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ORIGINAL ARTICLE

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Development and validation of diagnostic algorithms for the laboratory diagnosis of porphyrias

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Abstract

Porphyrias are rare metabolic disorders of the haem synthesis. They can present with acute neurovisceral attacks, cutaneous symptoms, or a combination of both. As they present with a wide variety of clinical symptoms, diagnosis is often delayed and correct interpretation of porphyria-related tests remains a challenge for many physicians. We developed and validated two algorithms for the laboratory diagnosis of porphyrias based on presenting symptoms. Based on a literature search and clinical/laboratory expertise, we developed algorithms for acute and cutaneous porphyrias. We validated these algorithms using all porphyria related laboratory test requests between January 1st 2000 and September 30th 2020 in UZ Leuven. In addition, we also evaluated our algorithm using samples from the European porphyria network (EPNET) external quality assessment scheme (2010–2021). Sensitivity of the algorithm for acute porphyria was 100.0% [74.9%–100.0%] (13 acute intermittent porphyria (AIP) and 1 variegate porphyria [VP]) with a specificity of 98.5% [91.0%–100.0%] (65 patients). Sensitivity of the algorithm for cutaneous porphyria was 100% [95.1%–100.0%] (7 VP, 59 porphyria cutanea tarda (PCT), 23 erythropoietic protoporphyria (EPP), 2 X-linked erythropoietic protoporphyria [XLEPP]) with a specificity of 93.9% [82.9%–98.5%]. There were no diagnostic samples of other types of porphyria. The algorithms correctly identified 18 of the 19 EPNET porphyria cases. One of the two hereditary coproporphyria cases was missed. The algorithms for acute and cutaneous porphyria showed high sensitivity and specificity and can be used to aid the clinician in correctly interpreting the laboratory findings of porphyria-related tests.

KEYWORDS

acute porphyria, algorithm, cutaneous porphyria, porphyria, sensitivity and specificity

1 | INTRODUCTION

The porphyrias are a group of rare (mostly) inherited, metabolic disorders, caused by a deficiency or gain of function of one of the enzymes of the haem synthesis pathway (Figure 1).[1–3] They present with acute neurovisceral attacks, skin lesions or a combination of both. Symptoms can be attributed to accumulation of the haem precursors delta-aminolevulinic acid (dALA), porphobilinogen (PBG), and porphyrins.[1]

Porphyrias can be categorized based on two different principles: (1) the organ in which haem precursors

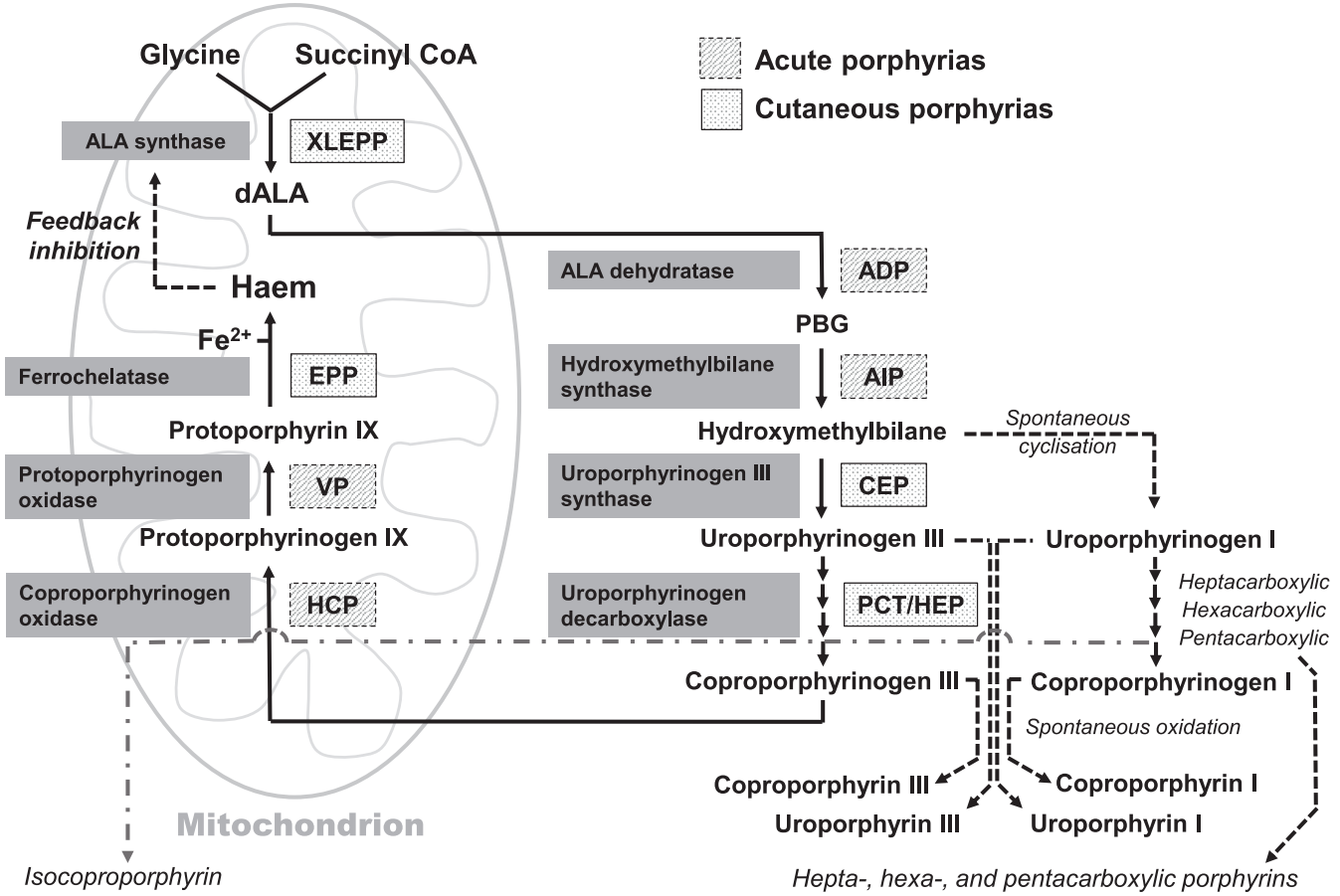

FIGURE 1 Haem biosynthetic pathway (adapted from Whatley and Badminton).[3] See Table 1 for the abbreviations of the different porphyria disorders. The full lines show the normal metabolic pathway and the dashed lines indicate side branches. In porphyrias the flux through these side branches can increase significantly (e.g., increased formation of uroporphyrinogen I and heptacarboxylic porphyrin in PCT)

accumulate, either erythropoietic or hepatic, and (2) whether they present with acute attacks or cutaneous symptoms.[1,4] For this manuscript, porphyrias will be categorized as presenting with acute attacks and/or cutaneous symptoms (Table 1).[1,5–9] Acute intermittent porphyria (AIP) and dALA dehydratase (ALAD) deficiency porphyria (ADP) only present with acute attacks. Hereditary coproporphyria (HCP) and variegate porphyria (VP) can present with either acute attacks or cutaneous symptoms. Porphyria cutanea tarda (PCT), erythropoietic protoporphyria (EPP), X-linked erythropoietic protoporphyria (XLEPP), hepatoerythropoietic porphyria (HEP), and congenital erythropoietic porphyria (CEP) only present with cutaneous symptoms.

