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ORIGINAL ARTICLE

Whole blood samples for adrenocorticotrophic hormone measurement can be stored at room temperature for 4 hours

Mette Christensen[a,b], Rikke Fogt Madsen[a,b], Line Rosengreen Møller[a,b], Cindy Soendersoe Knudsen[b] and Mie Hessellund Samson[b]

[a]Faculty of Health Science, VIA University College, Aarhus, Denmark; [b]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark

ABSTRACT

Introduction: The aim of this study was to investigate and compare the stability of adrenocorticotrophic hormone (ACTH) in whole blood stored on ice and at room temperature for up to 48 hours. This study differs from previous studies by a larger data material.

Materials and methods: EDTA-blood samples from 30 patients were collected, aliquoted and stored on ice or at room temperature for 0, 2, 4, 24, or 48 h before centrifugation, and the plasma was stored frozen until analysis. All samples were analyzed using an automated electrochemiluminescence immunoassay on cobas 6000 e601. The change in ACTH concentration was illustrated as ACTH recovery compared to standard conditions defined as samples stored immediately on ice, centrifuged and plasma frozen within 1 h. A change in ACTH concentration of more than 10% was considered to be of clinical relevance.

Results: The results showed no clinically relevant change in ACTH recovery for up to 4 h compared to standard conditions. For samples stored at room temperature for 4 h, a significant ($p < .0001$) relative mean change in ACTH concentrations of −4.3% was observed.

Conclusion: The comparison between samples stored at room temperature for up to 4 h and standard conditions showed that ACTH samples do not require cooling until centrifugation, if a mean difference in ACTH concentration of −4.3%, between the individual results, can be accepted.

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Introduction

Adrenocorticotrophic hormone (ACTH) is a stress hormone, which is synthesized and released by the pituitary gland. ACTH stimulates the production and release of steroid hormones, including cortisol. The measurement of plasma ACTH is a key step in making differential diagnoses in patients with hypothalamic-pituitary-adrenal disorders. ACTH is usually measured along with cortisol [1].

Pre-analytical factors influence measured concentrations of ACTH, as ACTH is unstable in whole blood due to proteolytic degradation [1]. Therefore, storage of the blood samples on ice until centrifugation is recommended by the kit manufacturer (Roche Diagnostics, Mannheim, Germany). However, previous studies have indicated that ACTH in whole blood might be stabile for up to at least 4 h at room temperature (RT) [2–4], which suggests that it might be unnecessary to store the samples on ice, if the samples are analyzed or the plasma frozen within 4 h. This would simplify the pre-analytical procedure, and provide a longer time period for collection and transport of ACTH blood samples prior to analysis. The present study aimed to expand the knowledge regarding the stability of ACTH as previous studies only included few samples (range from 10–22) [2–6].

We examined the change in ACTH concentration after 0, 2, 4, 24 and 48 h on ice and at RT. The time-points were chosen to cover desired collection procedures in clinical practice and to confirm that concentration of ACTH declines over time. Furthermore, the imprecision in the low end of the measurement range was examined, as also recommended by Talbot et al. [1]. EDTA plasma was used in this study, since this is the preferred material in the clinical laboratory, and because Evans et al. [7] demonstrated a better ACTH stability at higher temperatures in plasma compared to serum.

Materials and methods

Collection of samples

EDTA blood was obtained from patients having samples of blood taken for other reasons, following the guidelines of the department regarding collection of blood for quality evaluation purposes. No personal identifying information was obtained. The study was performed according to the hospital ethics guidelines, and in accordance with the ethical standards of the Central Denmark Region Committees on Biomedical Research Ethics and the Helsinki Declaration.

