

Improving the Laboratory Diagnosis and Differential Diagnosis of Various Forms of Anemia in Oncologic Diseases

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Abstract: **Objective:** to systematize the methods of laboratory diagnosis of the forms of anemia that occur in oncologic diseases and to improve the differential diagnostic algorithm. **Materials and methods:** an analytical review was conducted of data presented in international clinical guidelines (ESMO, ASCO/ASH), systematic reviews, and laboratory diagnostic recommendations; the diagnostic value of laboratory parameters (ferritin, transferrin saturation, soluble transferrin receptor, reticulocyte hemoglobin, C-reactive protein, hepcidin, lactate dehydrogenase, haptoglobin, vitamin B12, folic acid, and others) was comparatively assessed. **Results:** in oncology patients, anemia is often of mixed origin and may present simultaneously as anemia of inflammation, absolute and functional iron deficiency, chemotherapy-induced myelosuppression, blood loss, bone marrow infiltration, and hemolysis. The traditional ferritin cut-off (<30 ng/mL) loses sensitivity under inflammatory conditions; a combination of ferritin, transferrin saturation, the sTfR/log-ferritin index, and reticulocyte hemoglobin allows the different forms to be distinguished more reliably. A three-stage algorithm (screening – differentiation – etiological confirmation) is proposed. **Conclusion:** a multiparametric laboratory approach and a standardized algorithm help to identify the cause of anemia early, reduce unjustified transfusions and prescription of erythropoiesis-stimulating agents, and individualize treatment strategy.

Keywords: oncologic diseases, anemia, differential diagnosis, ferritin, transferrin saturation, soluble transferrin receptor, hepcidin, anemia of inflammation, functional iron deficiency.

INTRODUCTION

Anemia is one of the most common hematologic complications in oncology patients. According to systematic reviews, a considerable proportion of patients with malignant tumors already have reduced hemoglobin levels before treatment, and the frequency of anemia rises sharply during chemotherapy [1], [2]. The European Cancer Anaemia Survey (ECAS) followed more than 15,000 patients; anemia was present in about 39% of them at enrollment and was recorded in about 67% at some point during follow-up [3]. A decrease in hemoglobin impairs quality of life, worsens fatigue and functional status, and may adversely affect the effectiveness of radiotherapy and chemotherapy as well as overall survival [4].

In oncology patients, anemia rarely has a single cause. Its development involves inflammatory mediators released by the tumor itself (IL-6, TNF- α), hepcidin-mediated iron blockade, chronic blood loss, nutritional and vitamin deficiencies, tumor infiltration of the bone marrow, myelotoxic therapy, hemolysis, and kidney damage [5], [6]. Consequently, identical hemoglobin values may conceal different pathogenetic mechanisms, and their misinterpretation can lead to inadequate treatment (for example, prolonged oral iron in anemia of inflammation, or overlooking absolute iron deficiency) [7].

In practice, the greatest difficulty arises in interpreting iron metabolism parameters: inflammation

raises ferritin as an acute-phase protein and lowers transferrin saturation, which makes it hard to distinguish absolute iron deficiency from anemia of inflammation [8], [9]. Therefore, supplementing traditional screening parameters with modern markers (sTfR, reticulocyte hemoglobin, hepcidin) and combining them in a single algorithm is an urgent task.

Aim of the study — to comparatively evaluate the possibilities of laboratory diagnosis of the various forms of anemia in oncologic diseases and to improve a practical algorithm for differential diagnosis.

Objectives: (1) to classify the forms of anemia in oncology patients from the pathogenetic standpoint; (2) to assess the diagnostic value and limitations of the main laboratory parameters; (3) to develop a stepwise algorithm aimed at recognizing anemia of mixed origin.

MATERIALS AND METHODS

Study design. This work is analytical and review-based: it relies on a comparative analysis of data from published clinical guidelines, systematic reviews, and laboratory diagnostic recommendations, and on the formulation of a differential diagnostic algorithm based on them. The sources used were the ESMO (2018) and ASCO/ASH guidelines on the management of anemia in oncology patients, the British Society for Haematology guideline on the laboratory diagnosis of functional iron deficiency, and key reviews on anemia of inflammation and iron metabolism [10].

