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Antoine Poli, Hana Manceau, Anvi Laetitia Nguyen, Boualem Moulouel, Nathalie Dessendier, et al.. Quantification of urine and plasma porphyrin precursors using LC–MS in acute hepatic porphyrias: improvement in routine diagnosis and in the monitoring of kidney failure patients. *Clinical Chemistry*, 2023, 69 (10), pp.1186 - 1196. 10.1093/clinchem/hvad117 . cea-04318571

HAL Id: cea-04318571

<https://cea.hal.science/cea-04318571v1>

Submitted on 1 Dec 2023

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Quantification of Urine and Plasma Porphyrin Precursors Using LC–MS in Acute Hepatic Porphyrrias: Improvement in Routine Diagnosis and in the Monitoring of Kidney Failure Patients

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BACKGROUND: The quantification of delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) in urine are the first-line tests for diagnosis and monitoring of acute hepatic porphyrias (AHP). Ion-exchange chromatography (IEC), which is time- and staff-consuming and limited to urine, is still the preferred method in many specialized laboratories, despite the development of mass spectrometry-based methods.

METHODS: We describe a new LC-MS method that allows for rapid and simple quantification of ALA and PBG in urine and plasma with an affordable instrument that was used to analyze 2260 urine samples and 309 blood samples collected in 2 years of routine activity. The results were compared to those obtained with IEC, and urine reference ranges and concentrations in asymptomatic carriers were determined. Plasma concentrations were measured in healthy subjects and subgroups of symptomatic and asymptomatic AHP carriers.

RESULTS: In urine, the clinical decision limits were not impacted by the change of method despite discrepancies in low absolute concentrations, leading to lower normal values. Two-thirds of asymptomatic AHP carriers (with the exception of coproporphyrin carriers) showed an increased urine PBG concentration. Urine and plasma levels showed a good correlation except in patients with kidney disease in whom the urine/plasma ratio was relatively low.

CONCLUSION: We described an LC-MS based method for the routine diagnosis and monitoring of AHP that

allows for the detection of more asymptomatic carriers than the historical method. Blood analysis appears to be particularly relevant for patients with kidney disease, where urine measurement underestimates the increase in ALA and PBG levels.

Introduction

Porphyrias are inherited metabolic diseases caused by a defect in a specific enzyme in the heme synthetic pathway. Three autosomal dominant acute hepatic porphyrias (AHP) are defined by the occurrence of acute neurovisceral attacks: acute intermittent porphyria (AIP), the most frequent, variegata porphyria (VP), and hereditary coproporphyrin (HCP) (1, 2). Acute attacks are characterized by acute autonomic neuropathy with abdominal pain, dysautonomic symptoms, and, in the most severe cases, acute peripheral and central neuropathy (3). Their diagnosis is based on the measurement of 2 biomarkers in urine: delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) (4, 5), which are considered neurotoxic (6). Both metabolites are precursors of porphyrins, the tetrapyrrolic intermediates of heme biosynthesis. ALA results from the condensation of glycine and succinyl-CoA. Two ALA molecules react together to form a PBG molecule. Precursors accumulate strongly in biological fluids during an acute attack of porphyria (AAP) (7). PBG is pathognomonic of acute porphyrias, while ALA concentration can also be increased in lead poisoning (8).

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Received March 8, 2023; accepted July 10, 2023.

<https://doi.org/10.1093/clinchem/hvad117>

Elevated levels of ALA and PBG during AAP are due to hepatic induction of ALA synthase 1 (ALAS1), the first enzyme of the heme synthesis pathway, when heme demand increases. In such circumstances, the activity of the impaired enzyme downstream of ALAS1 in the pathway becomes limiting, leading to an accumulation of the upstream ALA and PBG metabolites (7).

To date, urine ALA and PBG measurements are the front-line tests when AAP is suspected. Historically, measurement was based on the use of a pyrrole-specific reagent, the Ehrlich reagent, allowing detection by colorimetry (9). ALA and PBG are first separated by ion-exchange chromatography (IEC), and then, ALA is converted to a pyrrolic derivative before reacting with Ehrlich reagent. This method is still used in many porphyria reference laboratories around the world but suffers from several limitations. The sensitivity of the method limits its application to urine only, excluding blood samples. This limitation can impact care in situations such as oligo- or anuria or kidney failure which is common in AIP (10).

Over the past 2 decades, liquid chromatography coupled with mass spectrometry (LC-MS) has become widely used in clinical metabolic biochemistry laboratories and has greatly facilitated the development of small-molecule quantitative methods. Several methods of ALA and PBG measurement using LC-MS have been described in the literature (11–14); however, to date, few laboratories have adopted this technology in routine practice (8, 15, 16). Indeed, except in university medical centers, laboratories specializing in porphyria may have limited access to mass spectrometry.

Based on a 2-year experience in the routine medical laboratory at the French Porphyria Centre, we present here a fast, economical, robust, and easy-to-handle method for the measurement of both ALA and PBG in biological fluids, which can be performed on a single quadrupole LC-MS.

Material and Methods

SAMPLES AND PATIENTS

All procedures were conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. Clinical and biological data presented in this article were collected as part of patients' routine care, and all samples were anonymized (online [Supplemental Fig. 1](#)).