CONTACT Rikke Fogt Madsen rikke_fogt@icloud.com Department of Clinical Biochemistry, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark

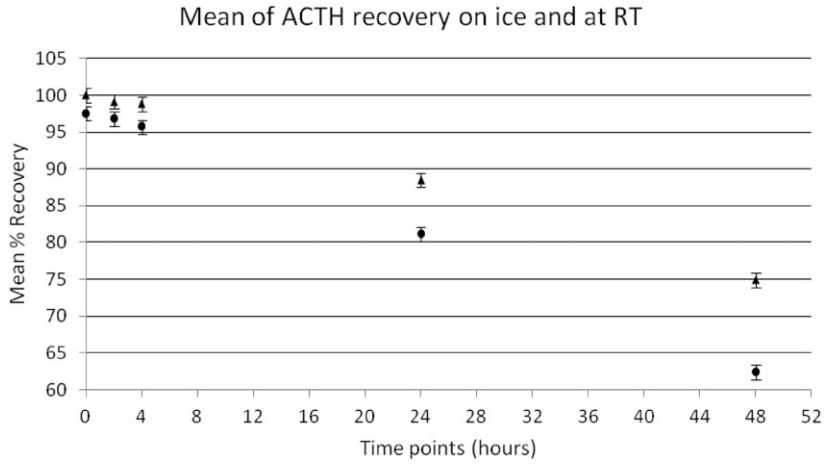

Figure 1. Mean % recovery from standard conditions as a function of time. The triangles and the circles illustrate the mean recovery of ACTH concentrations stored on ice and at RT, respectively. The results are expressed as mean ± SEM.

Blood samples were collected from 30 patients. In an attempt to cover a broad range of ACTH concentrations, samples were obtained from both patients suspected of diseases related to the hypothalamic-pituitary-adrenal axis ($n = 6$) as well as patients suspected of other diseases ($n = 24$).

A total of 60 mL K_2EDTA blood was collected from each patient; 30 mL were stored on ice (ice and water) immediately after collection, and the other 30 mL were stored at RT. Ten mL of the EDTA blood stored on ice and 10 mL stored at RT were centrifuged immediately after sampling (0 h). The remaining 20 mL of both samples were divided into aliquots and centrifuged after 2, 4, 24 and 48 h. The samples were centrifuged at 2300 *g* for 10 min, at 4 °C or at RT for the samples stored on ice or at RT, respectively. Plasma was stored frozen until analysis. In the following, the concentrations of those samples immediately stored on ice, centrifuged and analyzed, are referred to as 'standard conditions'.

To estimate the imprecision of the analysis in the low range of measurement, a plasma pool was collected according to standard conditions and divided into 20 aliquots and stored frozen until analysis.

Measurement

Samples with levels of hemoglobin >0.4 g/dL, of lipid >1500 mg/dL and bilirubin >25 mg/dL were excluded, because hemolysis, lipemia and bilirubinemia above these levels have been demonstrated by the manufacturer to influence ACTH measurements [8].

ACTH was measured in duplicates using an automated electrochemiluminescence immunoassay on cobas 6000 e601 (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. To estimate the imprecision, the aliquots ($n = 20$) were measured over two consecutive days.

Data analysis

A change in ACTH concentration >10% was considered clinically relevant and used as threshold for the evaluation of the stability of ACTH [3,7,9,10]. The stability of ACTH was evaluated relative to standard conditions. To compare the concentrations of the individual samples stored at RT to the concentrations at standard conditions, the difference (%) was calculated and illustrated in a modified Bland-Altman plot. Concentrations of samples stored at RT for 4 h were compared to concentrations at standard conditions with Student's paired *t*-test. The imprecision was expressed as CV%.

Results

Of the 30 samples, 28 had levels of ACTH within the reference range (7.37–63.0 ng/L). One measurement (5.95 ng/L) was below the reference range but within the measurement range. One sample was hemolyzed with hemoglobin >0.4 g/dL and was therefore excluded.

Decreasing concentrations of ACTH were observed for samples stored at both temperatures (Figure 1). The relative decline in the mean recovery after 4 h was 1.2 and 1.8% for samples stored on ice or at RT, respectively.

ACTH levels for samples stored at RT for 4 h were 95.7% compared to standard conditions (Figure 1). After 24 h, this recovery of ACTH had decreased to 88.5 and 81.1% for samples stored on ice or at RT, respectively, thus exceeding the acceptable limit of 10%.

Figure 2 shows, for the individual samples, the difference between the concentration of ACTH at RT and at standard conditions for 4 h. At 2 h (figure not shown) one ACTH sample exceeded the lower limit, and at 4 h, this sample was on the limit. Furthermore, Figure 2 illustrates that relative changes in ACTH concentration were independent of ACTH level.

