Impact of collection method and sample handling on measured levels of circulating ACTH

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Introduction: Cortisol, which is produced and released by the adrenal gland under the regulation of the adrenocorticotropic hormone (ACTH), plays a crucial role in the survival mechanisms involved in critical illness. A dissociation between high plasma cortisol and low ACTH in patients has been observed in the more chronic phase of critical illness induced by severe sepsis and multiple trauma [1]. In order to assess the potential confounding impact of errors in the measurement of ACTH, we evaluated the stability of ACTH during sample processing.

Methods: Two tests were performed: 1) two blood samples per patient were taken from 10 randomly selected patients at the surgical ICU. One sample was collected 'warm' (i.e. samples were collected at room temperature (RT), left for 60-90 minutes at RT followed by 24 hours at 4°C, centrifuged (10 minutes, 1000 rpm), and serum was stored at -80°C), the other sample was collected 'cold' (i.e. recommended collection procedure for ACTH detection: samples were collected on ice, centrifuged (10 minutes, 1000 rpm), and plasma was stored at -80°C). 2) For a second test, again, two blood samples were taken per patient (n = 10) at the surgical ICU. One sample was kept frozen until ACTH testing ('original'), while the other sample was defrosted several times at RT before analysis ('defrosted'). ACTH levels were measured using an ACTH RIA kit (BRAHMS) and the different sampling methods and sample treatments were compared using Bland-Altman statistics.

Results: No clinically relevant differences in ACTH levels were observed between 'cold' (28,2 $\pm$ 22,3 pg/ml) and 'warm' (24,3 $\pm$ 20,6 pg/ml) sampling (bias 3,8 $\pm$ 7,3 pg/ml) or between 'original' (22,1 $\pm$ 10,8 pg/ml) and 'defrosted' (22,3 $\pm$ 10,5 pg/ml) samples (bias 0,2 $\pm$ 1,9 pg/ml). Moreover, the differences in ACTH levels measured between the two collection methods or sample treatments were independent of the amount of ACTH present in the samples.

Conclusion: We showed that warm collection or frequent defrosting of samples does not induce a clinically significant error in ACTH levels measured, implying that non-ideally collected or treated samples can still be used for rough ACTH analysis.

References:

1. Vermes et al. J Clin Endocrinol Metab, 1995; 80:1238-42