SELECTION OF IRON METABOLISM TESTS
IN CASES WHERE IRON DEFICIENCY IS SUSPECTED

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I. INTRODUCTION

An iron deficiency (lack of iron) can be identified by measuring iron metabolism markers in the blood.

Testing for an iron deficiency is an essential stage in the aetiological investigation of anaemia.

In 1995, ANDEM, a national quality assurance agency, issued recommendations on laboratory tests to diagnose an iron deficiency. The main points were:

- to assess a possible iron deficiency it is necessary to measure serum ferritin, OR to measure serum iron in conjunction with the transferrin saturation coefficient;
- serum iron measurements can only be interpreted in conjunction with the determination of the transferrin saturation coefficient;
- screening for an iron deficiency does not require the simultaneous measurement of serum iron and ferritin.

However, in 2008, despite the recommendations issued by ANDEM and by other institutions, the measurement of serum iron in isolation and of serum iron in conjunction with ferritin accounted for 39% of laboratory tests prescribed in order to study iron metabolism according to data collated by the Health Insurance agency.

In order to ensure tests are prescribed according to medical criteria, in July 2009 the CNAMTS, the French national health insurance agency, asked the HAS to assess laboratory tests to be carried out in order to study iron metabolism, particularly in cases of iron-deficiency anaemia and inflammatory anaemia as well as iron overloads such as haemochromatosis. This request forms part of a review of laboratory tests which proposes that health expenditure should be optimised by limiting requests for tests which are no longer relevant due to the advancement of medical and scientific knowledge (medical-based control of the volume of services provided).

In 2005, the HAS issued professional recommendations on genetic haemochromatosis. As a result, this assessment does not deal with the subject of iron overloads, but focuses on the selection of iron metabolism tests when an iron deficiency is suspected. This study relates to the laboratory test procedure for diagnosing an iron deficiency and is not intended to identify the various clinical situations which may or may not call for an iron metabolism test.
II. CONTEXT

II.1 Laboratory tests available to study iron metabolism

The reference test for assessing the amount of iron in the body is the measurement of iron in the bone marrow after using a specific staining technique. This is possible by a bone marrow aspiration or biopsy, and is therefore an invasive process.

Tests which can be carried out using a venous sample are:

- determination of serum iron, although note should be taken of marked intra-individual variability over a 24-hour period;
- determination of transferrin, which carries iron in the plasma; two theoretical elements are calculated from this determination of transferrin by weight:
  - the total iron binding capacity of transferrin (TIBC);
    - TIBC (μmol/L) = transferrin (g/L) x 25, or
    - TIBC (mg/L) = transferrin (g/L) x 1.395;
  - the iron saturation coefficient of transferrin, which is the ratio between serum iron and the total iron binding capacity of transferrin (serum iron/TIBC);
- determination of soluble transferrin receptors, which reflects cell avidity for iron for erythropoiesis;
- determination of serum ferritin, which reflects iron reserves in the body.

II.2 Disorders concerned

Iron is used primarily for the synthesis of haemoglobin. Insufficient iron intake or incorrect use of iron leads to inadequate erythropoiesis, the ultimate manifestation of which is the onset of anaemia.

An iron deficiency is the most common nutritional deficiency world-wide. It affects in particular children, adolescents, pregnant women and women of child-bearing age. In France for example, 25% of women with child-bearing potential have an iron deficiency and 5% have anaemia. In fact, this population uses more iron due to their higher-level needs (pregnancy, growth), inadequate iron intake or high-level losses (gynaecological bleeding). In men or, generally speaking in elderly subjects, an iron deficiency is less common, and is usually explained by occult bleeding and may indicate lesions in the digestive tract (polyps, cancers, etc.).

From the biological point of view, the markers that indicate iron-deficiency anaemia are a reduction in serum ferritin (reserves) and serum iron, an increase in transferrin, a marked reduction in the transferrin saturation coefficient, and an increase in soluble transferrin receptors (indicating cell avidity).
Iron for erythropoiesis may also be lacking due to inflammatory mechanisms which may be observed, particularly in situations involving infections, cancers and chronic inflammatory conditions. These pathological situations lead to the production of cytokines, which repress erythropoietic precursors on a central level, and make iron unavailable by sequestering it in the cells of the mononuclear phagocyte system. This is not therefore a true deficiency. From the biological point of view, there is an increase in ferritin (a protein in the acute phase of inflammation), and a reduction in serum iron and transferrin (due to retention and catabolism respectively); the transferrin saturation coefficient is normal or drops, but to a lesser extent than in iron-deficiency anaemia. There is no increase in soluble transferrin receptors.