1.1 | Biochemical laboratory testing for porphyrias

Porphyria diagnosis is based on biochemical testing in three sample matrices: urine, blood, and feces (Table 2).[10–15] PBG and dALA are determined in urine, total porphyrins and their fractionation in urine and feces and plasma porphyrins and total erythrocyte protoporphyrins in blood.[2] It is important to always protect the samples from light as porphyrins (particularly protoporphyrins) and PBG are light sensitive.[2] In addition, enzyme activity of PBG deaminase, dALA dehydratase, and uroporphyrinogen decarboxylase can be determined in red blood cells.

Diagnostic testing should be guided by the clinical presentation.[2] If an acute porphyria is suspected, we measure total urinary porphyrins, dALA, and PBG on a random, preferably early morning, urine sample. There is discussion in the literature whether to include dALA as a first-line screening test for acute porphyria. Two published algorithms only recommend measuring dALA if PBG is negative and there remains a strong suspicion of acute porphyria,[2,16] while the algorithms by Di Pierro et al. and the Mayo Clinical Laboratories include dALA as first-line test.[17,18]

It is recommended to report the excretion of PBG, dALA, and urinary porphyrins per mmol creatinine in

TABLE 1 Overview of the main types of porphyria (adjusted from "The porphyrias" on EPNET website[1,5])

Disorder	Enzyme defect	Inheritance	Neurovisceral crises	Skin lesions		Epidemiology		
				Fragile skin, blisters	Acute photosensitivity	Prevalence Europe (/1 000 000)[6]	Typical age of onset	Gender
Acute porphyrias								
ADP	ALAD	AR	+	−	−	<0.01	Childhood, adolescent	−
AIP	PBGD[a]	AD	+	−	−	5.9 (5.0–7.2)	Adolescent, adult	F > M
HCP	CPOX	AD	+[b]	+[b]	−	<1	Adolescent, adult	F > M
VP	PPOX	AD	+[b]	+[b]	−	3.2 (2.4–4.0)	Adolescent, adult	F > M
Non-acute porphyrias								
CEP	UROS	AR	−	+	−	<1	Birth, infancy	M = F
PCT	UROD	Complex[c]	−	+	−	50–100	Adult	M > F
HEP	UROD	AR	−	+	−	<1	Childhood	M = F
EPP	FECH/ClpXP	AR[d]/AD[e]	−	−	+	9.2 (7.7–11.6)	Infancy, childhood	M = F
XLEPP	ALAS2	XL	−	−	+	<1	Infancy, childhood	M > F[f]

Abbreviations: AD, autosomal dominant; ALAS2, ALA synthase 2; AR, autosomal recessive; ALAD, delta-aminolevulinic acid dehydratase; ClpXP, ClpX protease; CPOX, coproporphyrinogen oxidase; F, female; FECH, ferrochelatase M, male; PBGD, porphobilinogen deaminase (hydroxymethylbilane synthase); PPOX, protoporphyrinogen oxidase; UROD, uroporphyrinogen decarboxylase; UROS, uroporphyrinogen III synthase; XL, X-linked.

[a]Other frequently used names are hydroxymethylbilane synthase and uro-1-synthetase.

[b]Skin lesions and neurovisceral crises may occur alone or together.[1]

[c]About 25% of cases are familial, inherited in an autosomal dominant pattern. 75% of cases are sporadic, caused by a genetic and environmental risk factors.[1,19]

[d]FECH-deficient EPP is an AR disorder which may appear "pseudodominant" in some families due to a low-expressing allele; the proportion of such families being determined by the population frequency of the IVS3-48C allele.[7]

[e]Recently, one family has been identified with a missense mutation in the *ClpX* gene which is inherited in an autosomal dominant way. The ClpX protease regulates ALAS activity.[8]

[f]The phenotype of XLEPP in heterozygous females ranges from asymptomatic to severe as in affected males due to random X-chromosomal inactivation.[9]

TABLE 2 Typical biochemical findings in symptomatic porphyria patients[10–13]

Porphyria	Urine	Plasma peak	Stool	Erythrocyte
ADP	dALA >> PBG, Copro III	Normal	Normal	ZnPP
AIP	PBG > dALA Uro >> Copro	(615–620 nm)[a]	Normal or slightly increased (uro)	Normal
HCP	PBG > dALA, Uro Copro	(615–620 nm)[b]	Copro >> proto, Ratio Copro III/I > 2	Normal
VP	PBG > dALA, Copro	624–627 nm	Proto > Copro III	Normal
CEP	Uro I (>>III), Copro I (>>III)	615–620 nm	Copro I (>>III)	ZnPP and Free PPIX Uro I, Copro I
PCT	Uro (I + III), Hepta (≥25%)[c]	615–620 nm	Hepta, Isocopro Normal ratio Copro III/I	Normal
HEP	Uro (I + III), Hepta	615–620 nm	Hepta, Isocopro Normal ratio Copro III/I	ZnPP
EPP	Normal	630–635 nm	Proto	Free PPIX
XLEPP	Normal	630–635 nm	Proto	ZnPP and Free PPIX

Note: Indicated haem precursors are increased in the indicated sample matrix.
Abbreviations: Copro (I or III), coproporphyrin (I or III); dALA, delta-aminolevulinic acid; Hepta, heptacarboxylic porphyrin; Isocopro, isocoproporphyrin; PPIX, protoporphyrin IX; Proto, protoporphyrin; Uro (I or III), uroporphyrin (I or III); ZnPP, zinc protoporphyrin.
[a]Plasma porphyrins are normal or only slightly elevated.[14]
[b]Plasma porphyrins are usually normal, but increased when blistering skin lesions develop.[14]
[c]In PCT, heptacarboxylic porphyrins are usually >25% of uroporphyrins in urine.[15]

urine.[2] Collection of a 24 h urine specimen is discouraged as it (1) unnecessarily delays diagnosis, (2) offers little advantage, (3) increases the risk of losses during the collection period, (4) increases the risk of light exposure, and (5) increases the risk of incorrect storage.[19] Urine PBG is strongly elevated during an acute attack (typically ≥4× upper reference limit [URL]).[2,20] If PBG is normal, this excludes an acute porphyria attack. It is important to measure both PBG and urine porphyrins, as urine PBG can rapidly return to normal within a few days after clinical presentation, especially in VP and HCP.

