Zinc Protoporphyrin in Anemia of Chronic Disorders

By Jan Hastka, Jean-Jacques Lasserre, Andreas Schwarbeck, Manfred Strauch, and Rüdiger Heilmann

Hemato fluorometric determination of zinc protoporphyrin (ZPP) is a screening method for the assessment of iron deficiency (ID). Chronic disorders are frequently accompanied by anemias of unclear origin, most probably caused by an impairment of iron metabolism. We investigated the relevance of ZPP for the detection of derangements of iron metabolism in anemias of chronic disorders (ACD). In 19 patients with ACD caused by chronic inflammatory non-neoplastic diseases, ZPP was determined and correlated with ferritin, transferrin saturation, and hemoglobin (Hb). Marrow sideroblast counts and semiquantitative grading of the marrow hemosiderin were performed in all patients to exclude ID and to show the decreased iron bioavailability. In all ACD patients who exhibited the typical laboratory findings of disturbed iron metabolism, such as hypoferremia, decreased transferrin saturation, decreased bone marrow sideroblasts, and increased marrow hemosiderin, strongly elevated ZPP levels were found (131 ± 23 μmol/mol heme). ZPP returned to normal after successful treatment of the underlying disease. This is shown in three patients with polymyalgia rheumatica. We conclude that the fluorometric determination of ZPP allows detection and quantification of derangements of iron metabolism associated with chronic inflammatory disorders. By recording the derangements quantitatively, ZPP allows monitoring of therapy of chronic inflammatory diseases.

PATIENTS WITH chronic systemic inflammatory disorders often develop an anemia that is associated with characteristic disturbances of iron metabolism. The typical findings are subnormal values of serum iron concentration and transferrin saturation, normal or increased ferritin, and an increased amount of storage iron.1-4 The hypoferremia has been suggested to represent a nonspecific defense mechanism, because the iron, which is necessary for optimal growth, is not available to the invading microorganism.5-9 The hypoferremia is probably caused by an impaired release of iron from tissue to plasma, from a decrease in the so-called early release pathway.10-12 The induction of apoferritin synthesis13 and liberation of lactoferrin from the neutrophil leukocytes14 are the two discussed pathophysiologic conceptions. The consequence of hypoferremia is a decreased iron supply to the red blood cell (RBC) precursors and a decreased synthesis of hemoglobin.1-3,12

In case of iron deficiency and lead poisoning16-22 zinc is incorporated into protoporphyrin IX instead of iron, and zinc protoporphyrin (ZPP) is produced instead of heme. According to its fluorescence properties, ZPP can be measured fluorometrically. The development of a front-face hemato fluorometer, which allows a rapid determination of the erythrocyte ZPP/heme ratio, made the routine use of this parameter possible.17,20,23-26

In normal persons without iron deficiency, ZPP is <40 μmol/mol heme; in patients with iron deficiency, ZPP levels are increased and show good correlation with the commonly used parameters of the iron metabolism.27 In a preliminary study, we could show that ZPP is a more sensitive parameter than ferritin for the detection of iron deficiency in erythropoietin-treated patients with end stage renal failure.28 In this study, we investigated the relevance of ZPP for the detection of derangements of iron metabolism in patients with chronic inflammatory diseases.

MATERIALS AND METHODS

The study was performed during 1988 to 1991 in the III. Med. Klinik and the Department of Nephrology in Mannheim. Nineteen anemic patients suffering from different chronic inflammatory disorders were examined. The group included patients with rheumatoid arthritis (n = 9), polymyalgia rheumatica (n = 3), polyarthropathy (n = 1), lupus erythematosus (n = 1), tuberculosis (n = 3), sarcoidosis (n = 1), and subacute infective endocarditis (n = 1). Only patients with typical anemias of chronic disorders (ACD)-criteria were included: serum iron level <10.7 μmol/L, transferrin saturation <25%, sideroblast count <30%, bone marrow hemosiderin >2 (6-point scale, 2 = normal). Exclusion criteria were iron deficiency, hemoglobin ≥10 g/dL, and blood transfusion within the last 4 months.

In all patients ZPP, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and serum levels of iron, ferritin, and transferrin were determined. The transferrin saturation (normal value 25% to 50%) was calculated.29 Iron levels (normal value 10.7 to 32.2 μmol/L) were determined with a multiinstrument (SMAC 11, Technicon, Tanytown, NY), transferrin (normal value 2 to 4 g/L) and C-reactive protein (CRP) (normal value <0.5 mg/L) by nephelometry and ferritin (normal value 34 to 310 μg/L for men and 25 to 210 μg/L for women) by enzyme-linked immunosorbent assay. Hb (normal value >13 g/dL for men and >12 g/dL for women), MCV (normal value 80 to 96 FL), and MCH (normal value 28 to 34 pg) were measured using an automatic cell counter (Model CC-180; Sysmex, Kobe, Japan). Erythrocyte sedimentation rate (ESR) (normal value <8/18 mm for men, <11/20 mm for women) was measured by the Westergren method, urinary excretion of the 6-aminolevulinic acid (6-ALS) (normal value <5.5 mg/day) by ion-exchange chromatography.

