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Mini Review

Revaluating serum ferritin as a marker of body iron stores in the traceability era

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Abstract

Serum ferritin is used for diagnosing iron-related disorders. However, most studies validating this application were performed before the introduction of the 2nd and 3rd WHO International Standards (ISs) to harmonize assay results. We revised the available literature to evaluate if consolidated clinical applications of ferritin and recommended cut-offs have been validated using ISs calibrated assays. All Medline retrieved reviews and individual studies performed since ISs availability were selected and analyzed according to predefined criteria. Concerning ferritin and iron deficiency (ID), only one review, including studies published before 1988, met established criteria. Results showed that ferritin can effectively rule out ID anemia in patients with or without inflammatory disease at cut-offs of 70 and 40 μg/L, respectively. From two studies using ISs calibrated assays that met inclusion criteria, no information emerged on which cut-off should be employed to obtain similar sensitivity. Regarding iron overload, even when the framework was restricted to hereditary hemochromatosis, no synthesis of scientific evidence, if any, about diagnostic accuracy of ferritin was available both before and after ISs introduction. Available evidence of the ferritin diagnostic effectiveness is limited to ID conditions. Recommended cut-offs for this application are, however, based on studies published from 1970 to the 1980s using non-harmonized assays.

Keywords: anemia; diagnosis; ferritin; harmonization; hemochromatosis; iron.

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Introduction

Ferritin is the main iron storage protein of the body with a critical role in iron homeostasis. In the clinical setting, measurement of circulating ferritin is used extensively in the diagnosis of iron-related disorders (1). The iron-free protein, apoferritin (MW 450 kDa) consists of 24 subunits, heavy (H, MW 21.0 kDa, 182 amino acids) and light (L, MW 18.5 kDa, 174 amino acids) monomers, forming a shell to hold ferric iron as ferrihydrite. Under physiological conditions the ratio between H and L monomers varies according to the tissue; L-subunit rich isoferritins are mainly found in liver and spleen (2). Although isoferritin profile is quite specific for each organ, it changes in the course of infections and inflammation, which affect the ratio between H and L monomers (3). Recent reports demonstrate that ferritin is also involved in other aspects of biology, such as cell activation, development, immunity and angiogenesis (3).

Intracellular iron-storing ferritin derives from apoferritin synthesized by the smooth endoplasmic reticulum and further partially converted in insoluble hemosiderin. Circulating ferritin derives from a small amount of apoferritin released from the rough endoplasmic reticulum and it differs from the intracellular form since glycosylated and poor in iron (2). In healthy subjects, blood ferritin concentrations are reported to reflect the amount and the changes of intracellular ferritin as confirmed by studies employing quantitative phlebotomy, radio-iron retention and bone marrow aspirate (BMA) (4–6). In particular, it is widely accepted that 1 μg/L of serum ferritin corresponds to approximately 8 mg of stored iron (5).

Since the 1970s, using radioimmunoassays (RIA), the measurement of ferritin in blood has been regarded as a sensitive index to diagnose iron deficiency (ID) and iron overload (IO) (1). However, some criticisms arose as results from these assays widely varied according to the isoferritin type of labelled ferritin, the specificity of antibodies, and the type of ferritin used as standard (7). As the poor comparability of analytical results is likely to cloud the interpretation of reported data, a project for the development of a reference material for ferritin was started in the 1980s in order to use it for achieving comparability between test results from different methods and laboratories (8, 9). The outcome of the project was the release at different times of three preparations, subsequently established as International Standards (ISs) by the World Health Organization (WHO) (10). The 1st IS for ferritin (liver, code 80/602) was established in 1985 (8). It was superseded by the 2nd IS for ferritin (spleen, code 80/578) in 1992.

