the STR index as the reference standard. The optimal threshold value was determined using the Youden index, derived from receiver operating characteristic (ROC) curve analysis. Diagnostic performance was further assessed by calculating the area under the ROC curve (AUC).

A 26 pg threshold for CHr had previously been proposed by Lungren, and in our study the sensitivity and specificity of iron-deficiency anemia detection at this level reached 92.3% and 100%, respectively [11].

We then compared the results of CHr and the gold standard STR index in terms of detecting IDA in a context of inflammation.

In a sensitivity analysis we separated patients below the age of 75 years and those aged 75 years and over, in order to assess the accuracy of CHr in the elderly.

3. Results

3.1. Patient Characteristics

A total of 174 patients were included, with 84 below the age of 75 and 89 aged 75 years and over. Their clinical and biological data are detailed in Table 1. The mean age was 72.5 (SD 16.9) years; 44% of them were female, with mean hemoglobin values at 9.5 (SD 1.3) g/dl and mean CRP at 94.1 (SD 76.0) mg/L.

Table 1. Clinical and biological characteristics of the patients.

	Patients less than 75 years old n=85	**Patients aged 75 years and over n=89**	**All patients n= 174**
Mean age (SD)	58.8 (14.8)	84.6 (5.2)	72.5 (16.9)
Female n (%)	42 (47)	34 (40)	174 (44)
Hemoglobin g/dl mean (SD)	9.4 (1.3)	9.6 (1.4)	9.5 (1.3)
Hematocrit % mean (SD)	29.2 (4.2)	29.2 (4.3)	29.2 (4.3)
MCV fl mean (SD)	83.3 (8.7)	87.7 (8.6)	85.7 (8.9)
MCHC g/dl mean (SD)	27.1 (3.7)	29.1 (3.2)	28.1 (3.6)
MCH pg mean (SD)	32.5 (1.8)	33.1(1.2)	32.8 (1.6)
Reticulocytes G/L	55.43 (24.0)	56.08 (26.0)	55.77 (23.9)
Serum iron ⍰m/L mean (SD)	5.84 (2.7)	7.75 (4.5)	6.84 (3.9)
Transferrin g/L mean (SD)	1.75 (0.4)	1.64 (0.5)	1.70 (0.5)
IBC ⍰m/L mean (SD)	44.01 (10.9)	41.20 (11.2)	42.55 (11.5)
Transferrin saturation % mean (SD)	14.19 (6.9)	22.63 (22.8)	18.58 (17.6)
Serum ferritin ⍰g/L mean (SD)	646.42 (789.9)	759.20 (1601.9)	704.42 (1271.6)
STR mg/l mean (SD)	5.79 (6.5)	3.82 (1.9)	4.78 (4.8)
STR/log ferritin index mean (SD)	2.52 (3.4)	1.73 (1.6)	2.12 (2.6)
CHr pg mean (SD)	26.4 (4.5)	28.8 (4.6)	27.7 (4.7)

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CRP mg/L mean (SD)	98.7(79.3)	89.7 (72.9)	94.1 (76.0)

MCV: mean cell volume; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; IBC: iron-binding capacity; STR: soluble transferrin receptor; CHr: reticulocyte hemoglobin content; CRP: C-reactive protein.

3.2. Correlations STR Index and CHr

Correlations between the STR index and CHr for patients under 75 years or 75 years and over are presented in Figures 1, and 2, respectively, at -0.39 (95%CI: -0.55; -019), p<0.001 and -0.46 (95%CI: -0.61; -0.28) p<0.001.