Urinary porphyrins are increased in all acute porphyrias during an attack, and fractionation can sometimes contribute to the final diagnosis (Table 2). The reddish-brown color of urine during an attack is attributed to the presence of porphyrins and porphobilin (a degradation product of PBG).[14] Uroporphyrins and coproporphyrins are formed via auto-oxidation of their respective uroporphyrinogens and coproporphyrinogens (Figure 1). Additional testing of plasma porphyrins can guide the diagnosis to VP, as a fluorescence peak between 624 and 627 nm on plasma diluted at neutral pH is specific for VP (Table 2).[10] PBG deaminase in erythrocytes is decreased in AIP, but this is not definite for AIP diagnosis as the activity range in affected individuals overlaps with the lower end of the activity range in unaffected individuals.[21] Furthermore, hydroxymethylbilane synthase (HMBS) mutations in exon 1 result in decreased enzyme activity in non-erythroid tissues, but do not affect enzyme activity in erythrocytes (non-erythroid form of AIP, 5% of all cases).[17,22]

To further differentiate the acute porphyrias, total porphyrins in stool should be determined, followed by fractionation if elevated.[14] In AIP, fecal porphyrins are normal or slightly elevated. In HCP, there is an accumulation of coproporphyrin III with an increased coproporphyrin III:I ratio of >2 (normally <0.8).[23] A similar increased fecal coproporphyrin III:I ratio is observed in variegate porphyria.[24] Of note, coproporphyrin III is the predominant coproporphyrin isoform in urine in all acute porphyrias and in non-porphyria patients.[24]

Depending on the cutaneous symptoms, different testing strategies have been proposed for blistering lesions and acute non-blistering photosensitivity.[2] If patients present with blistering skin lesions, measurement of plasma porphyrins and total urinary porphyrins followed by fractionation are typically recommended. If both tests are negative, PCT, VP, and HCP are excluded as cause of the blistering lesions. In a study by Woolf et al., all patients with PCT had a plasma peak between 617 and 623 nm and increased highly carboxylated porphyrins in urine with increased uroporphyrin I, uroporphyrin III, and heptacarboxylic porphyrin (typically ≥25% of the sum of uroporphyrin I and III).[2,15] Hepta-, hexa-, and pentacarboxylic porphyrins are formed by auto-oxidation of the corresponding porphyrinogen intermediates of the stepwise decarboxylation of uroporphyrinogen to

coproporphyrinogen by uroporphyrinogen decarboxylase (Figure 1).[25] Isocoproporphyrin in stool is typically increased in PCT and HEP.[17] Isocoproporphyrin arises by bacterial degradation of dehydro-isocoproporphyrinogen which is formed by the premature metabolism of the pentacarboxylic intermediate by coproporphyrinogen oxidase (Figure 1).[26] Dicarboxylic porphyrins in stool lack diagnostic significance.[17] If the plasma scan shows an emission peak between 624 and 627 nm, VP is the cause of the blistering skin lesions.[1,10] If cutaneous symptoms are due to underlying HCP, this is more difficult to establish. In most HCP cases, symptoms of an acute attack will be present as well, with a concordant increase in urinary PBG and total porphyrins. If there is no acute attack, total urinary porphyrins can be normal.[23] If there is strong clinical suspicion of HCP, stool can be examined.[23] In CEP, an increase in urinary uroporphyrin I and coproporphyrin I is expected, as well as a marked increase in plasma and erythrocyte porphyrins.[10]

If patients present with acute painful photosensitivity without blistering lesions, total erythrocyte protoporphyrins and plasma porphyrins should be determined.[2] Total erythrocyte porphyrins are significantly increased, typically ≥3000 μg/L RBC (reference range ≤800 μg/L RBC), and a plasma peak between 630 and 635 nm is characteristic for EPP and XLEPP.[10,13,27] EPP is characterized by a large increase in metal-free protoporphyrin (typically ≥85%), while there is an increase in both metal-free and zinc-chelated protoporphyrin in XLEPP.[10,12,28] Total erythrocyte protoporphyrins (predominantly zinc-chelated protoporphyin) are also increased in lead poisoning, iron deficiency, and disorders associated with disturbed incorporation of iron into protoporphyrin such as iron-refractory iron deficiency anemia (IRIDA), anemia of chronic disease, and X-linked sideroblastic anemia and ataxia.[17,29]

Besides the porphyrias, there are other causes of elevated intermediates of the haem synthesis.[30] There are two important conditions in which dALA is strongly elevated in urine, with no or only slightly elevated PBG. The first condition is lead poisoning.[30] Lead mainly inhibits ALAD, leading to high urine dALA levels. The second condition is type I tyrosinemia which leads to accumulation of succinylacetone, a structural analogue of dALA and therefore a competitive inhibitor of ALAD.[1,2] Both lead poisoning and tyrosinemia type I can exhibit the same symptoms as an ADP patient during an acute attack with strongly increased dALA and normal or slightly elevated PBG.[30] An important and common cause of elevated total erythrocyte protoporphyrins with increased zinc protoporphyrins is iron deficiency and other red blood cell disorders. Other causes of secondary elevation of porphyrins include several clinical conditions, such as liver disease and intake of certain medications.[30,31] End stage renal disease (ESRD) can also lead to acquired PCT due to a decrease uroporphyrinogen decarboxylase activity and accumulation of haem precursors.[32] PCT in ESRD may result from iron overload in combination with various susceptibility factors.