Bone marrow aspirates were obtained from the sternum or the posterior iliac crest. For the iron examination the aspirates were stained by the Prussian blue reaction and counterstained with hematoxylin. The RBC precursors were evaluated for the presence of iron granulas. Sideroblast count was determined in percent of erythroblasts (normal value 30% to 50%). The iron stores were assessed by a semiquantitative estimation of storage iron, according to a 6-point scale (0 = iron absent; 1 = iron decreased; 2 = normal amount of iron; 3 = iron increased; 4 = iron markedly increased; 5 = iron massively increased).

Blood samples for ZPP measurement were obtained by venipuncture and anticoagulated with EDTA. Measurements were performed
within 2 days of blood sampling. Samples that were not examined on the same day were stored in the refrigerator at 4°C.

ZPP was measured with the Aviv front-face hematofluorometer (Aviv Biomedical Company, Lakewood, NJ). The ZPP measurements were performed with washed erythrocytes; the methodologic details have been described elsewhere. Approval was obtained from the Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki.

RESULTS

Table 1 summarizes the parameters of iron metabolism in the 19 ACD patients analyzed. The mean ZPP was markedly increased (131 ± 23 μmol/mol heme, range 92 to 180 μmol/mol heme). The mean and the SD of Hb, MCV, and MCH were 8.9 ± 0.6 g/dL, 84.6 ± 3.7 fl, and 27.9 ± 1.3 pg, respectively. Serum iron (x̄ = 3.4 ± 1.1 μmol/L), transferrin (x̄ = 178 ± 24 mg/dL) and transferrin saturation (x̄ = 15.2 ± 3.8%) were decreased. The sideroblast counts were decreased in all patients (x̄ = 11.5% ± 3.8%; range 6% to 16%) and ferritin levels were markedly elevated (x̄ = 642 ± 212 μg/L). Also, the storage iron in the bone marrow was increased to grade 3 in 8 patients and grade 4 in 11 patients. Urinary excretion of the α-ALS was within the normal range in all patients (x̄ = 2.8 ± 1.2 mg/die; range 1.1 to 5.2 mg/die), which excluded hepatic porphyria and lead poisoning.

All of these parameters, including ZPP, returned to normal on successful treatment of the underlying chronic inflammatory disease. This is illustrated by three patients with polymyalgia rheumatica and ACD who showed a complete recovery after therapy (Table 2).

The clinical course of one of these three patients (patient no. 1) is described in the following in detail, to demonstrate the practical value of ZPP in treatment of chronic inflammatory diseases.

Patient no. 1. A 55-year-old woman presented with severe ACD in the course of a polymyalgia rheumatica. Treatment with 100 mg prednisone led to a continuous increase of Hb, which was accompanied by a decrease of ESR, ferritin, storage iron in the bone marrow, and ZPP and by an increase of transferrin saturation. With a gradual reduction of the prednisone dose to 60 mg, symptoms reappeared, whereas the ZPP was persisting at about 100 μmol/mol heme. An increase of the prednisone dose led to a disappearance of symptoms and a further decrease of ZPP. Other parameters, such as ESR, CRP, Hb, ferritin or transferrin saturation, were not sufficiently sensitive to reflect quick changes of disease activity. An episode of acute angina tonsillaris (days 60 to 70) led to another increase of ESR and CRP; ZPP was not influenced. After the angina subsided, ESR and CRP returned to normal (with the same prednisone dose). Because of stomach trouble, the patient was treated additionally with azathioprine, and the prednisone dose was reduced. Six months later the patient had completely recovered (Hb 16.0 g/dL, ZPP 37 μmol/mol heme, ESR 7/23, CRP 4 mg/L, Fe 11 μmol/L, transferrin saturation 29%, ferritin 25 μg/L).

DISCUSSION

Up to now, a complicated procedure was necessary to diagnose iron derangements in ACD. In addition to the commonly used "iron parameters," such as ferritin, serum iron, and saturation of transferrin, an examination of Prussian blue-stained marrow smears, including the estimation of storage iron and evaluation of the sideroblast count, had to be performed. Thus, the diagnosis was invasive, rather expensive, and required a hematologist. In spite of this extensive diagnostic workup, only severe ACD cases could be detected and a quantification was not possible. Therefore, the ACD diagnosis in most cases was not really proven but only postulated.

