Diplomarbeit

Acute Hepatic Porphyrias: Case reports at the Medical University Graz

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Abbreviations

5ALA	Delta-aminolevulinic acid
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALAD-Porphyria	Aminolevulinic acid dehydratase deficiency porphyria
ALAD	Delta-aminolevulinic acid dehydratase
ALAS	Delta-aminolevulinic acid synthase
ALAS1	Delta-aminolevulinic acid synthase 1
ALAS2	Delta-aminolevulinic acid synthase 2
cDNA	Complementary DNA
CEP	Congenital erythropoietic porphyria
CPOX	Coproporphyrinogen III oxidase
CYPs	Cytochromes P450
EPP	Erythropoietic protoporphyria
FECH	Ferrochelatase
GnRH	Gonadotropin releasing-hormone
HCC	Hepatocellular cancer
HCP	Hereditary coproporphyria
HMB	Hydroxymethylbilane
HMBS	Hydroxymethylbilane synthase
OLT	Orthotopic liver transplantation
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PCT	Porphyria cutanea tarda
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator-1alpha
PPIX	Protoporphyrin IX
PPOX	Protoporphyrinogen oxidase
RNAi	RNA interference
siRNA	Small interfering RNA
UROD	Uroporphyrinogen decarboxylase
UROIIIS	Uroporphyrinogen III synthase
VP	Variegate porphyria

XLDPP	X-linked dominant protoporphyria

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Zusammenfassung

Hintergrund

Porphyrien sind hauptsächlich genetisch vererbte Erkrankungen der, an der Hämbiosynthese beteiligten Enzyme. Die Untergruppe der Akuten hepatischen Porphyrien (AHPs) umfasst dabei die akut intermittierende Porphyrie (AIP), hereditäre Koproporphyrie (HCP), Porphyria variegata (VP) und ALA-Defizienz-Porphyrie (ALAD-Porphyria). Diese Erkrankungen sind durch akute Attacken mit teilweise sehr unspezifischen Symptomen wie Bauchschmerzen gekennzeichnet. Viele Patient*innen leiden auch zwischen den akuten Attacken an den Auswirkungen der Porphyrie. Trotzdem gibt es einige Patient*innen, die mitunter jahrelang an starken Beschwerden leiden, ohne eine Diagnose zu erhalten und viele teilweise invasive diagnostische Verfahren durchleben müssen.

Die Zahl der an der Medizinischen Universität Graz als tertiäres Zentrum behandelten Patient*innen mit AHP und deren Krankheitsverlauf sind noch nicht bekannt.

Zielsetzung

Ziel dieser Arbeit ist es, (i) die Anzahl der Patient*innen mit AHP zu ermitteln, die am Tertiärklinikum der Medizinischen Universität Graz diagnostiziert oder dorthin überwiesen wurden, (ii) ihren individuellen Krankheitsverlauf zu beschreiben und (iii) ihn mit dem in der Literatur beschriebenen Krankheitsverlauf zu vergleichen. Darüber hinaus soll untersucht werden, ob die Patient*innen für neue Therapieformen in Frage kämen und ob sie davon profitieren würden.

Methoden

Bei dieser Diplomarbeit handelt es sich um eine retrospektive Datenanalyse über Patient*innen, die seit 2004 an der Medizinischen Universität Graz mit einer Form von AHP diagnostiziert wurden, oder aufgrund einer AHP behandelt wurden.

Die Daten wurden mittels einer Abfrage des Medizinischen Instituts für Informatik der MEDOCS Datenbank durchgeführt. Als Suchparameter wurden die Begriffe AHP, AIP, VP, HCP und ALAD-Mangel Porphyrie verwendet, sowie akute hepatische Porphyrie, akut intermittierende Porphyrie, Porphyria variegata, hereditäre Koproporphyrie, und die ICD-10 Codes E80.1 und E80.2 in verschiedensten Kombinationen. Zusätzlich wurde eine ähnliche

Abfrage nach Patient*innen mit erhöhten Delta-Aminolävulinsäure (5ALA) und Porphobilinogen (PBG) Werten im 24h Urin durchgeführt.
Die Literaturrecherche wurde mittels PubMed, Google Scholar und Lehrbüchern der Inneren Medizin und Biochemie durchgeführt.

Ergebnisse

Insgesamt wurden fünf Patient*innen mit gesicherter AHP-Diagnose in die Datenanalyse eingeschlossen. Die Zeit vom ersten Auftreten der Symptome bis zur Erstdiagnose betrug bei vier der fünf Patient*innen mehr als ein Jahr. Drei Patient*innen wurden wegen ihrer Bauchschmerzen invasiv behandelt oder untersucht, ohne eine Diagnose zu erhalten. Vier dieser fünf Patient*innen zeigten zwischen den akuten Attacken Symptome, die mit AHPs in Verbindung gebracht werden können. Die Patient*innen verbrachten durchschnittlich 6,7 Tage im Krankenhaus zur Behandlung der akuten Anfälle.
Alle Patient*innen erhielten Hämin als Therapie für akute Attacken und zwei als prophylaktische Therapie. Nebenwirkungen der Hämin Therapie traten bei zwei von fünf Patient*innen auf. Eine Patientin starb an den Folgen der AIP.

Conclusio

Diese retrospektive Datenanalyse identifizierte seit 2004 fünf Patient*innen mit der Diagnose AHP am Tertiärklinikum der Medizinischen Universität Graz. Entgegen der Annahme, dass es sich bei AHP um eine Erkrankung mit rezidivierenden Attacken und beschwerdefreien Intervallen handelt, hatten vier von fünf Patient*innen einen chronischen Krankheitsverlauf mit Symptomen zwischen den Attacken. Dies steht im Einklang mit einem kürzlich veröffentlichten Bericht über eine große internationale Kohorte von AHP-Patient*innen. Eine prophylaktische Therapie mit Hämin lindert nicht alle Symptome und eine bessere Krankheitskontrolle mit neuartigen Therapien wie Small interfering RNA (siRNA) ist erwünscht.

Abstract

Background

Porphyrias are genetically or – less often - acquired diseases of enzymes involved in heme biosynthesis. The subgroup of acute hepatic porphyrias (AHP) includes acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) and aminolevulinic acid dehydratase deficiency porphyria (ALAD-Porphyria) and is characterized by acute attacks of overall unspecific symptoms dominated by abdominal pain. Many patients also experience symptoms between acute attacks. Due to the unspecific nature of symptoms and the rare occurrence of these diseases, the proper diagnosis of AHP is often missed and delayed. Thus, some patients remain symptomatic for years without receiving the correct diagnosis and therapy is withheld or – even worse – unnecessary diagnostic procedures are performed.

The number of patients treated at the tertiary hospital centre of the Medical University of Graz who suffer from AHPs and their disease course are not yet known.

Objective

The aim of this work is (i) to determine the number of patients with AHP, who have been diagnosed or referred to the tertiary hospital centre at the Medical University Graz, (ii) to describe their individual disease course and (iii) to compare it with the disease course reported in literature. In addition, the work aims to find out whether the patients would be eligible for new forms of therapy and whether they would benefit from them

Methods

This diploma thesis is a retrospective data analysis of patients who have been diagnosed or treated for AHP at the Medical University Graz since 2004. Data has been retrieved with the help of the Medical Institute for Informatics, who searched the clinical record system MEDOCS for the terms AHP, AIP, VP, HCP and ALAD-Porphyria, acute hepatic porphyria, acute intermittent porphyria, variegate porphyria, hereditary coproporphyria as well as for the ICD-10 codes E80.1 and E80.2 in various combinations. In addition, a similar query was carried out for patients with elevated delta-aminolevulinic acid (5ALA) and porphobilinogen (PBG) values in the 24-hour urine. The literature search was carried out in PubMed, Google Scholar and textbooks of internal medicines and biochemistry.

Results

A total of five patients with a definite diagnosis of AHP were included in the data analysis. The time from first onset of symptoms to initial diagnosis was more than a year in four of the five patients. Three patients underwent surgery for their abdominal pain without identifying a disease that would explain the symptoms. Four of these five patients showed symptoms in between acute attacks which can be linked to AHPs. The patients spent an average of 6.7 days in the hospital for treatment of acute attacks. All patients received hemin for acute attacks and two as prophylactic therapy. Side-effects of hemin therapy occurred in two out of five patients. One patient died from complications of AIP.

Conclusion

This retrospective data analysis identified five patients with the diagnosis of AHP at the tertiary hospital centre Medical University Graz since 2004. In contrast to the assumption that AHP is a disease with recurrent attacks and symptom-free intervals, four out of five patients had a chronic disease course with symptoms in between attacks. This is in line with a recent report on a large international cohort of AHP patients. Prophylactic therapy with hemin does not cease all of their symptoms and a better disease control with novel therapies, such as Small interfering RNA (siRNA), is desired.

1. Introduction

1.1 Historical background

A popular, but not confirmed, belief says that at the time of Hippocrates (approx. 400 BC) the first case of a porphyria has been documented. Porphyria is the Greek term for "purple pigment", which describes the colour change of body fluids from individuals with porphyria. In addition, the myth of vampires and werewolves could have its origin in the group of porphyria diseases. Individuals with porphyrias suffer from hypertrichosis, had reddish teeth due to the accumulation of heme precursors and avoid light, because it causes skin mutilation, which may have led people to come up with the legend of werewolves and vampires. There are also historically known persons who are thought to have suffered from porphyrias, such as James I and IV, George III, Frederick the Great and Kaiser Wilhem (1). Even Vincent van Gogh is thought to have had porphyria, explaining his episodic abdominal pain, seizures and hallucinations, which could also have had an influence on his works (2).

1.2 Heme

Porphyrins are essential parts of larger molecules required to execute their biological processes. The best-known example of a porphyrin containing macromolecule is heme. Heme is a porphyrin ring containing iron (Figure 1), with multiple biological functions. Its most prominent function is binding of oxygen in red blood cells. In erythrocytes the heme ring is integrated into globin chains to build hemoglobin. Beside hemoglobin, heme is sufficiently found in myoglobin and many enzymes such as cytochromes of the electron transport chain, catalase, and nitric oxide synthase (3).

There are different types of heme, which can be distinguished by their substituents on the porphyrin macrocycle (4). Heme b, from which other hemes are derived, represents the most common type and it is mainly found as hemoglobin in red blood cells (3).

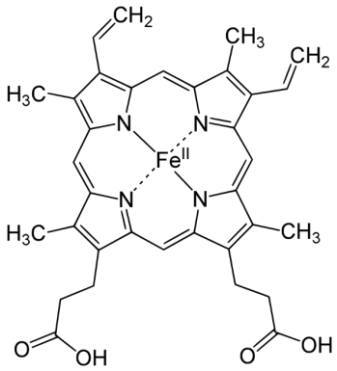

Figure 1: Structural formula of heme b (graphic license-free from Wikipedia: https://de.wikipedia.org/wiki/H%C3%A4me_(Stoffgruppe)).

1.2.1 Metabolism of heme

Most of the biosynthesis of heme occurs in the cytosol and mitochondria of erythrocytes in the bone marrow and hepatocytes in the liver (3). The initial reaction of the biosynthetic pathway of heme starts in the mitochondria and is catalysed by the delta-aminolevulinic acid synthase (ALAS), which is the rate-limiting enzyme (5, 6). There are two genes coding ALAS, which differ in their regulation, depending on the tissue in which the synthesis takes place. ALAS1 on chromosome 3, is ubiquitously expressed and ALAS2, located on chromosome X, is specific for erythropoietic cells. ALAS1 is responsible for the production of heme in the liver and is inhibited by the negative feedback of heme, the end product of the biosynthesis. ALAS2 in erythroid cells on the other hand is inhibited by the availability of iron and not by the presence of heme (7).