TABLE 3 Methods and reference intervals used for the evaluation of the algorithms

Test	Method and reference interval
ALA[a]	Quantitative ion exchange resin method
	<50 μmol/L[19]
	<46 μmol/24 h
PBG[a]	Quantitative ion exchange resin method
	<10 μmol/L[19]
	<9 μmol/24 h
Porphyrins (urine)	Total porphyrins (screening)[b]: ≤270 nmol/L
	If positive screening: HPLC analysis
	Coproporphyrins: ≤115 nmol/L
	Coproporphyrins: ≤280 nmol/24 h
	Uroporphryins: ≤24 nmol/L
	Uroporphryins: ≤40 nmol/L/24 h
Porphyrins (plasma)	Qualitative method (plasma fluorescence scanning)
Total erythrocyte protoporphyrins	≤800 μg/L RBC[28]
PBG deaminase	Enzymatic end point/ spectrofluorometric
	<6.0 nmol/L RBC.s: suggestive for AIP
	6.0–6.9 nmol/L RBC.s: intermediate result
	≥7.0 nmol/L RBC.s
Porphyrins (feces)	HPLC analysis
	Protoporphyrins: ≤170.0 nmol/g dry weight
	Coproporphyrins: ≤45.0 nmol/g dry weight
	Uroporphryins: ≤3.0 nmol/g dry weight
	Ratio coproporhyrin III/I: ≤0.8

[a]For 24 h urine collections, the result was considered abnormal if the result per liter or per 24 h was above the upper reference limit.
[b]Screening using a spectrophotometric screening method, followed by fractionation by high-performance liquid chromatography (HPLC) for positive samples.

1.2 | Rationale to develop diagnostic algorithms

Many clinicians have difficulties selecting and interpreting the laboratory tests when porphyria is included in the differential diagnosis.[33] Diagnostic algorithms could help improve the diagnosis of porphyrias. While a number of algorithms have been published, none of these have been validated using patient results or take account false positive or false negative results of some tests.[2,16,17,34] To improve the laboratory diagnosis of porphyria, we developed and validated diagnostic algorithms for acute and cutaneous porphyrias.

2 | MATERIAL AND METHODS

2.1 | Study design

This retrospective study was performed in the University Hospitals of Leuven (UZ Leuven, Belgium) after approval by the local ethics committee (MP012472 and S54187). UZ Leuven is one of the two Belgian porphyria centers recognized by the European Porphyria network (EPNET).

2.2 | Patient cohort

First, we identified all patients with porphyria in the patient database of the center for metabolic diseases of UZ Leuven. Next, a query was performed in the laboratory information system (LIS) of UZ Leuven for all porphyria related laboratory tests, requested between January 1st 2000 and September 30th 2020. For patients with laboratory values outside of the reference range for one or more porphyria related tests, the medical file was consulted for the final diagnosis (see Table 3 for the reference ranges).

2.3 | Development and validation of the algorithms

Based on a literature search (see introduction), the patient database and clinical/laboratory expertise, we developed algorithms for acute and cutaneous porphyrias. After the algorithms were finalized, they were validated. The sensitivity was determined using the patients diagnosed with acute or cutaneous porphyria in the database if diagnostic samples were available. The specificity was evaluated using 101 consecutive patients with one or more abnormal porphyria related test results but an alternative diagnosis according to their medical file. The patient selection was based on chronological occurrence of the results in the database, starting from the most recent results. All patients categorized as false positive or false negative were seen by a specialist in porphyria (a dermatologist of hepatologist specialized in porphyria) and the results were also reviewed by the clinical pathologist specialized in porphyria. In addition, the sensitivity was also validated with results available from the external quality program of EPNET (2010–2021).

2.4 | Statistics

Calculation of sensitivity and specificity was carried out in Microsoft® Excel 2016 (Redmond, WA). 95% confidence intervals of proportions were calculated using the modified Wald method on the GraphPad QuickCalcs Website (http://www.graphpad.com/quickcalcs/ConfInterval1.cfm, accessed December 2021).

3 | RESULTS

3.1 | Patient cohort

The LIS query identified 14 152 laboratory requests consisting of one or more laboratory tests requests of 8775 different patients. For 8705 lab requests from 7109 patients all porphyria related test results were within the reference range. One or more porphyria related tests were indicative for a possible underlying porphyria in 1285 patients. For about half of these patients ($n = 646$) no clinical information was available. These patients were excluded from further analysis. Of the remaining 639 patients, 222 were diagnosed with porphyria (Table 4), while an alternative diagnosis was made in 417 patients (Table S1).

3.2 | Development of the algorithm

The algorithm was developed based on a literature search and expert opinion. We decided to construct two algorithms based on the clinical presentation. For the acute porphyrias, the algorithm starts with the measurement of PBG, dALA and porphyrins in urine (Figure 2). According to literature and expert opinion, these were the three tests necessary for a first-line screening for an acute porphyria. If these tests are all negative and samples are collected during symptoms, an acute attack of porphyria can be excluded. Based on the followed path, plasma porphyrins by fluorescence scan, porphyrins in feces and PBG deaminase activity are additionally tested.

TABLE 4 Number of patients with different types of porphyria and sensitivity of the algorithms

Porphyria	Number of patients	With all required test results	Correct diagnosis via algorithm	Sensitivity [95% CI]
Acute	40	14	14/14	100% [74.9%–100%]
Cutaneous	178	91	91/91	100% [95.1%–100%]
AIP	35	13	13/13	100% [73.4%–100%]
VP[a]	21	7		
		Acute symptoms	1/1	100% [16.8%–100%]
		Cutaneous symptoms	7/7	100% [59.6%–100%]
HCP	2	0	-	-
ADP	0	0	-	-
EPP	73	23	23/23	100% [83.1%–100%]
XLEPP	2	2	2/2	100% [29.0%–100%]
PCT	89	59	59/59	100% [92.7%–100%]
CEP	0	0	-	-
HEP	0	0	-	-

Note: Only patients for whom all the required diagnostic test results for the respective algorithm were available were included for the calculation of the sensitivity.

[a]One patient with VP presented with both acute and cutaneous symptoms.

For the cutaneous porphyrias, the algorithm begins with the analysis of plasma porphyrins by fluorescence scan and porphyrins in urine in case of blistering lesions, and with plasma porphyrins by fluorescence scan and total erythrocyte protoporphyrins in case of acute, painful photosensitivity (Figure 3). If the two first-line tests are negative, an underlying porphyria as cause of the current cutaneous symptoms can be excluded. Then additional testing of fecal porphyrins and fractionation of erythrocyte protoporphyrins can be performed, based on the followed path.

