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Changes in the 24-h plasma cortisol rhythm in patients with cirrhosis

To the Editor:

We read with interest the recent study by Galbois and colleagues [1], which showed that the measurement of total serum cortisol considerably overestimates the prevalence of hepatoadrenal syndrome because the bound fraction of cortisol may be reduced in the presence of cirrhosis, while the more important free cortisol fraction is unchanged. We would like to report on how abnormalities in the 24-h rhythm of cortisol may also confound the diagnosis of adrenal insufficiency in this patient population.

Twenty outpatients with biopsy-proven cirrhosis [mean (±SD) age 59 ± 11 yr; Child's grade A: n = 14, B: 4, C: 2] and nine healthy volunteers (60 ± 14 yr) were studied in a sleep laboratory under light-controlled conditions [2]. Venous blood samples for the measurement of plasma free cortisol (PFC) concentrations were obtained hourly between 18:00 and 10:00 and two-hourly between 10:00 and 18:00. PFC was measured using a radioimmunoassay [3]. Absolute PFC values at 08.00 ('morning cortisol') were obtained for each subject. Means of the *five lowest*/*highest* PFC concentrations over the 24 h were also obtained. The mathematical difference between the mean of the *five lowest* and the *five highest* values, or *range*, was calculated. The *onset* and *offset* of the PFC rhythm were defined as the time points, on the rise and fall of the concentration–time curve, when the plasma concentrations equalled 25% of the peak concentration [4]; the time interval between rhythm *onset* and *offset* was termed peak *duration*.

Visual analysis of the 24-h PFC concentration–time curves (Fig. 1) revealed a morning 'double-peak' in both the healthy volunteers and the patients with cirrhosis. However, the timing of the PFC peak in the patients was considerably delayed (Fig. 1). This feature was more prominent in patients with Child's B/C cirrhosis (Fig. 2).

No differences were observed in mean PFC values at 08:00 between patients and healthy volunteers (212 ± 102 vs. 171 ± 67 nmol/L, p = 0.29), most likely reflecting the shape of the PFC concentration–time curves and the considerable variability of cortisol concentrations over the morning hours (Fig. 1). Similarly, no significant differences were observed in the average *five lowest/highest* PFC concentrations (51 ± 19 vs. 53 ± 12 nmol/L, p = 0.60; 248 ± 76 vs. 211 ± 37 nmol/L, p = 0.20) or the PFC *range* (196 ± 73 vs. 157 ± 34 nmol/L, p = 0.12).

A PFC rhythm *onset* could be identified in all subjects; it was significantly delayed in the patients compared with the healthy volunteers (05:24 ± 02:06 vs. 03:54 ± 01:00, p = 0.02, Fig. 1). In two (22%) healthy volunteers and seven (35%) patients the rhythm *offset* occurred after 18:00, beyond sampling time, thus the parameters *offset* and *duration* could not be obtained. In the 13 patients and seven healthy volunteers in whom these parameters were obtained, no significant difference was observed in the timing of the rhythm *offset* between the two groups but the *duration* of the peak was significantly shorter in the patients (7.0 ± 3.8 vs. 10.6 ± 2.3 h, p = 0.03).

Patients with Child's B/C cirrhosis had significantly lower 08.00 PFC values compared to their counterparts with Child's A cirrhosis (135 ± 42 vs. 246 ± 103 nmol/L, p = 0.04). In addition, they had a higher mean of the *five lowest* PFC concentrations (61 ± 12 vs. 48 ± 20 nmol/L, p = 0.05), a lower, although not significantly different, mean of the *five highest* PFC concentrations (208 ± 74 vs. 264 ± 72 nmol/L, p = 0.12) and, in consequence, a lower *range* (148 ± 75 vs. 217 ± 63 nmol/L, p = 0.04). In addition, a direct correlation was observed between the means of the *five lowest* PFC concentrations and the Pugh's score (R = 0.64, p <0.01).

Both the PFC rhythm *onset* and *offset* were delayed in patients with Child's B/C compared to those with Child's A cirrhosis (Fig. 2) but the differences did not reach statistical significance (*onset* 06:12 ± 01:48 vs. 05:06 ± 02:12, p = 0.28; *offset* 13:48 ± 02:42 vs. 12:00 ± 03:00, p = 0.37).

A considerable delay in the onset of the cortisol rhythm and abnormalities in the duration of the peak were observed in this patient series, in parallel with the degree of hepatic dysfunction. While the exact pathophysiology of cortisol timing alterations in patients with cirrhosis is unknown, they are most likely cerebral in origin. Cortisol, like melatonin, is a direct marker of the phase of the hypothalamic clock, which regulates the diurnal rhythm of circadian hormones in relation to light and dark cues. It has previously been shown that patients with cirrhosis exhibit abnormalities in the sensitivity of the hypothalamic clock to light [2], which may explain abnormalities in both melatonin and cortisol rhythms [5].