Comparison of results from RT at 4 h with standard conditions showed a statistical significant difference ($p < .001$). The imprecision (CV%) was calculated to 1.73% at a level of 8.4 ng/L.

Discussion

In this study, the effect of time and temperature on ACTH concentration in whole blood samples was investigated.

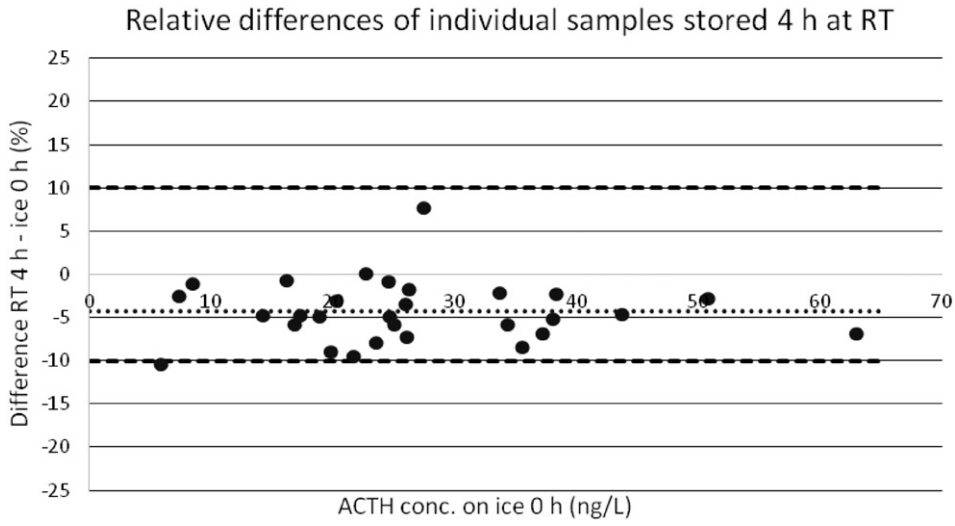

Figure 2. Difference between the concentration of ACTH in samples stored at RT for 4 h and samples analyzed at standard conditions as a function of the concentration at standard conditions. The mean difference between the two conditions is −4.3% at 4 h illustrated by a dotted line. The dashed lines illustrate the limit of clinical relevance (10%).

In spite of the described influence of proteolytic degradation, this study showed that ACTH may be considered stable for up to 4 h at RT, if a mean bias of up to −4.3% can be accepted. A limit of 10% for the acceptable bias is based on clinical considerations and used as limit in several other studies of ACTH [3,7,9,10].

Previous studies on ACTH stability suggests that a larger number of samples are necessary to confirm the findings [5,6]. This study does not only confirm several earlier findings on the stability of ACTH, it also strengthens the conclusions of the findings due to a larger data material.

In this study, 24 and 48 h was included to verify the instability of ACTH. The results from samples stored on ice and at RT after 24 h were outside the limit of 10% (Figure 1), confirming that the proteolytic degradation occurs over time in whole blood. Furthermore, the results also show a greater change in ACTH concentration at RT in comparison with concentration in samples stored on ice after 24 h.

A limitation to this study is the fact, that it includes only few samples with concentrations near the decision limits (reference range: 7.2–63.3 ng/L). But, as the level of ACTH concentration did not seem to influence the stability of ACTH (Figure 2), this does not affect the conclusions of this study.

The imprecision found in this study, that low concentrations of ACTH is considered acceptable, both clinical and by comparison to the imprecisions described by the manufacturer (3.5–5.4%).

The findings of this study are consistent with findings of other studies concerning the stability of ACTH in whole blood [2–4] although the other studies differed slightly in their conclusions. The studies have used different lengths of storage time before centrifugation, and found samples to be stable up to 17.5 h, but none of them showed that ACTH was unstable for less than 4 h [2–4].

Based on this and the findings in previous studies, we find no reason to store blood samples for ACTH measurement on ice, if it is possible to centrifuge the samples and analyze or freeze the plasma within 4 h. Likewise the decrease in ACTH concentration was considered unacceptable after 24 h for samples stored both on ice and at RT due to the 10% limit of clinical relevance. The relative change of ACTH concentration does not seem to be dependent on the concentration level.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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