Guidelines and Recommendations

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Algorithm of differential diagnosis of anemia involving laboratory medicine specialists to advance diagnostic excellence

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Abstract

Objectives: Anemia is a severe global public health issue. Testing practices for anemia suggest overuse of screening laboratory tests and misinterpretation of studies even in "easy-to-diagnose" underlying causes, leading to late diagnoses and missed treatment opportunities. We aimed to develop a complete and efficient algorithm for clinical pathologists and laboratory medicine physicians for the differential diagnosis of anemia.

Methods: Comprehensive literature search encompassing original articles, studies, reviews, gold standard books, and other evidence.

Results: We created a complex algorithm, primarily for clinical pathology/laboratory use, that explores all major and several rare causes of anemia in an efficient and evidence-based manner. The algorithm includes gold-standard diagnostic laboratory tests available in most clinical laboratories and indices that can be easily calculated to provide an evidence-based differential diagnosis of anemia.

Conclusions: The diagnostic strategy combines previously available diagnostic tests and protocols in an efficient order. Clinical pathologists following the algorithm can independently provide valuable diagnostic support for healthcare providers. Clinical pathologists providing complete differential diagnostic services with the proposed algorithm may create an opportunity for an advanced diagnostic service that supports diagnostic excellence and helps patients receive a timely diagnosis and early treatment opportunities.

Keywords: algorithm; anemia; differential diagnosis; efficiency

Introduction

Anemia is a severe global public health issue, one of the more common conditions healthcare providers (HCP) encounter daily. Self-reported testing practices for anemia suggest overuse of screening laboratory tests and misinterpretation of iron studies in the evaluation of even the most common form of anemias, the new-onset iron-deficiency anemia, leading to misdiagnosis [1].

WHO defines anemia in adults as a blood hemoglobin concentration of <13 g/dL or <130 g/L in men, <12 g/dL or <120 g/L in non-pregnant women, and <11 g/dL or <110 g/L in pregnant women. Based on WHO data published in 2008 [2], the global prevalence of anemia is 24.8 %, but there is a wide variation between geographical areas, age, and sex. The global prevalence of anemia among children under five is 42.6 % worldwide, compared to 29 % among non-pregnant women and 38.2 % among pregnant women [3].

Iron deficiency anemia (IDA) is the most common cause of anemia (IDA) globally [4], representing half of the anemic population [2]. However, numerous other causes may lead to anemia, such as other nutritional deficiencies (including folate, vitamin B12, and vitamin A deficiency), acute and chronic inflammation, parasitic infections, and inherited or

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acquired disorders of the hemoglobin synthesis, red blood cell production or red blood cell survival.

Hemolytic anemia is caused by a shortening in the survival of circulating red blood cells (RBCs) to a value of less than 100 days (normal lifespan between 110 and 120 days) [5], while regeneration cannot replace the lost volume. Hemolytic anemias may be hereditary or acquired, corpuscular (if the cause is a red blood cell disease) or extracorpuscular (if the environment is the cause of hemolysis). They may be divided into immune-retardant and non-immune-retardant subgroups, and classified as intravascular and extravascular hemolysis, based on the site of red cell destruction. In intravascular hemolysis, RBCs are destroyed within the vascular bed, while in extravascular hemolysis, RBCs with altered structure or membrane appearance are phagocytosed and degraded by macrophages in the liver and spleen.

In the case of mild hemolysis, hemoglobin concentrations may still be within the reference range, so it is possible to have a condition where hemolysis occurs without marked anemia. The destruction of RBCs can be compensated for some time by an increase of erythroid activity in the bone marrow as a result of increased erythropoietin production in the kidneys.

Laboratory tests classify anemias into microcytic (MCV <80 fL), normocytic (MCV 80–100 fL), and macrocytic (MCV >100 fL) groups based on the mean red blood cell volume (MCV) [6, 7].

Diagnostic algorithms combine laboratory and clinical data to elucidate the background of anemia. However, a thorough guideline covering all causes of anemia has still not been available for healthcare professionals. After several consecutive sessions, the Working Group on Guidelines and Algorithms of the Hungarian Society of Laboratory Medicine created a complex laboratory algorithm that identifies common and rare causes of anemia to advance diagnostic excellence and support efficient diagnostic workouts.

Our algorithm for the differential diagnostics of anemia is proposed with the mindset of the clinical pathologist. As such, beyond aiming to advance efficient diagnostic processes, our algorithm proposes a greater involvement of clinical pathologists in the diagnostic process. Clinical laboratories should provide an option for clinicians, family practitioners, and other HCPs to order a full-scale evaluation of anemia, enabling clinical pathologists to choose the necessary tests and the order of those.

