Diagnostic work up of anemic patients: role of iron deficiency

Abstract

Objectives: Diagnosing disturbances in iron metabolism can be challenging when accompanied by inflammation. New diagnostic tools such as the “Thomas-plot” (TP) (relation of soluble transferrin receptor [sTfR]/log ferritin to reticulocyte hemoglobin content [RET-He]) were established to improve classification of anemias. Aim of this retrospective study was to assess the added diagnostic value of the TP in anemia work up.

Methods: Patients from December 2016 to September 2018 with a complete blood count, iron status, RET-He and sTfR were manually classified into the four quadrants of the TP on basis of conventional iron markers. Manual and algorithm-based classifications were compared using cross tabulations, Box–Whisker-Plots as well as Receiver-Operating-Characteristics (ROC) to calculate the diagnostic accuracy using Area under the Curve (AUC) analysis.

Results: A total of 3,745 patients with a conventional iron status, including 1,721 TPs, could be evaluated. In 70% of the cases the manual classification was identical to the TP, in 10% it was deviant. 20% could not clearly be classified, mostly due to inflammatory conditions. In the absence of an inflammatory condition, ferritin was a reliable parameter to define iron deficiency (ID) (AUC 0.958). In the presence of inflammation, the significance of the ferritin index (AUC 0.917) and of the RET-He (AUC 0.957) increased.

Conclusions: The TP can be useful for narrowing down the causes of anemia in complex cases. Further studies with focus on special patient groups, e.g., oncological or rheumatic patients, are desirable.

Keywords: anemia; ferritin; iron deficiency; reticulocytes; sTfR.

Introduction

Iron deficiency is the most common cause of anemia in at least 50% of all cases [1]. The diagnosis in young patients without comorbidities is rather simple by determining the blood count and ferritin. However, both the diagnosis and the therapy of disorders in iron metabolism are challenging if they occur in the context of renal anemia or in combination with acute or chronic inflammatory diseases, infections, or malignant tumors [2]. This so-called “anemia of chronic disease” (ACD) is the second most frequent anemia after iron deficiency anemia (IDA), and probably the most frequent cause in hospitalized patients [3]. The pathophysiology is based on numerous immune-mediated responses that intervene in iron metabolism and lead to inadequate erythropoietin production, to diminished bone marrow response to erythropoietin, to cytokine-induced inhibition of erythropoiesis, to hepcidin-induced retention of iron in the reticuloendothelial system (RES), and to shortened erythrocyte survival [4–8]. Therefore, it may be difficult to distinguish IDA from ACD during acute-phase-reaction (APR), the conventional iron parameters (ferritin, transferrin, transferrin saturation (TSAT) being insensitive and only of limited use [2, 9, 10].

For a more differentiated diagnosis of functional iron deficiency (FID) in the context of ACD, additional hematological markers have been available for several years. These include the soluble transferrin receptor (sTfR) and, derived from ferritin and sTfR, the ferritin index as indicator of the storage iron reserves as well as the reticulocyte hemoglobin (RET-He) as indicator of the current iron requirements of erythropoiesis. If both biomarkers are combined, an algorithm-based diagram (“Thomas-plot” [TP]) is obtained.
for the classification and assessment of anemia. Four stages of iron metabolism can be identified (Figure 1) [9, 11].

The clinical symptoms of both ACD and IDA are identical and characterized by fatigue, physical weakness, and reduced mental performance [2]. Iron deficiency hinders the function of mitochondria, cell metabolism, enzyme activities, and the synthesis of neurotransmitters [12]. Furthermore, iron deficiency can cause increased susceptibility to infection and heart failure [13]. Numerous studies have concluded that iron substitution can improve symptoms, physical performance and quality of life with acceptable side effects [14, 15]. Especially in case of an existing ACD, the above-mentioned symptoms are often attributed to the underlying disease. Therefore, in many cases a further diagnosis regarding the presence of FID is not initiated [8]. The prevalence of a real iron deficiency in combination with ACD varies between 20 and 85% [2, 3, 16, 17].