Unfortunately, as the value of the latter was not traced to the 1st IS, a new traceability chain was practically established, making ferritin values 5%–10% higher if the 2nd IS was used for calibration (11). More recently, the 2nd IS was replaced by the 3rd IS for ferritin (recombinant, code 94/572), its evaluation and establishment being described in a publication in 1997 (9). This time the ferritin value assigned to the 3rd IS was traceable to the 2nd IS, so that the continuity between the two materials was warranted and the traceability chain having on the top the 2nd IS definitively established given the unlimited supply of the recombinant material. In 1999, requirements for assay traceability, as outlined in the European Community In Vitro Diagnostic Directive (EC IVDD), prompted manufacturers to align their analytic systems to the available higher-order 3rd IS, in turn traceable to the 2nd IS (12). However, the use of these preparations as reference required a change in the test calibration that gave origin to different results (11, 12). Because of this change, the inference and the validity of the large number of data from clinical studies performed before the implementation of traceability of ferritin results to 2nd and 3rd ISs should be confirmed. As recently pointed out (13), tracing back the calibration of routine assays to a reference material can actually modify the relation of analyte results to existing decision thresholds and this may invalidate some of the clinical decision-making criteria currently used. The purpose of this study was to critically revise the scientific evidence available in literature on the diagnostic accuracy of ferritin as a biomarker of body iron stores and evaluate if consolidated clinical applications and recommended cut-offs have been validated using 2nd–3rd IS calibrated assays.

Methods

Literature search

Concerning ferritin in ID, two Medline searches were conducted by PubMed, from 1966 up to December 2011, by considering as MeSH terms [ferritin] and [iron] or [iron disease] and "diagnosis" as subheading, with limits "Title/Abstract, Human Subjects, English''. The final aim of the search was to identify original articles and reviews in which serum ferritin measurement was investigated as diagnostic tool for ID. From the 582 papers initially identified, those focused on the effectiveness of iron supplementation were eliminated, and 229 papers were finally considered as potentially eligible for the study purpose. Among these papers, 65 reviews and 164 original papers were identified.

Concerning ferritin in IO, a first Medline search was performed with the previously mentioned "limits" but with MeSH terms [ferritin] and [iron overload] and "diagnosis" as subheading. This search allowed drawing 1379 papers, including 172 reviews. According to the study purpose, a preliminary screening of papers warned on the low specificity of the search, due to the high prevalence of works employing ferritin to describe baseline clinical data or as outcome for phlebotomy. According to the classification of IO (14), a second Medline search was performed by considering as MeSH terms [ferritin] and [hemochromatosis] and "diagnosis" as subheading. This research allowed drawing 652 original papers and 76 reviews.

The full text of all recruited papers was considered and reference lists from all reviews and primary articles were examined. This allowed to check the goodness of the Medline searches and to throw any possible additional relevant article. Given the limitation for the number of references to be reported in this minireview, the complete list of papers retrieved by Medline searches during the study is available on request to the corresponding author.

Selection criteria

The reviews focused on the use of ferritin for the diagnosis of ID and IO were classified as 'scientific overview' for their methodology according to the following criteria (15):

1. systematic search and selection of the studies,
2. methodological assessment of evidence,
3. synthesis of evidence by quantitative analysis.

The original papers were selected according to the following criteria:

1. studies carried out using ferritin assays calibrated with 2nd or 3rd IS or, if this information was not available, papers published from 1999, time of proposed implementation of the EC IVDD (16),
2. evaluation of ferritin diagnostic accuracy in comparison with the diagnostic gold standard method as primary or secondary aim,
3. data presentation to allow the calculation of diagnostic sensitivity at least.

In particular, for ID the BMA examination was retained as diagnostic gold standard method (17). For hemochromatosis, the framework was restricted to hereditary hemochromatosis (HHC), as the preliminary evaluation of the available literature revealed very few studies employing the diagnostic reference standards for IO (liver iron concentration after biopsy, magnetic resonance imaging, spectroscopy), whereas the use of genetic tests, detecting *HFE* gene mutations C282Y and H63D, is mandatory for diagnosing the hereditary condition. Recruited papers were excluded when considering serum ferritin concentrations to select patients, using serum ferritin concentrations in the diagnostic algorithm to classify patients, and/or other biochemical and hematological blood indices were employed as diagnostic reference standard method. The quality of original studies was finally evaluated according to the Standards for Reporting Diagnostic Accuracy (STARD) (18).