Materials and methods

In a comprehensive literature search conducted from October 1, 2022, to May 1, 2023, we reviewed and analyzed encompassing original articles, studies, reviews, gold standard books, and other manuscripts or guidelines that were published in English from PubMed, Agency for Healthcare Research and Quality reports, education-specific books, and other relevant databases. We also used UpToDate (www.uptodate.com) to find relevant publications for selected diseases. Publications through May 1, 2023 were considered.

Our inclusion criteria were the occurrence of the following keywords: “anemia”, “differential diagnostics”, “anemia algorithm”, “diagnostic guideline for anemia” in PubMed and Google search engines to identify publications with algorithmic diagnostic protocol suggestions in a scoping way. Then we looked up all the specific “end-diagnoses” present in our algorithm (e.g., vitamin B12 deficiency, thalassemia, MDS, infections, etc.) that account for anemia and searched on the current diagnostic guidelines/suggestions for that specific disease with the key words “diagnosis of …”.

We excluded guidelines and algorithms published more than 10 years ago and diagnosis of “hyperacute bleeding” in traumatic injuries or surgical interventions or “severe burn” cases.

We constructed the diagnostic flowchart in an iterative way to find an efficient route for the majority of the conditions that may cause anemia.

Results – description of the algorithm

Our algorithm also aims to serve as one complete guideline, and we made it available in one Figure (Supplementary Figure 1). For printing objectives, we dissected the algorithm into three separate Figures (Figures 1–3).

Reticulocyte production index

The automated hematology analyzers measure hemoglobin concentration as part of the complete blood count (CBC) panel. We follow the WHO guidelines in defining anemia as stated in the Introduction. At the same time or as a next step, reticulocyte number should be determined (Figure 1). This is a significant difference between our algorithm and some other guidelines, where reticulocyte count may not or may be considered to be measured later [8–15]. Due to the simplicity, low cost, and importance in all further anemia subgrouping, the absolute reticulocyte number should be assessed, and the derived reticulocyte percentage (Ret%) – which is sometimes wrongly referred to as reticulocyte count – and reticulocyte production index (RPI) should be calculated at this step. Ret% is the percentage of the absolute reticulocyte number and the red blood cell number. RPI is a marker adjusted for the level of anemia and is a more accurate reflection of erythropoiesis. RPI is calculated from reticulocyte percentage (Ret %), hematocrit (Hct), and a maturation time, based on the

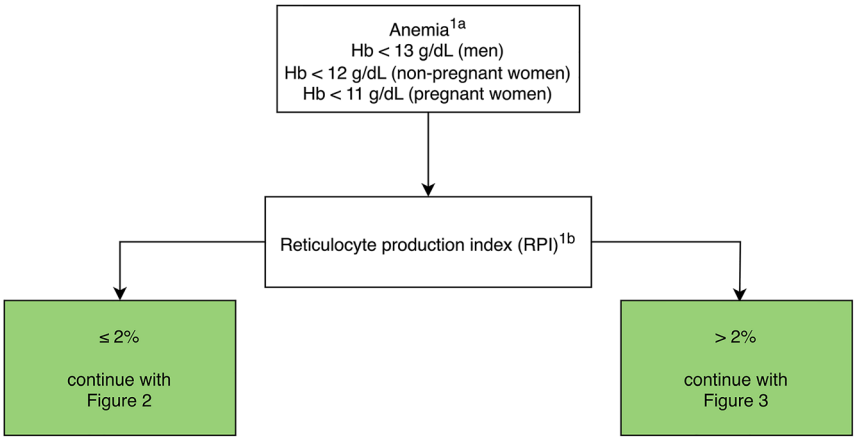

Figure 1: The first step in the differential diagnosis of anemia. Steps referring to: 1a, [44] 1b, [16, 17, 23].

$$\text{RPI} = \frac{\frac{\text{Hct}}{45} \times \text{Ret\%}}{\text{maturation time}}$$

equation [16], where maturation time is 1.0 day for Hct 35–40 %, 1.5 days for Hct 25–35 %, 2.0 days for Hct 15–25 %, and 2.5 days for Hct < 15 % [17].

Note, that the bone marrow response to anemia requires days to two weeks to increase reticulocyte number and RPI.