Aim of this study was to retrospectively record the prevalence of anemia at a maximum care hospital and to compare and evaluate the diagnosis using the conventional iron parameters and the TP. The rational for this study was that this comparison has so far mainly been made under study conditions with highly selected patients only or in retrospective studies with rather low case numbers. The heterogeneity of the patient cohort thus resembled a normal patient population. In this way, it was possible to evaluate whether the “novel” parameters are practicable in everyday clinical practice and whether they can add diagnostic and therapeutic value. In addition to improved patient outcome, this also includes economic aspects with regard to the use of erythrocyte concentrates, intravenous iron preparations, and erythrocyte stimulating agents (ESAs).

Figure 1: Diagnostic diagram (TP) with its four quadrants for the definition of different states of iron metabolism. The classification is performed according to method-specific cutoff values for ferritin index: 14 (Beckman Coulter Dx 800) and RET-He: 28 pg (Sysmex XN-2000).

Materials and methods

Subjects

This study was carried out at a maximum-care hospital in southwestern Germany (Marienhospital Stuttgart, Germany). Primarily, it included all patients who underwent blood testing at the Department of Laboratory Medicine during the period December 2016 to September 2018. This included in- and outpatients from all hospital departments. In addition, blood samples from external practices were analyzed at the Institute, including a nephrological practice with about 150 dialysis patients per quarter. Only data of adult patients were used and no further restrictions were made to obtain a realistic picture of the patient cohort. For data generation, a systematic and anonymized query of patient and laboratory data was carried out from the laboratory information system (LIS) LabCentre (i-solutions Health, Mannheim, Germany). The study protocol was approved by the Ethics Committee II of the University of Heidelberg (Faculty of Medicine Mannheim), Germany.

Methods

The following EDTA blood parameters were used for the analysis: Hemoglobin (Hb), erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), leukocytes, thrombocytes, and the reticulocyte-hemoglobin equivalent (RET-He). The blood count parameters were determined by the XN-2000 (Sysmex Corporation, Hamburg, Germany). In addition, the following serum parameters were evaluated: ferritin, transferrin and serum iron, from which the TSAT is derived, C-reactive protein (CRP), creatinine, lactate dehydrogenase (LDH), vitamin B12, and sTfR. The determinations of serum iron, LDH, creatinine, and CRP were performed by Architect ci8200 from Abbott (Wiesbaden, Germany). Ferritin, sTfR, and vitamin B12 were determined by an automated immunoassay using the UniCel Dx 800, and transferrin by nephelometry on the Immage (both Beckman Coulter, Krefeld, Germany). The ferritin index was calculated using the formula sTfR/decadic logarithm (log) of ferritin.

Data analysis was done with SPSS Statistics Version 25.0 (IBM, Chicago, IL, USA). Only the chronologically first complete data set of each patient was included in the analysis. The further classification and evaluation were based on the reference range values listed in Table 1. Patients were excluded from further evaluation if the ferritin level was higher than 1,000 ng/mL or the TSAT was over 50%. In these cases, it was not possible to further...
differentiate whether iatrogenic iron overload was present or whether it was part of an underlying hematological disease. Furthermore, cases with MCV > 96 fl without exclusion of a vitamin B12 deficiency and cases with a proven vitamin B12 deficiency were not considered. Patients with bi- or tricytopenia as an indication for the presence of underlying hematological disease or recent chemotherapy were also excluded. Each case was assigned manually to one of the four categories of the TP. According to WHO criteria, anemia in women was characterized by a Hb value <12 g/dL and in men <13 g/dL [18].

The investigator was blinded for the parameters sTfR, ferritin index and RET-He.