In the first step, succinyl CoA and glycine are catalysed to delta aminolevulinic acid (5ALA) via ALAS. The resulting 5ALA is secreted from the mitochondria into the cytosol, where delta-aminolevulinic acid dehydratase (ALAD) catalyses the condensation of two 5ALA molecules to form porphobilinogen (PBG). PBG already contains the pyrrole ring which is characteristic for the porphyria molecule. In the next step, four molecules of PBG are converted into hydroxymethylbilane (HMB) via hydroxymethylbilane synthase (HMBS), also called porphobilinogen deaminase (PBGD). Subsequently the enzyme uroporphyrinogen III synthase (UROIIIS) converts HMB to uroporphyrinogen-III, which is then processed in a further step by uroporphyrinogen decarboxylase (UROD) to coproporphyrinogen III. Coproporphyrinogen III is then taken back into the mitochondria and processed into protoporphyrinogen IX by coproporphyrinogen III oxidase (CPOX). Next, the protoporphyrinogen oxidase (PPOX) converts protoporphyrinogen IX to protoporphyrin IX (PPIX), which then is converted in a last step to heme by adding an iron

molecule via the enzyme ferrochelatase (FECH) (Figure 2 shows the principal enzymatic steps and its metabolites) (5, 6).

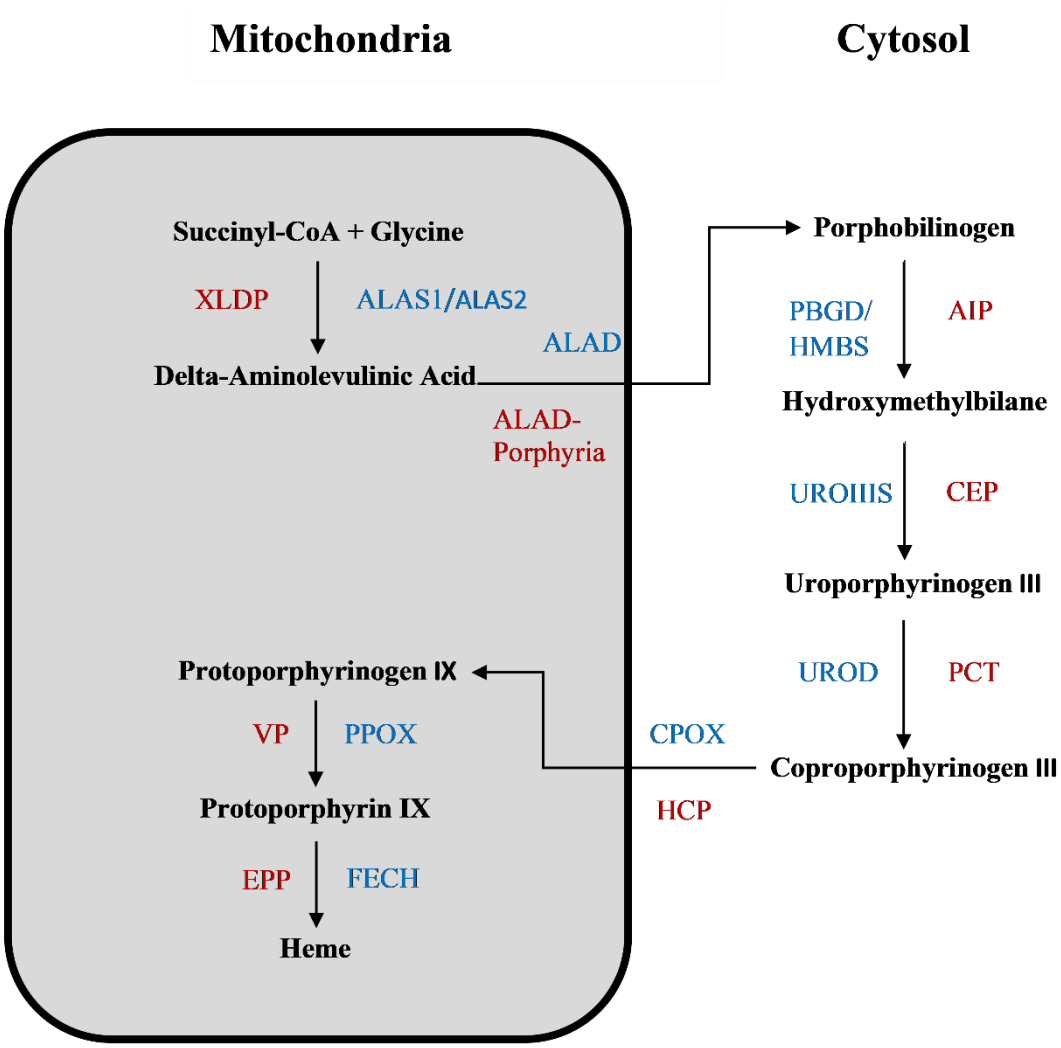

Figure 2: Biosynthetic pathway of heme. Adapted from (7). Red: Porphyrias related to the enzyme defect; XLDP=X-linked dominant protoporphyria. ALAD-Porphyria=Aminolaevulinic acid dehydratase deficiency porphyria. AIP=Acute intermittent porphyria. CEP=Congenital erythropoietic porphyria. PCT=Porphyria cutanea tarda. HCP=Hereditary coproporphyria. VP=Variegate porphyria. EPP=Erythropoietic protoporphyria. Blue: Enzymes of the biosynthetic pathway of heme; ALAS1=Delta-aminolevulinic acid synthase 1. ALAS2=Delta-aminolevulinic acid synthase 2. ALAD=Delta-aminolevulinic acid dehydratase. PBGD=Porphobilinogen deaminase=HMBS=Hydroxymethylbilane synthase. UROIIIS=Uroporphyrinogen III synthase. UROD=Uroporphyrinogen decarboxylase. CPOX=Coproporphyrinogen II oxidase. PPOX=Protoporphyrinogen oxidase. FECH=Ferrochelatase

Since heme can cause cellular damage in higher concentrations, its synthesis, which is highly variable, must be tightly regulated (3). In the liver heme synthesis is regulated by a negative feedback of the intracellular heme pool on the ALAS1 gene (7). If the concentration of heme is low the synthesis of ALAS1 is stimulated. On the other hand, if the intracellular heme and hemin (used for treatment of acute hepatic porphyrias (8)) concentration is high because they are not incorporated into proteins and start to accumulate, the heme synthesis stops (3).

In addition, ALAS1 activity can be induced by certain drugs (e.g. barbiturates) and by factors that stimulate the synthesis of hepatic cytochromes P450 (CYPs) such as alcohol or smoking, which then increases the demand for heme. Heme formed in the liver is mainly used for CYPs, which are involved in the metabolism of various chemicals such as drugs, vitamins or prostaglandins, to name just a few (9). In general, stimulation of CYP450 enzymes triggers the need for newly synthesized heme.

Moreover, inflammatory processes, fasting or conditions which lead to oxidative stress, induce ALAS1 and thus heme synthesis. During these stress conditions the enzyme heme oxygenase-1 is overexpressed, which increases heme degradation, lowers the concentration of the heme pool and therefore removes the negative feedback inhibition of heme on ALAS1. On a molecular level, heme does not directly inhibit ALAS1 function or its transcription, but it decreases its protein expression by decreasing the stability of ALAS1 messenger RNA. In addition, heme favours cytosolic breakdown of the mature enzyme and prevents mitochondrial shuttling of the precursor molecule. Glucose, which has been used for treatment of an acute porphyric attack, prevents the transcription of ALAS1, and therefore the overproduction of porphyrin precursors can be prevented by glucose (10).

ALAS2 is erythroid specific and regulated during erythroid differentiation. ALAS2 activity is increased by the hormone erythropoietin, which is released by the kidney at low oxygen levels and limited by iron availability. It is in contrast to ALAS1 not inhibited by intracellular heme and hemin concentrations (3).

1.3 Porphyrias

1.3.1 Definition

Porphyrias are most often genetic diseases of enzymes in the heme biosynthesis pathway. However, there also exist secondary porphyrias, where enzyme function is not genetically impaired, but enzyme function is affected by secondary causes such as lead poisoning, hepatic or haematological diseases. Eight different enzymes are involved in this biosynthetic pathway of heme and each of these enzymes can be limited in its function due to a mutation (Figure 2). Depending on the main site of enzyme expression, porphyrias can be divided into erythropoietic and hepatic porphyrias (Figure 3). Depending on the clinical manifestation they can be divided into acute and chronic forms (Figure 3) (11). Porphyrias that show neurological symptoms are called acute, because they typically manifest in an episodic

fashion, whereas porphyrias with cutaneous manifestation are called chronic, because their main clinical presentation, an increased photosensitivity, persists (12).

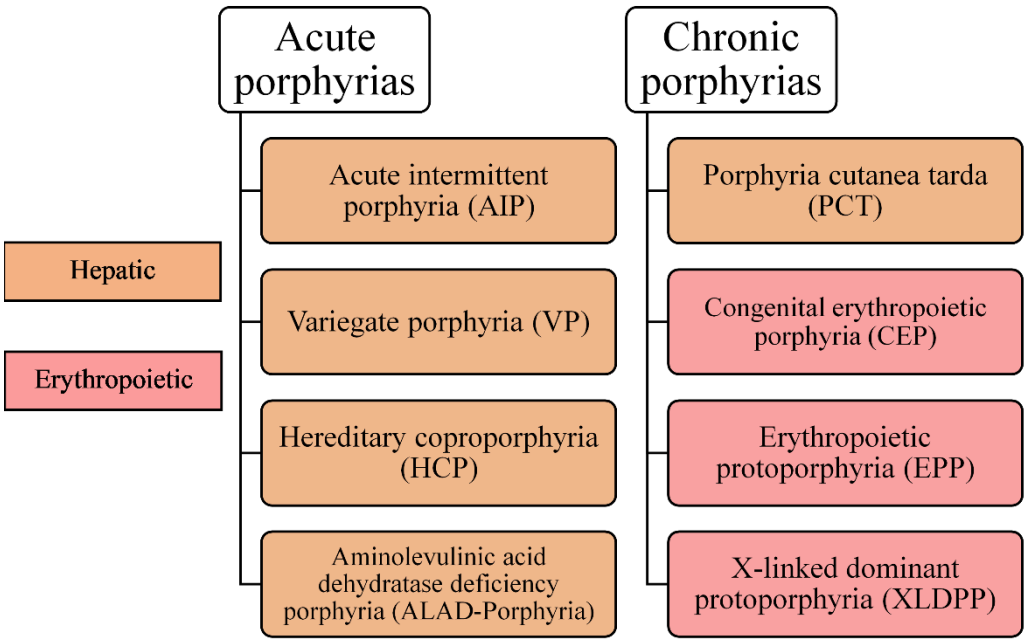

Figure 3: Classification of porphyrias in acute, chronic, hepatic and erythropoietic. Adapted from (13).

1.3.2 Pathophysiologic mechanisms of acute hepatic porphyrias

Heme can be synthesized in the mitochondria and cytosol for respiratory and redox processes ubiquitously in every human cell, but most of it is produced in erythropoietic cells for the synthesis of hemoglobin and in liver cells for the synthesis of cytochromes and hemoproteins (7). When the function of one of these enzymes is severely restricted (genetically or functionally), precursor molecules accumulate and enter the circulation. The high systemic concertation of the precursor metabolites may then cause clinical symptoms. Normally, only very small quantities are generated and can be sufficiently excreted in the urine via the kidneys, or the bile via the liver (14, 15).

There are four types of acute hepatic porphyrias (AHPs), which are caused by genetic reduction in the enzymatic function: acute intermittent porphyria (AIP), variegate porphyria (VP), and hereditary coproporphyria (HCP), which are all inherited in an autosomal dominant manner, and the delta-aminolevulinic acid dehydratase deficiency porphyria (ALAD-Porphyria), which is inherited in an autosomal recessive manner (16). Depending on the mutated enzyme, metabolites of the previous enzymatic steps accumulate (Table 1). The accumulated metabolites can be measured in urine, stool, erythrocytes or plasma of

patients allowing to conclude which enzyme is impaired and eventually make a diagnosis (7).

PORPHYRIA	URINE	STOOL	PLASMA	ERYTHROCYTES
AIP	PBG, 5ALA, Uroporphyrin	Coproporphyrin I	PBG, 5ALA, Uroporphyrin I	PBGD↓
VP	PBG, 5ALA, Copropoprhyrin III	Protoporphyrin, Coproporphyrin III	Porphyrinpeptide conjugate	-
HCP	PBG, 5ALA, Coproporphyrin III	Coproporphyrin III	Coproporphyrin	-
ALAD-PORPHYRIA	5ALA, Coproporphyrin	-	5ALA	Zinc Protoporphyrin

Table 1: Accumulated molecules in acute hepatic porphyrias. Adapted from (17). AIP=Acute intermittent porphyria. VP=Variegate porphyria. HCP=Hereditary coproporphyria. ALAD-Porphyria=Aminolaevulinic acid dehydratase deficiency porphyria. PBG=Porphobilinogen. 5ALA=Aminolaevulinic acid. PBGD=porphobilinogen desaminase

In AHPs porphyrin precursors like 5ALA and PBG accumulate in the liver. These metabolites can be systemically spilled over and damage neuronal structures which may be the reason for pain, neurological and psychiatric symptoms in such patients. In photocutaneous porphyrias the precursors accumulate either in liver cells or in cells of the bone marrow, depending on the subtype of porphyria (15). These porphyrins enter the bloodstream and eventually accumulate in the skin, where they get activated by sunlight. Porphyrins absorb light energy at a wavelength of about 400nm because of their electronic configuration and are thereby promoted to a singlet-excited state. While returning to their stable states they emit energy in form of light and energy for chemical reactions. This activation leads to an immune mediated reaction, releases free radicals and damages the lower dermis and basement membranes (18, 19).

Although all heme precursor molecules share a similar chemical structure the symptoms differ depending on their location, deposit, concentration, and solubility in cells and cell organelles. Therefore, depending on which enzyme is defective, porphyrias partially show different symptoms (14).

1.3.3 Clinical presentation of acute hepatic porphyrias

The penetrance of autosomal dominant inherited AHPs (AIP, VP and HCP) is low (20). About 80% of individuals with a mutation for AIP, VP or HCP never experience any acute attack and do not show any symptoms throughout their entire life (21).

Symptomatic patients can experience acute attacks showing many different non-specific symptoms that can even lead to a life-threatening crisis. Porphyric attacks may present with sudden and severe symptoms, such as abdominal pain, nausea and vomiting, tachycardia, excess sweating, constipation, hypertension, and behavioural changes such as anxiety, restlessness, and insomnia. Other signs of mental disturbance like depression, disorientation, hallucinations, paranoia, or confusion present in 20-30% of patients. However, these patients usually do not show any abnormalities in physical examination and x-ray analysis (7).
Dehydration and electrolyte imbalances like hyponatraemia due to inappropriate antidiuretic hormone secretion in 40% of cases and hypomagnesaemia can lead to convulsions and seizures. Those attacks can cause the death of a patient because of neurological complications, especially when medication, that is known to cause acute attacks of porphyria, is taken during an attack. Neurological complications include muscle pain in arms and legs, weakness beginning in proximal muscles, and even paresis, which can progress and cause tetraplegia and death, because of bulbar and respiratory paralysis (7).
Clinically, symptoms during acute attacks are comparable in severity among AHPs and cannot be distinguished from another (22). However, individuals with VP and HCP may additionally suffer from cutaneous manifestations. These manifestations present with subepidermal blisters, bullae, erosions and other symptoms such as scars and thickening due to chronic damage on sun-exposed skin, like hands and face (23).
Taken together, the leading symptomatic trias of patients with AHPs consist of abdominal pain, psychosis, and neuropathy. In a clinical setting however, AHPs are often overlooked because they represent rare diseases and very often patients only show mild symptoms (2).

The occurrence of acute attacks peaks in the third decade. Before puberty and after menopause the likelihood of an acute attack is low (7, 16). Women are more at risk of developing an acute attack than men. Most patients only experience one or a few attacks in their lives and can recover fully (7).
As mentioned above, the majority of individuals with a genetic defect remain asymptomatic and most of symptomatic patients only have one or a few attacks in their lifetime (21). 3-8% of patients suffer from recurrent attacks and some patients may also experience symptoms between acute attacks, which have a negative effect on their physical and emotional health, ultimately lowering their quality of life. Long-term side effects of AHPs include liver cirrhosis, hepatocellular carcinoma, chronic kidney disease, and systemic atrial hypertension (16).

Chronic porphyrias do not show an immediate onset of symptoms and skin affections are the predominating symptoms observed in these patients (17). (Table 2 summarizes the main symptoms in patients with acute and chronic porphyrias.)

Acute porphyrias	Chronic porphyrias
Abdominal pain	Cutaneous photosensitivity
Nausea	Skin fragility
Vomiting	Skin erosions
Constipation	Bullae
Diarrhea	Blisters
Pain in extremities	Vesicles
Headache	Milia
Chestpain	Atrophy
Backpain	Hypertrichosis
Paresis	Hyperpigmentation
Respiratory paralysis	Ocular pain
Behavioral changes	Photophobia
Confusion	
Hallucinations	
Depression	
Hyponatremia	
Convulsions	
Tachycardia	
Arterial hypertension	
Dark or reddish urine	
Cutaneous blistering (HCP, VP)	
Milia (HCP, VP)	

Table 2: Common symptoms of acute and chronic porphyrias. Adapted from (21, 8, 7). HCP=Hereditary coproporphyria. VP=Variegate porphyria.

1.3.4 Acute hepatic porphyrias

1.3.4.1 Acute intermittent porphyria (AIP)

AIP is an autosomal dominant disorder (15). It is the most common type of AHPs with disease-related mutations of about 1:1,700. These mutations show a penetrance of approximately 20% in families with AIP and only 1% in the general population (16).

In AIP hydroxymethylbilane synthase (HMBS), also called porphobilinogen deaminase (PBGD), the third enzyme in the biosynthetic pathway of heme, is defective and when it

comes to a hepatic stimulation of ALAS the precursor molecules (PBG, 5ALA) are synthesized at a higher rate and start to accumulate (24). Under normal conditions the activity of ALAS is low and the deficiency of HMBS is not sufficient to cause an accumulation of precursor molecules, therefore the disease remains latent. However, under certain conditions , the activity of ALAS can rise up to 100 times of its normal activity and subsequently overload HMBS, which shows a residual activity of about 50% compared to normal individuals (25).

The increase of ALAS activity can be caused by endogenous factors such as sex hormone fluctuations during the menstrual cycle or pregnancy, or exogenous factors like certain medications, alcohol, infections, and fasting (22) (Table 3 lists factors which can increase ALAS activity and therefore can lead to episodes of acute attacks). Those aforementioned factors can lead to an induction of cytochrome p450 which requires about 2/3 of the heme formed in the liver for its synthesis, which then leads to a decrease of the hepatic heme-pool. The reduction of the heme-pool increases the activity of ALAS (11).

A patient diagnosed with AIP, who received an allogenic liver transplantation has been cured from neurologic symptoms and showed normal urinary 5ALA and PBG levels within 24 hours, which suggests that the liver is the source of excess precursor molecules (21).

Results from experimental and clinical data show that 5ALA leads to acute attacks caused by direct neurotoxic effects. These neurotoxic effects are likely caused by modulations of the GABA-ergic system due to similar molecular structure to GABA and possibly the formation of free radicals and reactive oxygen species (22, 26). It is not exactly known, if PBG one the other hand is neurotoxic. There are also other hypotheses on the pathogenesis of symptoms in porphyrias. One of the hypotheses is that the deficiency of heme leads to a decrease of hemoproteins like cytochromes, which could have direct or indirect effects on the nervous system and therefore cause the typical symptoms (26).

Factors inducing acute attacks in AHPs	
Barbiturates	Clonazepam
Estrogens	Reductions in caloric or carbohydrate intake
Progestagens	Excess of alcohol
Sulfonamides	Smoking
Hydantoins	Exhaustion – emotional or physical
Sulfonamides	Metabolic stress (infections, surgery)
Pheytoin	Psychological stress
Ergots	Endogenous hormones
Diclofenac and possibly other NSAIDs	

Table 3: Factors inducing acute attacks in AHPs. Adapted from (21).

1.3.4.2 Variegate porphyria (VP)

In VP the enzyme protoporphyrinogen oxidase (PPOX), which is the seventh enzyme of the biosynthetic pathway, is defective due to a pathogenic heterozygous loss of function variant. This results in a reduction of its activity to about 50% of normal. PPOX catalyses the oxidation of protoporphyrinogen to protoporphyrin. VP is inherited in an autosomal dominant manner, with rare de novo variants and a low penetrance. Most individuals with a heterozygous variant of VP do not show any symptoms and are often not diagnosed. In Europe, the prevalence is estimated to be about half of that for AIP with about 3,2 individuals per 1,000,000 (23).

It is both an acute porphyria with attacks of neurological symptoms and, in contrast to AIP, a cutaneous porphyria with chronic blistering skin lesions such as subepidermal vesicles, bullae, and erosions especially in parts of the skin exposed to the sun like hands and face. Other chronic skin-related symptoms include facial hyperpigmentation, hypertrichosis, milia, scarring, thickening, and areas of decreased and increased skin pigmentation (23). In winter chronic cutaneous manifestations may improve and are less prevalent in people living in northern regions and in dark-skinned individuals in general.

Symptoms can occur at any age but appear rarely before puberty and in elderhood. They are most frequent in adulthood and women are more likely to develop symptoms than men (23). Chronic blistering in sun-exposed parts of the skin represents the most common manifestation of VP. These skin lesions are similar to skin lesions described in porphyria cutanea tarda (PCT), HCP, congenital erythropoietic porphyria (CEP), and hepatoerythropoietic porphyria (23).

Acute attacks share similar symptoms with AIP and other acute porphyrias such as abdominal pain, constipation, seizures, motor neuropathy, muscle weakness, and behavioural changes like anxiety (23).

1.3.4.3 Hereditary coproporphyria (HCP)

HCP is a rare AHP and inherited in an autosomal dominant manner manifesting in defects in the enzyme coproporphyrinogen III oxidase (CPOX), the sixth enzyme of the heme biosynthetic pathway, which converts coproporphyrinogen III to protoporphyrinogen IX (27).

Most people suffering from HCP have an affected parent. However, the penetrance of the CPOX genetic variant is also low and therefore individuals with a CPOX mutation do not necessarily have to show symptoms (12).

HCP is less common than AIP and VP. Patients with HCP show about 50% residual activity of CPOX. An increase from 10 to 200 times of the normal fecal excretion of coproporphyrinogen can be seen in individuals with HCP, as well as an increased urinary excretion of PBG, 5ALA and coproporphyrin III during an acute attack. Under UV light feces emit a typical red fluorescence, which represents an easily performed diagnostic test for acute attacks in HCP (27).

The accumulation of 5ALA and PBG can be explained by an allosteric inhibition of HMBS by coproporphyrinogen III and other molecules, which accumulate before the defective enzyme (10).

A slowly increasing low-grade abdominal pain that worsens over a period of days leading to nausea and vomiting is typical for an acute attack of HCP. The pain can sometimes be located in the back or extremities of patients and if not treated correctly patients might develop a motor neuropathy over the course of days or weeks. The neuropathy starts proximally in arms and legs as a weakness and as it develops it moves further distally. Some patients even experience respiratory insufficiency. In some cases it can be mistaken for an acute inflammation of the appendix, gallbladder or other organs (12).

Similar to VP and PCT, patients with HCP show chronic cutaneous manifestations, especially in sun-exposed areas of the skin. Bullae and fragility of the skin lead to depigmented scars. Excess hair growth can also be seen in patients with HCP. The risk of liver and kidney damage may be less than in AIP (12).

1.3.4.4 Aminolaevulinic acid dehydratase deficiency porphyria (ALAD-Porphyria)

ALAD-Porphyria is the rarest type of all four AHPs, with only eight documented cases worldwide. It is also called "Doss porphyria" named after M. Doss, the first to identify the disease, or "plumboporphyria", because symptoms and biochemical findings are similar to lead poisoning, due to the ability of lead to inhibit ALAD (28).

It is inherited in an autosomal recessive manner and effects the second enzyme of the heme biosynthetic pathway ALAD, which converts 5ALA to PBG. Due to a mutation, causing a defect in the enzyme, 5ALA accumulates within the red blood cells and hepatocytes of the liver. Accumulated 5ALA then can leak into the plasma and damage other tissues. Subjects with heterozygosity for ALAD-Porphyria show a residual activity in ALAD of about 50% and are asymptomatic but may be at risk for developing symptoms when exposed to factors, which further decrease ALAD activity, such as lead, which inhibits ALAD by displacing zinc. Usually patients show a compound heterozygous mutation of ALAD (29).

Because ALAD has a higher activity than the other enzymes of the pathway, a more severe deficiency, with about 5 percent of residual activity or less, is required to cause symptoms of ALAD-Porphyria (21).

Patients with ALAD-Porphyria have neurovisceral symptoms similar to other acute porphyrias, and do not show any cutaneous manifestations (29).

ALAD can be inhibited by many different factors other than an inherited genetic defect, and therefore the differential diagnosis should contain the other hepatic porphyrias and intoxications that diminish activity of ALAD such as lead poisoning, hereditary tyrosinemia, zinc deficiency, smoking, alcoholism, diabetes mellitus and chronic renal insufficiency (29).

1.3.5 Chronic porphyrias

1.3.5.1 Porphyria cutanea tarda/Hepatoerythropoietic porphyria (PCT/HEP)

Porphyria cutanea tarda (PCT) is the most common type of porphyria. The prevalence is 1 in 25.000 in the United States. It is a chronic disease characterized by mainly cutaneous manifestations and must be delineated of other cutaneous porphyrias such as the erythropoietic protoporphyria and congenital erythropoietic porphyria (18).

In PCT the fifth enzyme, uroporphyrinogen decarboxylase (UROD), of the heme biosynthetic pathway, which catalyses the conversion of uroporphyrinogen to coproporphyrinogen, is mutated. As in other porphyrias, the decrease in enzymatic activity leads to an accumulation of precursor molecules such as uroporphyrinogen (18).

Patients with PCT do not show any neurovisceral symptoms. Cutaneous manifestations include bullae, rupturing easily, blisters, sores, and vesicles. After the manifestations heal, areas with hypo- or hyperpigmentation can appear (17).

The skin manifestations can be accompanied by iron overload and liver disease and occasionally by hepatocellular carcinoma (18).

In patients with blistering in sun-exposed areas PCT should be considered. Other porphyrias such as VP and HCP can cause blistering as well, therefore biochemical tests might be necessary to confirm the diagnosis of PCT and differentiate between other porphyrias (17).

Preventive strategies consist of avoiding exposure to direct sunlight by wearing protective clothing and using sunscreen. Drugs and chemicals known to trigger or exacerbate PCT such as alcohol, smoking and oral contraceptive pills should be avoided as well (18).

A very effective treatment method for the achievement of remission is phlebotomy, or low-dose drug therapy with hydroxychloroquine or chloroquine, which can result in clearance of skin lesions and physiologic porphyrin levels (18). Phlebotomy is thought to help achieving remission by reducing iron blood. The preferred current therapy is chloroquine, which forms complexes with porphyrins, which can then be excreted more easily (30).

1.3.5.2 Erythropoietic protoporphyria (EPP)

Erythropoietic protoporphyria (EPP) is a cutaneous form of porphyria, characterized by painful photodermatosis (31). Males are affected as often as females and the prevalence ranges from 1:75000 to 1:200000 (32–34).

EPP results from an autosomal dominant inherited deficiency of ferrochelatase (FECH) the last enzyme of the biosynthetic heme pathway. If FECH activity is below 30% the precursor molecule protoporphyrin IX (PPIX) starts to accumulate in several tissues of the body including erythrocytes, skin and liver. An increase in PPIX can also be seen in X-linked dominant protoporphyria (XLDPP) (for further details see below) (31, 35).

Accumulated PPIX molecules in circulating erythrocytes released from the bone marrow are taken up by the liver and endothelium, including superficial skin vasculature, where, they absorb light radiation and enter an excited energy state (35). This energy gets released amongst other things by forming oxygen radicals that harm tissue and vessels as well as activate the complement system, which is thought to cause the skin damage seen in XLDPP and EPP (35).

Individuals suffering from EPP have first manifestations usually starting in early infancy or childhood. The initial symptoms include skin stinging, burnings sensations and prickling in

sun exposed areas of the skin. Repeated exposure to sunlight leads to permanent skin alterations, such as skin thickening and hyperkeratosis (31).

Cutaneous manifestations can be accompanied by hepatobiliary disease, as obstruction of bile flow and cholestasis, which may occur due to progressive accumulation of PPIX in bile (35).

Anaemia can be seen in 47% of patients and another 27% will develop abnormal serum aminotransferases (35).

To treat patients for their painful phototoxic reactions, which might not respond to analgesics, cold compresses and cold air can be used, as well as anti-histamines and oral corticosteroids. The most effective treatment however, consists of sun protection by wearing long sleeved clothing and gloves, as topical sunscreens cannot protect against phototoxic reactions, because they do not filter UVA and visible light (35).

1.3.5.3 X-linked dominant protoporphyria (XLDPP)

X-linked dominant protoporphyria (XLDPP) is a very rare form of porphyria resulting from gain-of-function mutations in the erythroid specific ALAS2 gene, which regulates the rate of erythroid heme biosynthesis. The gain-of-function mutation in ALAS2 results in a higher rate of 5ALA production. As FECH becomes the rate-limiting step of heme biosynthesis and cannot keep up with the increased amount of 5ALA, PPIX accumulates (35). Thus, XLDPP clinically presents similar to EPP with painful skin lesions on sun-exposed areas of the skin starting in infancy or childhood. The pain may occur within minutes of sun exposure and is preceded by tingling and itching of the skin. In contrast to other cutaneous porphyrias vesicles or bullous lesions are rather uncommon in XLDPP and EPP (35).

In XLDPP biochemical testing shows an elevation of total erythrocyte protoporphyrin. In contrast to EPP, which also shows an elevation of total erythrocyte protoporphyrin, a lower fraction of metal-free protoporphyrin is found as ferrochelatase activity is not limited. For confirmation of the diagnosis genetic sequencing of ALAS2 gene is used (35).

The most effective treatment for XLDPP is minimizing the exposure to sunlight and wearing protective clothing. Vitamin D should be supplemented as well, as the avoiding of sunlight-exposure might lead to vitamin D deficiency (36).

1.3.5.4 Congenital erythropoietic porphyria (CEP)

CEP, also called Günther´s disease, is a rare form of porphyria caused by mutations in the gene coding for uroporphyrinogen III synthase, resulting in a lower enzyme activity of uroporphyrinogen III synthase and an accumulation of uroporphyrin I and coproporphyrin I in erythrocytes, bone marrow, plasma and a deposition in the teeth, skin and other tissues (17). It is inherited in an autosomal recessive manner and characterized by photosensitivity and hematologic abnormalities. The severity of the clinical manifestations depends on the residual activity of uroporphyrinogen III synthase and ranges from fatal to only mild cutaneous lesions (37).

CEP clinically presents with neonatal jaundice, photosensitivity forming vesicles and bullae ultimately leading to secondary infections and chronic scarring. It can also present with severe hemolytic anaemia as porphyrins accumulate in erythrocytes causing hepatosplenomegaly and potentially hydrops fetalis in utero (17). Chronic hemolytic anaemia is common and can lead to bone marrow hyperplasia and increased porphyrin production. Patients can also have erythrodontia, a discoloration of teeth, and corneal scarring (37).

Treatment consists of avoiding sunlight exposure and wearing sun-protective clothing, as well as sunglasses. Secondary cutaneous infections should be avoided by use of topical antibiotics. In cases of symptomatic hemolytic anaemia erythrocyte cell transfusions might be necessary and in more severe cases of CEP bone marrow or hematopoietic stem cell transplantation can be performed (37).

1.4 Diagnosis

Rapid diagnosis and correct treatment are important, as delayed diagnosis and therapy particularly for AHPs can cause permanent neurological damage and even death (21). Unfortunately, the diagnosis is often made late, because the clinical manifestations of acute attacks are unspecific and can mimic acute encephalopathy or abdominal crisis of other origins (22). Not surprisingly, many patients have a long history of diagnostic work-up and even unnecessary explorative operations, before an acute porphyria is considered as differential diagnosis (21).

An acute porphyric attack should be suspected when patients present with unexplained abdominal pain, especially in combination with other symptoms like nausea, vomiting, constipation, neuropsychiatric symptoms, and hyponatraemia (7, 21).

Different tests for the diagnosis of AHPs are available, which can broadly be divided into first-line tests, that are frequently used for screening, (e.g. the Watson-Schwartz test or the Hoesch test), and second-line tests, which should be used as follow up test to make an exact diagnosis (21, 38). The first-line testing or screening should include a measurement of PBG, total porphyrin and creatinine levels using a spot urine sample, or - if available - PBG separately could be measured using a rapid method (38). An algorithm for diagnosis of AHPs can be seen in Figure 4. It is important to note, that urine samples must be protected from sunlight to prevent false negative results (22). In patients with advanced renal disease PBG can also be measured in plasma or serum samples. Plasma samples however show a lower elevation of PBG than urine samples of patients with normal renal function (38).

If urine PBG concentrations of a patient with symptoms exceeds 10mg/g creatinine and is about 5 times above the upper limit of normal, which does not occur in any other illness, the diagnosis of AIP, HCP or VP is confirmed (38).
Treatment can be started as soon as samples for second-line testing to further differentiate the diagnosis are collected (38).
In patients with AIP the excretion of PBG normally is 20 to 200mg/day, compared to 0 to 4mg/day in healthy individuals. 5ALA excretion is normally about half of that of PBG. Concentrations of 5ALA and PBG are lower in HCP and VP compared to AIP during attacks and decrease faster after an attack (38).

If urine PBG and porphyrin excretion of a symptomatic patient is measured at normal levels, all four types of AHPs can be excluded as a cause of concurrent or recent symptoms (38). Second-line testing is not recommended if first-line testing is negative, but if acute porphyria is still suspected the same test may be repeated if symptoms reappear (38).
Another reason for a negative first-line test is that the samples have been obtained after hemin treatment and porphyrin precursor levels are already reduced by hemin (38).

If urine PBG excretion of a symptomatic patient is only slightly elevated the result is difficult to interpret. It might suggest that symptoms are caused by something else than a genetic porphyria. If a porphyria is still suspected testing can be repeated if symptoms recur. Because PBG is not excreted in such high concentrations and decreases faster in HCP and VP than in AIP, porphyrins in stool and plasma can be measured, as these are the most sensitive biochemical tests (38).

If urine PBG excretion is normal and total porphyrins are elevated in symptomatic patients ALAD-porphyria, which is characterized by an elevation of 5ALA but not PBG may be present, or either HCP or VP, because PBG might have already normalized as samples were taken. However, increased excretion of porphyrins may also be nonspecific and can be caused by many other medical conditions (38). Thus, other causes of ALAD deficiency than ALAD-Porphyria must be excluded, especially lead poisoning and tyrosinemia type I, both of which can mimic symptoms of AHPs (21). In this case second-line testing is indicated (38).

To confirm and further differentiate between porphyrias second-line testing is required. For second-line testing the same urine sample, with elevated PBG concentration, should be used, and additionally blood and stool samples need to be collected. Using high performance liquid chromatography individual porphyrin levels can be measured, which provides further information for the exact diagnosis (38). Table 1 shows a list of accumulated heme precursor molecules for each type of AHP.

High coproporphyrin concentrations in urine for example can indicate HCP or VP and are not expected in AIP. An increase of only 5ALA and coproporphyrin III without an elevation of PBG levels is only seen in ALAD-Porphyria (38).

In plasma samples porphyrins are usually increased in some HCP patients with cutaneous symptoms and patients suffering from VP. A peak at 626nm in Fluorescence scanning of plasma samples is a specific finding for VP (38).

Porphyrin concentrations in stool are noticeably elevated in patients with HCP and VP, whereas in AIP only slight increases can be seen. In VP coproporphyrin III and protoporphyrin are about at the same level, in HCP coproporphyrin III is dominating. A coproporphyrin III to coproporphyrin I ratio exceeding 1.5 is a clear indication of HCP (38).

Most patient with AIP show about 50% residual activity of PBG deaminase in erythrocytes, which can be measured by enzymatic testing and helps to confirm the diagnosis of AIP (38). Enzymatic tests can also be used to diagnose other porphyrias based on their deficient enzymes (21).

In summary, after a positive first-line testing result a plasma fluorescence scan should be done to confirm a diagnosis of VP. If VP can be excluded, stool analysis including coproporphyrin III/I ratio, should be done next to further differentiate between HCP and AIP. In individuals that are not symptomatic, but are suspected to suffer from an AHP,

measuring PBG and porphyrins in urine, stool and plasma can still be useful, because PBG and porphyrins might still be elevated after symptoms have subsided (38).

After determining the exact type of acute porphyria, the mutations causing the defective enzyme can be identified using DNA testing. As soon as the mutation is identified asymptomatic family members can be tested as well, before having a first manifestation of the disease. Most mutations are family specific, therefore patients diagnosed with porphyria and their relatives should have genetic counselling, so that informed decisions can be made. Knowing about genetic mutations helps family members to avoid certain precipitating factors and in further sequence prevent an acute attack (21).

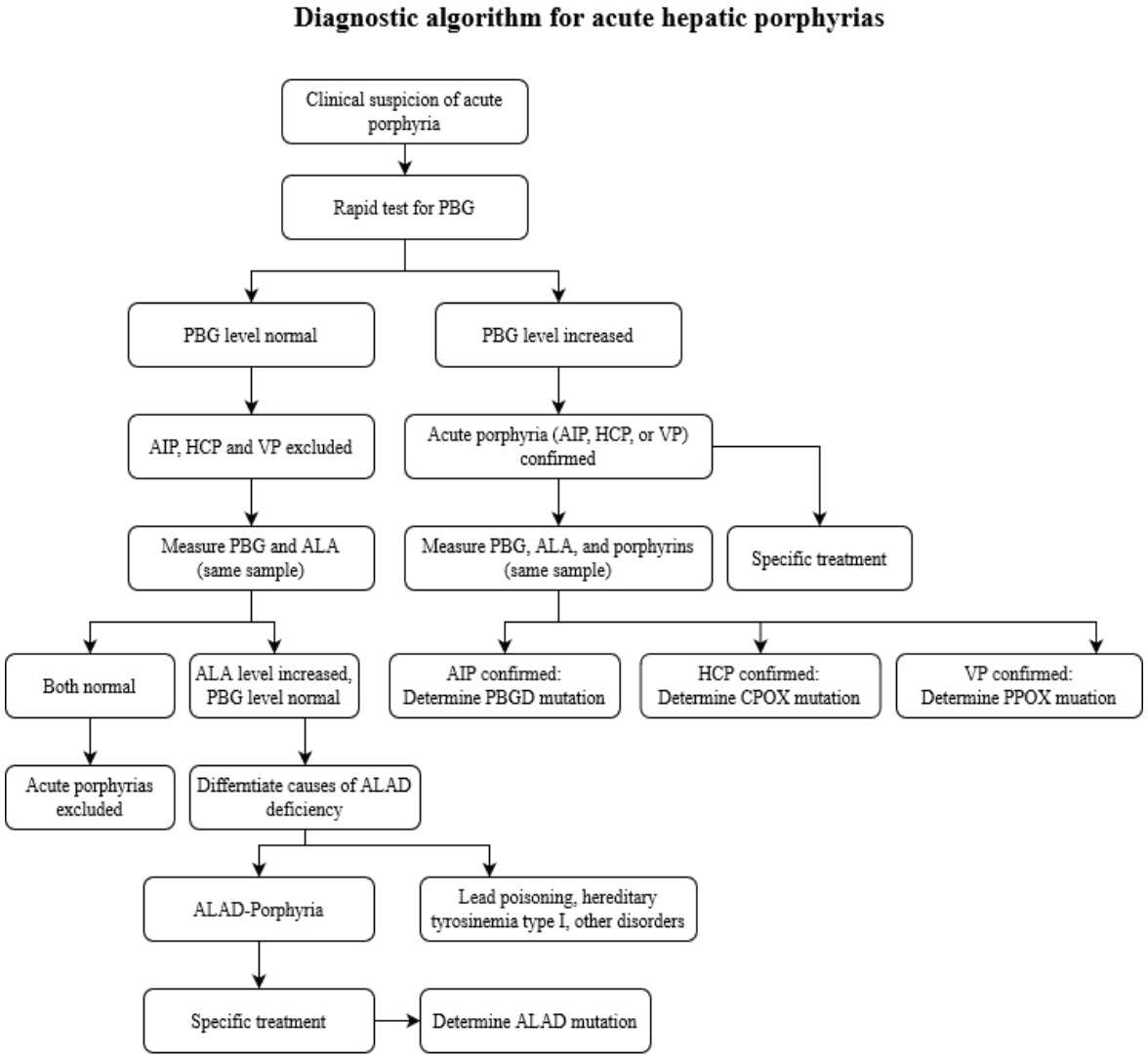

Figure 4: Diagnostic algorithm for acute hepatic porphyrias. Adapted from (21). ALA=5ALA=Delta-aminolevulinic acid. AIP=Acute intermittent porphyria. ALAD=Delta-aminolevulinic acid dehydratase. CPOX=Coproporhyrinogen III oxidase. HCP=Hereditary coproporphyria. PBG=Porphobilinogen. PBGD=Porphobilinogen deaminase. PPOX=Protoporphyrinogen oxidase. VP=Variegate porphyria.

1.5 Treatment

1.5.1 Management of acute attacks

The treatment of AHPs consists of 3 steps: Treating the acute attack, preventing an acute attack and long-term monitoring (38).

During an acute attack hospitalization is often required to treat severe pain, nausea and vomiting. Admission to the intensive care unit may also be necessary in the event of life-threatening complications (38). An important factor of the treatment of an acute attack is to identify and immediately discontinue medications and other possible factors that are known to precipitate acute attacks. This also applies to the prevention of future acute attacks (a list of precipitation factors is shown in Table 3) (8). Chronic symptoms like cutaneous lesions seen in HCP and VP can be prevented by avoiding sunlight exposure and wearing protective clothing (23, 12).

As painkillers opioids are necessary in most cases and small to moderate doses of a phenothiazine are used to treat nausea, vomiting, anxiety and restlessness. Electrolyte imbalances, typically hyponatraemia, need to be corrected via intravenous fluids. Hypertension and tachycardia should be treated using beta-adrenergic blocking agents (21).

1.5.1.1 Carbohydrate loading

Weak ALAS1 repression and a reduction of accumulating precursors can be achieved by carbohydrate loading. This therapy is most effective in malnourished patients or when fasting has contributed to the attack and is mediated in the liver by peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α) (38). Pathophysiologically, low glucose levels result in an upregulation of PGC-1α, which leads to an induction of ALAS1 (9).

Patients should eat a balanced diet and avoid fasting or dieting, however, a high carb diet is also not recommended, as it cannot prevent acute attacks (8).

Although carbohydrate loading generally has a relatively weak effect and there is no high quality evidence for efficacy of this treatment option, it has been used for decades as the standard treatment and still may suffice for mild attacks in patients with low narcotic requirements and without hyponatraemia or paresis, or if hemin is not available (38, 21).

In such cases patients should receive sucrose, glucose polymers or carbohydrate rich foods orally, if they do not have abdominal distension or ileus and tolerate oral treatment.

Otherwise, 10% glucose intravenously for a total of 300g up to 500g daily is the standard therapy, although it should be noted that a high dose of 10% glucose can trigger hyponatraemia (21).

1.5.1.2 Intravenous hemin

Currently, the most potent and effective treatment option for acute attacks is intravenous infusion of hemin. In Europe and South Africa, a hemin preparation called heme-arginate (Normosang®) is used for treatment. Hemin binds to circulating hemopexin and albumin and is then primarily taken up by hepatocytes, where it reconstitutes the intracellular heme pool leading to a down regulation of hepatic ALAS1. In a few days a reduction in precursor molecule excretion can be seen (38).

Hemin downregulates hepatic ALAS1 by reducing the synthesis of ALAS1, destabilizing the ALAS1 mRNA and inhibiting the uptake of the ALAS1 enzyme in the mitochondria (3). However, a reduction in 5ALA and PBG levels do not necessarily predict a clinical response. Hemin cannot be given orally and needs to be administered intravenously, as it is catabolized by the heme oxygenase system during intestinal absorption (21).

The standard dosage of intravenous hemin is three to four mg/kg bodyweight daily for four days or longer if patients do not respond within four days. It may take longer for patients to respond to therapy, if it is started late during an attack and advanced neuronal damage might be seen. Repeated treatment with hemin may lead to loss of venous access, chronicity of attacks, increased frequency of attacks, and can have adverse effects on the liver (38, 21).

Patients receiving heme-arginate (Normosang®) infusions over the course of many years are at risk of developing secondary hemochromatosis because of iron overload induced by this therapy with associated complications, such as liver fibrosis. Therefore, serum ferritin levels should be monitored and screening for complications of iron overload must be done (39).

Less common side effects are fever, aching, malaise, hemolysis, anaphylaxis and circulatory collapse (38).

1.6 Management of symptoms

The management of symptoms is important to relief discomfort and is targeted to the dominating symptoms (19).

Table 4 gives an overview of typical symptoms, which are associated with an acute porphyria attack, and their treatment options.

Clinical manifestation	Treatment
Abdominal, extremity or back pain	Opiates
Tachycardia	Beta-Blockers
Arrhythmia	Beta-Blockers
Hypertension	Beta-Blockers
Constipation	Lactulose
Vomiting	Chlorpromazine
Muscle weakness	Early activating physiotherapy
Respiratory muscle paresis	Mechanical ventilator
Pneumonia	Antibiotics except sulphonamides
Insomnia	Diazepam, Lorazepam
Anxiety	Diazepam, Lorazepam
Hallucinations	Chlorpromazine
Convulsions	Correction of hyponatraemia, Diazepam, Gabapentin
Hyponatraemia	Water restriction due to SIADH in mild cases, 3% saline infusion in severe cases
Fasting	Glucose infusion if unable to eat

Table 4: Treatment of symptoms of acute hepatic porphyrias. Adapted from (19).

1.6.1 Prevention of acute attacks

Individuals suffering from repeating acute attacks have a severe impact on their quality of life and management of the disease is very difficult. By reviewing all forms of current medication, searching for concurrent infections or underlying conditions and having a nutritional assessment, factors precipitating acute attacks should be eliminated. A possible iron deficiency should be treated, and smoking should be stopped. Women should monitor their cycle closely to see if attacks often begin during the luteal phase, which is common in women (38).

1.6.1.1 Intravenous hemin

Prophylaxis using heme-arginate (Normosang®) may be effective and is appropriate especially for preventing noncyclic attacks of AHPs (40).

Patients having 4 or more acute attacks per year are candidates for prophylactic or "on demand" infusions of hemin. Prophylactic infusions are given monthly, every two weeks or weekly to prevent recurrent attacks. However, heme is metabolized rapidly by the heme oxygenase system and therefore its immediate effects on ALAS1 might not last longer than one week (8).

Some patients experience prodromal attack symptoms before the onset of an acute attack. In this case heme can be administered “on demand” on an outpatient basis in order to prevent hospitalization and treat symptoms (8).

Patients receiving prophylaxis for a long time often need intravenous ports for venous access and sometimes require phlebotomies to address iron overload, resulting from frequent treatment with heme for a long period. After one or two years prophylactic treatment should be stopped, to see if still further needed (38, 41).

1.6.1.2 Cyclic attacks

In women with AHPs acute attacks can be related to their menstrual cycle. Attacks usually begin during the luteal phase at high progesterone levels. At the onset of the menstruation attacks subside. Menstrual cycle related attacks can be prevented by avoiding other precipitating factors, gonadotropin releasing-hormone (GnRH) analogues, low dose hormonal contraceptives or prophylactic hemin infusions (8).

Typical side effects of GnRH analogue treatment are hot flushes, decreased breast size, transient breast swelling or tenderness, decreased libido, vaginal dryness and irritation (41). Furthermore, treating with a GnRH analogue for more than 6 months can lead to osteoporosis, therefore a low dose of estradiol or a bisphosphonate should be added. To prevent bone loss treatment can also be changed to a low dose oral contraceptive. In the long term, gynecological follow-up examinations should be carried out, as a GnRH analogue in combination with estradiol increases the likelihood of developing endometrial cancer. After a year, treatment with GnRH analogs should be interrupted to see if it is still necessary, as premenstrual attacks rarely occur during a woman's entire reproductive life (38).

1.6.1.3 RNA interference

RNA interference (RNAi) is a new approach in the therapy of AHPs by using synthetic small interfering RNA (siRNA) to target affected genes in the heme biosynthetic pathway (42). The US Food and Drug Administration and the European Medicines Agency approved Givosiran, which uses RNAi to inhibit hepatic ALAS1 synthesis, for the treatment of adults and adolescents aged 12 and older with AHP (43).

Givosiran is a synthetic double-stranded siRNA that specifically targets ALAS1 in hepatocytes. Within the hepatocyte, the RNA is split into smaller fragments by cellular enzymes and then separated into single strands. The single strands then bind to the ALAS mRNA and prevent its translation (42). Givosiran is given once a month as a subcutaneous

injection (43). The advantage of RNAi over hemin is that it can potentially reduce the need and burden of frequent intravenous hemin infusions (44).

1.6.1.4 Gene therapy as future treatment options

1.6.1.4.1 PBGD complementary DNA (cDNA)

Another new approach in treating AIP is to correct the deficiency in HMBS (=PBGD) by using a viral vector to deliver a normal functioning HMBS gene to hepatocytes. A phase one clinical trial was not able to show efficacy in preventing acute attacks in AIP using this method yet but work is still continuing to increase delivered dose and transduction (38, 43).

1.6.1.4.2 PBGD mRNA

Another method for treating AIP is the packaging of human PBGD mRNA in lipid nanoparticles, which are then injected intravenously into the patient and taken up by hepatocytes. The endogenous translation system of hepatocytes then produces functioning PBGD proteins using the injected human PBGD mRNA. The advantages of this method over PBGD cDNA are the lower costs, a lower risk of causing an insertion mutation and that it is a less immunogenic strategy (43).

1.6.1.5 Liver transplantation

Orthotopic liver transplantation (OLT) is an effective treatment option for patients with intractable acute attacks not responsive to medication, recurrent attacks that severely affect the quality of life and life-threatening attacks. This approach can alleviate symptoms and normalize biomarkers within three days of transplantation (43).

However, as OLT is associated with morbidity and mortality it should be the last choice in treating AHPs. OLT can also not completely repair long-standing injury to motor nerves and the central nervous system (8). Especially patients with advanced motor neuropathy have an increased risk of postoperative complications (38).

1.6.2 Management during anaesthesia and surgery

As metabolic stress induced by surgery can favour the emergence of an acute attack, patients who will undergo surgical procedures should inform physicians about their diagnosis, so that pre-procedure evaluation can be done. A prophylactic hemin infusion can prevent a perioperative attack and might be considered in patients with recurring attacks (21, 8).

1.6.3 Management of acute hepatic porphyrias during pregnancies

Although sex hormones peak in the second and third trimesters, acute attacks are rare during pregnancy (22). Nevertheless, some women might have more frequent acute attacks during pregnancy and sometimes an AHP manifests for the first time during pregnancy. Fertility is usually not impaired in patients with AHPs, and pregnancies are generally well tolerated, but there is an increased risk of miscarriages in women with a history of recurrent attacks. Nausea and a decreased intake of calories, which happens especially during the first trimester of a pregnancy might precipitate an attack. If a patient develops an attack during pregnancy hemin can safely be administered as treatment and terminations of pregnancy are rarely necessary. After birth, monitoring should still be continued, because attacks can also occur in the postpartum phase (8).

Fertility inducing drugs and contraceptive methods such as oral contraceptives, hormone-releasing implants and intrauterine devices using only progesterone or a progestin, should be avoided, because they can precipitate acute attacks. Intrauterine devices without progesterone and mechanical barrier methods are considered safe (8).

1.6.4 Prognosis

Due to faster diagnosis, better treatment options and more knowledge about triggering factors, acute attacks nowadays occur in only 17% of patients with AIP instead of 49% and in only 14% of patients with VP instead of 38% compared to early 1980s (19).

If diagnosis is missed and precipitating drugs are administered a patient can still die from an acute attack. Although the chance for a fatal outcome is low, the fatality rate can be between 10% and 40% during an acute attack (19).

1.6.5 Follow-up management

Long term complications of AHPs include systemic arterial hypertension, renal and liver impairment, hepatocellular carcinoma, chronic pain, psychiatric symptoms, and peripheral neuropathy (43).

To prevent long-term complications continued monitoring is essential. Symptomatic patients should be followed once a year or more often, if they receive prophylactic treatments or have acute attacks in-between. After hospitalization for an acute attack a follow up visit should be done after one month to re-evaluate precipitating factors, preventive measures, and management of symptoms (38).

Patients suffering from chronic pain are at risk for opioid dependence and should be referred to a pain management specialist. Psychiatric evaluation and treatment for pain associated anxiety and depression should also be done to provide a long-term benefit for patients. Liver imaging is recommended at six to twelve month intervals for patients after the age of 50 with recurrent attacks or past symptoms, as an increased risk for hepatocellular cancer (HCC) in AHP patients is well documented (38).

2 Objective

AHP is a rare disease with a non-specific constellation of clinical symptoms, but a major negative impact of quality of life. The number and natural history of patients with AHP, which have been referred to the responsible tertiary clinical centre of the Medical University Graz is unknown.

The aim of this diploma thesis is to determine the number of patients with AHP and discuss their individual history in comparison with what is known from literature. In addition, we will discuss the new molecular precision treatment options and discuss, if the identified patients could benefit from these novel treatment strategies.

3 Methods

This diploma thesis is a retrospective data analysis of patients who have been diagnosed or treated with any form of acute hepatic porphyria at the University Hospital Graz in the last 16 years.

3.1 Data collection

We performed a search of the MEDOCS database from 2004 until 07/2020 via the Medical Institute for Informatics. As searching parameters, we used the terms: AHP, AIP, VP, HCP, ALAD deficiency porphyria, acute hepatic porphyria, acute intermittent porphyria, variegate porphyria, hereditary coproporphyria and the ICD-10 code E80.1 and E80.2 in various combinations. In addition, a similar search for patients with elevated 5ALA and PBG values in 24-hour urine was made. Cutaneous porphyries were already an exclusion in the primary search. All patients with a diagnosis other than acute hepatic porphyria, having a similar abbreviation or with a single porphyrinuria of a cause other than AHPs, were excluded, as well as patients with the diagnosis of porphyria cutanea tarda, pseudoporphyria, erythropoietic porphyria, dialysis associated porphyria and unconfirmed suspected cases. All patients who were identified in this query and met the inclusion criteria for the diploma thesis were compared and discussed regarding the course of their disease based on clinical reports and laboratory results.

The following patient data from the medical electronic documentation system (MEDOCS) of the LKH Graz was recorded:

- age at diagnosis

- sex
- date of initial manifestation
- date of initial diagnosis
- period from initial manifestation to initial diagnosis
- definite diagnosis
- date and cause of death (if already deceased)
- symptoms of acute attacks
- chronic symptoms between acute attacks
- diagnostics before initial diagnosis
- treatment before initial diagnosis
- family history
- therapy after initial diagnosis
- triggering attacks
- therapy side effects
- number of hospitalizations for acute attacks
- length of hospital stays
- laboratory values

3.2 Literature research

Reference literature, which includes reviews as well as original articles on acute hepatic porphyrias and related literature was searched via online databases such as PubMed and Google Scholar and studied in textbooks of internal medicine and biochemistry.

3.3 Ethic vote

Before collecting individual data, we retrieved a positive ethic vote for performing this retrospective data analysis (EK-Number 32-620 ex 19/20). No informed patient consent was required for retrospective data collection.

4 Results

The search carried out by the Medical Institute for Informatics in 07/2020, returned a total of 16 patients. Of these, two were excluded because they suffered from a non-acute hepatic porphyria. One had porphyria cutanea tarda the other one suffered from symptoms that suggested porphyria cutanea tarda and even had elevated blood porphyrin levels, but he did not have any further diagnostic steps carried out. Two more patients were excluded, because the abbreviation "AIP" referred to autoimmune pancreatitis. For one patient AIP was just listed in the differential diagnosis. Another patient was excluded because only once increased urinary porphyrins were detected and no definite diagnosis of AHP had been made. One patient was excluded because acute hepatic porphyria was ruled out in the later course and the patient did not appear for further investigations. Four patients had a definite diagnosis of AIP, but have been treated or transferred to the tertiary hospital centre of the Medical University Graz only once. For these four patients no further information on initial diagnosis, number of attacks, or in-between symptoms was available. Thus, these patients were excluded from our longitudinal analysis.

Overall, after excluding eleven patients, our search resulted in a collective of five patients in total, all suffering from AIP. Of these five patients:

- three patients underwent surgical procedures that were unnecessary in retrospect, because AHPs had been missed at initial presentations
- in four patients the diagnosis was delayed more than a year from initial manifestation of symptoms
- the patients spent an average of 6,7 days in hospital due to acute attacks.

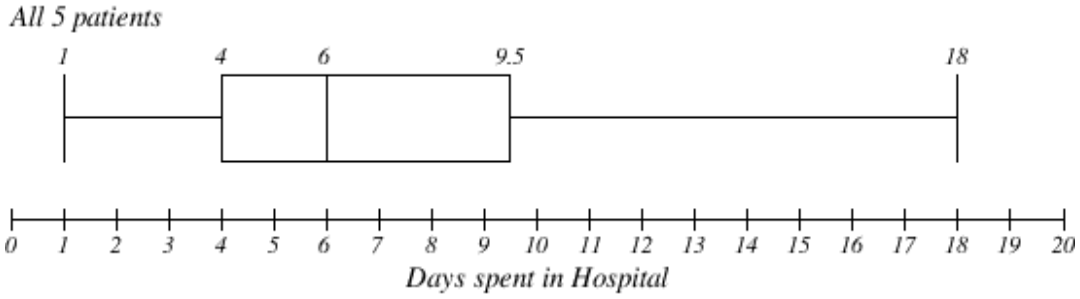

Figure 5: Days spent in hospital by all patients

- four of the final five patients suffer from chronic symptoms (psychosis, neuropathy and arterial hypertension in three out of five patients, any form of pain in two out of five patients and tachycardia in one out of five patients) between acute attacks.
- one patient died of complications related to AIP at the age of 33.
- the number of attacks during the observation period ranged from 3 to 32, corresponding to 1,8 attacks per year.
- the time between acute attacks ranged from 9 days to 9.3 years.
- all patients received heme-arginate (Normosang®) as treatment for acute attacks and two out of five also received heme-arginate (Normosang®) as prophylactic treatment.

All patients suffered from abdominal pain during their acute attacks. Neuropathy, psychosis (depression, behaviour change, exhaustion and delirium), nausea and vomiting, arterial hypertension and constipation occurred in 60% of the patients. 40% of patients had pain in other parts of the body and 20% had tachycardia and hyponatremia. (Figure 6)

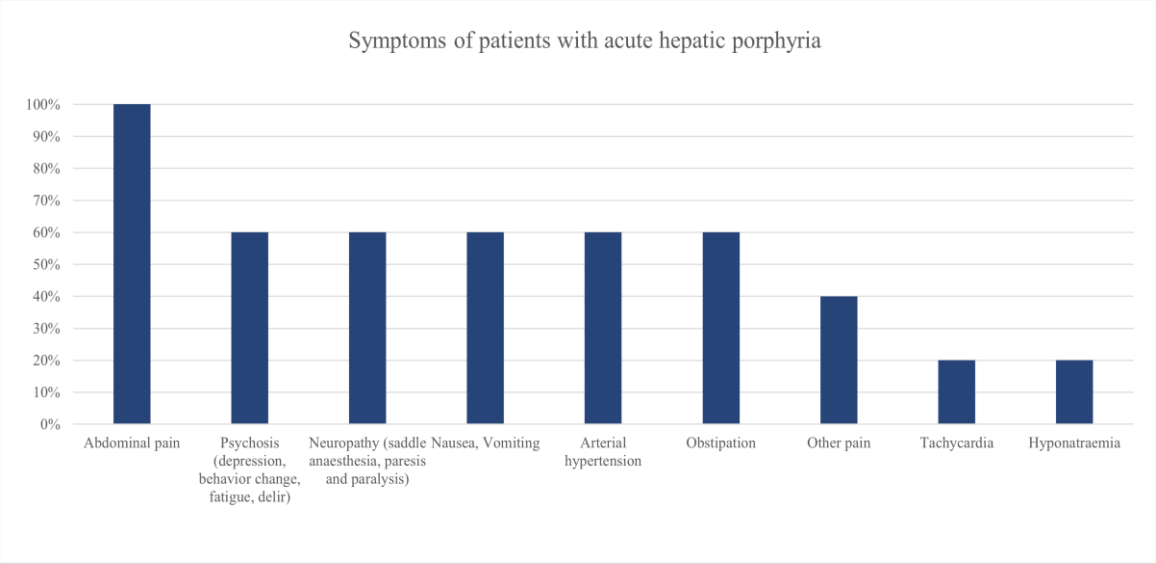

Figure 6: Distribution of symptoms

Between the acute attacks, four of the five patients in the data collection also suffered from chronic symptoms such as psychosis (depression and fatigue), neuropathy (saddle anaesthesia, paresis and paralysis), arterial hypertension, other pain (pain in the extremities, thoracic pain, whole-body pain) and tachycardia. (Figure 7)

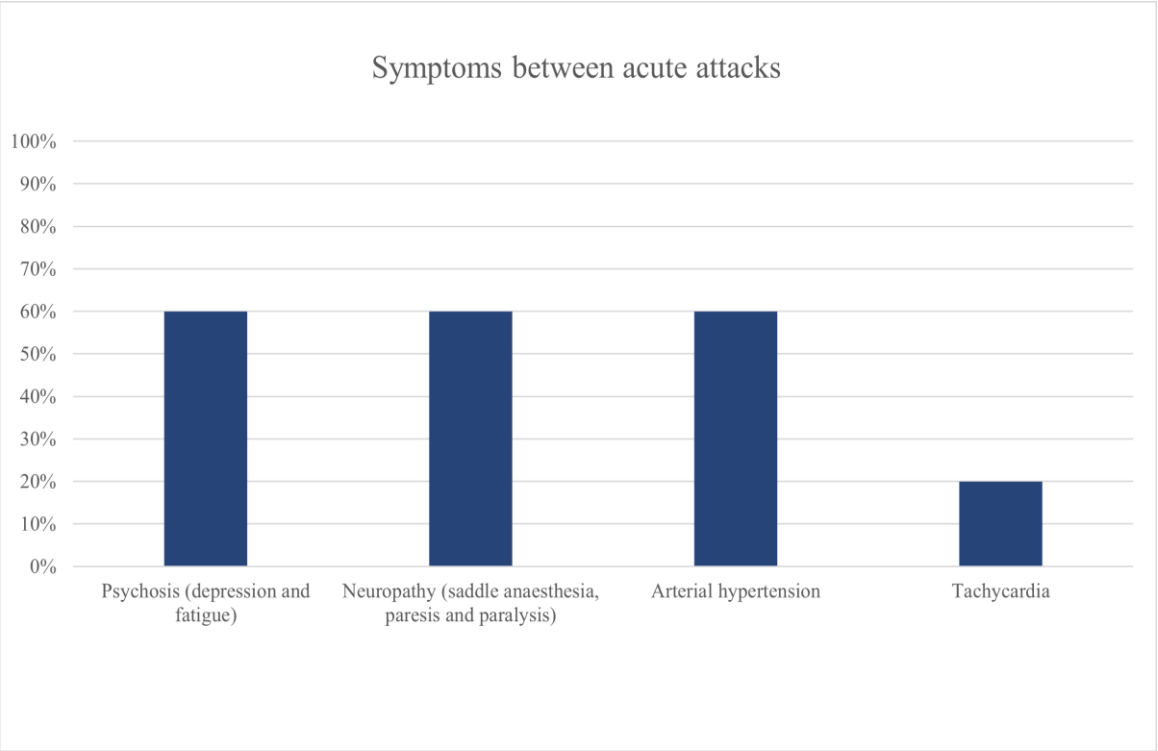

Figure 7: Distribution of symptoms between acute attacks

Table 5 shows an overview of the specific complaints and symptoms of the five patients.

Patients	Abdominal pain	Obstipation	Nausea, vomiting	Other pain	Hypo-natraemia	Delir	Depression	Fatigue	Neuropathy	Tachycardia	Art. Hypertension
Patient 1	X	X	X	X				X	X	X	X
Patient 2	X		X				X				X
Patient 3	X			X			X		X		X
Patient 4	X	X			X	X		X	X		
Patient 5	X	X	X								

Table 5: Table of symptoms per patient. Blue=Symptoms during acute attacks. Red=Symptoms between acute attacks.

4.1 Case reports

4.1.1 Patient #1

4.1.1.1 Initial manifestation

In 1993 the 27-year-old woman was admitted the first time to the hospital due to recurrent abdominal pain. A cause for her symptoms could, however, not be found. Two more similar episodes, all requiring in-hospital stay, occurred. During one of these further stays exploratory laparotomy had been performed, because a paralytic ileus was suspected, but could not be confirmed.

4.1.1.2 Initial diagnosis

15 months after first onset of symptoms, during her fourth visit to a hospital due to recurrent abdominal pain, AHP was suspected for the first time. Determination of porphyrins in her 24-hour urine (Ehrlich Probe PBG 200µmol/L) were clearly positive and AIP was diagnosed. Her medical records at the time of admission showed tachycardia up to 130bpm, high blood pressure of 180 systolic, as well as nausea, vomiting and obstipation.

4.1.1.3 Therapy

Immediate non-specific therapy consisted of parenteral nutrition, rehydration, a colon decompression tube and opioids for abdominal pain. As specific treatment heme-arginate (Normosang®) was administered for eight days. The patient was informed about potentially triggering factors, which should be avoided. After twelve days, the patient could be discharged from the hospital with the diagnosis of AIP.

However, the patient continued to be symptomatic.

During the attacks, the patient's urine was repeatedly reddish in colour and she had chronic symptoms such as fatigue, fever, pain in the limbs, especially in the upper extremities, neuromotor deficits in the hands with tremors and restricted mobility.

As a reason for her recurrent AIP attacks her menstrual cycle was suspected, which could be confirmed with the help of a cycle calendar later in the disease course. To reduce attacks progestin (Duphaston®), which interrupts the reproductive cycle was given. Despite this treatment and prevention of triggering factors she visited the hospital four more times over the next 15 months for recurrent attacks in the premenstrual period and had to be treated with intravenous glucose and heme-arginate (Normosang®). In 02/1995 progestin

(Duphaston®) was exchanged by buserelin (Suprecur®), a GnRH analogue and a year later switched to goserelin (Zoldalex®), to inhibit ovulation and minimize her premenstrual attacks. A side-effect of this anti-estrogen therapy is a reduction in bone density which was treated with bisphosphonates. With this therapy the patient stayed attack-free for only nine months. Then premenstrual attacks started to reoccur at intervals of every two to three months for the next three years until GnRH analogue treatment was discontinued and a heme-arginate (Normosang®) long-term therapy was started in 12/1999. The regimen consisted of a heme-arginate (Normosang®) infusion on two consecutive days every two weeks, which provided relief for the patient and significantly reduced the frequency of attacks. In 2007 decapeptyl, another GnRH analogue, was re-introduced as long-term therapy. From 12/1999, when heme-arginate (Normosang®) long-term therapy was started, until 12/2020 the patient "only" suffered from nine acute attacks, three of which were caused by infections.

4.1.1.4 Side effects of therapy

There occurred several direct and indirect side-effects due to the preventive long-term therapy with heme-arginate (Normosang®). Since heme-arginate (Normosang®) can only be administered intravenously, a chest port became necessary for the patient in order to administer the medication regularly, which she received in 01/1999. This chest port had to be replaced a total of ten times in the event of dysfunction and septic events and even caused a pneumothorax during replacement. Heme-arginate (Normosang®) long-term therapy regimen caused an iron overload with serum ferritin values of 5153µg/l and transferrin values of 91%. In addition to the acute attacks and the associated hospital admissions, the patient developed a porphyria-associated neuropathy with radial palsy, which was treated with electrotherapy.

4.1.1.5 Follow-up

The patient received heme-arginate (Normosang®) as long-term therapy. However, due to her chronic disease course and multiple complications, in particular related to the chest port, the patient´s therapy was switched to Givosiran, one of the novel siRNA treatment options, in June 2021.

4.1.1.6 Summary

Overall, from the diagnosis in July 1994 to November 2020, the patient suffered from a total of 35 acute attacks that required hospitalization. The patient's average length of stay in hospital was 6.2 days with a maximum of 14 days. In between attacks, the patient continues to remain symptomatic.

4.1.2 Patient #2

4.1.2.1 Initial manifestation

Since 2015, a then 47-year-old man has repeatedly suffered from severe recurrent abdominal pain with punctum maximum in the epigastrium, in combination with nausea, vomiting and loss of appetite. In July 2015, he was administered to a peripheral hospital with the same abdominal painful symptoms and even had to be further transferred to the intensive care unit, because of uncontrollable worsening of pain and nausea. AHP had already been suspected and for further clarification the patient was transferred to the internal medicine ward of the Medical University Graz after release from the intensive care unit. A 24-hour urine test for elevated porphyrin levels was carried out, but the 24-hour urine examination showed normal values of 5ALA and PBG. The diagnosis of porphyria was thus excluded. The patient was continued to be treated with opioids for the pain and was discharged from the hospital after a total of 22 days.

Five months later in December 2015 the patient was admitted to the hospital for another 18 days, because of identical symptoms. A 24-hour urine examination for AHP was again recommended, but not carried out.

Over the next 3 years, the patient was hospitalized another four times and treated with opioids, without a specific cause for the symptoms being found. In November 2018, when he was admitted for the fifth time, chronic pancreatitis was blamed as cause of his symptoms.

4.1.2.2 Initial diagnosis

A month later in December 2018, this diagnosis of pancreatitis was doubted. Porphyrin values in the urine were again determined and the PBG values were found to be increased, which indicated acute hepatic porphyria.

4.1.2.3 Therapy

Intravenous heme-arginate (Normosang®) therapy was started immediately, after which the patient was quickly relieved of pain. During the same hospital stay, however, further symptoms occurred even after the start of heme-arginate (Normosang®) therapy, which were blamed to be an acute flare-up of AIP. As such, the patient suddenly reported pain and sensitivity disorders in the right leg 8 days after the diagnosis. The patient also suffered from depression and arterial hypertension before the diagnosis, which could also possibly be linked to AIP. After 11 days the patient was discharged from the hospital in improved

condition. Since then, the patient was not admitted to the hospital because of an acute attack again. Aside from depression and arterial hypertension there are no reports on other symptoms which occurred or persisted during and after the attacks. No further prophylactic therapy with heme-arginate (Normosang®) has been necessary.

4.1.2.4 Summary

A total of nine hospital visits, eight of them inpatient, were necessary within three years and five months from the first appearance of symptoms to the final diagnosis of acute intermittent porphyria. The patient's average length of stay in hospital was 10 days.

No triggers for the acute attacks could be identified. Since the last admission in December 2018, the patient has not been admitted to the university hospital Graz anymore.

4.1.3 Patient #3

4.1.3.1 Initial manifestation

For this patient there are no reports on a clinical history before initial diagnosis. Therefore, we assume that she was correctly diagnosed at her first attack.

4.1.3.2 Initial diagnosis

In August 2002, at the age of 28, the female patient was first diagnosed with ovulocyclic AIP. Several of the patient´s relatives, including her father, also suffer from AIP and there is even one death in the family associated with AIP. Unfortunately, we were unable to extract data from her family, because the family members were not recorded by the medical system MEDOCS.

4.1.3.3 Symptoms

The patient suffered from symptoms that are believed to be related to AIP, such as depression, whole-body pain, abdominal pain with maximum punctum in the epigastrium and arterial hypertension (177/123). Later in 2004 she developed radial paralysis on her left side with additional development of wristdrop. According to her medical history this was the first episode of symptoms.

4.1.3.4 Therapy

Since the establishment of the diagnosis of AIP in August 2002 the patient was treated with heme-arginate (Normosang®) against acute attacks and with opioids against whole-body- and abdominal pain.

No reports of her disease course between August 2002 and January 2005 exist, but beginning from January 2005 the patient was admitted to the hospital because of acute attacks a total of three times. Then therapy with intramuscular GnRH-Analogues as long-term treatment was started to inhibit the patient's ovulatory cycle. Three months later in March 2005 the patient received a chest-port to further allow venous access for heme-arginate (Normosang®) infusions as peripheral venous access got continuously difficult.