Urine. Urine samples ($n = 2260$) were collected over a 2-year period (2020 to 2021) from the routine laboratory of the French Porphyria Centre. Method correlation was assessed using 217 urine samples for which ALA and PBG were measured both by IEC (RECIPE) and LC-MS (see below). In this batch, 40 pathological samples were selected for high PBG ($>9 \mu\text{mol}/\text{mmol}$ creatinine). Reference values were determined on 1565 samples sent to the laboratory for investigation of

possible AAP that did not lead to the diagnosis of porphyria. In asymptomatic porphyria carriers, ALA and PBG levels were determined in 158 urine samples from AIP, 60 from VP, and 14 from HCP. Moreover, 246 urine samples from AIP patients and asymptomatic carriers (AC, patients with an *HMBS* mutation who never had an AAP) were collected along with blood samples.

Blood. For the determination of plasma levels, specimens were taken from 37 healthy donors, 97 asymptomatic AIP carriers, and 116 symptomatic AIP patients. In AIP patients, blood was collected outside of an attack period in 85 AIP patients (104 samples) with a mild phenotype (less than one attack per year) and in 31 with a severe phenotype (at least one attack per year). In the AIP patient group, 30 samples were also collected during an AAP episode. Plasma samples were collected in EDTA tubes (centrifugation $2500\times g$, 10 min at room temperature). Blood samples collected in lithium-heparin, clot activator, and serum gel tubes were also tested during the development phase. All samples collected are summarized in a flowchart ([Supplemental Fig. 1](#)).

REAGENTS

Water and solvent (mass spectrometry, MS, grade) were purchased from Biosolve Chimie. 5-Aminolevulinic acid hydrochloride, 5-aminolevulinic acid- $^{13}\text{C}_5$, ^{15}N hydrochloride, and sulfosalicylic acid (SSA) were purchased from Sigma-Aldrich. Porphobilinogen and porphobilinogen- $^{13}\text{C}_2$ were obtained from Frontier and Isosciences, respectively. The AccQ-Tag Ultra Derivatization Kit Reagent was purchased from Waters.

CALIBRATION AND QUALITY CONTROL

A calibration scale was adapted depending on the biological fluid studied, by diluting stock solutions of ALA and PBG at $2 \text{ mmol}/\text{L}$ in 0.1 M HCl . For the urine assay, the calibration consisted of the following 5 concentrations: 0.5, 1, 5, 100, and $350 \mu\text{mol}/\text{L}$. For blood monitoring, the scale was shifted to lower concentrations: 0.05, 0.1, 0.5, 1, 5, and $25 \mu\text{mol}/\text{L}$. Calibration levels and reagent blanks were diluted in 0.1 M HCl . Calibration curves were built from the instrument response ratios of standards using $1/x$ weighted least-squares regression analysis. Curve fitting was linear for ALA and quadratic for PBG. In urine, 2 levels of Clincheck quality controls (RECIPE) were used: level 1 ALA and PBG (32.3 ; $7.9 \mu\text{mol}/\text{L}$, respectively); level 2 ALA and PBG (142.0 ; $69.7 \mu\text{mol}/\text{L}$, respectively). For plasma, 2 pools of samples were used as control: low normal pool (1.31 ; $0.26 \mu\text{mol}/\text{L}$, for ALA and PBG, respectively) and high pathological pool (13.91 ; $6.75 \mu\text{mol}/\text{L}$ for ALA and PBG, respectively).

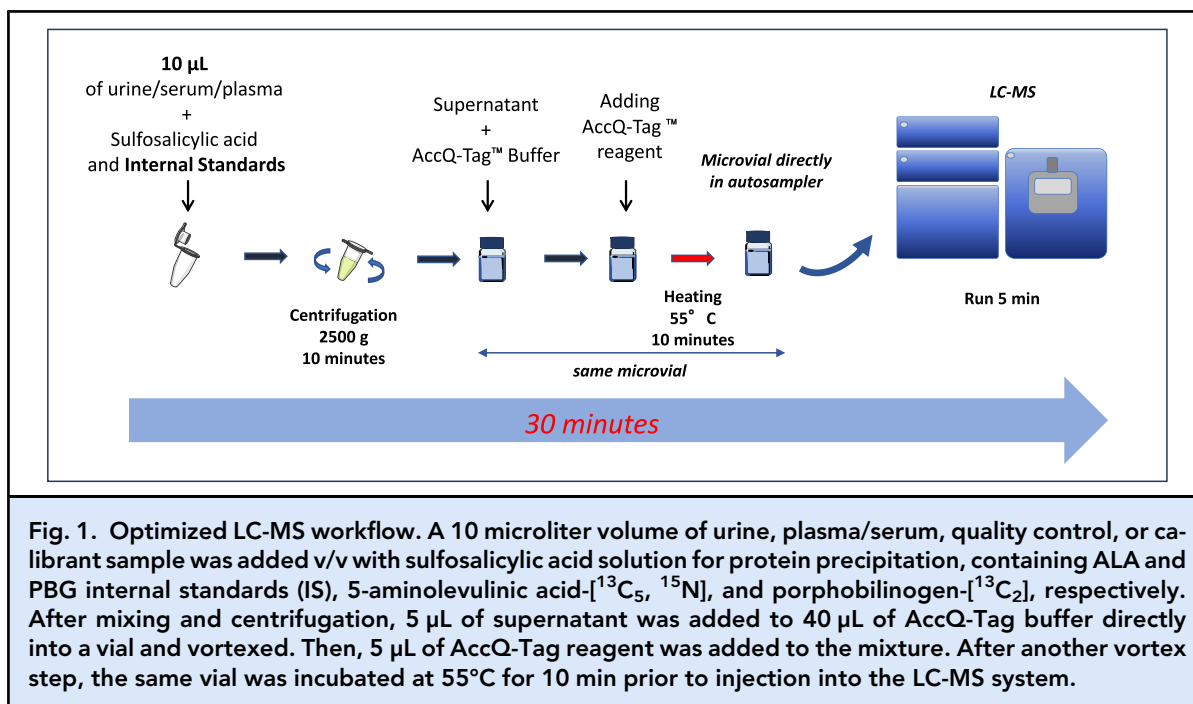


Fig. 1. Optimized LC-MS workflow. A 10 microliter volume of urine, plasma/serum, quality control, or calibrant sample was added v/v with sulfosalicylic acid solution for protein precipitation, containing ALA and PBG internal standards (IS), 5-aminolevulinic acid-[¹³C₅, ¹⁵N], and porphobilinogen-[¹³C₂], respectively. After mixing and centrifugation, 5 µL of supernatant was added to 40 µL of AccQ-Tag buffer directly into a vial and vortexed. Then, 5 µL of AccQ-Tag reagent was added to the mixture. After another vortex step, the same vial was incubated at 55°C for 10 min prior to injection into the LC-MS system.

LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

The method for urine sample analysis was developed and validated on an H-Class PLUS coupled with a single quadrupole QDa instrument (Waters) and compared to the historical IEC method. For the blood assay, both a single quadrupole and a triple quadrupole mass spectrometer Xevo TQ-MS (Waters) were used.

The analytes were separated on a BEH C18 column (2.1 mm × 50 mm) (Waters) containing particles 1.7 µm in diameter at 45 °C. Mobile phases were composed of 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). After injection of 2 µL of sample, a gradient elution program, detailed in the online [Data Supplement](#), was applied at a constant flow rate of 0.5 mL/min for 5 min ([Supplemental Table 1](#)). Derivatized analytes were detected in positive ionization mode by selected ion recording (SIR) or multiple reaction monitoring (MRM) depending on the kind of mass detector (single or triple quadrupole, respectively). The mass spectrometry parameters are reported in online [Supplemental Table 2](#).

STATISTICAL ANALYSIS

Correlations were assessed using a Pearson test. The methods were compared by a Bland–Altman analysis with determination of 95% limits of agreement. Medians or means were compared using a Mann–Whitney nonparametric test or Student parametric *t*-test when possible. Significance was set as two-sided

P < 0.05. All statistical analyses and graphs were performed using Prism 7 (GraphPad software).

Results

DEVELOPMENT AND ANALYTICAL VALIDATION OF ALA AND PBG ASSAYS IN URINE AND PLASMA FOR AAP SCREENING

Pretreatment of urine or plasma samples, to ensure protein precipitation and derivatization, was optimized using the recommendations of the derivatization reagent manufacturer as indicated in [Fig. 1](#), the aim being to achieve sufficient sensitivity and optimize ease of handling. The pipetting volumes, 10 µL and then 5 µL, were optimized to minimize volumes of biofluid and AccQ-Tag reagent, respectively. ALA and PBG concentrations could be easily measured in both urine and plasma within 30 min. This assay was initially developed and validated for urine monitoring on an easy-to-use single quadrupole, the QDa detector (Waters) coupled with an ultra performance liquid chromatography (UPLC). For both ALA and PBG assays, the lower limit of quantification (LLOQ) was determined to be 0.5 µmol/L, and the upper limit of quantification (ULOQ) reached 350 µmol/L (*R*² > 0.99 for ALA and PBG). No matrix effect was found when comparing calibration in urine and in 0.1 M HCl (online [Supplemental Table 3](#)). The limit of detection (LOD) was set at 0.1 µmol/L (signal-to-noise ratio, *S/N* > 3). The linearity range was mainly limited by PBG, which exhibits quadratic behavior over a wider

range. However, this range was sufficient to detect the specific rise in ALA (10-fold) and PBG (100-fold) concentrations in an AAP, both in urine and plasma. Intra-assay CVs were $\leq 4.9\%$ (ALA) and $\leq 4.6\%$ (PBG). Inter-assay CVs reached 3.5% and 4.2%, respectively (online Supplemental Table 4). ALA was stable in urine for 8 days at room temperature or 4°C in darkness or natural light. PBG was equally stable only in darkness at 4°C (online Supplemental Fig. 2).

VALIDATION OF ALA AND PBG ASSAYS FOR AHP FOLLOW-UP IN PLASMA

This LC-MS method allows for the measurement of both biomarkers in different matrixes, such as urine, serum, or plasma. To adapt the analytical scale to plasma monitoring, the calibration was shifted to lower concentrations: 0.05 to 25 $\mu\text{mol/L}$ ($R^2 > 0.99$ for ALA and

PBG). As for urine, serum and plasma did not induce matrix effects (Supplemental Table 3). To achieve sufficient sensitivity and validate the method, plasma tests were performed on a triple quadrupole coupled to UPLC. Inter- and intra-assay CVs, assessed in the same way as urine, were determined to be $\leq 7.06\%$ and 5.70% for ALA and $\leq 4.46\%$ and 11.02% for PBG, respectively (online Supplemental Table 5).

ALA and PBG concentrations of samples collected in lithium-heparin, EDTA, or clot activator tubes were not significantly different from the serum-separated tube (SST) with a gel separator (online Supplemental Fig. 3A and B). Regarding stability in whole blood samples, mimicking the shipping conditions, ALA and PBG levels did not show significant differences after storage at 4°C in the dark for 8 days. At room temperature, PBG and ALA concentrations decreased over time. The median

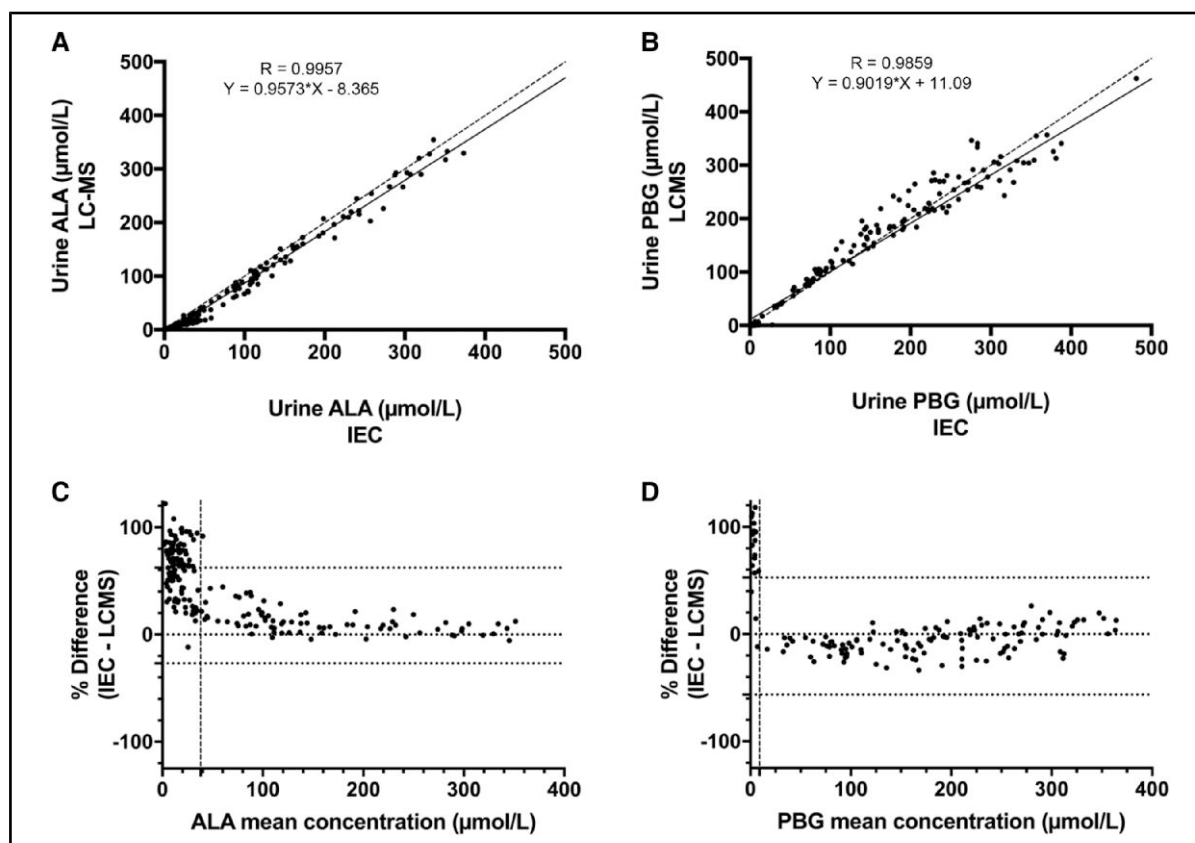


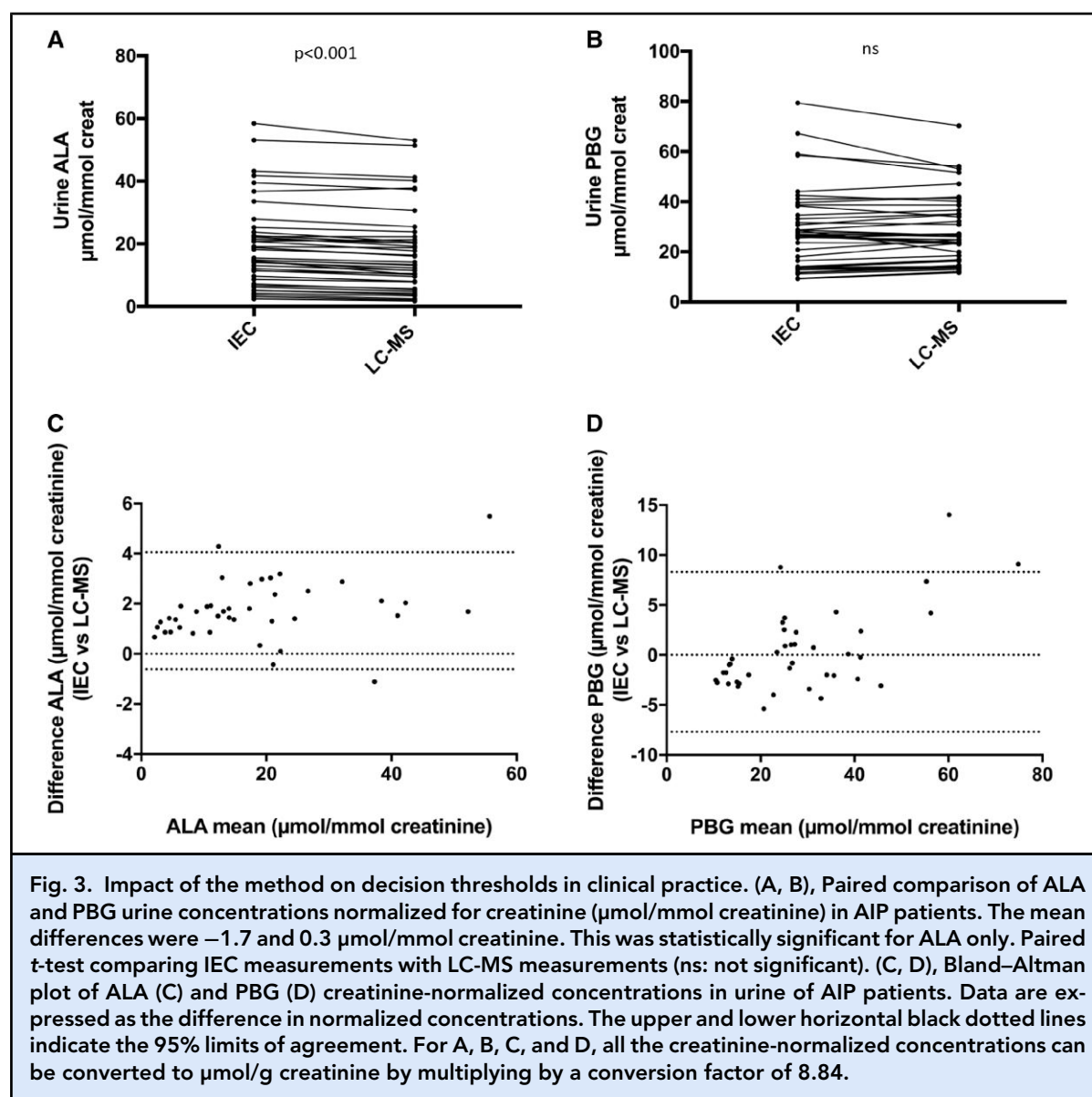
Fig. 2. Methods comparison in urine samples. (A, B), Correlation of urine concentrations of ALA (A) and PBG (B) between LC-MS and IEC. Pearson R and the linear regression equation are given on the graph. The dotted line is the reference line $y = x$. (C, D), Bland–Altman plot of ALA (C) and PBG (D) absolute urine concentrations. Data are expressed as a percentage of the difference between the 2 methods: $100 \times (\text{IEC} - \text{LC-MS}) / (\text{mean of concentrations from each method})$. The vertical dotted lines show the upper reference interval for the IEC method (ALA: 38 $\mu\text{mol/L}$; PBG: 9 $\mu\text{mol/L}$). The upper and lower horizontal black dotted lines indicate the 95% limits of agreement considering only values above the upper reference limit (IEC method).