3.3 | Algorithm validation: sensitivity

In a first step, results of all patients who were diagnosed with an acute or cutaneous porphyria were screened to see if there were diagnostic samples available in the period of the LIS query. For VP and HCP, we first determined with which symptoms the patient presented in order to run the patients through the right algorithm. Only patients for whom all the required test results when following the flowchart were available (except porphyrins in feces for acute porphyria) were used to validate the algorithms (Table 4). The sensitivity of the algorithm for acute porphyria was 100.0% [74.9%–100.0%] (13 AIP and 1 VP). Sensitivity of the algorithm for cutaneous porphyria was 100% [95.1%–100.0%] (7 VP, 59 PCT, 23 EPP, 2 XLEPP).

There were no diagnostic samples of other types of porphyria. For the HCP patients in the database ($n = 2$), no diagnostic samples were available. No patients with ADP or CEP were present in our database. Of the 73 patients diagnosed with EPP according to the medical records, six cases had only a tentative EPP diagnosis with inconclusive biochemical findings (according to the treating physician and the clinical pathologist) and inconclusive genetic findings (no biallelic pathogenic variants in FECH on molecular single gene testing). These six cases were excluded for the validation. Two of the patients diagnosed with VP ($n = 21$), were diagnosed via genetic familial screening and had never experienced any symptoms. These patients were also excluded for validation.

PBG deaminase activity was decreased (<7.0 nmol/L RBC.s) in 12 of the 13 patients diagnosed with AIP (92.3%) and normal in one AIP patient (8.0 nmol/L RBC.s; c.912+2t>c in intron 14 of the HMBS gene). In 91.5% of patients diagnosed with PCT, the percentage heptacarboxylic porphyrin was at least 25% of the sum of uroporphyrin I and III in urine. Total erythrocyte protoporphyrins were ≥3000 μg/L RBC in 20 of the 23 EPP patients. Three late-onset cases (start of symptoms at the age 23 years or later) had total erythrocyte protoporphyrins < 3000 μg/L RBC (2495, 1713, and 1413 μg/L RBC). Both XLEPP patients had total erythrocyte protoporphyrins ≥ 3000 μg/L.

After validation using the laboratory results of our database, we also validated the algorithm using the cases from the EPNET external quality assessment scheme in the period 2010–2021. The "acute attack" algorithm correctly identified all 4 porphyria patients presenting with acute symptoms (3 AIP and 1 HCP).

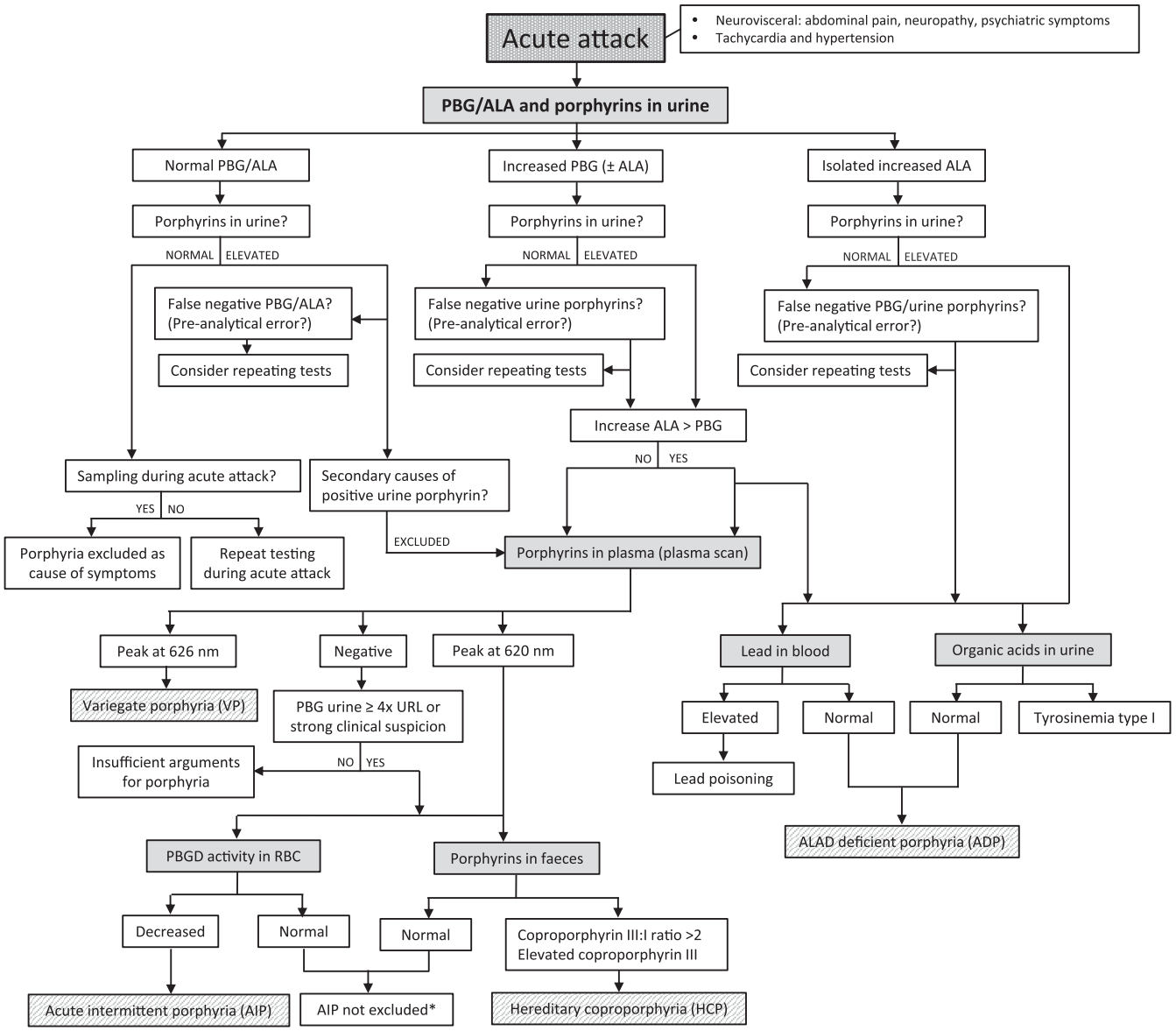

FIGURE 2 Diagnostic algorithm for acute porphyrias. *5% of AIP have a normal PBGD activity (including the non-erythroid form of AIP). MDS: myelodysplastic syndrome.