Criteria for anemia. Anemia was defined according to the World Health Organization criteria: hemoglobin below 130 g/L in men and below 120 g/L in non-pregnant women [11]. In oncology practice, severity is usually graded as mild (above 100 g/L), moderate (80–100 g/L), and severe (below 80 g/L).

Laboratory parameters evaluated. Complete blood count (hemoglobin, mean corpuscular volume — MCV, RDW, reticulocytes, blood smear morphology); iron metabolism (serum iron, total iron-binding capacity, transferrin saturation — TSAT, ferritin); functional and modern markers (soluble transferrin receptor — sTfR, sTfR/log-ferritin index, reticulocyte hemoglobin — Ret-He or CHr, percentage of hypochromic red cells, hepcidin); inflammatory markers (C-reactive protein — CRP, IL-6); hemolysis markers (lactate dehydrogenase — LDH, haptoglobin, indirect bilirubin, direct antiglobulin [Coombs] test); vitamin status (vitamin B12, folic acid, and, if necessary, methylmalonic acid and homocysteine); renal function (creatinine, glomerular filtration rate — GFR); and bone marrow examinations (aspiration and trephine biopsy) [12], [13].

Analysis criteria. For each parameter the following were assessed: (a) pathogenetic rationale; (b) susceptibility to the influence of inflammation and chemotherapy; (c) ability to distinguish between different forms of anemia; (d) practical applicability (cost, standardization, turnaround time). The results were summarized in the form of a table and an algorithm.

RESULTS

1. Pathogenetic forms of anemia in oncology patients

The analysis showed that it is appropriate to distinguish the following main forms of anemia in oncology patients [14], [15]:

- **Anemia of inflammation (anemia of chronic disease)** — under the influence of IL-6, hepcidin synthesis increases and the release of iron from macrophages and the intestine via ferroportin is blocked; the response to erythropoietin and red cell survival are reduced.
- **Absolute iron deficiency** — depletion of iron stores resulting from chronic blood loss (especially in gastrointestinal tumors), malabsorption, or impaired nutrition [16].
- **Functional iron deficiency** — iron stores are adequate, but iron is not delivered to

erythropoiesis; often observed in the setting of erythropoiesis-stimulating agents or inflammation.

- **Combined (mixed) anemia** — coexistence of the above mechanisms; in practice the most common and diagnostically the most complex variant.
- **Myelosuppressive anemia related to chemotherapy and radiotherapy** — direct toxic effects on erythroid progenitors (platinum compounds, taxanes, antimetabolites) and reduced erythropoietin production due to kidney damage.
- **Bone marrow infiltration** — displacement of erythropoiesis by metastases or hematologic malignancies (leukoerythroblastic blood smear picture).
- **Hemolytic anemia** — immune (autoimmune hemolytic anemia) in lymphoproliferative diseases, as well as microangiopathic hemolysis in DIC and thrombotic microangiopathy.
- **Vitamin B12 and folic acid deficiency** — in the setting of gastric and intestinal tumors, gastrectomy, and impaired nutrition; also under the effect of antifolate drugs.
- **Treatment-related myelodysplasia and pure red cell aplasia** — after alkylating agents and radiotherapy; pure red cell aplasia in thymoma and chronic lymphocytic leukemia.

2. Diagnostic significance of laboratory parameters

Ferritin. Although it is the most sensitive and specific indicator of iron stores, as an acute-phase protein it rises in inflammation and in the tumor process. In the general population, a value below 30 ng/mL is accepted for absolute iron deficiency; in oncology patients, a higher cut-off (often up to 100 ng/mL) is used because of inflammation, and in this range the sensitivity of ferritin decreases markedly [17]. Therefore, ferritin must never be interpreted in isolation but always together with CRP and TSAT.

Transferrin saturation (TSAT). A TSAT below 20% indicates insufficient delivery of iron to erythropoiesis, but it does not distinguish absolute deficiency (low ferritin) from functional blockade (normal or high ferritin). Because serum iron and TSAT vary during the day and in inflammation, it is recommended that the test be taken in the morning, in the fasting state.