PBG level reached 75% of the initial concentration on Day 3 with or without light protection. ALA was more stable, as the median level decreased below the threshold of 75% on Day 5 (light exposure) or Day 6 (in the dark) at room temperature (Supplemental Fig. 3C–E).

COMPARISON BETWEEN THE LC-MS AND IEC METHODS IN URINE

The LC-MS assay was compared with the standard IEC assay in urine. Absolute ALA concentrations showed a very high correlation ($R > 0.995$). The correlation was slightly lower for PBG ($R > 0.985$) (Fig. 2A and B). Both slopes of the linear regression were close to 1

(>0.9) with a significant bias (-8.365 for ALA, $+11.09$ for PBG). The Bland–Altman diagram highlighted the limitations of the correlations between the 2 methods. For ALA and PBG, very low concentrations were highly discordant with concentrations determined by IEC, reaching more than double the mean concentration (Fig. 2C and D). This deviation occurred below the upper reference limit of the column test (i.e., ALA: $38 \mu\text{mol/L}$; PBG: $9 \mu\text{mol/L}$) and therefore had no impact on interpretation and clinical management. Beyond these extremely low levels, the differences in concentrations were much smaller and more homogenous across the data set for both parameters.



The impact of the change in method on patient care was assessed based on creatinine normalized concentrations within the clinical interpretation range. ALA and PBG measurements were both used to diagnose an acute attack of porphyria and to monitor symptomatic AHP patients. In patients with urine PBG levels greater than 9 $\mu\text{mol}/\text{mmol}$ creatinine (Cr) (79.6 $\mu\text{mol}/\text{g}$ Cr), PBG concentrations did not show a significant difference, and 90% (36/40) of the differences did not exceed 5 $\mu\text{mol}/\text{mmol}$ Cr (Fig. 3A and B). ALA concentrations were significantly higher with the IEC method, but the 95% limits agreement of Bland–Altman analysis was very close, from -0.61 to 4.00 $\mu\text{mol}/\text{mmol}$ Cr (Fig. 3C and D).

REFERENCE INTERVAL OF HEME PRECURSORS IN URINE IN HEALTHY PEOPLE AND ASYMPTOMATIC CARRIERS

Considering the discrepancies observed in the low-concentration ranges between the 2 methods, we report urine ALA and PBG levels measured by LC-MS in a clinical context in which low values are relevant. The 95% range ALA concentrations from 1565 nonporphyric subjects covered 1.2 to 24 $\mu\text{mol}/\text{L}$ and 0.3 to 2.1 $\mu\text{mol}/\text{mmol}$ Cr (2.7 to 18.6 $\mu\text{mol}/\text{g}$ Cr) (Fig. 4A and B). For PBG, normal values were less than 1.4 $\mu\text{mol}/\text{L}$ and 0.2 $\mu\text{mol}/\text{mmol}$ Cr (1.8 $\mu\text{mol}/\text{g}$ Cr), without a detectable lower limit. In AIP AC ($n = 158$), ALA concentrations ranged from 1.68 to 451 $\mu\text{mol}/\text{L}$ with a median of 13.72 $\mu\text{mol}/\text{L}$, or 0.48 to 39.42 $\mu\text{mol}/\text{mmol}$ Cr with a median of 1.595. The distributions were similar in VP AC patients ($n = 60$), with ALA median of 16.6 $\mu\text{mol}/\text{L}$ [1.94–155.4] or 1.68 $\mu\text{mol}/\text{mmol}$ Cr [0.448–9.77]. In HCP patients ($n = 14$), the medians were lower than those in VP and AIP AC patients, with no significant difference from control individuals. In the 3 AHP, the median ALA concentrations of AC subjects were under the upper limit of normal (95%). Urine PBG levels were widely dispersed with a median and range of 5.90 $\mu\text{mol}/\text{L}$ [0–478.3] and 0.64 $\mu\text{mol}/\text{mmol}$ Cr [0–35.5] in AIP patients, 4.27 $\mu\text{mol}/\text{L}$ [0–151.3] and 0.36 $\mu\text{mol}/\text{mmol}$ Cr [0–21.4] in VP patients, and 0 $\mu\text{mol}/\text{L}$ [0–100.5] and 0 $\mu\text{mol}/\text{mmol}$ Cr [0–6.6] in HCP patients (Fig. 4A and B). In contrast to ALA, the median PBG concentrations were above the upper limit of normal, meaning that the majority of AC (65% of AIP, 68% for VP) exhibited high PBG concentrations. For comparison, 181 PBGs measured in AC by IEC during 2018 to 2019 showed that only 21% of the results were above the reference value. PBG levels in HCP carriers were lower, but significantly higher than those in controls (Fig. 4C and D).

ALA AND PBG PLASMA LEVELS: IMPACT OF CKD

Urine and plasma were collected simultaneously from AIP patients. The concentrations in the 2 fluids were

positively correlated with R at 0.78 and 0.70 for ALA and PBG, respectively (Fig. 5A and B). However, the presence of many outliers highlighted the limitations of these correlations. In Fig. 5C, the concentration ratio of ALA and PBG urine/plasma (U/P) is shown according to the stage of chronic kidney disease (CKD) using kidney disease improval global outcome (KDIGO) criteria (17). These results revealed a decrease in the U/P when the glomerular filtration rate drops. With normal renal function, the ratios were 10 and 8 for ALA and PBG, respectively. The decrease was significant from CKD stage 3, with a ratio of approximately 5. These ratios fell below 2 at CKD stages 4/5. Thus, the U/P could be used as a conversion factor (CF) to express a urine equivalent of a plasma concentration in normal renal conditions (glomerular renal function >90 mL/min/1.73 m^2) according to the formula [Urine] = CF \times [Plasma] (Fig. 5D). These results demonstrate the underestimation of heme precursor levels in CKD porphyria patients when measured in urine.

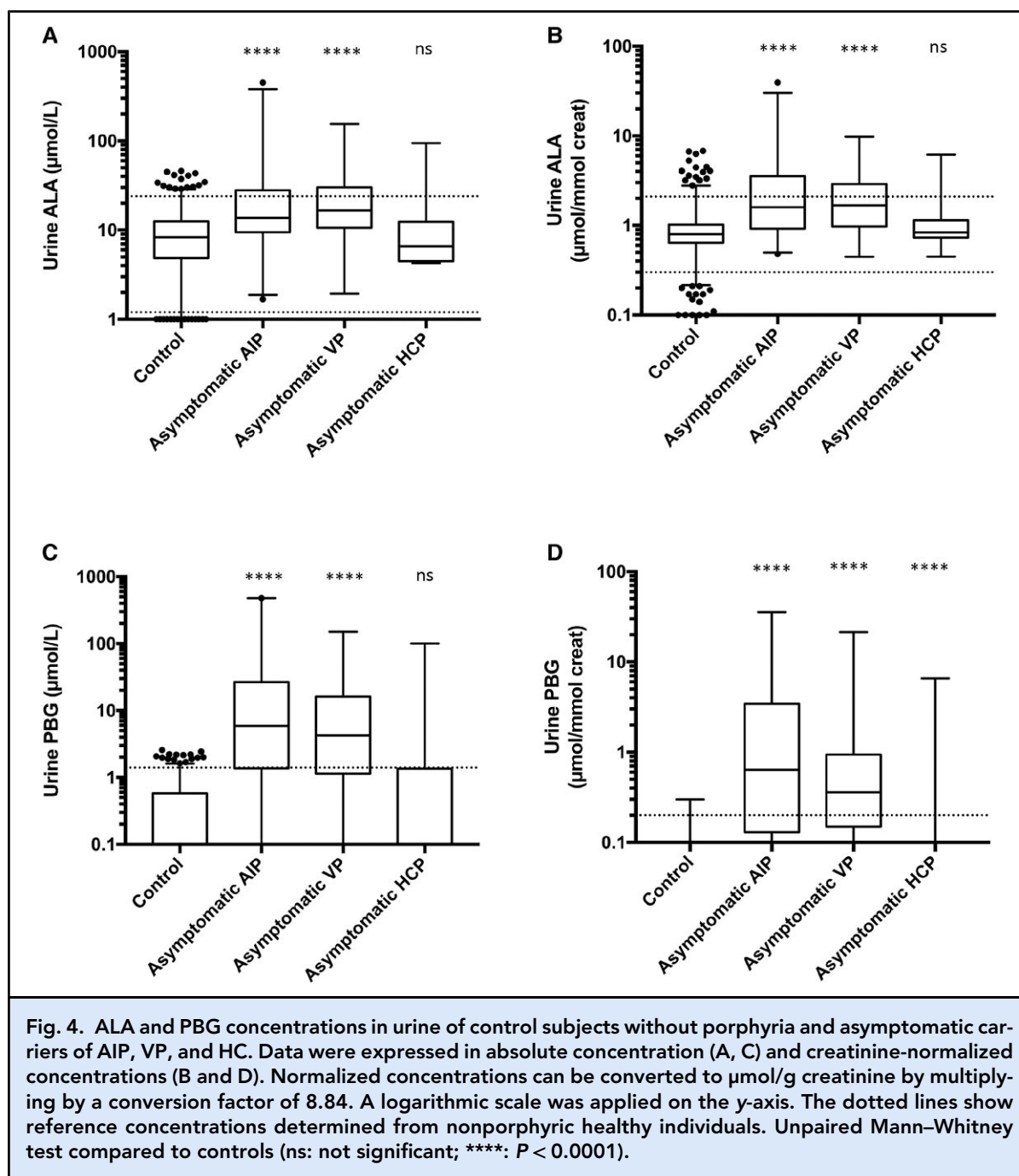
IN AIP PATIENTS, PLASMA LEVELS OF ALA AND PBG IN THE ASYMPTOMATIC PERIOD DEPEND ON THE SEVERITY OF THE DISEASE

In healthy individuals, the plasma concentration of ALA ranged from 0.05 $\mu\text{mol}/\text{L}$ to 0.14 $\mu\text{mol}/\text{L}$ with a median of 0.08 $\mu\text{mol}/\text{L}$. The reference range of 95% of the cohort was from 0.05 to 0.13 $\mu\text{mol}/\text{L}$. There was no detectable PBG in plasma in this control group (Fig. 6).

In patients, plasma ALA concentrations were significantly different between each AIP subgroup studied. The median ALA and PBG concentrations increased with the severity of AIP clinical presentation, with values of 0.38, 1.03, 2.34, and 4.14 $\mu\text{mol}/\text{L}$ (ALA) and 0.80, 2.42, 5.19 and 6.87 $\mu\text{mol}/\text{L}$ (PBG) for AC, mild patients, severe patients, and during an acute attack, respectively (Fig. 6). Unlike ALA, the difference in PBG concentrations between groups was not consistently significant, notably between AC and control on the one hand and severe patients and acute attack on the other hand. Furthermore, we noted that only 61% of AC had PBG concentrations above the upper limit of the normal range.

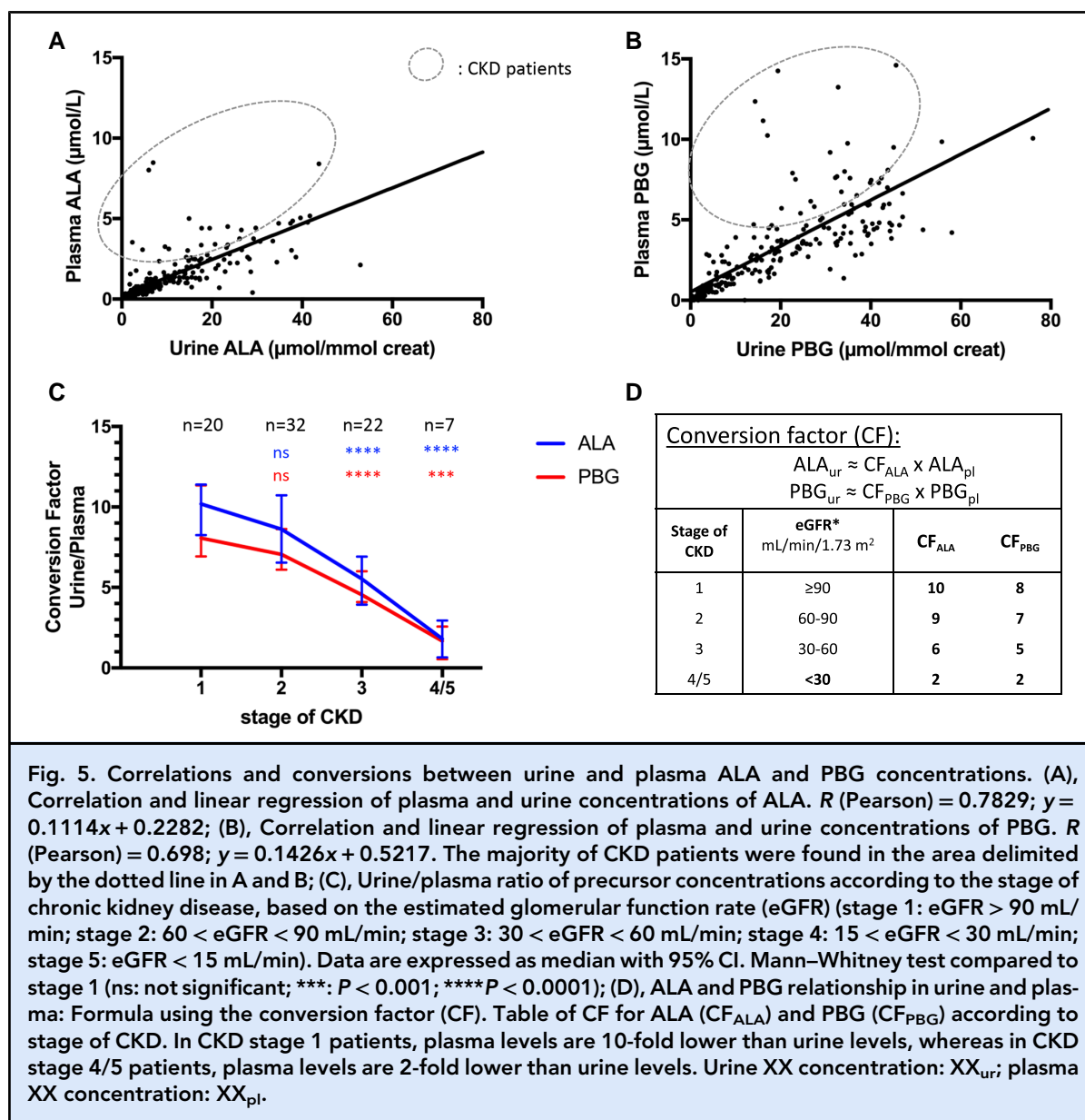
Discussion

Here, we present a rapid and semi-automated method to measure ALA and PBG to meet the daily routine analytical needs of a clinical laboratory. Previously described LC-MS assays highlighted sensitivity and ability to be performed on plasma, but routine clinical laboratory considerations were not taken into account. For



instance, adding a solid-phase extraction step prior to analysis greatly improves sensitivity but makes sample preparation more complex and not suitable for emergency hospital use (12, 18). Hydrophilic interaction liquid chromatography (HILIC) has also been promoted, allowing for the retention of highly polar ALA (13). During our method development, the trade-off between ease of handling and analytical performance led us

to optimize sample preparation using a protein precipitation step followed by fast but robust derivatization. Thus, ALA can be retained on a conventional reversed-phase separation while maintaining a high level of sensitivity by increasing ionization efficiency. This allowed for the elimination of a time-consuming extraction or classical derivatization (e.g., butylation), including a drying and incubation step. In addition, the coupling



of the UPLC and the easy-to-use MS detector increased the speed of analysis, automation, and connection to the laboratory information management system (LIMS). Finally, this method can be combined with amino acid profiles for which the derivatization process was initially developed (19, 20). In previously published methods, a better sensitivity has been described in urine (e.g., LLOQ = 0.044 $\mu\text{mol/L}$ in Agarwal et al.), but the linearity range of our method was suitable for use in clinical practice, both in urine and plasma samples (18).

Comparison with the historical IEC method showed a strong correlation at clinically relevant concentrations

but displayed discordance at low normal concentrations. This could be explained by the lack of specificity in the Mauzerall and Granick method (9). The switch in method had no impact on the diagnosis and follow-up of patients because concentration differences were negligible. However, this divergence in the lower levels shifted the lower reference interval downwards. Reference concentrations were mainly determined from nonporphyric patients. This reference cohort may appear to be different from healthy volunteers used in other studies. However, in the other published methods, the urine ALA concentrations ranged from 0.152 to 2.30 (18), 0.09 to 3.0 (14), and below 2.6 $\mu\text{mol/mmol Cr}$ (13), and the PBG

AIP patients when using urine measurement. To assess the exposure of the nervous system to ALA and PBG toxicity, the metabolite levels in blood may be more relevant than those in urine, especially in CKD patients. The prevalence of CKD, defined as eGFR < 60 mL/min per 1.73 m², in AIP patients was estimated to be 59% compared to 11% in AC patients (23). Thus, the measurement of circulating ALA and PBG is underestimated in more than half of AIP patients. In addition, during AAP, the occurrence of acute renal failure caused by ALA and PBG toxicity has been suggested (10). Therefore, screening for AAP could be impacted by underestimated precursor exposure. In the case of CKD complicated by acute renal failure during AAP, the usual threshold in urine defining an acute attack would become completely unreliable, and the efficacy of heme treatment would be more difficult to assess. As serum sodium levels must be monitored in AAP, measurements of ALA and PBG concentrations can be added easily to the same blood sample (4).

Finally, the availability of a new treatment, Givosiran, based on an interfering RNA approach, generates a new requirement for plasma monitoring in the follow-up of treated AHP patients (24). Several patients receiving Givosiran treatment presented with side effects undetected during the phase 3 study (25). These findings led to spacing of the injection based on the individual evaluation of heme precursor concentrations. Plasma monitoring would be more appropriate in those patients treated with Givosiran who frequently have impaired renal function (26). These plasma assays hence contribute to the development of a personalized medicine approach.

The diagnosis and follow-up of AHP can benefit from the spread of mass spectrometry observed in clinical laboratories over the last 20 years. This easy-to-handle and fast LC-MS/MS method, requiring a small sample volume, meets the needs of ALA and PBG assays, which must be performed routinely and often urgently. This method is compatible with amino acid profiles performed on many metabolomic platforms. In addition, mass spectrometry enables plasma testing, allowing a single sample to be used for multiple tests, as well as quantifying ALA and PBG in CKD and oligoanuric patients. The specific ALA and PBG measurements in plasma can be used as a complementary tool in the follow-up and personalized monitoring of severe patients for whom new therapies, such as Givosiran, are indicated.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: ALA, delta-aminolevulinic acid; PBG, porphobilinogen; AHP, acute hepatic porphyrias; IEC, ion-exchange chromatography; AIP, acute intermittent porphyria; VP, variegata porphyria; HCP, hereditary coproporphyria; AAP, acute attack of porphyria; AC, asymptomatic carriers; Cr, creatinine; U/P, urine/plasma; CKD, chronic kidney disease; CF, conversion factor.

Human Gene: *HMB*S, hydroxymethylbilane synthase.

Author Contributions: *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

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Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form.*

Research Funding: INSERM and Paris Diderot University, the Laboratory of Excellence, GR-Ex, Paris, France, supported this work. The labex GR-Ex, reference ANR-11-LABX-0051 is funded by the program "Investissements d'avenir" of the French National Research Agency, reference ANR-11-IDEX-0005-02.

Disclosures: *No authors declared any potential conflicts of interest.*

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: We would like to thank the HIROS INSERM Unit for methodological assistance and the CFP staff for their excellent

technical work.

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