The classification was carried out according to the following conditions:
- IDA (quadrant 3): ferritin levels <10 ng/mL in women and <15 ng/mL in men or ferritin levels <100 ng/mL and TSAT <15% with CRP <10 mg/L.
- ACD (quadrant 1): Hb diminished, MCV, MCH, and reticulocytes within the normal range, ferritin levels >10 or >15 ng/mL, TSAT >15 and <50%, CRP >10 mg/L or creatinine >1.3 mg/dL.
- FID/ACD (quadrant 4): MCV and/or MCH diminished, TSAT <15% and CRP/leukocytes within normal range, ferritin levels normal or elevated, additional renal failure or oncological disease as indicator for an ACD.
- IDA (quadrant 2): Hb within normal range, but ferritin levels <10 ng/mL in women and <15 ng/mL in men.
- Latent iron deficiency (Latent ID) (quadrant 2): Hb within normal range, but ferritin levels <10 ng/mL in women and <15 ng/mL in men.

If patients could not be classified on the basis of the above-mentioned criteria, they were classified as follows:
- Healthy or possible chronic disease without current anemia: Hb, MCH, MCV, ferritin, and TSAT within the normal range, CRP and/or creatinine normal or elevated.
- Unclear cases: ferritin levels normal or elevated, TSAT <15% and elevated CRP as indicator of an APR; MCV/MCH low or normal.

### Statistical analysis

Data was primarily analyzed descriptively to make statements on the prevalence and distribution of anemias in the cohort. The Kolmogorov-Smirnov test was used to assess normality, with normal distribution expressed as mean ± standard deviation (SD), and non-normal distributions as median and interquartile range (IQR) (25th and 75th percentile). Cross tabulations were used to compare and quantify the results of the manual allocations and those of the TP. Individual laboratory parameters were graphically displayed using Box-Whisker-plots. A distinction was made between the clear cases (manual allocation and TP identical) and the primarily ambiguous cases. For further evaluation of the extent to which a laboratory parameter can distinguish between IDA and ACD, the true-positive versus the false-positive cases were plotted in Receiver Operating Characteristics (ROC)-curves. Subsequently, the AUC was calculated including the 95% confidence interval. Again, the unclear cases were evaluated separately.

### Results

From December 2016 to September 2018, a blood count was determined in a total of 70,956 patients. About one third of

---

Table 1: Reference ranges of the laboratory tests applied in this study.

| Test              | Unit     | Sex | Reference range          |
|-------------------|----------|-----|--------------------------|
| Hemoglobin        | g/dL     | m   | 13.0–17.5                |
|                   |          | f   | 12.0–16.0                |
| MCV               | fl       | f   | 80–96                    |
| MCH               | pg       | m   | 28–33                    |
| Erythrocytes      | ×10^12/L | m   | 4.5–5.9                  |
|                   |          | f   | 4.1–5.1                  |
| Leukocytes        | ×10^9/L  | m   | 3.5–9.8                  |
| Reticulocytes     | %        | m   | 0.7–2.5                  |
|                   |          | f   | 10–204                   |
| Ferritin          | mg/dL    | m   | 15–275                   |
|                   |          | f   | 202–336                  |
| Transferrin       | mg/dL    | m   | 16–45                    |
| Transferrin satu. | %        |     | 39–149                   |
| Serum iron        | µg/dL    | m   | 40–120                   |
|                   |          | f   | 28                       |
| sTfR              | nmol/L   |     | 12.16–27.25              |
| Vitamin B12       | pg/mL    | m   | 187–883                  |
|                   |          | f   | 135–225                  |
| LDH               | U/L      | m   | 135–214                  |
|                   |          | f   | 135–214                  |
| Creatinine        | mg/dL    |     | 0.7–1.3                  |
|                   |          |     | (×88.4 = µmol/L)         |
| CRP               | mg/L     |     | <10                      |

m, male; f, female; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; Ret-He, reticulocyte-hemoglobin equivalent; sTfR, soluble transferrin receptor; LDH, lactate dehydrogenase; CRP, C-reactive protein.
the patients had anemia according to WHO criteria. In 2,211 of
the 20,589 patients with anemia (10.7%), further diagnostics
was carried out by determining the serum iron parameters; in
895 of these patients, the TP was requested. Patients without
anemia received further diagnostics in 1,534 of 50,367 cases
(3%). Overall, a total of 3,745 patients with determination of
iron parameters, including 1,721 patients with a TP, could be
included in the further evaluation. The median age of these
patients was 71 years (IQR 55–79, range 18–105 years), with
48% male and 52% female patients.

In about 80% of the cases, the nephrological practice
and the departments of internal medicine, including the
emergency room initiated further anemia diagnostics. The
nephrological practice requested by far the largest pro-
portion of all TPs (about 70%), followed by the depart-
ments of internal medicine (28%). In other
departments the TP was requested only sporadically.

According to the manual classification, ACD was the
most common cause of anemia with 16%, followed by IDA
with 10%. ACD with suspected FID was very rare at 0.3%.
However, at this stage of the analysis it was not possible to
discriminate how many cases might have been overlooked
based on the conventional iron parameters. In 764 of 3,745
cases (20.4%), no clear allocation was possible on the basis
of the conventional iron parameters. In 294 of these 764
cases (38.5%), a further differentiation was initiated using
the TP. 30% of the patients did not have anemia and about
20% of the cases had to be excluded. This was mainly due
to abnormal laboratory values, which could not be inter-
preted without additional clinical information. The num-
ber of TPs to be evaluated thus decreased from 1,721 to
1,459 (Table 2).

All tested laboratory parameters showed a non-normal
distribution. The individual results, subdivided into ane-
mia categories based on the manual allocation and blinded
for the parameters of the TP, are presented in Table 3. The
characteristics of the ambiguous cases were of particular
interest. These were anemic patients (Hb median 10.2 g/dL,
IQR 8.9–11.7) with normocytic (MCV median 83.5 fL, IQR
79.3–87.9), rather hypochromic (MCH median 27.6 pg, IQR
26.0–29.3) anemia. The ferritin was within normal range or
slightly elevated (median 188 ng/mL; IQR 47.25–252), the
TSAT was decreased or in the low-normal range (median
14.5%; IQR 10.1–21.2). However, both parameters proved
not to be useful because of additional inflammatory con-
ditions (CRP median 30 mg/L; IQR 8.8–82.1).

After classification on the basis of the conventional
iron parameters, all 1,459 cases were assigned to a quad-
rant in the TP using the ferritin index and RET-H₂ (Figure 2).

Cross tabulation was used to compare the results of the
manual and algorithm-based classification (Table 4). In
1,014 of 1,459 cases (about 70%) the manual allocation was
identical to the TP. Apart from the 294 cases (20%) that
could not be clearly classified manually, the remaining 151
cases (10%) did not fall into the same category.

After applying the TP to the ambiguous 294 cases, ACD
was found in 142 cases (48.3%), latent ID in 80 cases (27.2%)
IDA in 43 cases (14.6%) and FID/ACD in 29 cases (10%).

Box–whisker plots were used to illustrate the different
values of the four anemia categories for ferritin, TSAT,
sTfR, and ferritin index (Figure 3A–H). While the ferritin
values were, as expected, lower in the context of iron
deficiency (A), the values in the unclear cases were almost
always within the normal range or even elevated (B).
Therefore, the ferritin value did not allow a reliable
assignment to an anemia category due to larger ranges and
resulting overlaps. While the TSAT with normal CRP
differed between iron deficiency conditions and ACD (C),
the differences vanished across all categories during an
APR (D). The distribution of medians and IQRs of the sTfR
were similar in both groups; increased in iron deficiency
and in the normal range in ACD (E, F). There was no overlap
in the IQR range within the categories, regardless of the
presence of an APR. In order to differentiate reliably be-
tween IDA and ACD, the ferritin index was used as “gold
standard” in the present study. Consequently, there were
clear differences between iron deficiency conditions and
ACD in this respect (G, H).