The "cutaneous symptoms" algorithm correctly identified 15 of the 16 patients with cutaneous symptoms (3 VP, 5 PCT, 4 EPP, 1 XLEPP, 1 HCP, 1 EPP or XLEPP). One patient with HCP was missed as dALA, PBG and porphyrins (urine) were normal. The only diagnostic biochemical abnormality was the coproporphyrin III:I ratio in feces. This is not unexpected as patients in the subclinical or in the latent phase of HCP have normal or only mildly increased dALA, PBG, and porphyrins (urine).[23] One case of HCP presenting with acute and cutaneous symptoms was identified by both algorithms and one case of HCP was excluded because no feces sample was available as required by the algorithm.

3.4 | Algorithm validation: specificity

—Of the 417 patients with an alternative diagnosis based on the clinical information, the 101 most recent patients who presented with acute and/or cutaneous symptoms suggestive of porphyria (65 with acute and 49 with cutaneous symptoms) were selected to estimate the specificity of the algorithms. Patients for whom not all the required test results when following the flowchart were available (except porphyrins in feces for acute porphyria) were excluded for the validation. The specificity of the "acute attack" algorithm was 98.5% [91.0%–100.0%] and the specificity of the algorithm for cutaneous porphyria was 93.9% [82.9% - 98.5%]. For the "acute attack" algorithm,

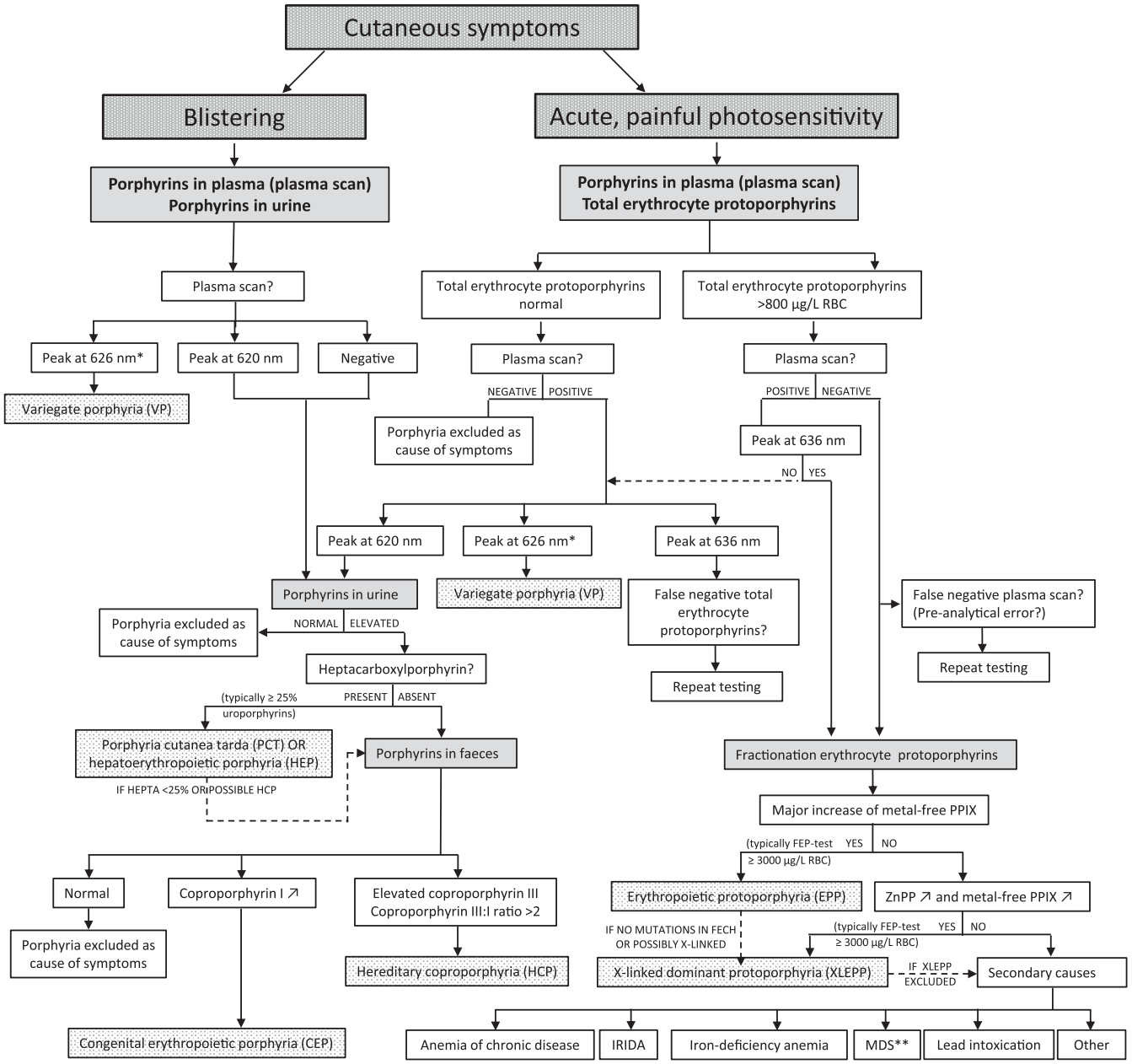

FIGURE 3 Diagnostic algorithm for cutaneous porphyrias. *The sensitivity of plasma scan is 100% for symptomatic VP patients, but less than 85% in asymptomatic family members VP patients.[35] **% zinc-chelated protoporphyrin depends on % clonal cells

there was one false positive result for a patient who presented with vomiting and diarrhea in which the algorithm diagnosed the patient with ADP (dALA 70 µmol/L, PBG 9 µmol/L, uroporphyrins 44 nmol/L, 462 nmo/L coproporphyrins) but the clinician diagnosed the patient with functional dyspepsia. For the "cutaneous porphyria" algorithm, there were three false positive results. The first case was a 2-year-old girl who presented with a rash on the arms and face, in which the algorithm showed a diagnosis of EPP (total erythrocyte protoporphyrins 1758 µg/L RBC), but the dermatologist contributed the rash to an acute cytomegalovirus infection. In the second false positive case, a 30-year-old woman presented with liver function disorder and hypersensitivity on sun exposed skin. Results of the algorithm showed a diagnosis of EPP (total erythrocyte protoporphyrins 1758 µg/L RBC), but this was genetically not confirmed and the woman was finally diagnosed with "sun allergy." In the last case, a 40-year-old woman was diagnosed with PCT based on the algorithm (uroporphyrins 1991 nmol/L of which 47% heptacarboxylic porphyrins, and coproporphyrins 32 nmol/L), but the porphyria specialist in our centre who followed the patient considered this not a PCT case due to the absence of skin lesions, but attributed the elevation in

uroporphyrins and heptacarboxylic porphyrins to underlying liver disease.

