Usense internal report #2

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Use of an innovative *in vitro* medical device for the measuring of Porphobilinogen and Total Urinary Porphyrins in the detection of porphyrias and specifically Acute Hepatic Porphyria.

Introduction

The burden of rare disease

A rare disease is defined as a disease or condition that impacts fewer than 1 in 2000 people (30) or less than 200 000 people in the USA (31). Although rare diseases are individually uncommon, collectively constitute a substantial burden on individuals, families, and healthcare systems, demanding increased attention and innovative approaches to diagnosis and treatment. With an estimate of over 10 000 rare diseases existing, individuals affected make up a considerable portion, potentially accounting for up to 10% of the global population (32). Diagnosing rare diseases presents a considerable challenge due to their inherent complexity and limited understanding of these conditions. Rare diseases often exhibit diverse and highly non-specific symptoms, making accurate diagnosis a daunting task (33). Additionally, the scarcity of information and expertise in the medical community about these conditions further complexifies the diagnostic process. Consequently, many patients with rare diseases experience delayed diagnosis, which can have profound implications on their survival chances and overall quality of life. Delayed diagnosis deprives patients of timely access to appropriate treatments and interventions, limiting disease management. This delay not only increases the risk of complications but also prolongs the emotional and physical burden on patients and their families.

A specific use case around Porphyrias and Acute Hepatic Porphyria

Genetic Disease Family

Porphyrias encompass several distinct types, each resulting from a specific enzyme deficiency within the pathway:

- Porphyria cutanea tarda;
- Acute hepatic porphyria;
- Erythropoietic protoporphyria.

These 3 types regroup a group of 9 metabolic disorders each implying defect in a heme biosynthesis pathway enzyme and associated with different symptoms (13).

In addition to the detection of this family of genetic disease, we would like to special focus on one specific condition that exemplifies the challenges and complexities inherent in rare disease management, Acute Hepatic Porphyria (AHP). AHP belongs to the family of genetic disorders, Porphyrias, caused by the defect in an enzyme involved in heme synthesis and leads to the accumulation of heme precursors having toxic effects on the organs (29). Our focus on this specific disease is due to the fact that :

- The pathology doesn't display any differential symptoms which implies a very difficult diagnosis;
- The diagnosis is based in the vast majority on the presence of one specific biomarker, porphobilinogen.

Epidemiology

It is currently estimated that AHP affects 1 case per 100,000 individuals (34), however, more recent results indicate that the disease could be much more prevalent than previously thought, with 1 in 1700 European, but with a low clinical penetration (of 1%-2%) (35). Nevertheless, even if rare, the impact of AHP on affected individuals is major.

Symptoms and diagnosis

AHP manifests with intermittent attacks of severe abdominal pain, often accompanied by other signs such as nausea, mental confusion, hyponatremia, hypertension, tachycardia, and muscle weakness. Due to these symptoms being non-specific and mimicking other more common disorders diagnosis is frequently missed or delayed, often for years (36). The diagnosis delay is estimated to range between 7,4 years (37) to 15 years (34) on average. Each day that passes leaving the disease undiagnosed puts patients at risk of potentially serious life-threatening neurological manifestations during acute attacks. These attacks can induce seizures, acute encephalopathy, delirium, hallucinations, psychosis, and even death. A long-term follow-up of AHP patients showed that 31% of patients died due to acute attacks (38). But even beyond these attacks, it is well known that AHP evolves with frequent and disabling chronic symptoms present in more than 65% of the patients, most of them related to neurological and psychiatric disturbances like severe chronic pain (abdominal, neuropathic, or diffuse myalgia), anxiety and mood disorders, fatigue, sleep disorders (especially insomnia), and muscle weakness (39). Regrettably, novel treatment options exist for this disease such as Givosiran, proving effective for the treatment and prevention of acute porphyria attacks (40). But many patients are deprived of these benefits due to a lack of diagnosis. Indeed, the diagnosis of AHP, and especially acute attacks, requires a comprehensive approach that involves:

- Clinical evaluation: due to the non-specific nature of the symptoms, clinical identification of the attacks can be challenging. It usually involves a thorough clinical history review in search of recurrent symptoms with repeated, frequently non-diagnostic medical evaluations. The disease is also most frequent in young women and family history should be explored (35).

- Laboratory testing: However due to the lack of specificity, clinical evaluation alone is not suited to identify acute attacks. There is a medical consensus that AHP attacks are characterized by an accumulation and a consequent increased urinary excretion of 2 non-porphyrin precursors δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). The increase of the 2 biomarkers, and especially PBG, is a clear sign of an acute attack. Furthermore, the increase in Total Urinary Porphyrins (TUP) are of great diagnostic importance for the presence of overall porphyrias (41).
- Genetic analysis: Once a diagnosis of AHP is biochemically confirmed, gene sequencing is required to identify the mutation in an index case and to determine the specific AHP (42).

Objectif and approach

The gold-standard method for diagnosing acute attacks of AHP involves the measurement of porphyrin precursors and related metabolites during an acute episode. Namely, the quantitative estimation of the urine PBG and ALA which plays a crucial role in the identification of the disease. The associated laboratory methods include column chromatography with spectrophotometry or mass spectrometry.
The measure of these 2 analytes must be completed by quantification of porphyrins with methods including High-Performance Liquid Chromatography (HPLC) with fluorescence detection or mass spectrometry (43) (44). It's important to collect samples during the acute attack as levels of these metabolites may be lower or normal between episodes (45).

In this paper we aim to compare the use a new and innovative *in vitro* diagnostic medical device for the biological measurement of biomarkers essential in the detection of porphyrias and AHP, namely:

- Total Urinary Porphyrins;
- Porphobilinogen.

This innovative device will enable healthcare professionals to easily and accurately measure the relevant biomarkers crucial for early diagnosis and effective monitoring of these diseases.

Material and Methods

Description of the approach

In this study, we will compare a novel approach based on an innovative *in vitro* diagnostic medical device for measuring 2 key biomarkers:

- Porphobilinogen;
- Total urinary porphyrins.

The quantitative levels of these 2 biomarkers will be challenged against the gold standard methods. This new approach aimed to provide a faster, more accessible and point-of-care alternative for assessing these biomarkers in the diagnosis of AHP during acute attacks.
The gold standard method used in clinical practice, involved labor-intensive laboratory procedures, specialized equipment, and trained personnel. We aim to provide analytical data to show that a miniaturized portable medical device is able to give back similar results.

Description of the Usense's JIMINI

Description of the hardware and technologies

Our medical device represents an innovative breakthrough in the field of urinalysis. The device utilizes a computer-implemented method for measuring biomarkers in urine samples, offering a rapid and cost-effective solution for assessing various health parameters.

The hardware incorporates:

- State-of-the-art optical spectrometers and fluorimeters that acquire high-resolution optical spectra of urine samples over a broad range of wavelengths (ranging from infrared to ultraviolet spectrum);
- Conductivity measurement tools that allow the acquisition of electro-analytical data with values ranging from 0 Hz to 1 Hz.

These technologies **Figure 1** are embedded in a small and portable device as displayed in **Figure 2**.

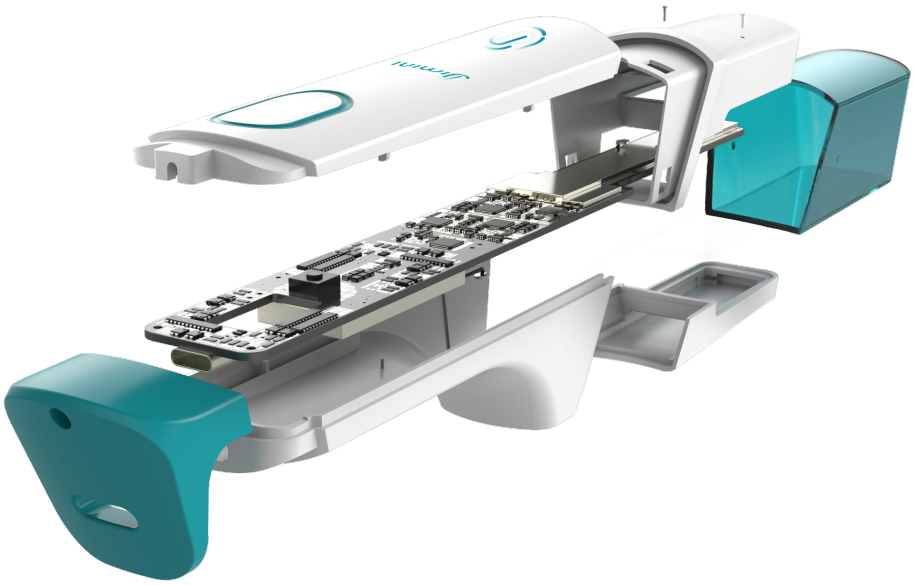

Figure 1: View of the Jimini's technologies

Figure 2: View of the Jimini

These spectra are then processed using advanced algorithms and biomarker models trained on reference data to estimate the values of specific biomarkers. The device provides accurate and real-time results, enabling healthcare professionals to quickly assess the health status of patients. Its portable and user-friendly design allows for point-of-care testing, making it suitable for various healthcare settings. By streamlining the measurement process and eliminating the need for expensive laboratory equipment, our device offers a cost-effective and efficient alternative to traditional methods. With its potential to enhance early diagnosis and facilitate timely interventions, our medical device holds significant promise in improving patient outcomes and revolutionizing urinalysis practices.