AUCs revealed that the ferritin index showed the highest
diagnostic accuracy (AUC 0.969) in the clear cases,
followed by ferritin concentration (AUC 0.958) and TSAT
(AUC 0.931). However, in the uncertain cases, ferritin (AUC
0.691) and TSAT (AUC 0.628) only played a minor role.

| Table 2: Results of the manual classification based on the con-
| conventional iron parameters (left columns: absolute number [n] and
| percentage [%], right columns number of patients with TP). |
| Manual classification based on the conventional iron parameters | Total | Thomas-plot carried out |
| | n | % | Yes | No |
| IDA | 356 | 9.5 | 127 | 229 |
| Latent ID | 82 | 2.2 | 46 | 36 |
| ACD | 617 | 16.5 | 377 | 240 |
| FID/ACD | 12 | 0.3 | 9 | 3 |
| No anemia | 1,123 | 30.0 | 606 | 517 |
| Ambiguous | 764 | 20.4 | 294 | 470 |
| Excluded | 791 | 21.1 | – | – |
| Total | 3,745 | 100 | 1,459 | – | – |
Table 3: Overview of the tested laboratory parameters, subdivided according to the manual classification to an anemia category, blinded for Ret-H_{f} and sTfR.

|                | Hb, g/dL | Erythrocytes, ×10^{12}/L | MCV, FL | CRP, ng/mL | Ferritin, ng/mL | Transferrin, mg/dL | TSAT, % | sTfR, mmol/L | Ferritin index, sTfR/log ferritin |
|----------------|----------|--------------------------|---------|------------|----------------|-------------------|--------|-------------|-----------------------------|
|                | Median   | Pct                       | Median  | Median     | Median         | Median            | Median | Median      | Median                     |
| IDA            |          |                          |         |            |                |                   |        |             |                            |
| n=67           |          |                          |         |            |                |                   |        |             |                            |
|                | 10.0     | 8.3                       | 4.0     | 3.5        | 77.5           | 72.1              | 3.7    | 1.3         | 10.5                       | 6.5                         | 304.0 | 265.0 | 7.02 | 4.27 | 34.07 | 26.12 | 32.84 | 22.88 |
| ACD            |          |                          |         |            |                |                   |        |             |                            |                            |
| n=73           |          |                          |         |            |                |                   |        |             |                            |                            |
|                | 11.0     | 9.8                       | 3.7     | 3.3        | 89.7           | 86.3              | 5.7    | 1.9         | 129.0                      | 58.8                        | 192   | 154.0 | 25.8 | 20.90 | 17.06 | 14.17 | 8.66  | 6.65  |
| Functional ID/ACD |        |                          |         |            |                |                   |        |             |                            |                            |
| n=12           |          |                          |         |            |                |                   |        |             |                            |                            |
|                | 10.1     | 8.5                       | 3.7     | 3.1        | 87.1           | 75.0              | 4.0    | 1.8         | 36.2                       | 29.9                        | 237   | 219.0 | 12.2 | 7.87  | 21.62 | 19.74 | 13.31 | 10.79 |
| Latent ID      |          |                          |         |            |                |                   |        |             |                            |                            |
| n=82           |          |                          |         |            |                |                   |        |             |                            |                            |
|                | 13.1     | 12.5                      | 4.7     | 4.4        | 84.9           | 82.4              | 1.4    | 0.7         | 11.5                       | 9.4                         | 299.0 | 265.0 | 13.7 | 11.20 | 22.0  | 18.58 | 19.12 | 16.42 |
| Healthy/No current anemia |      |                          |         |            |                |                   |        |             |                            |                            |
| n=1123         |          |                          |         |            |                |                   |        |             |                            |                            |
|                | 13.9     | 13.2                      | 4.7     | 4.4        | 87.6           | 85.0              | 1.7    | 0.8         | 84.7                       | 45.5                        | 233   | 212.0 | 28.6 | 22.90 | 16.44 | 14.08 | 8.83  | 7.27  |
| Ambiguous cases|          |                          |         |            |                |                   |        |             |                            |                            |
| n=764          |          |                          |         |            |                |                   |        |             |                            |                            |
|                | 10.2     | 8.9                       | 3.8     | 3.3        | 83.5           | 79.3              | 30.1   | 8.8         | 108.0                      | 47.44                       | 194   | 153.0 | 14.5 | 10.10 | 22.49 | 17.55 | 12.15 | 8.62  |
| Excluded       |          |                          |         |            |                |                   |        |             |                            |                            |
| n=791          |          |                          |         |            |                |                   |        |             |                            |                            |
|                | 10.6     | 8.7                       | 3.6     | 3.0        | 89.7           | 84.3              | 8.2    | 2.3         | 197.0                      | 76.2                        | 179   | 137.0 | 33.2 | 18.60 | 17.46 | 13.28 | 8.23  | 5.74  |

