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Diagnostic work up of anemic patients: role of iron deficiency

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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-H_e]) 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-H_e 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-H_e (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-H_e) as indicator of the current iron requirements of erythropoiesis. If both biomarkers are combined, an algorithm-based diagram (“Thomas-plot” [TP]) is obtained

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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 DxI 800) and RET-H_e: 28 pg (Sysmex XN-2000).

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 rationale 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).

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-H_e). 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 DxI 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-H_e.

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.

- 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.
- 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.

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.

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

	Unit	Sex	Reference range
Hemoglobin	g/dL	m	13.0–17.5
		f	12.0–16.0
MCV	fL		80–96
MCH	pg		28–33
Erythrocytes	×10 ¹² /L	m	4.5–5.9
		f	4.1–5.1
Leukocytes	×10 ⁶ /L		3.5–9.8
Thrombocytes	×10 ⁶ /L		130–400
Reticulocytes	%		0.7–2.5
Ferritin	ng/mL	m	15–275
		f	10–204
Transferrin	mg/dL		202–336
Transferrin saturation	%		16–45
Serum iron	μg/dL	m	40–120
	(×0.1791 = μmol/L)	f	39–149
	pg		>28
sTfR	nmol/L		12.16–27.25
	(×0.0737 = mg/L)		
Vitamin B12	pg/mL		187–883
LDH	U/L	m	135–225
		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-H_e, reticulocyte-hemoglobin equivalent; sTfR, soluble transferrin receptor; LDH, lactate dehydrogenase; CRP, C-reactive protein.

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 2: Results of the manual classification based on the 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,
			n	n
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	

IDA, iron deficiency anemia; ACD, anemia of chronic disease; FID, functional iron deficiency.

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 proportion of all TPs (about 70%), followed by the departments 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 interpreted without additional clinical information. The number 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 anemia 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 conditions (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 quadrant in the TP using the ferritin index and RET-H_e (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 between 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 3: Overview of the tested laboratory parameters, subdivided according to the manual classification to an anemia category, blinded for Ret-H_e and sTfR.

	Hb, g/dL		Erythrocytes, ×10 ¹² /L		MCV, fL		CRP, ng/mL		Ferritin, ng/mL		Transferrin, mg/dL		TSAT, %		sTfR, nmol/L		Ferritin index, sTfR/log ferritin	
	Median	Pct	Median	Pct	Median	Pct	Median	Pct	Median	Pct	Median	Pct	Median	Pct	Median	Pct	Median	Pct
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	47.45	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
	12.9		4.3		95.6		34.5		468.0		222.0		56.70		25.21		11.62	

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.

Figure 2: Thomas-plot of all 1459 cases. Cutoff value for the ferritin index: 14, cutoff value for RET-H_e: 28 pg. Quadrant upper left: ACD, quadrant upper right: latent ID, quadrant lower right: IDA, quadrant lower left: FID/ACD.

Table 4: Cross tabulation comparing the manual and automatic classification based on the TP.

Manual classification based on conventional iron parameters	Thomas-plot-based classification				
	IDA	Latent ID	ACD/No anemia	FID/ACD	Total
IDA	77	50	0	0	127
Latent ID	5	39	2	0	46
ACD	1	35	341	0	377
FID/ACD	0	5	1	3	9
No anemia	0	52	554	0	606
Ambiguous	43	80	142	29	294
Total	126	261	1040	32	1459

IDA, iron deficiency anemia; ACD, anemia of chronic disease; FID, functional iron deficiency. Italicized numbers: Manual classification identical to the TP. Underlined numbers, Manual classification different from TP.

The diagnostic accuracy of the other parameters (transferrin, iron, MCV, MCH) was low in both cases. In contrast, RET-H_e (AUC 0.957) became more important in the group of ambiguous cases. Together with the ferritin index (AUC 0.917), both showed the highest diagnostic accuracy for distinguishing IDA from ACD during an APR. The diagnostic accuracy of sTfR (AUC 0.88 and 0.9) was similar in both groups (Table 5).

Discussion

Aim of this study was to evaluate the diagnostic work up of anemias retrospectively using conventional iron parameters and novel hematological markers, in particular the TP. The evaluation of routine laboratory diagnostics at a

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.

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%

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	0.958	0.948–0.969	0.691	0.600–0.782
TSAT	0.931	0.912–0.949	0.628	0.513–0.742
sTfR	0.884	0.858–0.911	0.897	0.860–0.933
RET-H _e	0.883	0.859–0.908	0.957	0.933–0.982
Transferrin	0.856	0.823–0.889	0.571	0.469–0.673
Serum iron	0.852	0.825–0.878	0.596	0.481–0.711
MCH	0.831	0.8–0.863	0.711	0.603–0.819
MCV	0.83	0.797–0.862	0.67	0.56–0.781

AUC, area under the curve; CI, confidence interval; sTfR, soluble transferrin receptor; TSAT, transferrin saturation; Ret-H_e, reticulocyte-hemoglobin equivalent; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

[26] or 20 % [27]. This might be due to the bias with regard to the high proportion of nephrological patients, but also to an incomplete diagnosis in 470 other ambiguous cases. In 29 of the 294 uncertain cases (10%), an ACD with FID would have been overlooked without the TP. Assuming that in the other unclear cases, approximately further 10% were not detected, the expected proportion would be around 50 additional cases with ACD and FID.

Although the data query generated numerous data sets, in only 10.7% of cases with anemia a further and complete diagnosis regarding iron metabolism was requested. Reasons for the low diagnostic rate remain unclear. In some cases, it may be due to the fact that mild anemia (Hb 10–12 g/dL) without clinical symptoms are accepted, especially in elder patients [28]. In other cases, the cause of the anemia may already have been known or the diagnostic work up of anemia did not belong to the respective medical specialty. While the diagnosis of anemia was largely limited to the field of internal medicine, the proportion of TPs performed by the nephrological practice alone was 70%. Consequently, the significance of the results referring to the general clinical utility of the TP is limited. The proportion of TPs carried out in oncological (1%) and in rheumatological patients (7%) was extremely low. Especially with regard to an expected ACD with additional FID in around 20% [9], a higher proportion of these patient groups would have been desirable. In recent years, the frequent transfusion of erythrocyte concentrates and in return the rather restrained use of ESAs and of intravenous iron in oncological patients has been criticized. This is probably partly due to diagnostic uncertainties. International guidelines support the use of ESAs in tumor patients undergoing chemotherapy [29, 30]. A more frequent application of the TP could lead to a more efficient

diagnosis and therapy, but further studies with focus on these patient groups are needed.

The TP has already been discussed as the gold standard for the diagnosis of complex iron anemias [31]. However, the standardization of the analytical methods is still ongoing and warrants the use of method-specific cutoff values for classification. The introduction of a recombinant 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-H_e) which adds another uncertainty to the classification [33].

The determination of RET-H_e 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.

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