Cut-off value for RPI for optimal bone marrow response of accelerated RBC production varies in the literature between 2 and 3 %. We found two references for cut-off value of 2 % [8, 9], and two other references for 2.5 % [18, 19]. Five references suggested that optimal bone marrow response is at RPI >3 % while inadequate response is at RPI <2 %, leaving an undefined zone of RPI between 2 and 3% [20–24]. RPI cut-off is suggested at 3 % by three references [25–27]. In order for the algorithm to be sensitive for hemolysis, and to avoid an undefined "grey zone" of RPI, we accepted the cut-off value of 2 %. We also included a "runaway ramp" for cases of RPI above 2 % when causes listed for cases below 2 % of RPI should also be considered. Based on RPI, continue with Figure 2 (≤2 %) or Figure 3 (>2 %).

Anemia with RPI ≤ 2 %

If the RPI is ≤2 %, further classification of anemia is based on the mean red cell volume (MCV) (Figure 2). If MCV is <80 fL, the anemia is microcytic. Since iron deficiency is the most common cause of microcytic anemias, it is advised to continue the investigation towards iron deficiency anemia (IDA). IDA is likely if ferritin concentration is low, serum iron concentration is low, transferrin concentration is high, and transferrin saturation is low. As serum iron concentration is influenced by several physiological factors (diurnal rhythm, monthly cycle in women, stress, etc.), serum iron concentration alone is not acceptable to confirm iron deficiency. As the reference range of these parameters may vary according to age, sex, race, and method, results outside the reference range used in each laboratory are indicative. We also note that neither low ferritin with normal transferrin saturation, nor borderline low ferritin with low transferrin saturation exclude IDA [1].

If iron deficiency is not confirmed, calculation of the Mentzer index is recommended; automatic calculation can be set up in the laboratory information system (LIS). The Mentzer index is an easily accessible quotient of the MCV (in fL) and RBC count (in millions per microliter or $\times 10^{12}$/L). If the Mentzer index is below 13, hemoglobin electrophoresis or hemoglobin analysis by HPLC should be performed to measure the HbF and HbA_2 ratios of total hemoglobins. If the HbF >1 % or HbA_2 >3.5 %, beta thalassemia is likely, and a genetic background investigation is warranted. For this purpose, sequencing of the most frequently abnormal regions of the beta globin gene in the actual population is recommended. If HbF ≤1 % and HbA_2 ≤3.5 %, alpha thalassemia and other hemoglobinopathies are considered. Genetic testing is also recommended to identify these cases.

If the Mentzer index is ≥13, serum creatinine concentration should be determined. Based on serum creatinine, age, and gender, eGFR is calculated [28]. If eGFR is ≥60 mL/min/1.73 m^2, chronic inflammation or other chronic diseases (e.g., autoimmune disease, chronic endocrine disease) or malignancy are likely to contribute to anemia. In such cases, it is recommended to determine the soluble transferrin receptor (sTfR) concentration in serum, from which the sTfR/ferritin index can be calculated (automatically by the LIS) by dividing the sTfR concentration (mg/L) by the logarithm of the ferritin concentration (ng/mL). The sTfR/ferritin index is superior to sTfR (AUC 0.87 vs. 0.74, p<0.0001) [29]. The suggested cut-

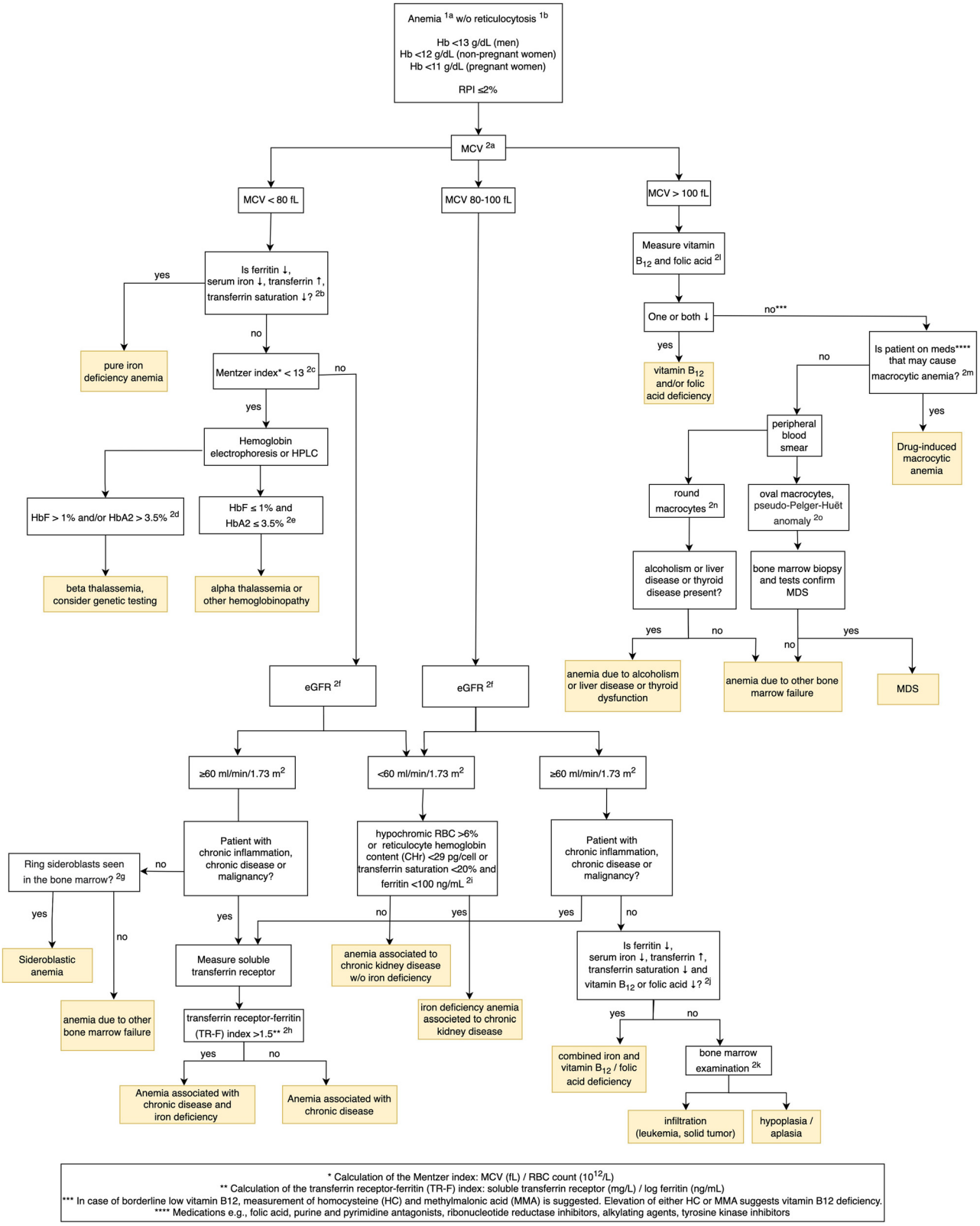

Figure 2: Algorithm for the evaluation of anemia without reticulocytosis. eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA2, hemoglobin A2; HbF, fetal hemoglobin; MCV, mean red cell volume; MDS, myelodysplastic syndrome; RBC, red blood cell; RPI, reticulocyte production index. Steps referring to: 1a, [44] 1b, [16, 17, 23] 2a, [45] 2b, [46, 47] 2c, [48] 2d, [49–51] 2e, [52, 53] 2f, [10] 2g, [54] 2h, [33, 55] 2i, [56, 57] 2j, [46, 47, 58] 2k, [59–61] 2l, [58] 2m, [62] 2n, [63] 2o, [62, 64, 65].