A genetic predisposition was finally proven in April, when blood samples were sent to a special laboratory, which determined a residual porphobilinogen deaminase activity of about 47% of normal, due to a mutation.

From September 2005 the GnRH-Analogue (Decapeptyl) injections were discontinued, as they had no effect on reducing the occurrence of acute attacks and there was a desire to have children.

Since December 2005, the patient received intravenous prophylactic heme-arginate (Normosang®) infusions as long-term treatment in an interval of 2 weeks through her chest port.

4.1.3.5 Side effects of therapy

In January 2007, the patient underwent a chest port revision, because of malfunction. In June, while the patient received her prophylactic heme-arginate (Normosang®) infusion, she suddenly got fever. Further investigations showed that the patient had developed a pleural empyema, which made a surgical decortication, a thoracic suction drainage and a transfer to the intensive care unit necessary. After 49 days the patient discharged herself from the hospital at her own request.

The day after her discharge the patient developed a febrile infection again, which ultimately lead to sepsis and once more required a hospitalization. During her stay in hospital, she developed a muscular weakness with increasing symptoms of tetraplegia.

A measurement of the nerve conduction velocity showed axonal demyelinating which caused tetraplegia and could be attributed to AIP. Repeated heme-arginate (Normosang®) infusions did not lead to any improvement of her condition anymore and the tetraplegia progressed further, resulting in central neuropathy, cranial nerve involvement and multiple asystoles.

4.1.3.6 Death

After a positive resuscitation, her condition still worsened, and the patient died in mid-November 2007 of multiple organ failure.

4.1.3.7 Summary

The date of the first manifestation is not known, but since the specific diagnosis of AIP, the patient received inpatient treatment for acute attacks at the tertiary hospital centre of the Medical University Graz 18 times from 2004 to 2007. On average, the patient spent 5.8 days in the hospital for her acute attacks. In November 2007, she died at the age of 33 from multiple organ failure associated with AIP in the intensive care unit.

4.1.4 Patient #4

4.1.4.1 Initial manifestation

In March 2010, the male patient suffered from symptoms that may be related to porphyria for the first time, when he had very severe abdominal pain and was admitted to the hospital for two days. The treatment only consisted of an ultrasound examination and a colonoscopy, during which a small polyp was removed.

From the first onset of abdominal pain in March 2010, the patient had other symptoms through the years, such as constipation, fatigue, and delirium, until a diagnosis of AIP in November 2019 was made.

4.1.4.2 Initial diagnosis

In November 2019, the 54 years old male patient was referred to the hospital by the family doctor, after a spinal disc surgery, because of high-grade hyponatraemia (110mM/L) of unknown cause with additional states of confusion and hallucinations. The patient repeatedly had reddish discolorations of his urine, which were falsely interpreted as a urinary tract infection. Other medical examinations revealed paralysis of the bowel, the cause of which could also not be identified.

The spinal disc surgery was performed, because the patient suffered from radicular pain for more than 6 months and additionally developed saddle anaesthesia, although there was no morphological correlate for it, according to neurosurgery.

During his stay, a 24-hour urine examination was carried out, where an increase in PBG was determined, diagnosing AHP.

4.1.4.3 Therapy

After the establishment of the diagnosis in November 2019 the patient was referred again to the neurosurgical department as his worsening pain was interpreted as recurrent prolapse. Treatment with heme-arginate (Normosang®) infusions were not started until later in December 2019, when he was transferred to the Clinical Department of Gastroenterology and Hepatology after a gastroenterological consultation. In February 2021, the patient suffered another acute attack due to a COVID-19 infection.

4.1.4.4 Genetic testing

In February 2020, a genetic analysis was done, which showed a pathogenic mutation and helped to make a definite diagnosis of AIP. The patient´s cousin's daughter also suffers from AIP.

4.1.4.5 Summary

From the first onset of symptoms that can be linked to AHP until the patient got his diagnosis it took nine years and eleven months and 3 admissions to the hospital with an average stay of 5 days. In addition, the patient underwent disc surgery, although neurosurgery showed that there was no morphological correlate for his radiculopathy.

4.1.5 Patient #5

4.1.5.1 Initial manifestation

In December 2017, the then 44-year-old female was admitted to the hospital because of severe abdominal pain, vomiting, nausea, and obstipation. During diagnostic work-up of her deteriorating abdominal pain and obstipation a laparoscopy examination has been performed but could not establish a diagnosis. After 4 days the patient could be discharged from the hospital with improved symptoms.

According to her statements, the patient has been suffering from similar complaints for years, but never had to be hospitalized before. It may be concluded that the patient may repeatedly have had minor acute attacks for years.

4.1.5.2 Initial diagnosis

The patient was next admitted to the department of internal medicine at the Medical University of Graz six months later in May 2018 when she was suffering from massive hyponatraemia after vomiting for days. Her serum sodium concentration was 99 mmol/l at the time of admission.

She was immediately treated with infusions of sodium and potassium to balance out her electrolytes. Later during her stay, acute hepatic porphyria was suspected, and measurement of the porphyrins in the 24-hour urine was carried out, which turned out to be positive for total porphyrins (322µg/L), PBG (57,5mg/L) and 5ALA (45,3mg/L), thus indicating AHP.

4.1.5.3 Therapy

Immediately after the diagnosis was established therapy with heme-arginate (Normosang®) and glucose infusions was started. After 13 days of treatment, the patient was discharged from the hospital in improved condition. Long-term therapy with heme-arginate (Normosang®) was not considered and alcohol and metamizol were suspected as possible triggers for the acute attacks.

4.1.5.4 Genetic testing

A genetic analysis showed a pathogenic mutation in the gene coding for PBG-Desaminase, having a residual activity of 66%, which confirms the diagnosis of AIP.

4.1.5.5 Follow-up

Since the Diagnosis in June 2018 the patient was administered to the hospital one more time because of an acute attack, where she was treated with heme-arginate (Normosang®) as well as glucose infusions and could be discharged after 7 days. Since then, she has not been administered to a hospital due to an acute attack or any other cause associated with porphyria.

4.1.5.6 Summary

At the time of her diagnosis in June 2018, the patient claims that she has had these symptoms for several years. The exact date of the initial manifestation is unknown. Since the diagnosis, the patient has been in hospital three times until today (December 2020), with an average stay of 8 days.

5 Discussion

AHPs are rare diseases and difficult to diagnose. Therefore, single centre experience is low and only a few multi-centre reports with sufficient numbers of AHP patients exist (see reference 16). The retrospective search for AHP patients at the tertiary referral hospital centre of the Medical University Graz spanning the last 16 years revealed a total number of five patients which had been admitted to the hospital for symptomatic AHP several times. Four more patients with a likely diagnosis of AHP had been one-time treated, but no longitudinal medical record was available. Given the low penetrance of a symptomatic disease course, a total of five AHP patients with recurrent acute attacks is a plausible number.

One big challenge in the diagnosis of AHP are common and unspecific symptoms, which often result in a delayed diagnosis (16, 22). Many patients complain about recurrent abdominal pain or constipation (45), which may be misleading to perform other diagnostic tests, although the proper diagnostic tests for AHP can be carried out easily and quickly (21). As a result, patients sometimes have to go through many unnecessary, sometimes invasive, diagnostic procedures and operations (45, 21).

In our case series the average time from initial manifestation to initial diagnosis was more than a year and patients had to be referred to the hospital several times. Moreover, two of our patients had to undergo laparoscopic surgery for the abdominal complaints due to misinterpretation of symptoms.

AHPs, even if called acute, are chronic genetic diseases, caused by mutations in genes, which are part of the heme-biosynthesis pathway (16). Although AHPs are mainly defined by their acute attacks, many patients suffer from chronic symptoms between the acute attacks (16). These chronic symptoms between acute attacks comprise chronic hypertension, relapsing constipation, permanent neurologic damage, depression, and other symptoms that are either directly or indirectly attributable to the AHPs and reduce the quality of life for patients significantly (16).

Our retrospective data analysis confirms that most patients with recurrent acute attacks also suffer from chronic symptoms during acute attacks. These chronic symptoms consisted of abdominal pain (in 100% of patients), psychosis (in 60% of patients) and neuropathy (in 60% of patients). Nausea, vomiting, art. hypertension and constipation also occurred frequently (in 60% of patients).

Symptoms that persist beyond acute attacks have a great impact on the quality of life of these patients but are rarely linked to AHPs and are rarely mentioned in the literature. Furthermore,

the currently available therapies, which mainly consist of hemin infusions, represent an effective therapy against acute attacks and alleviate acute symptoms, but they are usually not used for chronic complaints between the attacks (16).

If acute attacks re-occur, hemin can be administered as prophylactic therapy (38). However, repeated hemin therapy may have considerable side effects. Hemin can only be administered intravenously, (16) and for prophylactic therapy it must be administered every or every two weeks (21). Since it is toxic to the veins, repeated administration requires a permanent central line (38).

Two of our patients had severe complications due to repeated insertion of central lines. Patient 1 of the case report series had a replacement of her chest port ten times and developed a pneumothorax during one intervention. Patient 3 developed a pleura-empyema and had to be transferred to the ICU. Chronic hemin infusion also results in chronic iron overload which can lead to serious damage to vital organs (39). One of the patients had ferritin levels above 5.000ng/ml. It is also speculated, that repeated hemin infusions can have adverse effects on the liver and might even increase the frequency of attacks in the long run (46).

No patients were found with chronic liver disease, cirrhosis or HCC that can be linked to AHP, but the small number of patients may simply be too low to detect rarer complications of AHPs.

For those female patients, whose attacks are triggered by hormone fluctuations during the menstrual cycle, ovulation inhibitors can be used as prophylaxis. However, this therapy may negatively affect bone density and, since most female patients are in child-bearing age, can compete with the desire to have children (8).

New drugs, which selectively inhibit ALAS1 and thus further accumulation of toxic heme precursors give hope for future treatment without side effects of the heme therapy. One of these new drugs is Givosiran, a siRNA molecule, directed to degradate ALAS1 mRNA. This drug appears also to prevent or alleviate chronic symptoms (42, 38, 43).

They can be administered as long-term therapy and have to be injected subcutaneously only once per month. The reported side-effects profile appears to be excellent in the long term (43). However, since AHPs are very rare diseases, these drugs have orphan drug status, and pricing may become a relevant issue.

5.1 Limitations

The main limitation of our retrospective data analysis is the low number of patients who were eligible for the analysis, due to the rarity of AHPs. The aim was to precisely analyse the few patients in the data collection and to compare their disease history with the disease courses in the literature. Due to the very limited number of patients, we could perform our individual analysis very detailed. However, numbers are too low to draw statistically relevant conclusions from our data collection.

Another limitation of the study was the poor data situation on patients who were eligible for the analysis, especially if the first manifestation of the disease was more than 20 years ago. Furthermore, it was not possible to view documents from other hospitals outside of Styria, which resulted in a loss of data primarily from patients who have their place of residence outside of Styria.

Since this was a purely retrospective data collection without contact to patients, no direct questioning could be performed.

5.2 Conclusion

Although AHPs are extremely rare diseases with a low penetrance and a presumably high number of unreported cases, they are well described in the literature, especially regarding acute attacks, and treatment. However, the awareness of the disease, particularly when occurring as a symptomatic trias of abdominal pain, neurological and psychiatric symptoms can still be improved. Some patients still need to undergo unnecessary diagnostics, even invasive interventions. In addition, the understanding of AHP as a disease with a continuous chronic course between acute attacks is still underdeveloped and unmet by the current therapy. The retrospective data analysis showed that four of the five patients suffer from chronic manifestations of the disease, such as fatigue, pain and depression, even between the acute attacks and despite repeated heme-arginate (Normosang®) therapy.

As of today, with Givosiran, a novel precise siRNA therapy, there is a new treatment option available in Austria, which could minimize chronic symptoms and the occurrence of acute attacks. Since it is an orphan drug with high costs it remains open, how many patients will be treated with these new compounds. However, as our analysis shows, patients with recurrent AHPs require many hospital stays either for acute attacks or the complications of current treatment and therefore have impaired life quality. A cost-efficiency calculation of the new drug versus the current treatment concept unfortunately does not exist, yet.

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