Pct, 25th and 75th percentile. MCV, mean corpuscular volume; CRP, C-reactive protein; TSAT, transferrin saturation; sTfR, soluble transferrin receptor; IDA, iron deficiency anemia; ACD, anemia of chronic disease.

**Discussion**

The evaluation of routine laboratory diagnostics at a hospital aimed to study the diagnostic work-up of anemic patients retrospectively using conventional iron parameters and novel hemoglobin markers, in particular the TP. The diagnostic accuracy of the other parameters (transferrin, iron, ferritin) was lower in both cases. In contrast, the diagnostic accuracy of the ferritin index (ACD/No ACD) was lower in both cases. The diagnostic accuracy of the TP (ACD/No ACD) was similar in both groups (Table 5).

**Figure 2:** Thomas-plot of all 1459 cases. Cross-tabulation comparing the manual and automatic classification based on the TP.
maximum care hospital over a period of 20 months seemed appropriate for this purpose. Due to the retrospective design of this study, the power of this study may be limited to heterogenous collection times, particularly to TSAT, since the calculation is influenced by nutritive and circadian fluctuations of serum iron [19].

The classification into causes of anemia was made by a single expert in analogy to other studies with varying degrees of definition of the different cohorts [20]. Like in other studies, our classification was based on pre-defined inclusion and exclusion criteria with clear cutoff values [21, 22]. Such an approach seemed to be the most appropriate for optimizing objectivity. Some authors insist on the comparison with the iron status in bone marrow as the gold standard for the evaluation of iron parameters [23]. However, studies with this comparison have only been able to include a maximum case number of 176 patients only due to the invasiveness and costs [24]. In addition, it is repeatedly criticized that even the definition of the iron status in the Berlin blue-stained bone marrow smear is heavily examiner-dependent and therefore not objective [23, 25]. In a study comparable to the one presented here, the classification of patients was carried out by two independent investigators in order to increase objectivity without performing a bone marrow staining [26]. Yet, it remained unclear which criteria were used as a basis for their evaluation.

Similar to the findings in the literature, ACD was the most common cause of anemia in the clinical setting, followed by IDA [3]. The proportion of anemic patients with ACD and FID (quadrant 4) was very rare with a total of 32 cases (4%) only, whereas in other studies the proportion was ~10%.

Figure 3: Box-Whisker plots representing the different values for ferritin, TSAT, sTfR, and ferritin index in the four anemia categories. Distinction between the clear cases (manual assignment and TP identical) (left panel: A, C, E, G) and the ambiguous cases (right panel: B, D, F, H). The boxes represent the 25th and 75th percentiles, whiskers the 5th and 95th percentiles. Please note the logarithmic scale for ferritin and ferritin index.
Table 5: AUC and 95%-CI of the individual laboratory parameters to differentiate between IDA and ACD, divided into clear (manual classification and TP identical) and ambiguous cases.

|                | Clear cases   | Ambiguous cases |
|----------------|---------------|-----------------|
|                | AUC           | 95%-CI          | AUC           | 95%-CI          |
| sTfR/log ferritin | 0.969         | 0.96–0.979      | 0.917         | 0.882–0.951     |
| Ferritin       | 0.985         | 0.984–0.986     | 0.897         | 0.860–0.933     |
| TSAT           | 0.949         | 0.940–0.958     | 0.859         | 0.825–0.887     |
| sTfR WHO reference standard would increase the diagnostic value of sTfR and consequently of the ferritin index [32]. The reticulocyte hemoglobin can be estimated by two different principles (CHr and RET-He) which adds another uncertainty to the classification [33].

The determination of RET-He alone in patients undergoing dialysis is able to reduce the use of intravenous iron compared to the determination of ferritin and TSAT only [34]. However, 791 patients had to be excluded from further evaluation in our study because the overall decisive factor in the assessment of iron metabolism is not the isolated consideration of laboratory values but the inclusion of all clinical information on diagnoses and previous therapies.

In conclusion, in a majority of cases, the conventional iron parameters appear to be sufficiently effective in everyday clinical practice for the diagnosis of iron deficiency conditions (70% identical allocation). However, in at least 20% the TP proved to be useful for narrowing down the causes of anemia in complex cases. In 10% of our patients, it provided additional information with medical consequences. Further studies with focus on special patient groups at risk for iron deficiency, e.g., oncological or rheumatic patients, are desirable.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study protocol was approved by the Ethics Committee II of the University of Heidelberg (Faculty of Medicine Mannheim), Germany.

References

1. Onkopedia. Onkopedia leitlinien. Eisenmangel und Eisenmangelanämie 2018. Available at: https://www.onkopedia.com/de/onkopedia/guidelines/eisenmangel-und-eisenmangelanameie/@view/html/index.html. [Accessed 1 Sep 2019].

2. Camaschella C. Iron-deficiency anemia. N Engl J Med 2015;372: 1832–43.
3. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352:1011–23.
4. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta 2012;1823:1434–43.
5. La Ferla K, Reimann C, Jelkmann W, Hellwig-Burgel T. Inhibition of erythropoietin gene expression signaling involves the transcription factors gata-2 and nf-kappab. FASEB J 2002;16:1811–3.
6. Cazzola M, Ponchio L, de Benedetti F, Ravelli A, Rosti V, Beguin Y, et al. Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. Blood 1996;87:4824–30.
7. Papadaki HA, Kritikos HD, Valatas V, Boupas DT, Eliopoulos GD. Anemia of chronic disease in rheumatoid arthritis is associated with increased apoptosis of bone marrow erythroid cells: improvement following anti-tumor necrosis factor-alpha antibody therapy. Blood 2002;100:474–82.
8. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. Blood 2019;133:40–50.
9. Thomas L, Thomas C, Heimpel H. Neue Parameter zur Diagnostik von Eisenmangelzuständen: retikulozytenhämoglobin und seine ratio to serum ferritin in the diagnosis of iron deficiency. Med Klin 2003;98:1068–72.
10. Camaschella C. New insights into iron deficiency and iron deficiency anemia. Blood Rev 2017;31:225–33.
11. Thomas C, Kirschbaum A, Boehm D, Thomas L. The diagnostic plot. Med Oncol 2006;23:23–36.
12. Muckenthaler MU, Rivella S, Hentze MW, Galy B. A red carpet for iron metabolism. Cell 2017;168:344–61.
13. Cleland JG, Zhang J, Pellicori P, Dicken B, Dierckx R, Shoaib A, et al. Prevalence and outcomes of anemia and hematric deficiencies in patients with chronic heart failure. JAMA Cardiol 2016;1:539–47.
14. Anker SD, Comin Colet J, Filippatos G, Willenheimer R, Dickstein K, Drexler H, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. N Engl J Med 2009;361:2436–48.
15. Haddad S, Wang Y, Galy B, Korf-Klingebiel M, Hirsch V, Baru AM, et al. Iron-regulatory proteins secure iron availability in cardiomyocytes to prevent heart failure. Eur Heart J 2017;38:362–72.
16. Cappellini MD, Comin-Colet J, de Francisco A, Dignass A, Doehner W, Lam CS, et al. Iron deficiency across chronic inflammatory conditions: international expert opinion on definition, diagnosis, and management. Am J Hematol 2017;92:1068–78.
17. Punnnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997;89:1052–7.
18. World Health Organisation. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity 2011. Available at: https://www.who.int/vmnis/indicators/haemoglobin/en/ [Accessed 31 Aug 2019].
19. Heimpel H, Riedel M, Wennauer R, Thomas L. Die plasmasaeisenbestimmung – nützlich, unnötig oder irreführend? Med Klin 2003;98:104–7.
20. Skikne BS, Punnnonen K, Caldon PH, Bennett MT, Rehu M, Gasior GH, et al. Improved differential diagnosis of anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the stfr/log ferritin index. Am J Hematol 2011;86:923–7.
21. Shin DH, Kim HS, Park MJ, Suh IB, Shin KS. Utility of access soluble transferrin receptor (stfr) and stfr/log ferritin index in diagnosing iron deficiency anemia. Ann Clin Lab Sci 2015;45:396–402.
22. Sueka G, Kandarin G, Tubung J. Role of soluble transferrin receptor and transferrin receptor-ferritin index to detect iron deficiency anemia in regular hemodialysis patients. Open Access Med J Med Sci 2019;7:97–102.
23. Braga F, Infusino I, Dolci A, Panteghini M. Soluble transferrin receptor in complicated anemia. Clin Chim Acta 2014;431:143–7.
24. Hanif E, Ayyub M, Anwar M, Ali W, Bashir M. Evaluation of serum transferrin receptor concentration in diagnosing and differentiating iron deficiency anaemia from anaemia of chronic disorders. J Pakistan Med Assoc 2005;55:13–6.
25. Koulouzidis A, Said E, Cottier R, Saeed AA. Soluble transferrin receptors and iron deficiency, a step beyond ferritin. A systematic review. J Gastrointestin Liver Dis 2009;18:345–52.
26. Leers MP, Keuren JF, Oosterhuis WP. The value of the thomas-plot in the diagnostic work up of anemic patients referred by general practitioners. Int J Lab Hematol 2010;32:572–81.
27. Thomas C, Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clin Chem 2002;48:1066–76.
28. Ludwig H, Van Belle S, Barrett-Lee P, Birgegard G, Bokemeyer C, Gascon P, et al. The european cancer anaemia survey (ecas): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. Eur J Canc 2004;40:2293–306.
29. Bohlius J, Bohlke K, Castelli R, Djulbegovic B, Lustberg MB, Martino M, et al. Management of cancer-associated anaemia with erythropoiesis-stimulating agents: asco/ash clinical practice guideline update. J Clin Oncol 2019;37:1336–51.
30. Aapro M, Bokemeyer C, Djulbegovic B, Lustberg MB, Martino M, et al. Management of cancer-associated anaemia with erythropoiesis-stimulating agents: asco/ash clinical practice guideline update. J Clin Oncol 2019;37:1336–51.
31. Bohlius J, Bohlke K, Castelli R, Djulbegovic B, Lustberg MB, Martino M, et al. Management of cancer-associated anaemia with erythropoiesis-stimulating agents: asco/ash clinical practice guideline update. J Clin Oncol 2019;37:1336–51.
32. Harms K, Kaiser T. Beyond soluble transferrin: old factors gata-2 and nf-kappab. FASEB J 2002;48:1066–76.
33. Ludwig H, Van Belle S, Barrett-Lee P, Birgegard G, Bokemeyer C, Gascon P, et al. The european cancer anaemia survey (ecas): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. Eur J Canc 2004;40:2293–306.
34. Bohlius J, Bohlke K, Castelli R, Djulbegovic B, Lustberg MB, Martino M, et al. Management of cancer-associated anaemia with erythropoiesis-stimulating agents: asco/ash clinical practice guideline update. J Clin Oncol 2019;37:1336–51.
35. Aapro M, Bokemeyer C, Djulbegovic B, Lustberg MB, Martino M, et al. Management of cancer-associated anaemia with erythropoiesis-stimulating agents: asco/ash clinical practice guideline update. J Clin Oncol 2019;37:1336–51.