The determination of ZPP is a simple screening procedure for the detection of iron deficiency. In our present
study, we demonstrate that ZPP can further be applied to detect and describe the pathophysiologic defect, ie, impaired iron bioavailability underlying the anemia of chronic inflammatory disorders.

The 19 patients with chronic inflammatory diseases in our study showed anemias that were accompanied by hypoferremia, decreased saturation of transferrin, increased ferritin levels, and increased storage iron in the bone marrow. The inadequate supply of iron to the erythroid marrow in spite of increased hemosiderin was reflected by a decreased count of sideroblasts. These derangements of iron metabolism, which prove an impaired iron bioavailability, are the typical derangements of iron metabolism in chronic inflammatory disorders.

As a consequence of the impaired iron supply for the erythropoiesis, all our ACD patients showed markedly increased ZPP levels despite the increased bone marrow hemosiderin. These results demonstrate that the increased incorporation of zinc into protoporphyrin IX is independent of the cause of the impaired iron supply, because any disturbance in the iron metabolism ultimately leads to a decreased production of Hb and an increase of ZPP. The pathway that leads to the production of ZPP instead of heme in the case of iron deficiency works also in the presence of the impaired iron bioavailability in ACD. By this means, ZPP determination allows the rapid detection and quantification of derangements of iron metabolism in chronic inflammatory disorders.

ZPP is not a simple parameter of iron deficiency but rather a screening parameter of iron deficient erythropoiesis, surveying all steps of the iron metabolism from iron uptake to its utilization. Thus, the ZPP determination can serve as a kind of “end point control” of the whole iron metabolism, which detects real iron deficiency as well as derangements of iron metabolism in chronic inflammatory and neoplastic diseases, in sideroblastic disorders, eg, myelodysplasias, or after lead poisoning (Fig 1). The only possible source of error recognized so far seems to be the congenital erythropoietic porphyria and the protoporphyria caused by the fact that free erythrocyte porphyrin is fluorescent at a wavelength closely related to the wavelength of ZPP, resulting in measurement of falsely high ZPP levels in the test system used. However, these extremely rare diseases do not play a significant role in clinical practice and can easily be excluded by typical signs in patient history and laboratory findings.

Whereas lead-intoxicated patients show the most increased ZPP levels, up to 1,000 μmol/mol heme, the values observed in chronic inflammatory disorders are less increased and, even in the most severe cases, range below 200 μmol/mol heme. In patients with myelodysplastic syndromes we observed even in refractory anemia with ring sideroblasts.
levels below 80 amol/mol heme. The different ZPP levels measured in the different sideroachrestic anemias indicate that the derangements of iron metabolism are probably induced by different pathomechanisms. Thus, ZPP could be a tool to classify the sideroachrestic anemias according to the degree of ZPP synthesis. The significance of such classification requires further study.

In chronic inflammatory disorders, the degree of the disturbances of iron metabolism depends on the severity and activity of the underlying disease. By recording these disturbances quantitatively through ZPP determinations, chronic diseases and their response to therapy can be monitored inexpensively, quickly, and atraumatically. The normalization of the iron metabolism after a successful therapy of the underlying disease is accompanied by a normalization of ZPP. This may be of clinical relevance in patients with chronic infectious diseases such as tuberculosis.

It was reported recently that ZPP has a moderate inhibitory effect on the in vitro erythropoiesis. After addition of ZPP, a decrease in bone marrow erythroid colony-forming units and erythroid burst-forming units growth has been observed. This could be an additional effect in the multifactorial pathogenetic mechanisms, as illustrated by the arrows. ZPP detects real iron deficiency as well as derangements of iron metabolism with impaired iron availability caused by chronic inflammatory diseases, myelodysplasias, or lead poisoning.

In contrast to conventional inflammatory parameters, such as ESR or CRP, ZPP values are not influenced by acute inflammations. In patients with acute inflammatory diseases, such as bronchitis, influenza infections, pneumonia, meningitis, enteritis, angina tonsillaris, and erysipelas without iron deficiency, normal ZPP levels were measured.

We suggest that the ZPP determination can be used in routine practice as a simple method to detect and quantify derangements of iron metabolism in chronic inflammatory disorders.

REFERENCES
34. Lamola AA, Yamane T: Zinc protoporphyrin in the erythrocytes of patients with lead intoxication and iron deficiency anemia. Science 186:936, 1974
Zinc protoporphyrin in anemia of chronic disorders

J Hastka, JJ Lasserre, A Schwarzbeck, M Strauch and R Hehlmann

Updated information and services can be found at:
http://www.bloodjournal.org/content/81/5/1200.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml