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Pitfalls in Erythrocyte Protoporphyrin Measurement for Diagnosis and Monitoring of Protoporphyrias

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Abstract

BACKGROUND—Laboratory diagnosis of erythropoietic protoporphyria (EPP) requires a marked increase in total erythrocyte protoporphyrin (300–5000 μg/dL erythrocytes, reference interval <80 μg/dL) and a predominance (85%–100%) of metal-free protoporphyrin [normal, mostly zinc protoporphyrin (reference intervals for the zinc protoporphyrin proportion have not been established)]; plasma porphyrins are not always increased. X-linked protoporphyria (XLP) causes a similar increase in total erythrocyte protoporphyrin with a lower fraction of metal-free protoporphyrin (50%–85% of the total).

CONTENT—In studying more than 180 patients with EPP and XLP, the Porphyrias Consortium found that erythrocyte protoporphyrin concentrations for some patients were much higher (4.3- to 46.7-fold) than indicated by previous reports provided by these patients. The discrepant earlier

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reports, which sometimes caused the diagnosis to be missed initially, were from laboratories that measure protoporphyrin only by hematofluorometry, which is intended primarily to screen for lead poisoning. However, the instrument can calculate results on the basis of assumed hematocrits and reports results as “free” and “zinc” protoporphyrin (with different reference intervals), implying separate measurements of metal-free and zinc protoporphyrin. Such misleading reports impair diagnosis and monitoring of patients with protoporphyria.

SUMMARY—We suggest that laboratories should prioritize testing for EPP and XLP, because accurate measurement of erythrocyte total and metal-free protoporphyrin is essential for diagnosis and monitoring of these conditions, but less important for other disorders. Terms and abbreviations used in reporting erythrocyte protoporphyrin results should be accurately defined.

Erythropoietic protoporphyria (EPP)[8] is due to the inherited deficiency of ferrochelatase, the enzyme that catalyzes the chelation of iron with protoporphyrin IX to complete heme biosynthesis (1–3). The disease was not clearly described until 1961 (4). Increased erythrocyte and plasma protoporphyrin levels in EPP cause painful photosensitivity and impair quality of life (5). Since 2009 the Porphyrias Consortium has collected more than 180 patients with EPP and X-linked protoporphyria (XLP), and confirmed their diagnoses both biochemically and by DNA studies (3, 6). During this effort we found that erythrocyte protoporphyrin reports by 2 major clinical laboratories in the US are misleading and impair diagnosis of patients with EPP and subsequent monitoring of their porphyrin levels. This is concerning because EPP is the third most common porphyria and the most common in children, and it is often associated with long delays in diagnosis (1, 5). Furthermore, longitudinal monitoring of erythrocyte protoporphyrin is important because rising protoporphyrin concentrations may predict development of severe hepatic complications requiring liver transplantation (7).

Few patients with EPP or XLP seen at the Porphyrias Consortium centers had copies of laboratory reports documenting their original diagnoses. But among patients who had such records, 15 had current protoporphyrin concentrations that were much higher (4.7- to 46.7-fold) than those reported by other laboratories at the time of diagnosis (Table 1). Such marked increases over time are not reported in EPP in the absence of liver complications (1, 2), which were not present in any of these patients. The diagnosis was initially missed in 2 of these patients as a result of spuriously low results. All of the earlier reports were from either Quest or LabCorp, which currently offer only hematofluorometry for assessing erythrocyte protoporphyrin concentrations. Ordering physicians were not aware that this method is unsuitable for diagnosis of EPP or XLP, or that the protoporphyrin concentrations in these conditions are expected to be much higher (approximately 300–5000 μg/dL erythrocytes) than were reported. The diagnosis of protoporphyria was made in all but 2 of these patients because the inaccurate values (4.3- to 46.7-fold lower compared to later values) were still abnormal, and physicians with no information on the test methods and little experience with porphyrias accepted the results as evidence of protoporphyria. Because

[8]Nonstandard abbreviations: EPP, erythropoietic protoporphyria; XLP, X-linked protoporphyria; FEP, free erythrocyte protoporphyrin; OSHA, Occupational Safety and Health Administration; ZPP, zinc protoporphyrin.

the reported values did not reflect true protoporphyrin concentrations, they were useless for monitoring of these patients.