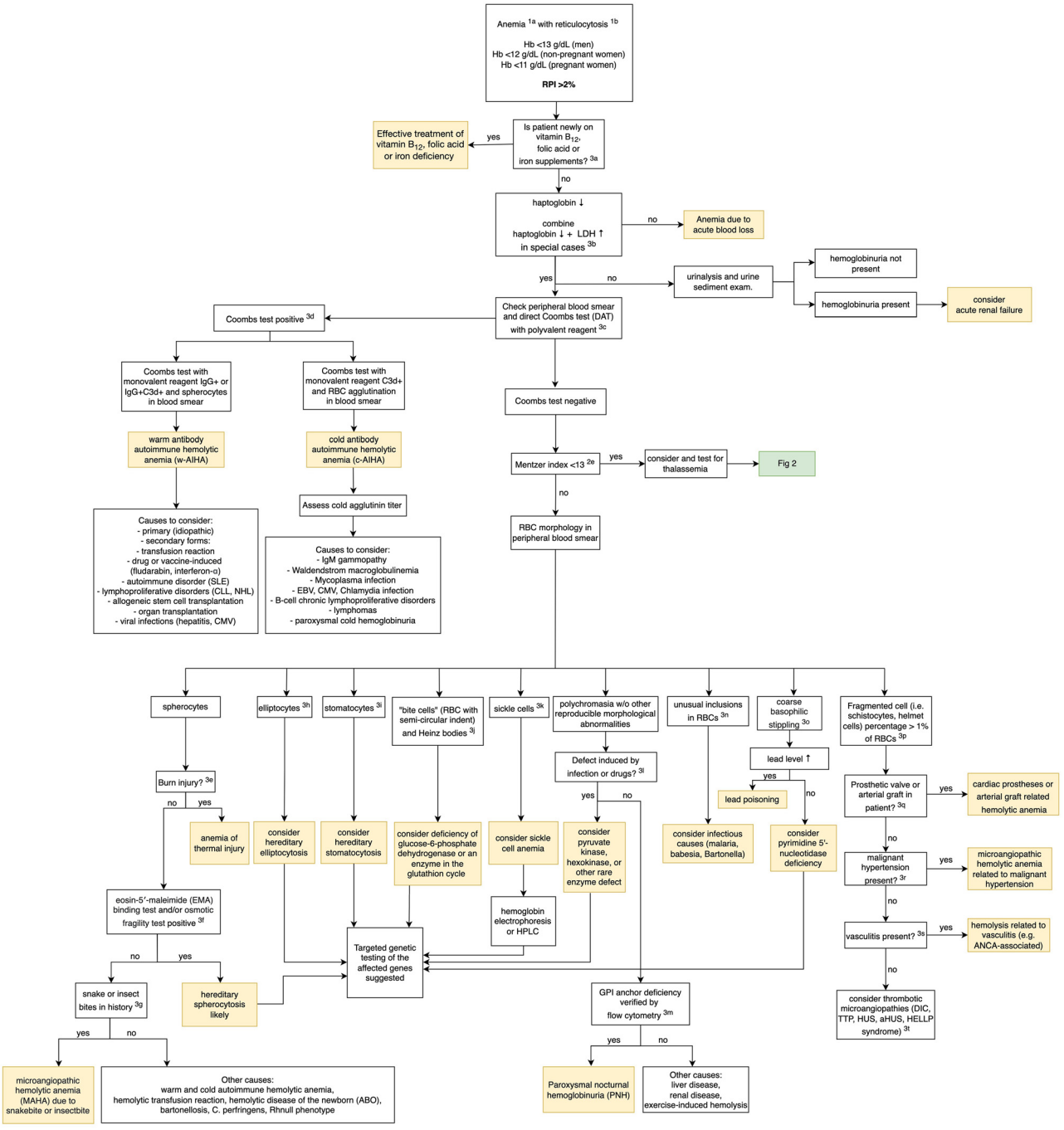

Figure 3: Algorithm for the evaluation of anemia with reticulocytosis. ANCA, antineutrophilic cytoplasmic antibody; aHUS, atypical hemolytic uremic syndrome; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; EBV, Epstein-Barr virus; eGFR, estimated glomerular filtration rate; GPI, glycosylphosphatidylinositol; Hb, hemoglobin; HELLP, hemolysis, elevated liver enzymes and low platelets; HPLC, high performance liquid chromatography; HUS, hemolytic uremic syndrome; IgG, immunoglobulin G; C3d, degradation product of complement 3; MCV, mean red cell volume; MDS, myelodysplastic syndrome; RBC, red blood cell; NHL, non-Hodgkin lymphoma; RPI, reticulocyte production index; SLE, systemic lupus erythematosus; TTP, thrombotic thrombocytopenic purpura. Steps referring to: 1a, [44] 1b, [16, 17, 23] 3a, [66–70] 3b, [36, 37] 3c, [71] 3d, [72] 2e, [48] 3e, [73, 74] 3f, [75, 76] 3g, [77] 3h, [78, 79] 3i, [80] 3j, [81] 3k, [82, 83] 3l, [84, 85] 3m, [86, 87] 3n, [16, 88], 3o, [89] 3p, [81] 3q, [90] 3r, [91] 3s, [81] 3t, [81].

off value varies between 1.03 and 1.8 in the literature, we suggest the average 1.5 for cut-off value [29–33]. If the sTfR/ferritin is >1.5, iron deficiency associated with chronic disease is likely; if it is ≤1.5, anemia associated with chronic disease without iron deficiency is likely. After excluding chronic disease, chronic inflammation and solid tumor, bone marrow examination should be performed to search for sideroblastic anemia [34]. In this case, a smear of bone marrow aspirate should be stained with Prussian blue for ring sideroblasts. Ring sideroblasts are erythroblasts with at least five siderotic granules in the cytoplasm, occupying at least one-third of the circumference of the nucleus [34]. The presence of ring sideroblasts confirm sideroblastic anemia. In the absence of ring sideroblasts, anemia due to other bone marrow failure is likely.

If eGFR is <60 mL/min/1.73 m^2, renal failure is possible. Moderately decreased eGFR values are recommended to be confirmed by cystatin-based eGFR calculation [28]. In this case, check for iron deficiency by determining the proportion of hypochromic RBCs or the reticulocyte Hgb (CHr), depending on the type of hematology analyzer. Iron deficiency anemia associated to chronic kidney disease can be confirmed if the hypochromic RBC is >6 % (HYPO% on ADVIA Siemens analyzer), or reticulocyte Hgb is <29 pg (all analyzers) [10], or transferrin saturation is <20 % and ferritin is <100 ng/mL. If these conditions are not met, iron deficiency is unlikely, and the diagnosis is simply anemia associated to chronic kidney disease.

The first step in the investigation of normocytic anemia (MCV 80–100 fL) is the measurement of serum creatinine and calculation of eGFR. If eGFR is <60 mL/min/1.73 m^2, the renal anemia of the patient should be investigated. If the patient with normocytic anemia has an eGFR ≥60 mL/min/1.73 m^2, the patient should be assessed for chronic inflammation, chronic disease (e.g., autoimmune disease, chronic endocrine disease), or malignancy. If any of them is present, consider anemia is due to iron deficiency associated with the chronic disease and investigate as described above. If chronic inflammation, chronic diseases, and malignancy are excluded, we evaluate whether iron deficiency is combined with vitamin B12/folic acid deficiency. In combined iron and B12/folic acid deficiency, the ferritin concentration is low, the serum iron concentration is low, the serum transferrin concentration is high, the transferrin saturation is low, and the vitamin B12 or folic acid concentration is low. If this combined deficiency cannot be confirmed, evaluate the bone marrow smear with panoptic staining assessing for infiltrating malignant cells (leukemias, multiple myeloma, bone marrow metastasis of solid tumor) and hypoplasia/aplasia (aplastic anemia, pure red cell aplasia). For a more precise indication of a bone marrow test, it is advised to look for blasts in the peripheral smear, which is readily available from the original blood sample. However, circulating tumor cells of a solid tumor are so few that they are not detectable by a standard peripheral blood smear analysis.

In macrocytic anemia (MCV >100 fL), serum vitamin B12 and folic acid concentrations are first to be measured. If the folic acid concentration is close to the lower limit of the reference range, measure the folic acid content of the red blood cells, as this may be the only detectable sign of folic acid deficiency. In case of borderline low vitamin B12, measurement of homocysteine and methylmalonic acid (MMA) may be suggested, elevation of either suggests B12 deficiency [35]. However, borderline low vitamin B12 levels usually do not result in anemia. If vitamin B12 and folic acid deficiencies are excluded, check for recently recommended/administered medications of the patient that may cause macrocytic anemia, such as antagonists of folic acid (methotrexate), purine (6-mercaptopurine, azathioprine, acyclovir) and pyrimidine (fluorouracil, zidovudine), ribonucleotide reductase inhibitors (cytosine arabinoside, hydroxyurea), alkylating agents (cyclophosphamide) and tyrosine kinase inhibitors (sunitinib, imatinib). In the absence of such medications, evaluate the shape of macrocytes in peripheral smear. Causes of round macrocytes include liver disease, alcoholism, or thyroid disease. If these are ruled out, anemia is due to other bone marrow failure. If the macrocytes in the peripheral smear are oval, bone marrow tests, including morphological examination of the smear and MDS-directed cytogenetic and FISH studies may reveal and confirm MDS. If MDS is ruled out, macrocytic anemia is associated with other bone marrow failure, including acquired forms such as primary myelofibrosis or inherited diseases (e.g., Fanconi anemia, Diamond-Blackfan anemia).

Anemia with RPI >2 %

If RPI is greater than 2 % (Figure 3), check patient's medical history for recently administered new medication (iron, vitamin B12, folic acid) for a previously confirmed deficiency anemia. In these cases, peripheral reticulocytosis is a normal bone marrow response. However, in the absence of supplementation, look for signs of intravascular hemolysis. Common markers for intravascular hemolysis are low haptoglobin, high LDH, and elevated unconjugated bilirubin levels; LDH and bilirubin being less specific for hemolysis. Haptoglobin alone showed high sensitivity and specificity (83 and 96 %, respectively) in distinguishing hemolytic and non-hemolytic conditions, providing 87 % probability of

predicting hemolytic disease when the serum haptoglobin level falls below 25 mg/dL (0.25 g/L) [36]. Combining LDH and haptoglobin only slightly increases sensitivity (92 %) and does not increase specificity (90 %) for ruling out hemolysis [37]. Low haptoglobin may result from hepatic insufficiency and in the rare cases of congenital ahaptoglobinemia. Therefore, we suggest that a combination of LDH and haptoglobin is only necessary with a history of hepatic insufficiency.

If the results do not suggest hemolysis, the guiding diagnosis is acute anemia after blood loss, with a time lag of 1–2 days after hemorrhage [6].

If hemolysis is confirmed, a urinalysis with urine dipstick and sediment examination should be performed to detect hemoglobinuria. Hemoglobinuria is defined as detectable hemoglobin on the urine test strip exam without red blood cells in the urine sediment. Hemoglobinuria increases the risk of acute renal failure. Rarely, hemoglobin casts are present.

In addition to urinalysis, hemolysis necessitates further testing including peripheral smear staining and evaluation, and a Coombs test (direct antiglobulin test, DAT) with polyvalent reagent. A positive Coombs test with polyvalent reagent confirms immune hemolysis, and the Coombs test should also be performed with the monovalent reagent. If IgG antibodies and C3d complement are detected by the DAT, and spherocytes are detected in the smear, warm antibody autoimmune hemolytic anemia (w-AIHA) is confirmed. Antibodies of warm AIHA may develop idiopathically (primary) or may be secondary due to transfusion complication, drugs (fludarabine, interferon-alpha), autoimmune disease (SLE), lymphoproliferative diseases (CLL, NHL), allogeneic stem cell transplantation, organ transplantation, or viral infections (hepatitis, CMV). If the Coombs test with monovalent reagent shows no IgG coverage, but C3d coverage of RBCs, and the RBCs in the smear are agglutinated, then cold agglutinin is the cause of hemolysis, and the cold agglutinin titer should be determined. This phenomenon may occur in IgM gammopathies, Waldenström macroglobulinemia, Mycoplasma infection, EBV, CMV, Chlamydia infection, chronic lymphoproliferative B cell disease, lymphoma, paroxysmal cold hemoglobinuria.

If the Coombs test with polyvalent reagent provides a negative result, calculate the Mentzer index. If the Mentzer index is <13, screening for thalassemia is recommended. For Mentzer index ≥13, further testing is based on the dominant pathological morphology of RBC in the peripheral smear.

In the presence of spherocytes in the smear and the patient has a history of burns or large skin deficiencies, the diagnosis is hemolysis associated with thermal injury. If medical history excludes thermal injury, congenital spherocytosis is suspected, and a flow cytometric test with eosin-5-maleimide staining and/or an osmotic resistance test of red blood cells is recommended. In case of a positive test result, it is reasonable to test for mutations in the suspected genes using molecular biology methods. If both tests are negative, check medical history for snakebite or insect bite to confirm hemolysis due to these causes. If medical history does not include bites, we should consider other diseases causing spherocytosis in addition to Coombs negativity, such as autoimmune hemolysis (with warm or cold antibodies), hemolytic complication after transfusion, AB0 neonatal hemolytic disease, bartonellosis, *C. perfringens* infection, Rh-null phenotype.

In case of the high rate of elliptocytes or stomatocytes in the smear, hereditary elliptocytosis or stomatocytosis is considered and genetic testing is warranted. If contracted, 'bite' or 'blister' RBCs and Heinz bodies in the RBCs are identified in the smear (with supravital staining), a glucose-6-phosphate dehydrogenase or glutathione cycle enzyme deficiency is likely; targeted genetic testing can confirm the enzyme deficiency.

The presence of sickle cells in the smear suggests sickle cell anemia and hemoglobin electrophoresis or HPLC can determine the proportion of the abnormal variant of hemoglobin. However, regardless the extent of the abnormality, targeted genetic testing with sequencing of the beta-globin gene is required to detect the characteristic mutation.

If the smear shows polychromasia without other reproducible morphological abnormalities, consider infection or drug exposure-induced hemolysis; the underlying enzyme defects (pyruvate kinase, hexokinase, or other rare enzyme deficiencies) can be verified by targeted genetic testing.

If hemolysis was not initiated by infection or drug administration, test for the absence of the glycosylphosphatidylinositol (GPI) anchor molecule by flow cytometry in both white blood cells and RBCs. If the absence of the GPI anchor is confirmed, the diagnosis is paroxysmal nocturnal hemoglobinuria. If the flow cytometry test is negative, other causes such as liver disease, kidney disease, hemolysis due to extreme physical activity (exercise-induced [march] hemoglobinuria) should be considered.