4 | DISCUSSION

To our knowledge, this is the first time that diagnostic algorithms for acute and cutaneous porphyria have been developed and validated with an analysis of the sensitivity and specificity. A few algorithms have previously been published, but these do not take into account false positive or false negative results of some tests.[2,16,17,34] In two of these algorithms, a distinction is made between active blistering skin lesions and acute photosensitivity as presenting clinical symptoms to guide a different testing strategy as is the case in our algorithm.[2,16]

There is discussion in the literature whether to include dALA as a first-line screening for acute porphyria in addition to PBG and urinary porphyrins. Two algorithms in literature claim it is not necessary to add dALA to the testing strategy as ADP is extremely rare. They claim testing for dALA should only be done if PBG is negative and there remains a strong suspicion of acute porphyria.[2,16] Algorithms proposed by Di Pierro et al. and the Mayo Clinical Laboratories start with both dALA and PBG in addition to urinary porphyrins.[17,18] We decided to also include dALA as first-line test because an isolated increase in dALA can guide diagnosis to lead poisoning and type I tyrosinemia which can present with similar symptoms as acute porphyria.[4] While all 14 patients diagnosed with acute porphyria in our cohort had a PBG ≥4× URL (if dALA increased, always PBG > dALA), there was also a patient with lead intoxication with a major increase of dALA (>10× URL) but only a mildly increased PBG (1.4× URL) and an isolated increase of urinary coproporphyrins (986 nmol/L), and a patient in follow-up for tyrosinemia type I with dALA 4.6× the cut-off and a normal PBG (0.88× URL) and normal urinary porphyrins.

Porphyria diagnosis highly depends on biochemical investigation.[3] Porphyria as the cause of clinical symptoms can only be established if increased haem precursor concentrations are demonstrated. Genetic testing should not be used to rule out porphyria as it cannot identify mutations in all biochemically and clinically diagnosed cases. Genetic testing alone cannot be used to establish the diagnosis of autosomal dominant porphyrias (AIP, VP, and HCP) as these disorders are characterized by a low clinical penetrance. Finding a pathogenic variation does not confirm an active porphyria. However, familial screening by genetic testing can identify patients at risk of developing an acute porphyria. These patients can get appropriate counseling about precipitating factors in order to prevent the occurrence of an acute attack. This, however, is outside of the intended scope of our algorithms.

The strength of our study is that the algorithms were validated using patient data. All algorithms available in the literature are constructed based on a literature search and expert opinion, but there is no mention of validation of the algorithms with clinical patient samples.[2,16,17,34] We tried to estimate both sensitivity and specificity of our developed algorithms. They showed a sensitivity of 100%. However, it should be taken into account that the sensitivities for the different porphyria types were estimated based on very limited patient numbers (resulting in wide confidence intervals). The algorithms showed high specificity as well, with only one false positive result for the "acute porphyria" algorithm and three false positive results for the "cutaneous porphyria" algorithm. In these four cases, no final porphyria diagnosis was made by the clinician when clinical findings and familial history were taken into account.

There are a number of limitations in our study. First, in the porphyria centre in UZ Leuven, there are no patients who were diagnosed with ADP, CEP or HEP in the last 20 years. Two patients with HCP are in follow-up, but were not diagnosed in UZ Leuven, so no diagnostic test results were available to run through the algorithms. This means that the sensitivity of the algorithm for these four types of porphyria could not be validated using diagnostic samples. This is not unexpected as HCP, ADP, HEP, and CEP are the rarest forms of all the porphyrias. There were three cases of HCP in the EPNET external quality assessment scheme in the period 2010–2021. One case was excluded for further analysis as no feces sample was available. The algorithms correctly identified one patient with HCP presenting with acute and cutaneous symptoms (for both), but missed one patient in whom only porphyrins in feces were abnormal.

A second limitation of our study is that the patient database was constructed based on laboratory results of the past 20 years. This means that diagnostic samples were not available for all the patients, which led to smaller patient cohorts for algorithm validation. A third limitation is the fact we did not use reference values normalized for creatinine in urine since creatinine was not systematically measured during the last 20 years. It is recommended to report excretion per mmol creatinine in urine and preferentially use a morning urine sample to increase sensitivity.[2] There were to our knowledge no known missed cases during the study period, although missed cases might have remained undetected. A fourth limitation is the fact that we used the results of patients previously diagnosed with porphyria in our

center. In order to further validate the algorithms given the retrospective nature of our study, we also used the results of 20 cases of the EPNET external quality assessment scheme as an independent set of samples.

5 | CONCLUSION

In this study, we tried to optimize the biochemical laboratory diagnosis of the porphyrias by developing diagnostic algorithms for patients with acute attacks and patients presenting with cutaneous symptoms. Both algorithms showed high sensitivity and specificity and can be used to aid the physician in correctly interpreting the laboratory findings of porphyria related tests. However, in order to obtain optimal interpretation of the results, it is recommended that clinicians ensure that samples are sent to porphyria expert laboratories and to always provide clinical information with the samples.

AUTHOR CONTRIBUTIONS

Pieter Vermeersch devised the study, collected data, and drafted the manuscript. Stefanie Lefever collected data and drafted the manuscript. Nele Peersman collected data. Wouter Meersseman and David Cassiman critically revised the algorithms. All authors critically reviewed the data and the manuscript.

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CONFLICT OF INTEREST

David Cassiman has received speaker fees and consultancy fees from Alnylam. The other authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data of this retrospective study are not publicly available due to ethical restrictions. The raw data of individual participants contain information that could compromise the privacy of participants.

ETHICS STATEMENT

This study was approved by the local ethics committee (MP012472 and S54187) of the University Hospitals of Leuven (UZ Leuven, Belgium).