The patients with decompensated cirrhosis had significantly lower PFC values at 08.00 and less variation in PFC values over the 24 h compared to their compensated counterparts. These findings might relate to: (i) adrenal insufficiency *per se*; (ii) abnormalities of cortisol metabolism, which could be responsible for the increase in the low PFC values; or (iii) sympathetic–parasympathetic imbalance, which could affect the patients' ability to modulate cortisol responses to external stimuli.

The strength of the present series is that cortisol 24-h rhythms were estimated under 'ideal' conditions, namely: (i) the measurement of PFC, which is not affected by albumin concentrations and, (ii) the exercise of tight controls for light exposure, which is known to phase-reset the cortisol rhythm, thus affecting its timing, via the hypothalamic clock [5]. The weakness is that most of the patients studied had compensated cirrhosis, whereas adrenal insufficiency is observed most frequently in those with decompensated disease and sepsis. However, the characteristics of the cortisol rhythms in the six patients with Child's B/C cirrhosis suggest that even more pronounced rhythm timing/amplitude abnormalities might be present in patients with more severe liver dysfunction.

It has recently been suggested that morning basal cortisol could be useful to identify patients with cirrhosis at high risk for adrenal insufficiency [6]. However, it seems extremely unlikely that isolated morning total or even free plasma cortisol

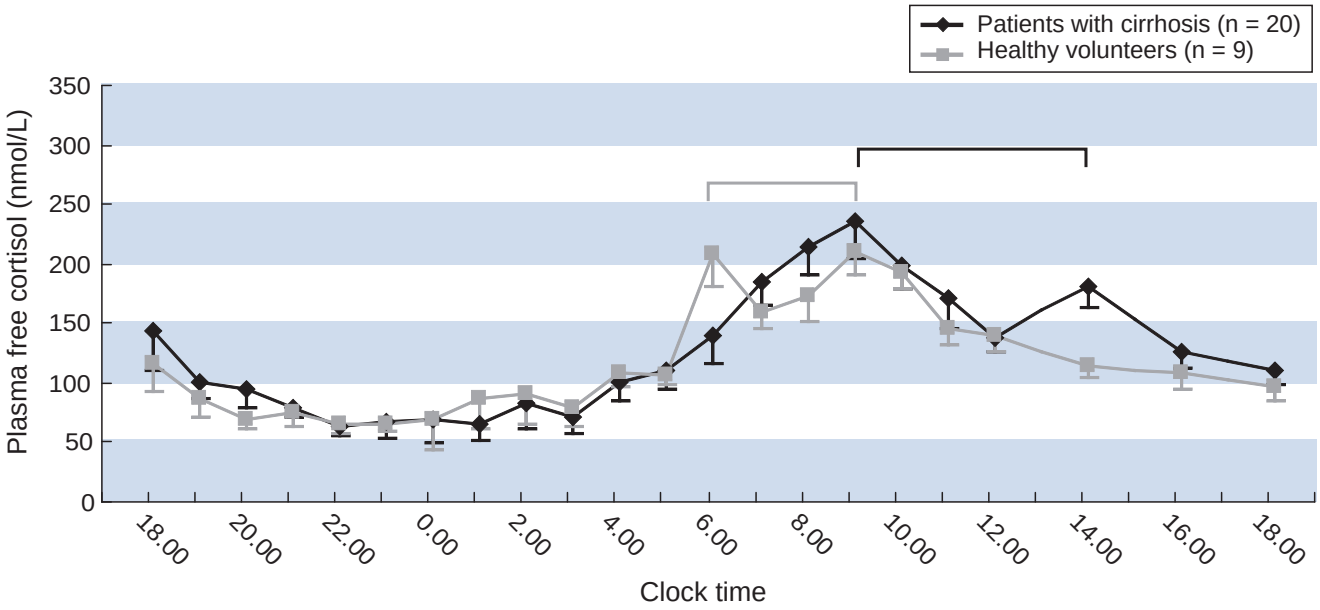

Fig. 1. Plasma free cortisol (PFC) 24-h concentration-time curves in healthy volunteers (grey squares) and patients with cirrhosis (black rhomboids). Healthy volunteers showed a morning PFC double peak (06.00 and 08.30 – grey bar) and patients with cirrhosis showed a delayed PFC peak (10.00 and 13.00 – black bar).