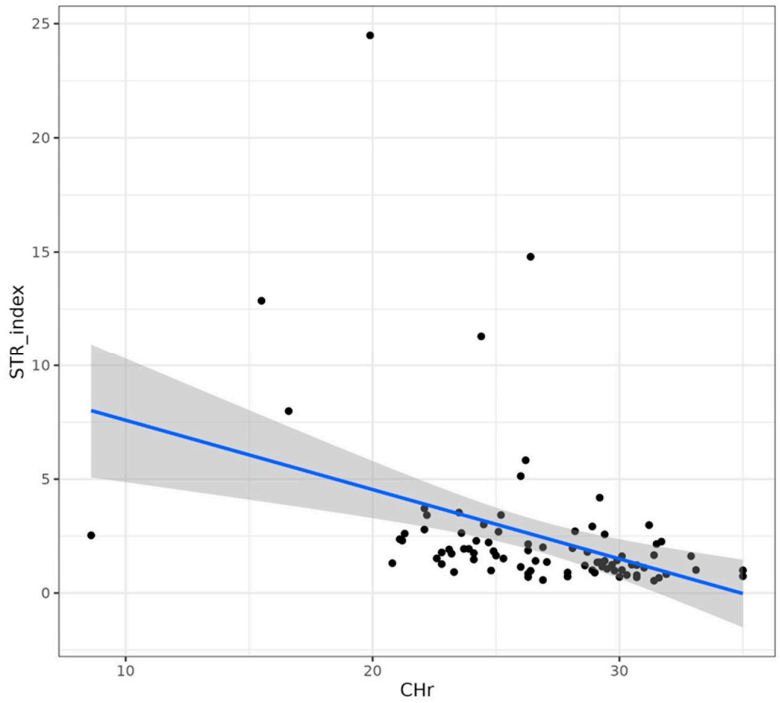

Figure 1. Correlation between the STR index and CHr in patients below the age of 75 years (n=84). Correlation coefficient: -0.39 (95%CI:-0.55; -019), p<0.001.

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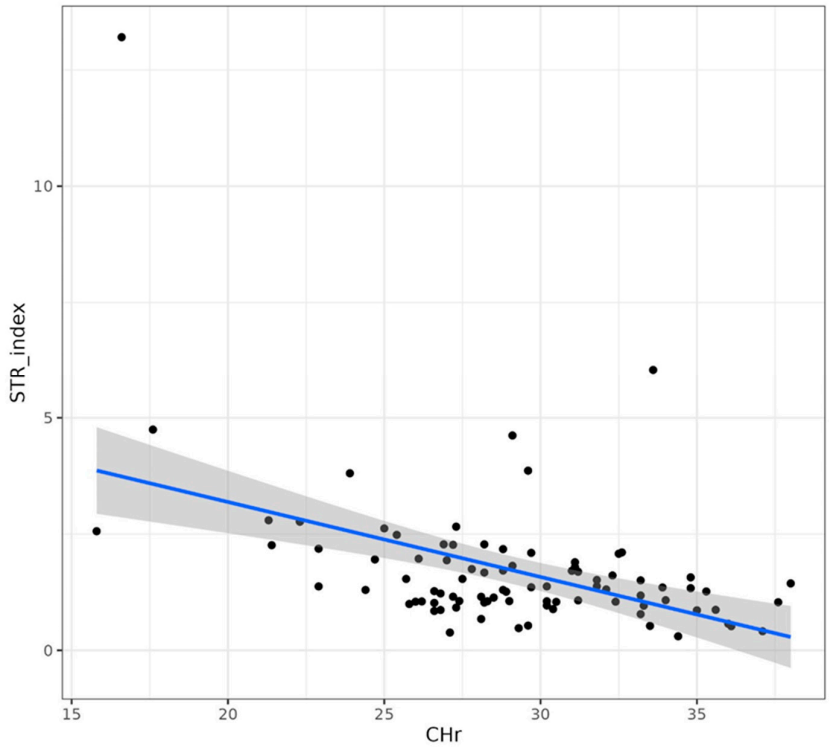

Figure 2. Correlation between the STR index and CHr in patients aged 75 years and over (n=89). Correlation coefficient: -0.46 (95%CI: -0.61; -0.28) p<0.001.

3.3. Diagnostic Accuracy

Evaluations of best CHr thresholds for IDA diagnosis in the whole population are presented in Table 2.

Table 2. Accuracy of CHr in the diagnosis of mixed anemia, inflammatory syndrome and iron deficiency, compared with the gold standard, the STR index.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Threshold	AUC
All patients	0.71	0.78	0.44	0.92	26.25	0.79
Patients Under below the age of 75 years	0.73	0.69	0.46	0.88	26.25	0.76
Patients aged 75 years and over	0.67	0.94	0.62	0.95	25.2	0.81

The different ROC curve analyses used to assess the diagnostic performance of CHr in detecting iron-deficiency anemia are presented in Figures 3, 4, and 5.

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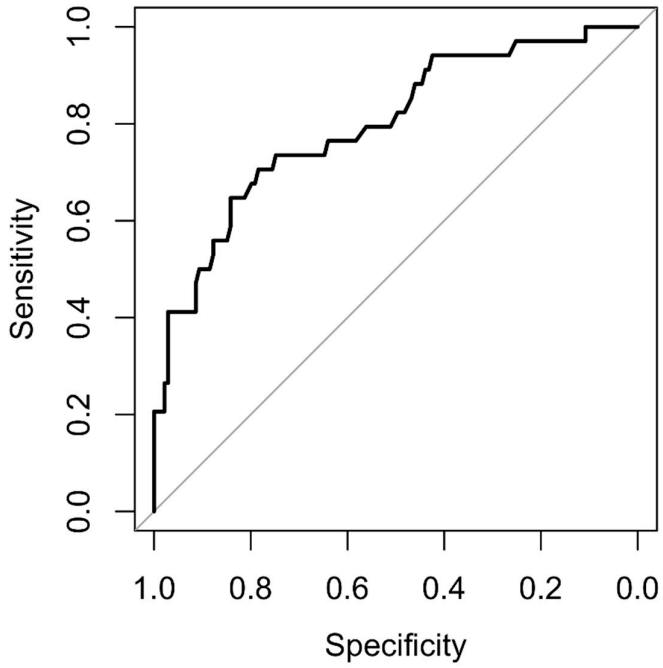

Figure 3. ROC curve for all patients: Area under the curve: 0.79 95% CI: 0.71-0.88.

