A guide to the diagnosis of porphyria: suggested methods and case examples

Christiaan Sies, MSc, MNZIMLS, Scientific Officer; Christopher Florkowski, MA, MD, MRCP (UK), FRACP, FRCPA, Chemical Pathologist
Clinical Biochemistry Unit, Canterbury Health Laboratories, Christchurch

Abstract
The objective of this review is to highlight the importance of the laboratory in the diagnosis of porphyria by providing the correct screening/diagnostic tests and offering interpretation of results.

Porphyria (por-fi're-ah) is due to a disturbance of porphyrin metabolism, characterised by marked increases in the formation and excretion of porphyrins or their precursors. Two main clinical manifestations may occur together or separately, potentially life-threatening attacks beginning with abdominal pain, and/or photosensitization of the skin. As the porphyrias may present in ways that mimic other disorders, and because different types share identical clinical features, accurate diagnosis requires the correct selection, performance and interpretation of laboratory tests.

An overview of porphyria is given, and several screening tests are discussed. Case examples are used to highlight the diversity and complexity of this group of diseases.

Key words: Acute intermittent porphyria (AIP), variegate porphyria (VP), hereditary coproporphyria (HCP), erythropoietic protoporphyria (EPP), porphyria cutanea tarda (PCT), congenital erythropoietic porphyria (CEP), porphobilinogen (PBG), Aminolevulinic acid (ALA).


Introduction
The porphyrias are uncommon disorders of haem biosynthesis and their effective management requires prompt and accurate diagnosis. Porphyria may present:

1. As an acute attack with abdominal pain and/or neurological symptoms.
2. With photosensitive skin lesions, skin fragility, erythema or bullae, leading to scarring and pigmentation.
3. As a combination of 1 and 2.
4. In the latent phase, e.g. as a relative of a known patient, or a patient presenting with a history of porphyria-like symptoms.

This article describes methods for the determination of urinary porphobilinogen, urine and faecal total porphyrins, and porphyrins in erythrocytes, as suitable tests for use in non-specialist laboratories. It also highlights the need for accurate screening tests that are followed up with definitive diagnostic tests. A similar article written by the same authors has recently been published in the New Zealand Medical Journal (1), and focuses on the clinical indicators for the investigation of porphyria rather than the laboratory methodology of this article.

Why is diagnosis the laboratory's responsibility?
The porphyrias form a heterogeneous group of inherited or acquired disorders of haem biosynthesis, and they are often missed or wrongly diagnosed, either as a result of being overlooked by the medical clinician or by incorrect or inappropriate laboratory testing. A partial deficiency of one of the seven enzymes in the haem biosynthetic pathway causes characteristic clinical and biochemical features. These disorders are due to specific alteration in the pattern of accumulation of porphyrin and porphyrin precursors (Figure 1). Each type of porphyria is defined by a unique pattern of accumulation and excretion of these haem precursors, as well as a reduction in the relevant enzyme activity and an associated genetic mutation (in all types except acquired PCT). Correct interpretation of the appropriate biochemical investigation is essential for accurately diagnosing and managing the porphyrias, as clinical features alone are not sufficiently specific either to confirm a diagnosis or to distinguish between the various forms.

Analogies can be drawn between this group of diseases and that of the current meningitis epidemic sweeping the country. Meningitis with its flu-like symptoms can only be accurately diagnosed by the use of biochemical and microbiological investigation of cerebral spinal fluid. Clinically its early symptoms are misleading and often assumed to be flu. Similarly the type of porphyria can only be defined by detailed biochemical investigations.

Raised total porphyrins in the presence of skin lesions don’t necessarily indicate a diagnosis of PCT, and it is the laboratory’s responsibility to make this known to the referring clinician.

The following case demonstrates how misleading porphyria can be:
A 25-year-old lady of Pacific Island descent was initially seen by her general practitioner and found to have blistering and fragile skin on sun exposed skin. She was referred to a dermatologist for further investigation. She had no other significant medical history, but gave a family history of PCT. A presumptive clinical diagnosis of PCT was made. Fortunately, a full biochemical investigation was then undertaken. Raised urinary PBG, high performance liquid chromatography (HPLC) analysis of urine and faeces and the presence of a specific plasma fluorescence peak indicated acute VP. Genetic analysis identified a previously unreported mutation. Mutation and biochemical analysis was offered to the extended family; some of who had been assigned a diagnosis of PCT (probably erroneously without biochemical porphyrin studies), though to date this offer has not been accepted. If a full biochemical investigation had not been undertaken this lady would have been labelled along with the rest of her family as PCT, a much different disorder, where the patient does not suffer from potentially life-threatening acute attacks.