Because ferrochelatase in bone marrow reticulocytes can utilize metals other than iron, it catalyzes the formation of zinc protoporphyrin from most of the protoporphyrin remaining after completion of hemoglobin synthesis. But ferrochelatase deficiency in EPP limits formation of both heme and zinc protoporphyrin (8), so most of the protoporphyrin that accumulates remains metal free. In other erythrocyte disorders with intact ferrochelatase activity (e.g. lead poisoning, iron deficiency, hemolytic anemias, and anemia of chronic disease), excess protoporphyrin accumulates mostly as zinc protoporphyrin (9–11). The diagnosis of EPP requires a marked increase in total erythrocyte protoporphyrin and a predominance (85%–100%) of metal-free protoporphyrin. XLP, a less common and quite recently described porphyria with the same clinical phenotype, is due to gain-of-function mutations of the erythroid form of δ-aminolevulinic acid synthase, the first enzyme in the heme biosynthetic pathway; it causes a similar increase in total erythrocyte protoporphyrin but with a somewhat lower fraction of metal-free protoporphyrin (50%–85% of the total) (3, 12, 13).

Acid extraction methods (14–16) remove zinc from protoporphyrin, and therefore measure both metal-free and zinc protoporphyrin. Expressing results of these methods as “free erythrocyte protoporphyrin,” abbreviated FEP, became inappropriate with the discovery in 1974 that erythrocyte protoporphyrin is mostly zinc protoporphyrin both in health and in many erythrocyte disorders, in contrast to EPP, in which it is indeed mostly metal free (8, 17). Extraction with other solvents such as ethanol or acetone (17–20) and chromatographic separation methods (21) were developed to determine the proportions of zinc and metal-free protoporphyrin in erythrocytes.

Hematofluorometers were developed in the early 1970s as convenient, portable instruments to screen for lead poisoning and iron deficiency using a drop of blood (22). These instruments are tuned to measure the molar ratio of zinc protoporphyrin to heme by front surface fluorescence, but do not measure metal-free protoporphyrin. The instruments can display microgram per deciliter concentration units using assumed hematocrits, which are specified differently by the Occupational Safety and Health Administration (OSHA) (hematocrit 42, result reported as “zinc protoporphyrin”) and the CDC (hematocrit 35, result reported as “erythrocyte protoporphyrin”) (14, 15). The terms “free erythrocyte protoporphyrin” and “FEP” are also used, even though total and metal-free protoporphyrin are not measured. Results of both calculations (for OSHA and CDC compliance) are often reported, as “FEP” and “ZPP” (zinc protoporphyrin) (with different reference intervals), implying separate measurements of metal-free and zinc protoporphyrin.

Hematofluorometry is still offered as a cost-effective alternative to blood lead measurement that is accepted by regulatory authorities. The Quest website states that their method for this test is hematofluorometry, that it is for “the assessment of iron deficiency anemia and lead poisoning” and does not list this as a porphyria related test. LabCorp states their method is hematofluorometry, and that “free” protoporphyrin is “noncomplexed, nonheme protoporphyrin.” This is incorrect, because zinc-complexed protoporphyrin is measured. It is

unlikely that physicians and clinical laboratories consult these websites before outsourcing tests for EPP, and these details are not apparent at the ordering interface. ARUP Laboratories offers both hematofluorometry for screening for lead exposure and an erythrocyte protoporphyrin measurement for EPP by an acid solvent extraction method. But instead of fractionating metal-free and zinc protoporphyrin, they suggest plasma fluorescence scanning (ordered separately) (23) for confirming that an increase in total erythrocyte protoporphyrin is due to EPP. In our experience, plasma porphyrins are not always increased in EPP, even when erythrocyte protoporphyrin is markedly increased.

On the basis of these observations, we suggest that laboratories offering erythrocyte protoporphyrin measurements should do the following:

1. Prioritize testing for EPP and XLP. These measurements are essential for diagnosis and monitoring of these conditions but are less important for diagnosis of lead poisoning, iron deficiency, and other erythrocyte disorders.
2. Offer and report individual determinations for total, metal-free, and zinc protoporphyrin.
3. Recognize that ordering physicians generally lack experience in porphyrias and testing methodology and make clear at the ordering interface, as well as on the website or in the test manual, (*a*) the method used and (*b*) whether the test is appropriate for diagnosis and monitoring of EPP.
4. Use only the following 3 terms as test names and for reporting results: (*a*) “total erythrocyte protoporphyrin” (or “total erythrocyte porphyrins”), (*b*) “erythrocyte zinc protoporphyrin,” and (*c*) “erythrocyte metal-free protoporphyrin.”
5. Terms and abbreviations that are imprecise or misleading, such as “protoporphyrin,” “free protoporphyrin,” “EP,” “EPP,” and “FEP,” should not be used unless accurately defined.

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Table 1

Delays in accurately measuring erythrocyte protoporphyrin in 16 patients with protoporphyrias as indicated by much higher concentrations when they enrolled in Porphyrias Consortium (PC) studies.[a]

		Submitted report					Enrollment result	
				Value				
Patient	Testing interval	Test name	Laboratory	µg/dL	µmol/ mol heme	Reference interval	Value, µg/dL	Fold difference from submitted report
1	29 years	Protoporphyrin	Bio Science[b]	94		<50	4391[c]	46.7
2	22 years	FEP	Illegible	181		<35	984[c]	5.4
3	13 years	Protoporphyrin Free Erythro	SmithKline Beecham[b]	213		<35	926[c]	4.3
4	13 years	Erythrocyte Protoporphyrin	Metpath[b]	75		<50	1527[c]	20.4
5	6.1 years	Protoporphyrin (FEP)	LabCorp	437		0–34	-	9.2
5	6 years	Erythrocyte Protoporphyrin	Quest		908	<70	4009[d]	-
6	2 years	Erythrocyte Protoporphyrin	Quest		687	<70	3242[c]	-
7	11 months	Erythrocyte Protoporphyrin	Quest		243	<70	2835[c]	-
8	9 months	Erythrocyte Protoporphyrin (EP)	Quest		223	<70	1195[c]	-
9	6 months	Erythrocyte Protoporphyrin	Quest		109	<70	2521[c]	-
10	3.1 months	Protoporphyrin (FEP)	LabCorp	72		0–34	643[c]	8.9
7	3 months	Protoporphyrin (FEP)	LabCorp	110		0–34	2835[c]	25.8
11	2 months	Erythrocyte Protoporphyrin (EP)	Quest		255	<70	2151[c]	-
12	1.3 months	Erythrocyte Protoporphyrin (EP)	Quest		255	<70	2225[c]	-
13	1.2 months	Protoporphyrin (FEP)	LabCorp	145		0–34	4703[c]	32.4
14	20 d	Plasma Total Porphyrins	Quest		253	<70	1931[c]	-
15	11 d	Protoporphyrin (FEP)	LabCorp	74		0–34	888[d]	12
16	None	Protoporphyrin, FEP/ZPP	Quest		127	<70	1687[c]	-

[a] Simultaneous measurements in 2 laboratories in the 16th patient clearly indicate that differences in laboratory methods are responsible. All patients shown were able to provide their original laboratory reports (patients 5 and 7 each provided 2 reports) for comparison with current findings. Test names did not distinguish total, zinc-chelated, or metal-free protoporphyrin, and often implied that total or metal-free protoporphyrin was measured.

[b] Acquired by Quest.

Enrollment results were from:

[c] the Porphyria Center at the University of Texas Medical Branch; or

[d] Mayo Medical Laboratories (reference range <80 μg/dL for both).