Apart from these “typical” situations, interpretation of the iron balance is more difficult in clinical contexts, which involve an iron deficiency and a change in iron markers due to other causes, such as: elevated ferritin levels irrespective of iron reserves in inflammation, hepatic and muscular cytolysis, poorly-controlled diabetes, chronic alcoholism, hyperthyroidism, certain metabolic syndromes; a reduction in erythropoiesis due to an erythropoietin deficiency and common disorders associated with chronic renal impairment; lower transferrin levels in cases of malnutrition, for example.

III. APPRAISAL METHOD

III.1 Analysis of the literature

The first selection of documents focused on recommendations and consensus conferences relating to the use of iron markers for the aetiological diagnosis of anaemia and/or the diagnosis of an iron deficiency.

The analysis did not include the prevention of an iron deficiency or iron-deficiency anaemia, the monitoring of treatment (using iron or an erythropoiesis-stimulating agent), and iron overload, according to the context.

As the conclusions of the Recommendations were consistent, firstly in relation to serum ferritin and secondly serum iron and transferrin to determine the transferrin saturation coefficient, the analysis of the literature was restricted to summary documents.

The bibliographical review was completed with an analysis of the original literature only for soluble transferrin receptors, to which little reference was made in summaries of the literature.

This selection identified 30 summary publications and 25 original articles on soluble transferrin receptors.

III.2 Consultation of the professions and specialties concerned

This analysis of the literature was completed by arguments put forward by experts at a meeting of the working group, in order to compare these data with their experience and with the practices of the professionals concerned. The minutes of this meeting were then validated by the members of the group.
Members of the group were as follows: 1 clinical haematologist, 2 laboratory 
haematologists, 1 clinical geneticist, 2 general practitioners, 2 gastroenterologists, 
1 obstetric gynaecologist, 1 geriatrician, 1 paediatrician, 1 specialist in internal 
medicine and 1 nephrologist. The expert appointed by the French Society of 
Rheumatology, responsible for an INSERM unit, specialising in the characterisation 
of iron metabolism and iron overloads was not able to attend the meeting of the 
working group, but also contributed by re-reading and commenting on the report 
and the minutes of the meeting of the working group.

IV. RESULTS OF THE APPRAISAL

IV.1 Summary literature

Conclusions drawn from the summary literature (30 publications) are based on a 
limited number of studies, which are old, and are not always ranked.

They are however relatively consistent as far as the following points are 
concerned:

- **Serum ferritin** is the marker to be measured in order to diagnose an iron 
deficiency.

The French recommendation by ANDEM, published in 1995, suggested measuring 
ferritin or the transferrin saturation coefficient.

- In addition to ferritin, the combination of **serum iron and transferrin** (making it 
possible to calculate the **transferrin saturation coefficient**) is recommended 
in situations involving inflammation (in particular in cases of cancer, chronic 
inflammatory intestinal disorders), cases of chronic renal impairment or when 
the serum ferritin result is not conclusive (normal or high level even though iron 
deficiency is strongly suspected). Furthermore, in such situations, the ferritin 
levels to be met in order to diagnose an iron deficiency are higher.

- **Serum iron alone** and the combination of **serum iron + ferritin (without 
transferrin)** are not recommended at any time.

- For pregnant women and children, there is no clear-cut consensus regarding 
the approach to the diagnosis of iron deficiency: no iron marker tests, or tests 
indicated if there is no response to a trial treatment with iron, or tests with iron 
markers which are firstly serum ferritin (combined with the transferrin saturation 
coefficient in one publication).

- **Soluble transferrin receptors** are mentioned only in the two WHO 
recommendations, in association with serum ferritin.