What to test for:
Various testing scenarios have been published (2), but these usually rely on the examining clinician having a good knowledge of porphyria and how it may present. This unfortunately is not usually the case, as not only are the presenting conditions extremely variable, and most clinicians would be lucky to see one presenting case in their professional lives, due to their relative rarity. PCT the most common form of porphyria has an estimated incidence of 1 per 25,000 people; AIP, 1-2 per 100,000; HCP, EPP&VP, 1 per 250,000 (3). The authors therefore suggest the full profile of samples:

- Urine: Fresh random urine is preferred to 24-hour collections;
this is ideally collected during an acute episode if acute porphyria is suspected.

- Faeces: A random 10 g sample of faeces is required.
- Blood: Whole blood (EDTA or heparinised).

Specimens should be protected from light and received by the laboratory within 24 hours. The collection and processing of all three sample types eliminates the need for repeat visits to GPs and allows for a quicker concise diagnosis. If an acute porphyria is suspected, but a patient does not have current symptoms, sample request forms should be sent home with the patient, to be acted on when symptoms return. During latent porphyria some or all screening tests may be normal.

The following case demonstrates how easily porphyria may be overlooked:

A 52-year-old lady of South African descent was taken by her family to an accident and emergency department with severe and unexplained migraines. She had no history of migraine and was extremely distressed. After a full examination, no obvious cause could be found, though the severe pain abated after analgesia. She was admitted for observation overnight and discharged into the care of her general practitioner the next day, no diagnosis was made. Her GP, also South African, thought of VP as a possible cause. A full laboratory investigation was undertaken; this found raised levels of ALA, PBG, total urine and faecal porphyrins, and a diagnostic plasma fluorescence peak. Confirmatory mutation analysis detected the p. R59W mutation. Follow-up found that the patient’s mother and two sisters had some years earlier been diagnosed in South Africa with VP, and that her acute migraine episode occurred several days post dental surgery anaesthetic.

VP is relatively common in the South African white population, estimated at 3 per 1000. New Zealand has over the last years had a large influx of immigrants from South Africa: 14,727 new immigrants between 1996 and 2001 (Census 2001 statistics), a 130 % increase. Therefore, laboratories around the country should be seeing the associated diagnosis of as many as 40 new VP cases.

Peripheral lab tests:

Urine Porphobilinogen (PBG) screen.

PBG is raised in patients during or in the days following an attack of acute hepatic porphyria, such as AIP, VP or HCP. PBG levels may return to normal between attacks (latent phase) or remain slightly raised. A normal level of PBG in urine collected during an acute attack of abdominal pain (the most common clinical indicator of an acute hepatic porphyria, such as AIP, VP or HCP) may not be enough to confirm the diagnosis of porphyria. PBG levels may remain slightly raised for several days after the attack.

If PBG is present in abnormal levels (4).

Urine total porphyrins.

Urine can be a complex mixture of porphyrins and therefore an accurate measure of the total porphyrin is not possible without knowing the relative amounts of the various porphyrins. However, these problems do not affect the clinical usefulness of this assay, any abnormal results should be followed up with an investigation by HPLC.

Reference interval:

Urine total porphyrins: < 300 nmol/L

Urine porphyrin/creatinine: < 35 nmol/mmol creatinine (4).

Concentrations will be increased in patients with current symptoms of PCT, VP, HCP, AIP and CEP.

Faecal total porphyrins.

Faecal porphyrin concentrations are increased in hepatic porphyrinas: PCT, HC, VP, EPP, gastrointestinal bleeding and very high meat diets, but not in AIP.

Spectrophotometric method (6): A small sample of faeces is homogenised in concentrated hydrochloric acid and then extracted with ether to remove interfering coloured compounds. On addition of water, coloured compounds, such as carotenoid and chlorophyll derivatives, remain in the ether phase whereas porphyrins partition into the acid aqueous phase. The aqueous phase is then scanned on the spectrophotometer in the same manner as used for the urine samples. There is a direct correlation between the Soret peak height and the concentration of coproporphyrins and protoporphyrins. These can be expressed as concentration per dry weight of faecal material.

Reference interval:

Faecal total porphyrin: < 200 μmol/kg dry weight of faeces (4).

Biochemical laboratories around the country are rapidly becoming fully automated, as a result of which most laboratories would no longer be performing manual tests that would require spectrophotometry. The measurement of urine and faecal porphyrins should be used to justify the maintenance or replacement of a spectrophotometer along with other urgent tests, such as: CSF xanthochromia and urinary VMA.

Whole blood.