Results

Ferritin as a marker of ID

Sixty-five reviews related to the accuracy of serum ferritin for ID diagnosis were identified, but only one met the criteria of a "scientific overview" (17). The synthesis of evidences

obtained in this systematic review, including 55 studies published since 1972 up to 1988, was later reported in the following and more recent narrative reviews, in none of which the systematic analysis was, however, updated (19–22). The main result of the overview by Guyatt et al. was the demonstration of diagnostic effectiveness of serum ferritin, measured by RIA, for detection of ID anemia across an exhaustive population of anemic patients with and without inflammatory, liver or neoplastic diseases (17). By aggregating data across different studies, the estimation of likelihood ratio showed that, in the general population, an effective rule in for ID anemia can be obtained at a ferritin decision level of 15 μg/L, whereas an effective rule out may be achieved by adopting thresholds of 40 μg/L for general population and 70 μg/L for patients with inflammatory/liver disease, respectively.

Concerning the four original papers published after the ISs release and employing BMA as diagnostic reference standard (23–26), only two met the third selection criterion (estimate of diagnostic sensitivity) (24, 26) (Table 1). Other papers only provided the estimation of ferritin mean concentrations in the studied patients (23, 25) and employed for ID diagnosis an algorithm including other laboratory tests in addition to serum ferritin (23). The two selected studies were focused on the capability of ferritin to discriminate [by estimating the area under the receiver operating characteristic (ROC) curve (AUC) and/or by calculating diagnostic sensitivity/specificity] patients with ID anemia in malignancies and benign hematologic diseases (24, 26). One of these studies resorted to a cross-sectional design by considering retrospectively patients with available BMA estimation (26). In spite of the inclusion of patients with inflammatory comorbidities, these authors employed lower limits of the value distribution in a healthy population partitioned by gender, resulting in a very insensitive but highly specific ability of ferritin to predict BMA iron findings (26). The other study adopted a decision level determined by ROC analysis, balancing sensitivity and specificity of ferritin in determining the absence of stainable iron in the BMA (24). From the two papers meeting inclusion criteria, no information emerged, however, on which cut-off should be employed to obtain high sensitivity and an effective rule out of ID anemia.

Ferritin as a marker of HHC

Among the 76 reviews on serum ferritin as a test for HHC diagnosis, only two met the first criterion established for a 'scientific overview' (27, 28). These two papers carried out a systematic evaluation of the available evidence up to 2004 (27) and up to 2009 (28), respectively, to update the previously published guideline on the screening of HHC in primary care (29). These overviews answered to several questions concerning prevalence, risk for complications and death, usefulness of serum markers, effectiveness of therapeutic strategies, and cost-benefit of the screening for HHC. In particular, they identified a broad number of studies addressed to evaluate: 1) serum ferritin performance in the screening of HHC; 2) serum ferritin concentrations in response to therapeutic phlebotomy; 3) serum ferritin change over time in asymptomatic patients; and 4) predictive value of serum ferritin in predicting severe HHC complications. However, no synthesis of the available evidence about the diagnostic accuracy of the test by quantitative analysis was provided because of several limitations affecting study designs. Major issues were: 1) the lack of an independent comparison between serum ferritin measurement and a standard reference diagnostic method for HHC; 2) the application of the diagnostic standard method to a limited proportion of patients; and 3) the subjective adoption of widely different serum ferritin thresholds, ranging from 200 μg/L to 500 μg/L. Under these conditions, the estimate of the biomarker diagnostic parameters (sensitivity/specificity and predictive values) is likely to be considered biased (27, 28).

The remaining reviews were primarily addressed to clinicians suggesting pragmatic approaches to HHC diagnosis. Basically, serum ferritin elevations were considered suggestive for HHC only when associated to an increase of the transferrin saturation (27, 28).

Among 652 original papers reporting on the use of serum ferritin measurement in the framework of hemochromatosis, 45 were identified for possible inclusion, as they evaluated ferritin in the context of HHC defined by genetic tests and, in some cases, by liver biopsy. These studies were classified into three main categories, according to the purpose to investigate the clinical value of the biomarker concentrations:

Table 1 Features and main results of the experimental studies published from 1999, evaluating serum ferritin in the diagnosis of iron deficiency anemia.

Year (ref.)	Type of patients (no.)	Company/assay	Threshold, μg/L	Available outcome for ferritin	Diagnostic parameters
2002 (23)	Elderly (49)	Beckman Access	15 F, 24 M	Mean concentrations, diagnostic algorithm	None
2002 (24)	Undergone BMA (78)	Beckman Access	50	Sens, Specs, AUC	Sens 42.3%, Specs 93.6%, AUC 0.66 (±0.14)[a]
2000 (25)	Anemic rheumatoid arthritis (30)	PerkinElmer AutoDELFIA	10 F, 20 M	Mean concentrations	None
1999 (26)	Undergone BMA (145)	NA	10 F, 22 M	Sens, Specs	Sens 25%, Specs 99%

[a]95% confidence interval. AUC, area under ROC curve plotting Sens vs. (1-Specs) for all possible test thresholds; BMA, bone marrow aspirate; F, females; M, males; NA, not available; Sens, sensitivity; Specs, specificity.

Table 2 Features and main results of the experimental studies published from 1999, evaluating serum ferritin for screening hereditary hemochromatosis.

Year (ref.)	No. of subjects	Assay	Threshold, µg/L	Available outcome for ferritin
2008 (30)	101,168	Roche latex immunoturbidimetry	200 F, 300 M 900[a]	Mean concentrations for different genetic variants; association with genetic variants
2008 (31)	29,699	NA	1000[a]	Mean concentrations for patients with ferritin >1000 µg/L vs. <1000 µg/L
2006 (32)	44,098	Roche latex immunoturbidimetry	200 F, 300 M	Mean concentrations for different genetic variants

[a]Threshold proposed by the authors to better classify subjects. F, females; M, males; NA, not available.

1. as risk factor for HHC comorbidities (i.e., liver fibrosis and cirrhosis, arthropathy) (n=6);
2. for differentiating genetic variants of HHC (n=16);
3. in the HHC diagnostic pathways (e.g., screening programs) (n=23).

Among the 23 studies in the third group, only three were preliminary considered eligible as potentially aiming to evaluate the accuracy of ferritin in HHC screening (Table 2) (30–32). However, these studies failed to meet most of our selection criteria and of STARD recommendations. In particular, they did not provide an estimate of biomarker diagnostic parameters (e.g., sensitivity and specificity), but the only available outcome was the percentage of subjects positive to the test in individuals with different genetic variants, using thresholds suggested by the assay manufacturer. The two more recent studies also applied higher ferritin thresholds to identify a more effective strategy for screening subjects at higher risk for serious clinical manifestations of HHC (30, 31).

Discussion

Since the commercial availability of RIA methods, several studies have reported that serum ferritin can be a suitable biomarker for iron-related disorders. However, an accurate clinical assessment greatly depends on the comparability of test results among different assays, which in turn allows an effective application of guidelines established by scientific or professional bodies often advocating use of specific decision limits for diagnosis and therapeutic intervention (33). There is an international agreement on the fact that, to be accurate and comparable, results must be traceable to higher-order references (34). Accordingly, the project of harmonization of ferritin immunoassays started in 1980s and its major outcome was the establishment in 1996 of a consistent reference material, represented by the 94/572 IS preparation (9, 10). Although the calibration of some commercial assays is still possibly not fully aligned to this IS, a recent trial has shown a good comparability between ferritin results obtained by different assays, which was achieved with the implementation of calibration traceability to the recombinant L ferritin 3rd IS (11).

There are several examples showing that the adoption of the traceability approach to harmonize assay results in clinical laboratories can substantially modify the relation of analyte results to existing decision thresholds (13). Without adequate (re)validation of recommended decision limits, this situation can impair the interpretation of the results and, paradoxically, worsen the patient's outcome. In the case of ferritin, the knowledge about the clinical validity of the test and the decision-making criteria used by physicians are based on data that were method-dependent, generated from 1970 to the 1990s with routine tests that were not harmonized (17, 29). As reported before, tracing back the calibration of commercial ferritin assays to ISs could modify the analyte results and invalidate the clinical decision-making criteria currently used. Thus, to maintain the accumulated clinical experience, the relationship to the previous "home-made" calibration systems should be established and, if necessary, clinical decision-making criteria (meaning clinical cut-offs) should be adjusted accordingly to ensure that patient care remains consistent despite the changes.