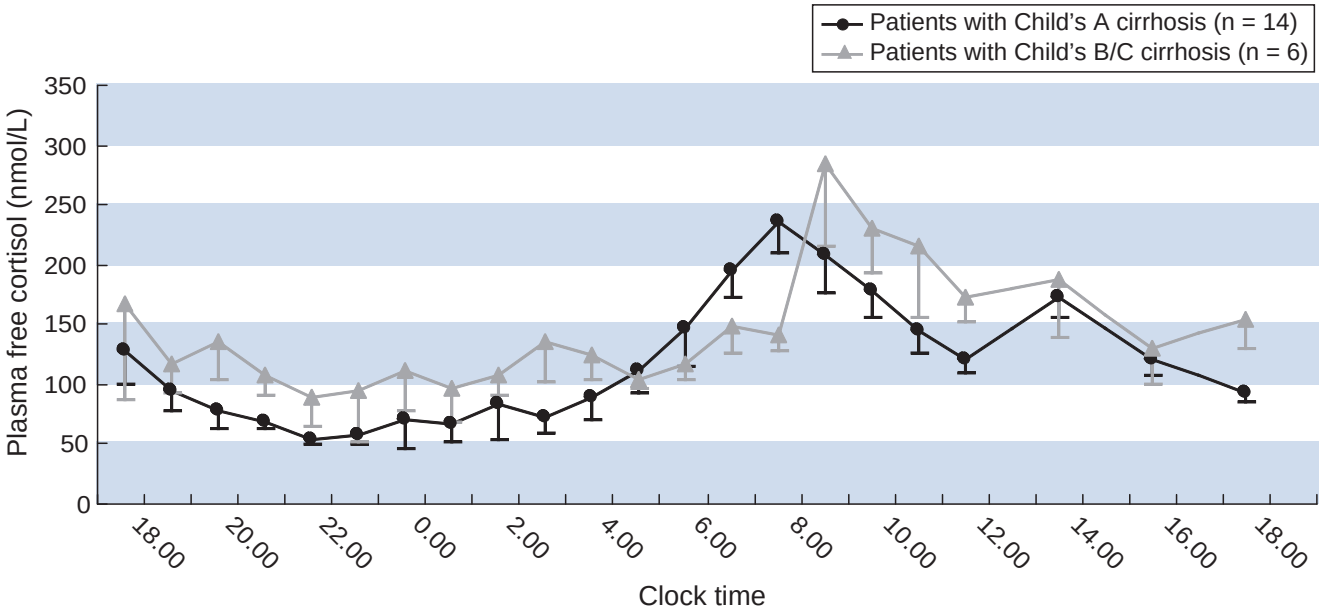

Fig. 2. Plasma free cortisol (PFC) 24-h concentration-time curves in patients with Child's A cirrhosis (black circles) and patients with Child's B/C cirrhosis (grey triangles). Patients with Child's B/C cirrhosis showed a more prominent delay in the timing of the cortisol peak compared to their counterparts with Child's A cirrhosis, although statistical significance was not reached.

samples could be used as a stand-alone diagnostic index because of the observed delay in cortisol rhythm onset and the shape of the cortisol peak. This, together with the recently published data on the potential drawbacks of measuring plasma total cortisol in this patient population [1], indicates that the diagnosis of hepatoadrenal syndrome might be more complex than previously believed. It may, for example, necessitate a free cortisol corticotropin test and, possibly, an assessment of the circadian phase using, for example, an evening melatonin measurement. Further studies are needed to devise a model whereby baseline/stimulated cortisol concentrations can then be adjusted for the patients' circadian phase.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Adrenal insufficiency: Diagnosis in patients with liver cirrhosis is difficult

Reply to the Letter to the Editor

Reply to Montagnese et al.:

We were very interested by the letter of Montagnese et al. published in this issue of the Journal of Hepatology [1]. Many recent publications led us to believe that adrenal insufficiency, assessed by serum total cortisol assays, was very common in patients with liver cirrhosis [2–7]. In fact, cortisol transport proteins (cortisol binding globulin and albumin) are often decreased in patients with cirrhosis, leading to a reduced bound fraction of cortisol, whereas free cortisol concentration, which is the active fraction of cortisol, remains unchanged. We recently showed that serum total cortisol assays (performed at 8 AM before and after a corticotropin injection) largely overestimate adrenal insufficiency prevalence in patients with cirrhosis, especially in those with serum albumin $\leqslant$25 g/L [8].

Interestingly, Montagnese and colleagues assessed the 24-h rhythm of cortisol in patients with cirrhosis using plasma free cortisol assays, avoiding the bias of the reduced cortisol transport proteins. They report that patients with Child-Pugh B/C cirrhosis have significantly lower plasma free cortisol concentrations and that their rhythm onset and offset are (not significantly) delayed compared to patients with Child-Pugh A cirrhosis. This delay could make the diagnosis of adrenal insufficiency more complicated in patients with severe cirrhosis.

In our study, we found that adrenal insufficiency assessed by salivary cortisol (an accurate reflection of free cortisol) was lower than previously reported with serum total cortisol, but not rare (8/88: 9.1%) [8]. All eight patients with adrenal insufficiency were classified as Child-Pugh C. It is unlikely that our results are partly explained by the delayed cortisol rhythm of patients with Child-Pugh B/C cirrhosis highlighted by Montagnese and colleagues. In fact, the maximal plasma free cortisol concentration seems very close to 8 AM in the study of Montagnese, which is the moment when we collected saliva and blood samples to assess cortisol concentrations. These interesting results should be confirmed by a larger study (Montagnese and colleagues included 4 patients with Child-Pugh B and two patients with Child-Pugh C) to determine if this delay has reliable consequences on the diagnosis of adrenal insufficiency in patients with cirrhosis.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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