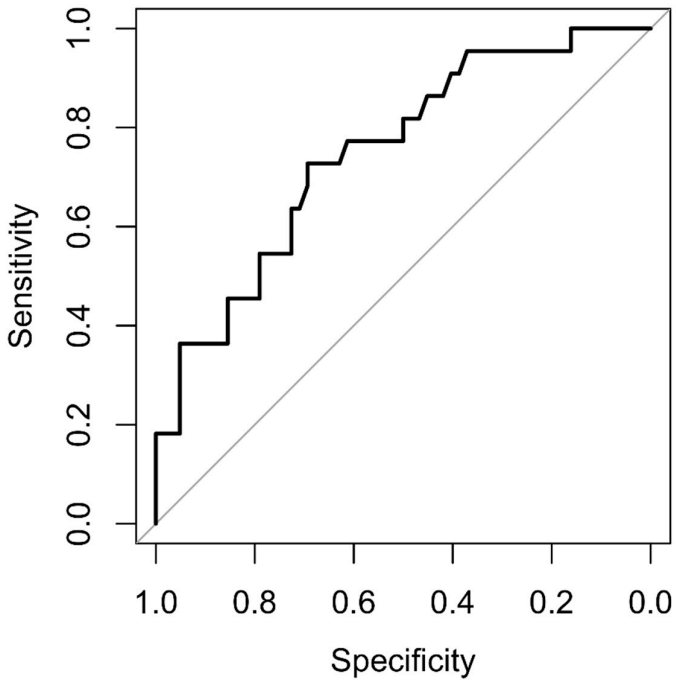

Figure 4. ROC curve for patients below the age of 75 years. AUC: 0.75; 95% IC: 0.63-0.87.

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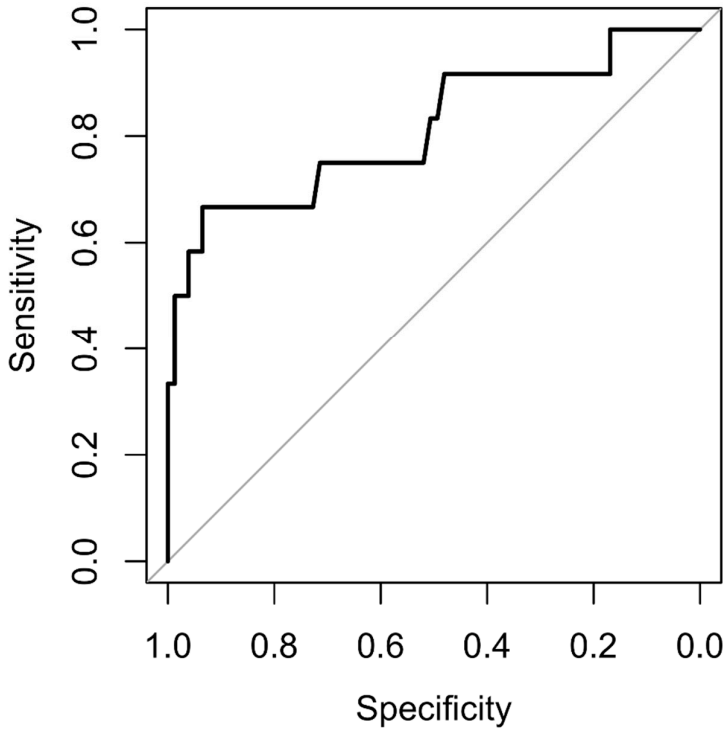

Figure 5. ROC curve for patients aged 75 years and over. AUC: 0.81; 95% CI: 0.65-0.97..

The optimal threshold value for the entire population, determined by maximizing the Youden index, was 26.25 pg. At this threshold, sensitivity and specificity reached 71% and 78%, respectively. The corresponding PPV and NPV were 44% and 92%. The AUC of the ROC curve was 0.79 with 95% CI: [0.71-0.88], suggesting fair diagnostic accuracy.

3.4. Sensitivity Analysis

In the older population, the mean age was 84.6 years (SD 5.2) and 40% of the subjects were female, with mean hemoglobin values at 9.6 (SD 1.4) g/dl and mean CRP at 87.7 (SD 72.9) mg/L.