Soluble transferrin receptor (sTfR) and the sTfR/log-ferritin index. sTfR increases in erythroid cells under conditions of iron deficiency and is almost independent of inflammation. A high sTfR/log-ferritin index (usually above 2) indicates absolute iron deficiency, and a low value (below 1) indicates anemia of inflammation; intermediate values are consistent with a mixed variant. Limitation: sTfR is also elevated by increased erythroid activity (hemolysis, erythropoietin therapy, myeloproliferative processes), and in addition, reagents and cut-off values are not standardized between laboratories.

Reticulocyte hemoglobin (Ret-He/CHr). It reflects, in near real time, the iron supply of recently produced red cells; it is one of the most useful parameters for detecting functional iron deficiency and is available as a routine parameter on many hematology analyzers (values depend on the type of analyzer). Values below about 28–29 pg point to iron-restricted erythropoiesis.

Hepcidin. The central mediator in the pathogenesis of anemia of inflammation; it rises in inflammation and falls in absolute iron deficiency. Although its diagnostic value is high, its widespread use is currently limited by the lack of methodological standardization and by high cost, so it is used mainly in research and specialized centers.

Red cell indices and blood smear. A decrease in MCV (microcytosis) is consistent with iron deficiency, whereas an increase (macrocytosis) is consistent with B12/folate deficiency, chemotherapy (antimetabolites), myelodysplasia, or reticulocytosis; an elevated RDW is an important sign of mixed origin, and when microcytic and macrocytic populations coexist the MCV may remain "normal". On the smear, schistocytes indicate microangiopathic hemolysis, while teardrop-shaped red cells and immature granulocytes indicate bone marrow infiltration.

Hemolysis and vitamin markers. Reticulocytosis, increased LDH, decreased haptoglobin,

increased indirect bilirubin, and a positive direct antiglobulin (Coombs) test confirm a hemolytic origin. In the hypoproliferative type of erythropoiesis (chemotherapy, infiltration, renal failure), reticulocytes are disproportionately low relative to the severity of anemia. When vitamin B12 deficiency is suspected and the serum B12 value is borderline, measuring methylmalonic acid and homocysteine clarifies the picture.

Table 1. Main laboratory features of the forms of anemia in oncology patients (summarized)

Form of anemia	MCV	Ferritin	TSAT	sTfR / Ret-He	Additional features
Absolute iron deficiency	N or ↓	<30 (<100 in inflammation) ng/mL	<20%	sTfR ↑, Ret-He ↓; index >2	RDW ↑, thrombocytosis; signs of blood loss
Functional iron deficiency	N or ↓	100–800 ng/mL (N/↑)	<20%	Ret-He ↓, hypochromic red cells ↑	In the setting of inflammation or erythropoiesis-stimulating agents
Anemia of inflammation	N or ↓	N/↑	↓ (<20%)	sTfR N; index <1	CRP ↑, hepcidin ↑, low reticulocytes
Mixed (inflammation + iron deficiency)	N or ↓	30–100 ng/mL	↓	sTfR ↑; index 1–2	CRP ↑, RDW ↑
B12 / folate deficiency	↑	N/↑	N	—	Low B12, MMA/homocysteine ↑, hypersegmented neutrophils, LDH ↑
Hemolytic (autoimmune),	N/↑	N/↑	N	—	Reticulocytosis, LDH ↑, haptoglobin ↓

microangiopathic)					bin ↓, indirect bilirubin ↑, Coombs +, schistocytes
Bone marrow infiltration	N	N/↑	N	—	Leukoerythroblastic picture, teardrop red cells, LDH ↑, cytopenias
Myelosuppressive (chemo-/radiotherapy)	N/↑	N/↑	N/↓	Reticulocytes ↓	Multilineage cytopenias, temporal relation to treatment cycles

Note: N — normal; ↑ — increased; ↓ — decreased. MMA — methylmalonic acid. Ferritin cut-off values depend on the presence of inflammation and should be clarified in accordance with the laboratory's guidance.