If unusual RBC inclusions are detected in the smear, an infectious background is likely: most common disorders are malaria, babesiosis, and Bartonella infection.

Coarse dense basophilic stippling in several RBCs suggest lead poisoning; measure serum lead level. Zinc protoporphyrin levels may indicate chronic lead toxicity. If lead poisoning is excluded, consider 5′ nucleotidase enzyme deficiency, which can be confirmed by targeted molecular biology testing.

If the proportion of fragmented cells (schistocytes) in the smear is over 1 %, consider mechanical damage to the red blood cells by cardiac protheses or arterial grafts.

Mechanical damage to the RBCs due to increased shear stress is the most widely occurring etiology of cardiac prostheses-related hemolytic anemia (CPHA) [38]. In the absence of such implants, malignant hypertension and vasculitis should be considered. Once these have been excluded, consider and investigate for thrombotic microangiopathies: disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), atypical hemolytic uremic syndrome (aHUS), hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome.

Discussion

Anemia is a frequent condition, and the range of underlying diseases is wide, presenting a diagnostic challenge. Available guidelines either focus on a particular segment of the possible underlying causes, such as gastrointestinal evaluation of iron deficiency anemia [12, 39, 40] or on the management of anemia and blood transfusion recommendations [41]. Guidelines developed for HCP specialists focus on testing relevant to their specialty.

We propose a complex algorithm for the differential diagnostics of anemia in adults from the clinical pathologist's perspective. In comparing of our algorithm with other current guidelines or approaches, we highlight the importance of the reticulocyte production index, a powerful and underrepresented marker in the differential diagnostics of anemia. Some guidelines include reticulocyte-related parameters, of which some start with reticulocyte count [13, 42], others use reticulocyte count or RPI at a later step [13–15]. There are guidelines that do not include such parameters [12, 43]. We suggest the use of the reticulocyte production index and we bring that to the start of our algorithm (Figure 1).

Guidelines often start with MCV in the algorithmic steps [14]; we suggest discrimination based on MCV only if reticulocytosis is not present.

Clinical diagnosticians following current guidelines must schedule multiple patient visits with phlebotomy at each visit. This approach is not convenient for the patient, poses a healthcare risk, and dramatically increases the time for diagnosis. We suggest an "anemia-directed laboratory investigation" lab order with one phlebotomy, allowing clinical pathologist to perform all the necessary tests to evaluate the underlying cause of anemia.

Clearly, there are disadvantages to the proposed diagnostic workflow. First, the laboratories' ordering options and the financing structure may need to be modified to implement the proposed diagnostic process.

The guideline prerequisites that the clinical pathologists have access to a detailed medical history of the patient. That may not be available in certain laboratory service structures. Private laboratory services may easily solve the challenge of direct communication with the patient via telecommunication technologies. In inpatient setting, the clinical pathologist can have access to the health information system of the hospital.

Our algorithm is unsuitable for cases with hyperacute bleeding, e.g., in traumatic injuries or surgical interventions, as it may take 1–2 weeks for a normal bone marrow response to increase the reticulocyte production index. However, those cases do not require a complex laboratory evaluation. Furthermore, several steps in the evaluation of anemia may be confounded by blood transfusion in a posttransfusion period; however, this is not unique to our algorithm.

The advantages of the anemia-oriented investigation panel overcome the challenges in numerous scenarios. Deeper involvement the clinical pathologist in the diagnostic process is beneficial when HCPs require the special expertise with advanced testing opportunities. Requiring fewer patient-physician encounters, the new diagnostic workflow may be more time-efficient than previous diagnostic workflows. An earlier diagnosis may spare unnecessary treatments (e.g., multiple blood transfusions spared for patients having spherocytosis with an early splenectomy; anti-complement therapy started in PNH, etc.). The decreasing rate of unnecessary treatments save further costs, along with several other beneficial effects (e.g., better health outcomes, less malpractice issues).

Clinical pathologists and laboratory medicine physicians offering advice for their healthcare provider colleagues can now enjoy the help of a complex algorithm. The algorithm can also be used by healthcare providers for cases that are not covered by their previously used guidelines.

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Author contributions: EA led the Working Group on Guidelines and Algorithms of the Hungarian Society of Laboratory Medicine, ZH created the study design, and participated in creating the algorithm. ZH and GT wrote the manuscript. GT participated in the comprehensive literature research and the review of current guidelines and fostered

its publication for international use. PA-SZ, MT-F, JK, BK, and EA participated in developing the algorithm and reviewing and editing the publication draft. All authors have read and agreed to the published version of the manuscript.

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