Red blood cell porphyrin levels may be raised in a number of types of porphyria (EPP, CEP, HEP and VP), but they may also be raised in a number of non-porphyria related conditions. In lead poisoning, ferrochelatase activity is inhibited and results in the formation of zinc protoporphyrin; in anaemia, resulting from iron deficiency, there is insufficient iron to create haem, thus zinc protoporphyrin is once again formed.

Front surface illumination method (Buchler Hematofluorometer): Light from a tungsten halogen lamp is directed through a 424 nm interference filter onto the bottom surface of a slide carrier, the slide carrier holds three slides, two of which are fixed within the instrument. These are the background check slide and an internal reference; the third slide holds oxygenated blood. As the operator moves the slide carrier through the instrument, each slide is exposed to the excitation light for 2 seconds. Re-emitted light from each of the slides is focused through another filter onto a photomultiplier tube. The photomultiplier measures the intensity of the background, reference and sample fluorescence, and from this the concentration of
Figure 1. The haem biosynthetic pathway shown in bold, the type of porphyria (or disease) shown in italics, the enzymes that catalyse the pathway shown on the right.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Intermediate</th>
<th>Enzyme</th>
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<tbody>
<tr>
<td>Aminolaevulinate dehydratase deficiency porphyria (ALAD)</td>
<td>Glycine + succinyl-CoA</td>
<td>δ-aminolaevulinic acid synthase</td>
</tr>
<tr>
<td>Acute intermittent porphyria (AIP)</td>
<td>δ-aminolaevulinic acid</td>
<td>Aminolaevulinate dehydratase</td>
</tr>
<tr>
<td>Congenital erythropoietic porphyria (CEP)</td>
<td>Porphobilinogen</td>
<td>Porphobilinogen deaminase</td>
</tr>
<tr>
<td>Porphyrina cutanea tarda (PCT)</td>
<td>Hydroxymethylbilane</td>
<td>Uroporphyrinogen III synthase</td>
</tr>
<tr>
<td>Hereditary coproporphyria (HCP)</td>
<td>Uroporphyrinogen III</td>
<td>Uroporphyrinogen decarboxylase</td>
</tr>
<tr>
<td>Variegate porphyria (VP)</td>
<td>Coproporphyrinogen III</td>
<td>Coproporphyrinogen oxidase</td>
</tr>
<tr>
<td>Erythropoietic protoporphyria (EPP)</td>
<td>Protoporphyriogen IX</td>
<td>Protoporphyriogen oxidase</td>
</tr>
<tr>
<td></td>
<td>Protoporphyrin IX</td>
<td>Ferrochelatase</td>
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Figure 2. Fluorescence emission scans (excitation at 405 nm) of diluted plasma from (a) a normal subject and patients with (b) porphyria cutanea tarda, (c) variegate porphyria, or (d) erythropoietic protoporphyria.
erythrocyte protoporphyrin or zinc protoporphyrin can be calculated. **Reference interval:**

- **Zinc protoporphyrin:** 6-40 μg/100 mL.
- **Erythrocyte protoporphyrin:** 6-36 μg/100 mL.

Alternatively, there are plasma and whole blood total porphyrin methods available using fluorometric methods, and spectrophotometric methods for total porphyrins on whole blood (4).

In the event that any of the above screening tests are raised or positive, all three samples should be referred to a reference laboratory that can perform quantitative analysis of urinary ALA and PBG, high performance liquid chromatography (HPLC), urine and faecal samples, plasma fluorescence scanning (see Figure 2), enzyme and or mutational analysis. Raised screening test results must not be considered diagnostic of porphyria or used to classify a certain type of porphyria.

The following case emphasizes the need for all three sample types:

- **The index case:** a 6-year-old male was referred to a paediatrician after what the GP described as “remarkable photosensitivity on minimal light exposure”. By the time the child was referred and seen by the paediatrician, urine and faecal screening tests were normal, but erythrocyte protoporphyrins were raised at 40 (g/100 ml (6-36). Follow-up investigation showed slightly abnormal HPLC patterns on urine and faeces, and a specific plasma fluorescence peak consistent with EPP.

This presentation of photosensitive burning pain on sun exposed hands and feet are typical of EPP, as are the normal urine and faecal screening tests. Often, raised blood erythrocyte protoporphyrins are the only marker of this disease.

**Quality assurance.**

It is good laboratory practise to participate in external quality assurance schemes. This is not only an ISO requirement, but also possibly the only time when a laboratory will have the opportunity to become familiar with positive specimens. RCPA-AACB provides a good scheme for total urine and faecal porphyrins and a semi-quantitative analysis of PBG. Internal quality assurance material is only routinely available for urinary porphyrins from organisations such as Bio-Rad or Chromsystems.