The main purpose of this paper was to critically revaluate the scientific evidence supporting ferritin as diagnostic marker of altered iron storing in the traceability era. According to the theory of metrological traceability, it was actually impossible to consider all the three ferritin ISs released by the WHO as part of the same traceability chain. Although both 1st and 2nd ferritin IS were value-assigned using the same method, i.e., measurement of ferritin protein content by the Lowry method, this happened in an independent way, 7 years apart, so that no continuity was established between the two ISs (10). Consequently, a new traceability chain was practically created when the 2nd IS replaced the 1st IS upon exhaustion of its stocks, with a bias of approximately 5%–10% between the two calibrations (11). For this reason, we tried to identify and select only studies that used ferritin assays calibrated with 2nd or 3rd WHO IS (the latter being the only reference material available today) or, if this information was not available, papers published from 1999, time of release of the EC IVDD requiring demonstration of assay traceability to available higher-order reference materials and/or methods (16). We are, however, aware that the second criterion could not permit to identify with certainty which clinical data can be considered to have been generated using 2nd or 3rd IS standardized assays, even because some manufacturers still currently claim calibration alignment to the 1st IS, even though it was sold out 20 years ago.

Concerning the use of serum ferritin for diagnosing ID anemia, the critical evaluation of the available scientific literature has revealed the need to update evidences in view of the harmonization of immunoassays. Despite the overview by Guyatt et al. (17) relies on a very good methodology, it is rather questionable to continue to use in the current clinical practice the cut-offs derived from studies carried out from 1970 to the 1980s, when worrying differences in ferritin results from different assays and a remarkable inter-laboratory variability were reported (7). Although such a situation implies the basic inability to define common critical decision levels and, consequently, a consistent risk for misclassifying patients, all recent reviews on the topic (19–22) and the 2011 guideline of the British Society of Gastroenterology (35) resort to Guyatt's recommendations on ferritin measurement to rule out ID anemia in the general population without any critical update.

In the perspective to confirm the diagnostic decision-making criteria of ferritin measurement for ID using 2nd or 3rd IS calibrated assays, a low number of studies were found meeting the established criteria for this purpose. The majority of the recruited papers did not use BMA as diagnostic reference method for classifying patients. From 2002, most studies considered serum ferritin as the diagnostic standard for ID, replacing BMA after the proposal of a clinical pathway that was shown to improve the diagnosis of ID and of underlying gastrointestinal lesions (36). This pathway employed serum ferritin as single diagnostic test and a subsequent endoscopic evaluation for patients with marker concentrations <45 μg/L (36). No further tests (e.g., serum transferrin iron saturation, red cell protoporphyrin, reticulocite count) were introduced in the proposed diagnostic algorithm since the reported negligible additional predictive value contributed by these tests to ferritin determination. Probably, the large amount of clinical evidence on patients with suspected ID accumulated up to 1990s, together with the contemporary report of technical limitations in BMA evaluation (37), definitively supported the practical introduction of ferritin as basic biomarker for ID detection without any further validation or criticism. It was, therefore, impossible to find robust information revalidating the diagnostic efficacy (including recommended cut-offs) of ferritin for ID detection, using assays traceable to the 2nd IS for its measurement.

Regarding the HHC framework, despite the high number of papers evaluating the use of serum ferritin, the available evidence does not support the appropriateness of ferritin measurement in the screening of this clinical condition. Several observational studies have suggested a poor diagnostic sensitivity of the test, as a consistent ferritin increase may be only detected in a proportion of C282Y homozigotes and ferritin concentrations can be normal even years after the genetic diagnosis (38). In addition, the characteristics of subjects (sex, age, and ethnicity) may complicate the relationship between *HFE* genotypes and iron-loading status (32). Accounting that the specificity of ferritin is also threatened by several conditions (e.g., inflammatory, metabolic, neoplastic), current guidelines state that only a persistency of markedly elevated ferritin concentrations (e.g., >1000 μg/L) associated to an increase in transferrin saturation may require *HFE* genetic tests (28). In this framework, a marked elevation of ferritin could identify those patients at higher risk for serious clinical manifestations (e.g., liver cirrhosis) (39).

In conclusion, available evidence of the ferritin diagnostic effectiveness is limited to ID conditions. Recommended cut-offs for this application are, however, based on studies published from 1970 to the 1980s. The lack of more recent studies using assays traceable to the available reference materials may prevent from their optimal use in current clinical practice. Regarding IO status, even when the framework is restricted to HHC, no definitive evidence about the diagnostic accuracy of ferritin is available, both before and after the introduction of ISs calibrated assays.

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