3. Proposed differential diagnostic algorithm

Based on the results of the analysis, a three-stage approach is proposed instead of reliance on a single parameter:

Stage I — screening and morphological orientation. Determine the presence and severity of anemia (complete blood count, reticulocytes, blood smear, CRP, renal function). The initial direction is chosen on the basis of MCV, RDW, and reticulocyte response: microcytic/normocytic/macrocyclic, and hypo- or hyperproliferative type. In cases of hemoglobin below 100 g/L, an unexpected or rapid decline, or anemia that is not consistent with the course of treatment, the work-up proceeds to the next stage.

Stage II — differentiating iron status. Ferritin, TSAT, and CRP are assessed simultaneously. (a) Ferritin below 30 ng/mL and TSAT <20% indicate absolute iron deficiency, with a search for the source (especially gastrointestinal blood loss). (b) Ferritin above 100 ng/mL, TSAT <20%, and high CRP indicate anemia of inflammation or functional deficiency. (c) When ferritin is 30–100 ng/mL, the sTfR/log-ferritin index and Ret-He are additionally determined: an index >2 and/or decreased Ret-He indicate superimposed absolute deficiency, an index <1 indicates pure anemia of inflammation, and an intermediate value indicates a mixed variant.

Stage III — etiological confirmation. In cases that are not explained by, or are inconsistent with, the iron parameters, the following are carried out: B12/folate, a hemolysis panel (LDH, haptoglobin, bilirubin, Coombs test), a hematologist's review of the blood smear, the temporal

relationship to chemotherapy cycles and, when indicated, bone marrow aspiration and trephine biopsy (infiltration, myelodysplasia, pure red cell aplasia).

The practical significance of the algorithm is directly related to treatment tactics: iron preparations in absolute iron deficiency (often intravenous in oncology patients); intravenous iron and/or erythropoiesis-stimulating agents, per guidelines, in functional deficiency and anemia of inflammation; replacement in B12/folate deficiency; etiological treatment in the hemolytic variant; and transfusion is considered in severe symptomatic anemia. Erythropoiesis-stimulating agents are used, as indicated, mainly during chemotherapy when hemoglobin is below 100 g/L, taking into account thromboembolic risk and the goal of treatment (curative or palliative).

DISCUSSION

The data obtained confirm that anemia in oncology patients is not a homogeneous condition and that each of its forms requires a different diagnostic and therapeutic approach. The most important clinical problem is reliably distinguishing absolute and functional iron deficiency from anemia of inflammation under inflammatory conditions. When used alone, traditional parameters (serum iron, ferritin, TSAT) change in inflammation, which increases the risk of misdiagnosis. Under such conditions, additional parameters such as the sTfR/log-ferritin index and Ret-He improve discriminative ability; however, their cut-off values are not standardized, so each laboratory should establish reference intervals from its own data.

The advantage of the proposed algorithm lies in its adaptability to existing laboratory capabilities: the Stage I and II parameters are available in most district- and regional-level laboratories, while Stage III provides for referral to specialized centers. This makes it possible to implement the algorithm in oncology institutions under the conditions of the Republic. In addition, timely identification of anemia of mixed origin may reduce unjustified red cell transfusions and ineffective oral iron intake, and may help in choosing erythropoiesis-stimulating agents appropriately.

Limitations of the study. Because the work is analytical and review-based, the sensitivity and specificity of the proposed algorithm must be tested prospectively in an independent clinical cohort. There are no uniform international cut-off values for ferritin, TSAT, and sTfR; tumor location, chemotherapy regimen, and renal function affect the parameters. Hepcidin testing is also limited in routine practice. Future studies should focus on validating the algorithm in a local patient cohort and on assessing its cost-effectiveness.

CONCLUSION

Anemia in oncology patients is often multifactorial and of mixed origin, and distinguishing the pathogenetic forms (anemia of inflammation, absolute and functional iron deficiency, myelosuppression, infiltration, hemolysis, vitamin deficiency) determines treatment tactics. Although assessing ferritin and TSAT together with CRP is sufficient for initial differentiation, supplementing them with the sTfR/log-ferritin index and reticulocyte hemoglobin at borderline values (ferritin 30–100 ng/mL) increases diagnostic reliability. Introducing the three-stage algorithm (screening – differentiation of iron status – etiological confirmation) into practice may help reduce unjustified transfusions and inadequate iron and erythropoiesis-stimulating agent therapy; it is recommended that it be tested in a prospective clinical study.

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