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REFERENCES

1. Puy H, Gouya L, Deybach JC. Porphyrias. *Lancet*. 2010;375:924-937.
2. Woolf J, Marsden JT, Degg T, et al. Best practice guidelines on first-line laboratory testing for porphyria. *Ann Clin Biochem*. 2017;54:188-198.
3. Whatley SD, Badminton MN. Role of genetic testing in the management of patients with inherited porphyria and their families. *Ann Clin Biochem*. 2013;50:204-216.
4. Karim Z, Lyoumi S, Nicolas G, Deybach JC, Gouya L, Puy H. Porphyrias: a 2015 update. *Clin Res Hepatol Gastroenterol*. 2015;39:412-425.
5. EPNET. The Porphyrias. 2021. Accessed December 20, 2021. https://porphyria.eu/content/porphyrias
6. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. *J Inherit Metab Dis*. 2013;36:849-857. doi:10.1007/s10545-012-9544-4
7. Elder G, Gouya L, Puy H, Badminton M, Deybach J. The molecular genetics of erythropoietic protoporphyria. *Cell Mol Biol (Noisy-Le-Grand)*. 2009;55:118-126.
8. Whitman JC, Paw BH, Chung J. The role of ClpX in erythropoietic protoporphyria. *Hematol Transf Cell Ther*. 2018;40:182-188. doi:10.1016/j.htct.2018.03.001
9. Balwani M, Naik H, Anderson KE, et al. Clinical, biochemical, and genetic characterization of north American patients with erythropoietic protoporphyria and x-linked protoporphyria. *JAMA Dermatol*. 2017;153:789-796.
10. Szlendak U, Bykowska K, Lipniacka A. Clinical, biochemical and molecular characteristics of the main types of porphyria. *Adv Clin Exp Med*. 2016;25:361-368.
11. Deacon AC, Elder GH. Front line tests for the investigation of suspected porphyria. *J Clin Pathol*. 2001;54:500-507. doi:10.1136/jcp.54.7.500
12. EPNET. Laboratory Diagnosis. n.d. Accessed December 20, 2021. https://porphyria.eu/content/laboratory-diagnosis
13. Lecha M, Puy H, Deybach JC. Erythropoietic protoporphyria. *Orphanet J Rare Dis*. 2009;4:19. doi:10.1186/1750-1172-4-19
14. Anderson KE. Acute hepatic porphyrias: current diagnosis and management. *Mol Genet Metab*. 2019;128:219-227.
15. The challenges of testing for and diagnosing porphyrias. *Commun Mayo Ref Serv Publ*. 2002;27:1-10.
16. Rigor J, Pinto SA, Martins-Mendes D. Porphyrias: a clinically based approach. *Eur J Intern Med*. 2019;67:24-29.
17. di Pierro E, de Canio M, Mercadante R, et al. Laboratory diagnosis of porphyria. *Diagnostics*. 2021;11:1-24. doi:10.3390/diagnostics11081343
18. Porphyria (Acute) Testing Algorithm. MayoClinic. 2019. Accessed June 15, 2021. https://www.mayocliniclabs.com/~/media/

it-mmfiles/special-instructions/Porphyria_Acute_Testing_Algorithm.pdf
19. Badminton MN, Whatley SD, Sardh E, Aarsand AK. Porphyrins and the porphyrias. In: Rifai N, Horvath AR, Wittwer CT, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 6th ed. Elsevier; 2018:776-799.
20. Linenberger M, Fertrin KY. Updates on the diagnosis and management of the most common hereditary porphyrias: AIP and EPP. *Hematol Am Soc Hematol Educ Progr*. 2020;2020:400-410. doi:10.1182/HEMATOLOGY.2020000124
21. Anderson KE, Lobo R, Salazar D, et al. Biochemical diagnosis of acute hepatic porphyria: updated expert recommendations for primary care physicians. *Am J Med Sci*. 2021;362:113-121.
22. Whatley SD, Roberts AG, Llewellyn DH, Bennett CP, Garrett C, Elder GH. Non-erythroid form of acute intermittent porphyria caused by promoter and frameshift mutations distant from the coding sequence of exon 1 of the HMBS gene. *Hum Genet*. 2000;107:243-248. doi:10.1007/s004390000356
23. Kühnel A, Gross U, Doss MO. Hereditary coproporphyria in Germany: clinical-biochemical studies in 53 patients. *Clin Biochem*. 2000;33:465-473.
24. Kühnel A, Groß U, Jacob K, Doss MO. Studies on coproporphyrin isomers in urine and feces in the porphyrias. *Clin Chim Acta*. 1999;282:45-58.
25. Luo J, Lim CK. Order of uroporphyrinogen III decarboxylation on incubation of porphobilinogen and uroporphyrinogen III with erythrocyte uroporphyrinogen decarboxylase. *Biochem J*. 1993;289:529-532.
26. Cooper CL, Stob CM, Jones MA, Lash TD. Metabolism of pentacarboxylate porphyrinogens by highly purified human coproporphyrinogen oxidase: further evidence for the existence of an abnormal pathway for heme biosynthesis. *Bioorg Med Chem*. 2005;13:6244-6251. doi:10.1016/j.bmc.2005.06.051
27. Porphyria (Cutaneous) Testing Algorithm. MayoClinic. 2018. Accessed June 15, 2021. https://www.mayocliniclabs.com/~/media/it-mmfiles/special-instructions/Porphyria_Cutaneous_Testing_Algorithm.pdf
28. Gou EW, Balwani M, Bissell DM, et al. Pitfalls in erythrocyte protoporphyrin measurement for diagnosis and monitoring of protoporphyrias. *Clin Chem*. 2015;61:1453-1456. doi:10.1373/clinchem.2015.245456
29. Bekri S, D'Hooghe M, Vermeersch P. X-Linked Sideroblastic Anemia and Ataxia. n.d. Accessed April 2, 2022. https://www.ncbi.nlm.nih.gov/books/NBK1321/
30. Stölzel U, Doss MO, Schuppan D. Clinical guide and update on porphyrias. *Gastroenterology*. 2019;157:365-381.e4.
31. Mayo Clinic. Aminolevulinic Acid, Urine. n.d. Accessed March 30, 2022. https://www.mayocliniclabs.com/test-catalog/overview/57350#Clinical-and-Interpretive
32. Pallet N, Karras A, Thervet E, Gouya L, Karim Z, Puy H. Porphyria and kidney diseases. *Clin Kidney J*. 2018;11:191-197. doi:10.1093/ckj/sfx146
33. Sood G, Anderson KE. Acute Intermittent Porphyria: Pathogenesis, Clinical Features, and Diagnosis. UpToDate. 2020. Accessed November 2, 2021. https://www.uptodate.com/contents/acute-intermittent-porphyria-pathogenesis-clinical-features-and-diagnosis
34. Schulenburg-Brand D, Katugampola R, Anstey AV, Badminton MN. The cutaneous porphyrias. *Dermatol Clin*. 2014;32:369-384.
35. Hift RJ, Davidson BP, van der Hooft C, Meissner DM, Meissner PN. Plasma fluorescence scanning and fecal porphyrin analysis for the diagnosis of variegate porphyria: precise determination of sensitivity and specificity with detection of protoporphyrinogen oxidase mutations as a reference standard. *Clin Chem*. 2004;50:915-923.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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