IV.2 Original literature on soluble transferrin receptors

The 25 articles included in this appraisal are case studies with a number of 
methodological limitations (level of evidence 4). They do not put forward any 
decisive arguments in favour of the use of soluble transferrin receptors for the 
diagnosis of an iron deficiency.
It appears that different units are used in the various studies, with no connection between them (mg/L, U/L, nmol/L).

In a situation involving inflammation in adult subjects, sensitivity and specificity values for soluble transferrin receptors are very variable (from 51 to 100% and 60 to 100% respectively), when quantification of bone marrow iron is the reference method.

In order to diagnose iron deficiency with no particular inflammatory context, and by comparison with serum ferritin and any other haematological parameters, the sensitivity of soluble transferrin receptors varies between 22 and 85%, with specificity varying between 63 and 100%.

In the case of children, studies were conducted mainly in areas where infections are highly prevalent or in populations seriously affected by inflammatory diseases. Compared to serum ferritin and other haematological parameters, the results are very variable, with sensitivity values between 54 and 100% and specificity values between 49 and 92%.

Sensitivities and specificities of the soluble transferrin receptors/log ferritin index appear to be better than those for soluble receptors alone.

IV.3 Review of the position of health professionals

The position of the working group has been concordant with the conclusions drawn from the critical analysis of the literature.

The main conclusions adopted by the working group, by consensus, are as follows:

- in order to investigate an iron deficiency, the marker to be measured is serum ferritin regardless of the clinical situation (apart from pregnancy and for children under 6 months of age);

- it is essential to standardise reference values for the measurement of serum ferritin;

- if applicable, in a complex medical situation, it may be useful to measure serum iron and transferrin in order to calculate the transferrin saturation coefficient, together with markers of inflammation, markers of malnutrition or specific tests required in the clinical context. In this situation, serum iron and transferrin must be determined with the patient fasted;

- the investigation must however start with a haemogram;

- iron markers should not be measured in situations involving acute inflammation. In the event of repeated measurements, it is preferable for these to be carried out in the same laboratory;

- the measurement of serum iron alone and the combination of serum iron + ferritin is not indicated in any circumstances;

- soluble transferrin receptors are indicated only in rare situations in specialist haematology.
V. CONCLUSIONS AND FUTURE PROSPECTS

V.1 Conclusions

In order to identify an iron deficiency, the markers to be measured are:

- as a priority: serum ferritin; a lower ferritin level confirms the diagnosis of an iron deficiency, and it is not necessary to measure other iron markers in such cases;

- in situations involving inflammation, chronic renal impairment or when the serum ferritin result is not conclusive (normal or high level even though iron deficiency is strongly suspected): serum iron combined with transferrin (allowing the transferrin saturation coefficient to be calculated) may be helpful for diagnostic purposes;

- consideration should be given to the need to take account of the clinical context and to carry out an initial haemogram.

The measurement of serum iron alone or the combination of serum iron + ferritin without transferrin is not indicated for the diagnosis of an iron deficiency.

The measurement of soluble transferrin receptors is limited to rare situations in a specialist environment.

Furthermore, it seems to be necessary to meet certain sampling standards: samples for the measurement of iron metabolism markers should not be taken during or immediately after an episode of acute inflammation; if repeated measurements are required, it is preferable for them to be carried out in the same laboratory; if serum iron and transferrin are to be measured, samples must be collected in the morning with the patient fasted.

There is no consensus regarding the interpretation of results for iron metabolism markers during pregnancy and in children.

V.2 Future prospects

This assessment report focuses on the approach to laboratory tests for diagnosing an iron deficiency, and may be combined with a study to identify various clinical situations, which may or may not include an iron metabolism analysis. In fact, although coding data do not indicate the clinical situation associated with the tests, it is nevertheless possible that in some situations the determination of iron markers is not justified (information provided by certain members of the working group) and could be reviewed.

The working group stressed the need to standardise serum ferritin reference values indicated by medical testing laboratories, and data from manufacturers producing assay kits, the references for which were prepared using unspecified populations and a limited number of samples. At present, the lower limits for serum ferritin used to identify an iron deficiency can vary considerably from one kit to another and from one laboratory to another, and may hinder interpretation. It would be beneficial to define these thresholds, in particular according to the clinical context (age, gender, presence of inflammation).