Description of the biomarker estimation models

Providing that the number of samples is still limited for more complex methods, we opted for a combination of signal processing and plain statistical modeling.

Total porphyrin estimation

In the case of the total porphyrin estimation, we expect the molecule to fluoresce at a specific frequency, provided a photic stimulation with a wavelength around 405nm. The current model uses the Beer-Lambert law, under which, for a specific wavelength, in an homogenous matrix, the logarithm of the electromagnetic radiation attenuation is linearly related to the compound concentration.
For this model, the absorption spectrum is first normalized by its value at the stimulation frequency, followed by a double derivative Savitzky-Golay filter. The value at 620 nm is then extracted from the spectrum.
Finally, this estimation of the attenuation is fit to the gold standard porphyrin concentration using a k-fold cross validated linear regression.

Porphobilinogen estimation

To estimate the porphobilinogen concentration, we rely on the spectral difference observed in a urine sample before and after a heating procedure. This model follows a procedure similar to the total porphyrin estimation, except that the model is applied on the difference between the two spectra, and the frequency at which we observe the attenuation (490 nm).

Study design

Goal

The objective of the study was to compare the biological results obtained from our device with the results obtained by a gold-standard method in a laboratory setting for 2 biomarkers:

1) Porphobilinogen (PBG);
2) Total Urinary Porphyrins.

We conducted a comparative analysis by collecting samples from a cohort of AHP patients during acute attacks. The samples were analyzed using both the medical device and the gold standard method. Statistical analysis was performed to evaluate the error between the true value calculated by the gold-standards and the predicted value of our device. The findings of

this study will shed light on the clinical utility of the new approach for measuring PBG and total urinary porphyrins.

Localization

The study was conducted in partnership with the “Reference Center for Porphyrias and Rare Anemias of Iron Metabolism” (Centre de référence des porphyries et anémies rares du métabolisme du fer) of the hospital Louis-Mourier in Colombes, FRANCE.

Data collection

The study was performed on 586 records (98 individual adults) for porphyrin estimation, and 1400 records (78 individual adults) for porphobilinogen.

Comparative method principle

Quantification of Total Urinary Porphyrins

The method used for the quantification of total urinary porphyrins is acidification and spectrophotometry. The porphyrins are solubilized by acid which strongly absorb around 400 nm (Soret band). The concentration of porphyrins is calculated based on the peak height relative to the baseline (difference in absorbance) (46).

The material used was a SHIMADZU UV-1800 spectrophotometer.

Quantification of Porphobilinogen

PBG was assessed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Urine samples (700 µL) were vortexed for 30s mixed with 300 µL formic acid (6 M), and centrifuged at 10,000g for 5 min. The supernatant was transferred in a 96-well plate and 5 µL was injected for high-performance liquid chromatographic tandem mass spectrometric analysis. 10 µL mesoporphyrin was used as an internal standard.

Results and Discussion

Results

For this use case, we set specific detection thresholds for total porphyrin (350nMol) and porphobilinogen (50uMol), transforming the problem into a classification task (absence, presence). To facilitate the choice of the algorithmic detection threshold, **figure 3** (center) represents sensitivity, specificity and balanced accuracy against the detection threshold. The product team can then choose the performance most suited for the use case by lowering or increasing the algorithmic threshold to increase sensitivity or specificity. The confusion matrix (right) is calculated for the threshold with the highest balanced accuracy.

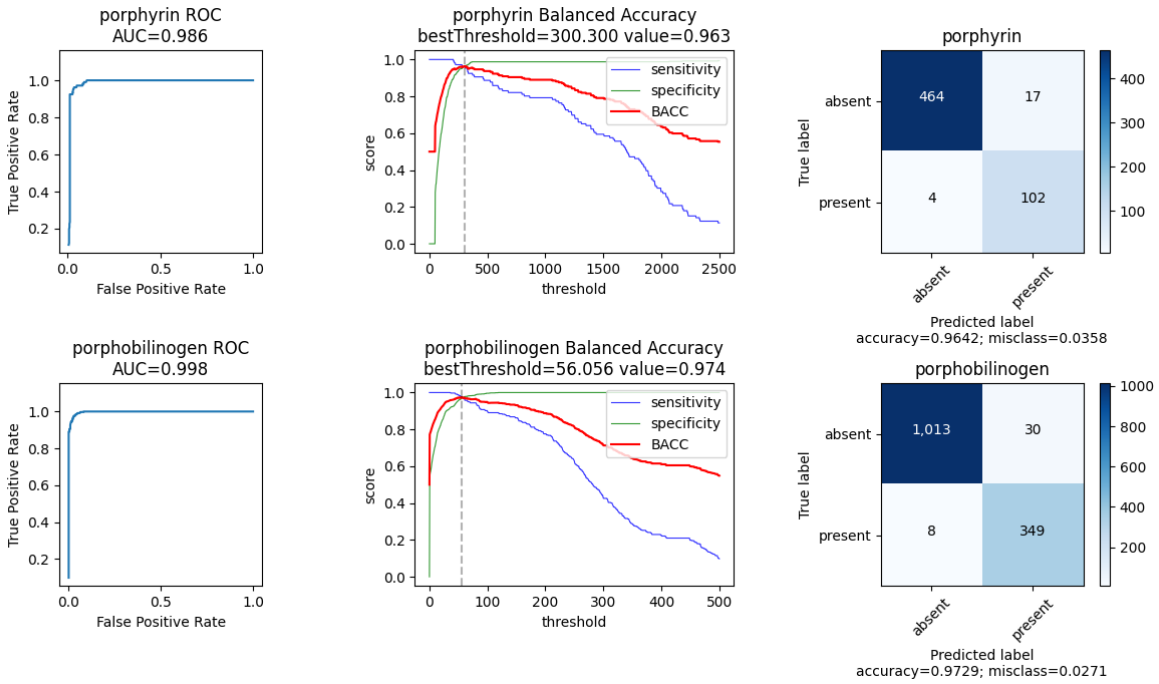

Figure 3: ROC curves, threshold selection curves and confusion matrices for porphyrin (top) and porphobilinogen (bottom)

The results obtained show Jimini's very high specificity and sensitivity for the detection of total porphyrins and PBG. Thus, the presence of porphyrins above the threshold of 350nmol/L, a threshold commonly used in clinical practice, shows a true positive rate of 86% and a true negative rate of 99%. Similarly, the presence of PBG above the 50µmol/L threshold, an AHP crisis threshold, shows a true positive rate of 92% and a true negative rate of 99%.

Discussion

In this study, we focused on the specific condition of AHP within the broader family of genetic disorders known as Porphyrias. Diagnosing these diseases is challenging due to the diverse nature of symptoms that often mimic more common disorders, resulting in missed or delayed diagnosis.

Our study aimed to compare the performance of a novel *in vitro* diagnostic medical device developed against the gold standard methods for detecting two essential biomarkers:

- Porphobilinogen;
- Total Urinary Porphyrins.

The current research was designed as an analytical study to develop and validate point-of-care technology for AHP diagnostics. The results obtained show that the current tool is sufficiently robust to be deployed within health centers and could allow better orientation of certain patients carrying the genetic mutation and above all relieve the reference center by facilitating on-site measurements, at the patient bedside. Moreover, results of our study revealed that our device provides significantly comparable results to the gold standard methods. These findings highlight the reliability and accuracy of our device in detecting these critical biomarkers, thereby demonstrating its potential as a valuable tool in the diagnosis and management of AHP.

The introduction of our innovative device into clinical practice has the potential to universalize the diagnostic pathway for AHP and other rare diseases. The portability and user-friendly design of our device enable healthcare professionals to perform point-of-care testing, facilitating timely diagnosis and interventions. By providing rapid and accurate results, our device streamlines the diagnostic process limiting the very important diagnostic wandering that exists today for these diseases. This device could lead to earlier interventions of existing and efficient treatments (47), improved patient outcomes, and a reduced economic burden on the healthcare systems. Early diagnosis has the potential to improve patient outcomes, prevent disease progression, and reduce healthcare costs associated with delayed or incorrect diagnoses.

The use of this type of medical device in research, clinical studies or screening campaigns in the general population could thus be of major interest. The use of categorical measurements (presence/absence) also facilitates medical interpretation. Compared with Lab gold-standard measurement of total porphyrin and PBG, the JIMINI system reported here is significantly more cost-effective, operable by less-skilled health workers and well suited for point-of-care settings, health clinics and rural areas.

Conclusion

In conclusion, our study demonstrates the promising capabilities of our innovative *in vitro* diagnostic medical device for the measurement of biomarkers in the diagnosis of AHP and overall porphyrias. The device's comparability to gold standard methods, ease of use, and potential for point-of-care testing make it a valuable tool in improving the diagnosis and management of rare diseases.

While we focused in this article on only two specific biomarkers, it's important to note that this is only a first step. Indeed, the described device has the capability to detect many more biomarkers, including those associated with infections, nutrition, hydration, and metabolism. This broad range of biomarkers enables its use in routine healthcare practice. Our ambition is to integrate the screening of these rare genetic diseases into routine analysis, as we believe that the prevalence of these diseases may be much higher than described in the literature. By incorporating the analysis of these biomarkers into routine procedures, we can proactively identify individuals at risk and provide timely interventions, contributing to improved patient care and potentially uncovering a more accurate prevalence rate for these conditions.

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