Clinical Nutrition 36 (2017) 186–190

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Validation of a point-of-care instrument for bedside glutamine screening in the intensive care unit

Linn Pettersson*, Sara Rydén, Marie Smedberg, Inga Tjäder, Olav Rooyackers, Jan Wernerman

Department of Anesthesiology and Intensive Care Medicine, K32, Karolinska University Hospital Huddinge, Karolinska Institutet, 141 86 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 19 December 2014
Accepted 21 October 2015

Keywords:
Critical illness
Plasma glutamine
High pressure liquid chromatography
Enzymatic assay
Nutrition
Bland–Altman plot

SUMMARY

Background: A point-of-care instrument developed for measuring glutamine levels in cell cultures was validated for bedside use in the ICU setting and compared with a standard HPLC technique to measure plasma glutamine. The aim was to evaluate the instrument for absolute measurements and for screening purposes.

Methods: Consecutive blood samples were obtained from one hundred adult ICU patients 3–5 days apart during their ICU stay. Each sample was divided into 3 aliquots, out of which two were used for analyses of plasma and whole blood glutamine by the point-of-care instrument, and one was used for analysis of plasma glutamine concentration by the gold standard HPLC technique. Comparisons were performed by Bland–Altman analyses.

Results: Comparison of the initial plasma sample of each subject (n = 100), between the point of care instrument and HPLC analysis revealed a systematic bias of −221 μmol/L. Comparisons between plasma and whole blood on the point-of-care instrument revealed comparable results. After pragmatic adjustments for the measured bias and hematocrit, whole blood analyses during ICU stay (n = 316) compared with HPLC plasma analyses showed a line of identity of −34 μmol/L and limits of agreement between 288 and -355 μmol/L.

Conclusion: When compared to the HPLC gold standard in particular, the lines of agreement indicate that the point-of-care instrument is not suitable for quantitative plasma or whole blood glutamine concentration measurements. For screening purposes the instrument may be useful in order to identify patients with hypoglutaminemia and hyperglutaminemia and the tested accuracy was high enough for safe supplementation of glutamine to patients with low plasma values measured with the device. The point-of-care instrument may also serve as a screening tool for scientific studies.

1. Introduction

Glutamine supplementation to critically ill patients has been a promising concept. There is a good physiological rationale in that glutamine availability is critical for rapidly dividing cells like enterocytes and immune cells, whose functions are believed to be important during critical illness. Moreover, the observation that a low plasma glutamine at ICU admittance is associated with an unfavourable outcome [1,2], has led to the hypothesis that glutamine supplementation in critical illness possibly could have positive effects on the ICU clientele. Simultaneously when hyperglutaminemia is present, beneficial effects of further supplementation is not to be expected and may be even harmful.

A large number of studies on glutamine supplementation have demonstrated that glutamine appears to reduce mortality, infectious complications and ICU as well as hospital length of stay. Although heterogeneous with respect to patient selection, glutamine dosage and length of treatment, meta-analyses of those studies have shown beneficial effects that have led to a recommendation for glutamine supplementation in current guidelines [3–5]. However, a recent large multicentre study has demonstrated possible harm from high doses of glutamine [5,6].

It is unfortunate that the initial hypothesis, that critically ill patients with hypoglutaminemia may benefit from glutamine

* Corresponding author. Department of Anesthesiology and Intensive Care Medicine, K32, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. Tel.: +46 858580000, +46 768008984.
E-mail address: linn.pettersson@karolinska.se (L. Pettersson).

http://dx.doi.org/10.1016/j.clnu.2015.10.008
0261-5614/

supplementation, never has been properly tested. However, future studies that aim to address this specific question will have to utilize a fast and preferably bedside method for glutamine analysis.

The gold standard for determining plasma glutamine concentration is by HPLC (high pressure liquid chromatography). This is the technique most commonly used by accredited hospital laboratories. HPLC is time-consuming and therefore it is usually not possible to determine plasma glutamine concentration within a clinically relevant time frame. Enzymatic assays, that are possible to include in automated systems for analysis, have thus far not been adapted to the automated instruments used by clinical chemistries for emergency analyses.

On the other hand, there are commercially available point-of-care (POC) instruments for measurement of glutamine concentration designed for use in laboratories working with cell cultures. In cell culture media it is often desirable to have a glutamine concentration of 3–5 mmol/L. Hence the instruments are designed to give accuracy in a cell culture medium and within that interval.

In order to assess the usefulness of a POC instrument for bedside plasma glutamine analysis in critically ill patients we performed a validation study comparing the POC analyzer with the gold standard HPLC for analyses of plasma and whole blood glutamine. The aim was to validate the POC device for glutamine screening purposes in the ICU as well as for measuring absolute plasma glutamine values.

1.1. Patients and methods

Adult patients (>18 years of age) admitted to the general ICU of the Karolinska University Hospital Huddinge were eligible for inclusion. Exclusion criteria were absence of informed consent, readmission after having been included in the study at an earlier stage or ICU discharge prior to screening. The ICU serves the entire hospital with medical and surgical admissions, including transplantations, hematological and infectious diseases, but excluding traumas, burns, neurosurgery and cardiac surgery. The study protocol was approved by the Regional Ethical Review Board in Stockholm. Before obtaining written informed consent, patients (or next of kin), were informed verbally and in writing about the study and possible risks involved.

After consent a first sample of 10 mL was obtained in an EDTA tube. It was divided into 3 aliquots: out of which two were immediately used for analyses of plasma and whole blood glutamine by the POC instrument and the last plasma was frozen at −80 °C for not more than 6 months, pending HPLC analysis. This procedure was repeated every 3rd to 5th days until ICU discharge. Samples were obtained via arterial lines when existing, otherwise via central venous lines.

During the study period (May 2013–November 2013), IV glutamine supplementation was included in the nutritional protocol of the ICU. Supplementation was added to the nutrition given to all adult patients, (except for patients with liver insufficiency), at the time-point when 50% of the caloric target was reached. The supplementation was given as a continuous IV infusion for 24 h every day.

The POC instrument (Bioprofile Basic, NOVA Biomedical, Waltham, Massachusetts, U.S.A.) extracts a 500 μL aliquot from any liquid sample presented. The liquid will be exposed to two membranes. The first one converts all glutamate to H_2O_2 by way of glutamate oxidase and the end product is then quantified by electrochemical detection. The second membrane contains both glutaminase and glutamate oxidase which degrades all glutamine to glutamate and the total glutamate (from both glutamine and glutamate) is detected from the H_2O_2 formation. The instruments' software will give glutamate and glutamine concentrations on a digital display, where the glutamine will be the total glutamate minus the initial glutamate content. The instrument only analyzes the extracellular fluid of the sample, and therefore whole blood measurements will have to be adjusted for the actual hematocrit. The device is automatically calibrated against 2 glutamine standards every 4 h and manually tested with 2 quality controls daily. The instrument maintenance includes changing reagents every 2nd week, changing membranes every 2nd month and changing sensors twice a year. In the instrument specification the manufacturers claim linearity in the range 200–6000 μmol/L, which we repeatedly validated in the lower part of that range as described below. Plasma glutamine analyses were done using an HPLC technique including an on column derivatization with ortho-phtaldialdehyde/3-mercaptopropionic acid (OPA/3-MPA) as described before [7,8].

Before starting the study protocol we performed standard curves of physiological concentrations of high quality glutamine (USP reference standard, Sigma–Aldrich) dissolved in PBS, (phosphate buffered saline), (Fig. 1). In order to elucidate recovery of glutamine on the POC device we spiked plasma obtained from a healthy subject with a known amount of glutamine standard (385 μmol/L). Both the un-spiked and spiked plasma were analysed 8 times. The un-spiked plasma had a concentration of 474 ± 24 μmol/L and the spiked plasma 849 ± 21 μmol/L, yielding a recovery of 97%. In addition, these results showed a coefficient of variation of the POC instrument of 5.0% and 2.4% in the un-spiked and spiked plasma samples, respectively. Repeated measurements (n = 8) of a whole blood sample gave a coefficient of variation of 21%.

From the known variability in plasma glutamine concentration at ICU admission [2] we chose to include not less than 100 patients. The pre-testing of the instrument was evaluated by linearity and recovery. The comparison of different samples and analyses were done by Bland–Altman plots defining lines of identity and limits of agreement (±2 standard deviations). All calculations were made with the Microsoft Office Excel 2007 program.

2. Results

The included patients (n = 100) had a median age of 64 years (range 19–88), 58% were male and their median SAPS III (Simplified Acute Physiology Score) at admission was 61 (range 32–98). Some patients were sampled repeatedly during their ICU stay, resulting in a total of 316 collected samples. As stated above, intravenous glutamine supplementation was sometimes given at sampling.

Plasma glutamine concentration values from first samples of all patients (n = 100) analysed by the POC instrument and by HPLC are

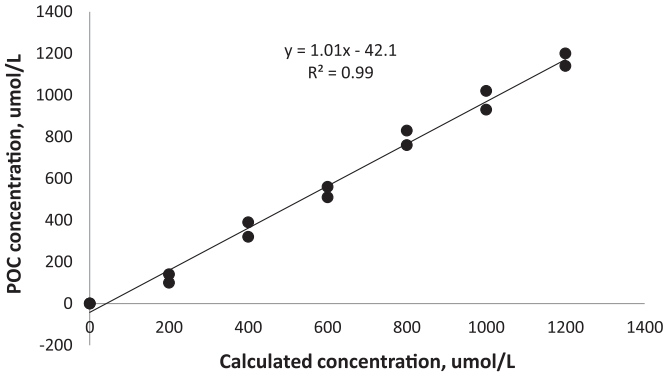

Fig. 1. A dilution series where crystalline glutamine was dissolved in PBS and analysed by the point-of-care instrument in duplicate is illustrated, showing good linearity in the physiological range.