The optimal threshold value, determined by maximizing the Youden index, was 25.2 pg. At this threshold, sensitivity and specificity reached 67% and 94%, respectively. The corresponding PPV and NPV were 62% and 95%. The AUC of the ROC curve was 0.81 with 95% CI: [0.65-0.97], suggesting good diagnostic accuracy.

Among patients below the age of 75 years, the optimal threshold value was 26.25, the AUC of the ROC curve was 0.75 with 95% CI: [0.63-0.87]; sensitivity reached 73%, specificity 69%, PPV 46% and NPV 87%..

4. Discussion

This study demonstrated that CHr shows fair diagnostic accuracy in detecting iron deficiency in a context of inflammation. It also suggests that the test performed better in older patients, particularly those aged over 75 years, within a hospitalized population.

The optimal threshold appeared to vary with age: a cutoff point of 26.25 pg produced the best performance in patients below 75 years of age, whereas a lower threshold of 25.2 pg was more appropriate among those aged 75 years and older.

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To the best of our knowledge, this is the first analysis to have involved such a large number of subjects. It addresses an important challenge, as the presence of iron deficiency and an inflammatory syndrome should prompt a search for a neoplastic cause [12].

The main finding of the study was a very good NPV with the CHr assay which eliminated the hypothesis of associated iron deficiency in cases of inflammatory anemia.

In the general population [11], the CHr assay is effective in the presence of underlying comorbidities such as renal failure and inflammatory bowel disease. CHr values also appeared to remain consistent regardless of age, when children and adults are compared [11].

Few data are available in the literature on the elderly, and the findings have remained somewhat inconclusive. Analyses of CHr levels were not superior to MCV or MCH regarding the diagnosis of IDA when screening for iron deficiency in elderly anemic hospitalized patients, but selected patients were different; very few patients had a chronic inflammatory syndrome in the study by Joosten et al.[8] and the patients were younger in the Karlsson study [13].

The AUC of the ROC curve in the 75 year and over population was better than in the control group below the age of 75, thus confirming the usefulness of this evaluation in the former population. In previous studies in adults [7], the AUC that detected iron deficiency with CHr had been 0.90.

In this particular situation - elderly subjects, a context of inflammatory anemia - the CHr assay can be useful and safe, making it possible to rule out underlying iron deficiency.

The choice of gold standard for the diagnosis of IDA in a context of chronic inflammation has been a subject of debate because, as mentioned above, there is no single biological parameter that can be used to diagnose iron deficiency with certainty. Ferritin cannot be the gold standard, as it is one of the first proteins to be affected by inflammatory syndrome, and apart from extreme values, a ferritin assay is not discriminating. The only indisputable gold standard is an osteomedullary biopsy, which is invasive and therefore difficult and costly to perform as a routine procedure.

In an elderly population, several parameters may influence CHr results when trying to diagnose IDA in a context of inflammation.

STR has been shown to constitute an indicator of iron deficiency that is unaffected by concomitant chronic disease and inflammation, and several authors questioned whether it should be used as a reference in the context of inflammation. Punnonen et al. [9] found an ROC curve with an AUC of 0.98, suggesting excellent test performance, a result that was confirmed by a more recent meta-analysis [14].

The STR Index was subsequently shown to be superior to the STR assay in detecting iron deficiency in patients with anemia and inflammation [3], so we therefore chose to compare the results obtained with CHr against this parameter.

CHr appears to have other advantages; for example, this assay seems to detect iron deficiency earlier and the findings correlate well with evolution of the deficiency [11].

One of the limitations of our study was the relatively small number of patients included. The mean hemoglobin levels were relatively moderately lower but reflected real-life data. In all cases, these rates were well below those published in the literature, with averages of around 30 in the two other studies of elderly patients [8,13]. Another limitation was the lack of data on the evolution of CHr levels, particularly in relation to hemoglobin levels, and as a function of the duration of inflammation. We deliberately included patients with a chronic inflammatory syndrome, in order to answer the real-life question.

5. Conclusions

In conclusion, in an elderly population with anemia, the inclusion of CHr in the assessment of iron deficiency is simple, inexpensive, rapid and practical. It can be determined as part of a routine complete blood count at little incremental cost, and a good NPV value can exclude the diagnosis.

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VL. Supervision: RD, RL. Writing-original draft RD, JL, PC, VL, RL. Guarantor: RD.. All authors have read and agreed to the published version of the manuscript.".

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by our Ethics Committee: CLEA september 2025.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.
Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CHr	reticulocyte hemoglobin content
IDA	iron deficiency anemia
STR	soluble transferrin receptor
MCV	mean cell volume
MCHC	mean corpuscular hemoglobin concentration
MCH	mean corpuscular hemoglobin
PPV	positive predictive value
NPV	negative predictive value
AUC	area under the curve

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