**The acute porphyrias.**

The three most common acute porphyrias, AIP, VP and HCP, are inherited as autosomal dominant disorders with clinical presentation after puberty. Many patients who inherit the enzyme abnormality remain asymptomatic during their lifetime. Abdominal pain is the commonest symptom of an acute attack. Nausea, vomiting, constipation, neuropathies and psychiatric symptoms may also accompany this. Hormonal changes (including menstrual cycle), drugs (lists of safe and unsafe drugs are available) and nutritional factors may aggravate the disorder (7). The skin symptoms of VP and HCP are: bullae, hyperpigmentation, and increased skin fragility. These abnormalities are due to accumulated free porphyrins in the skin, which absorb light and photodynamically damage cells.

**The rare acute porphyrias.**

The three rare porphyrias are congenital erythropoietic porphyria (CEP), hepato-erythropoietic porphyria (HEP) (the homozgyous dominant form of type II PCT), and aminolevulinc acid dehydratase (ALAD) deficiency. All may present in childhood, and for CEP and ALAD are autosomal recessive.

Approximately 1 % of acute attacks of porphyria may be fatal (5). Most patients experiencing an acute attack will require admission to hospital, where only drugs known to be safe in porphyrria should be prescribed. Oral or intravenous glucose and haem arginate is the mainstay of treatment. They inhibit synthesis of ALA, resulting in clinical and biochemical remission. Long-term, patients should be educated in the precipitating factors and should wear a Medic Alert bracelet. They should also be given an information booklet and/or encouraged to consult a support group web site (9,10).

**The non-acute porphyrias or cutaneous porphyrias.**

The two non-acute porphyrias seen in New Zealand are PCT, which probably accounts for 80% of all cases of porphyria, and EPP. PCT may be familial (autosomal dominant) or acquired. The disease may be precipitated by a number of factors including: excessive alcohol consumption, oral contraceptives, Hepatitis C, and haemochromatosis. The skin symptoms seen in PCT are indistinguishable from those of VP and HCP. Clinical symptoms of EPP however, include a sense of burning, oedema and itching, and are comparable to a severe case of sunburn. EPP is also autosomal dominant and usually presents in early childhood.

The cutaneous porphyrias are treated by the avoidance of sunlight and barrier protection. Additional options are venesecion to deplete excess iron stores and oral chloroquine to increase urinary porphyrin excretion. For PCT, the avoidance of alcohol and oestrogens is also suggested. For EPP, red cell porphyrins and liver function tests should be checked every 6 months, as about 10 % of patients may develop liver disease and about 4 % die of rapid and fatal liver failure (11).

**Family studies.**

The importance of investigating relatives of patients with AIP, VP and HCP can hardly be over-emphasised. Every effort should be made to identify gene carriers before they develop symptoms, so that they can be warned to avoid known precipitants of acute porphyria. The first priority is the accurate diagnosis of the index case. This may involve enzyme tests and or genetic mutation analysis in addition to the full profile of tests discussed earlier. With this knowledge, the rest of the family can be investigated, beginning with the siblings and parents. The family is then followed from the oldest generation down. If a person is shown not to have inherited the enzyme defect, then their descendents need not be tested. These family members can be confident that they do not have porphyria. For young children (pre-puberty), gene analysis is the only reliable method of confirming or excluding porphyria, as even the enzyme assays are unreliable for this age group. Furthermore, the other porphyria screening tests are unreliable in this age group and should not be used. If porphyria is suspected, a full family study should be undertaken. Testing the urine of a 2-month old baby because great grandmother “had porphyria” can neither support nor disprove a diagnosis of porphyria.

The following case highlights a scenario where family studies should be undertaken.

A 39-year-old female was biochemically diagnosed as having AIP. On investigation her mother had previously (1970’s) been diagnosed as VP, presumably incorrectly. However, she has managed to avoid any acute episodes and is still in good health at age 60. The index case’s maternal grandfather and great aunt both died relatively young at ages 42 and 36 respectively, quite possibly from the complications of porphyria. There are 8 other family members who would benefit from a full family study, including the index case’s young child.

**Conclusions.**

A clear concise diagnosis of an index case presenting with porphyria is essential. This then allows for follow-up family studies that can identify at risk family members. Those that are excluded of carrier status avoid the need for screening of future generations of that particular family branch. The input of a reference laboratory with experience of all the detailed procedures is invaluable.
References


Correspondence: C. Sies, Clinical Biochemistry Unit, Canterbury Health Laboratories, PO Box 151, Christchurch. Email: Chris.Sies@cdhb.govt.nz