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ORIGINAL INVESTIGATION

Cortisol levels in fingernails, neurocognitive performance and clinical variables in euthymic bipolar I disorder

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ABSTRACT

Objectives: Neurocognitive impairment has been found in bipolar patients. Hypercortisolemia is one possible cause but there has been no agreement on this. Previous sampling methods assessed only acute cortisol levels, whereas the association between cortisol and psychopathology might be better understood by investigating chronic levels. Fingernails are a novel method for measuring chronic cortisol concentration (CCC). Here, we measured CCC in euthymic bipolar disorder I (BD-I) patients and healthy controls using fingernails to investigate whether differences in CCC influenced neurocognitive performance. We also investigated whether differences in clinical illness variables influenced CCC in euthymic BD-I patients.

Methods: A previous study demonstrated neurocognitive impairment in euthymic BD-I patients. The current study included a portion of this sample: 40 BD-I versus 42 matched controls who provided fingernail samples.

Results: There was no statistically significant difference in CCC between controls and BD-I ($P = .09$). Logistic regression analyses revealed that euthymic bipolar I subjects with more than five years of current euthymia had decreased odds of having higher fingernail cortisol concentration (>71.2 pg/mg) compared to those with less than 1.5 years ($P = .04$). There was no association between CCC and cognitive impairment in all domains before and after adjustment for age and sex.

Conclusions: The current evidence suggests CCC is not a trait biomarker in euthymic BD-I (BD-I). Longer periods of stability in affective disorders are associated with lower CCC. Fingernail cortisol does not seem to be implicated in neurocognitive impairment and BD-I. Future studies may investigate CCC in different illness phases of BD-I.

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1. Introduction

Several studies have confirmed that between 5% and 34% of patients with bipolar disorder (BD) show cognitive impairment that persists even after clinical recovery (Winokur et al. 1969; Johnstone et al. 1985; Gitlin & Swendsen 1995; Martinez-Aran et al. 2002). This result has been observed not only in bipolar I but also in bipolar II patients. Furthermore, this association is independent of IQ, medical comorbidities, substance misuse, alcohol (Iverson et al. 2009), cultural background and language (Cheung et al. 2013).

Even though this association between cognitive impairment and BD has been demonstrated, the cause is unknown. Several factors have been postulated, including hypercortisolemia (Buchanan et al. 2006; Buchanan & Tranel 2008; Wolf 2009). However, it is not clear whether there is a close association between cortisol levels and cognitive impairment in BD, nor whether this finding may be a trait or state illness biomarker. One explanation for the heterogeneous results found in studies of cortisol levels in BD may be the different specimens that have been used to measure its levels. In particular, the methods have assessed acute levels of cortisol over hours or days, which may be highly variable and may not reflect long-term, chronic cortisol concentration (CCC). However, quite recently two new specimens have been introduced and validated for measuring CCC, hair (van Holland et al. 2012) and fingernails (Izawa et al. 2015).

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Based on the uncertainties shown in previous literature, we hypothesised that if hypercortisolemia is linked to neurocognitive changes in BD, this would be reflected in higher cortisol levels in bipolar disorder I (BD-I) and relationships between cortisol and measures of cognitive function. Specific hypotheses were (1) the CCC are increased in euthymic BD-I (BD-I) in comparison to healthy controls when measured by fingernail specimens; (2) clinical illness variables are related to increased CCC among subjects with euthymic BD-I; and (3) increased CCC in fingernails would be associated with neurocognitive impairment and euthymic BD-I subjects and healthy controls.

2. Methods

Subjects who had taken part in a previous study were used (Cheung et al. 2013). The Cheung et al. (2013) study had a case–control design, consisting of 104 individuals (18–64 years), including those with lifetime BD-I ($n = 52$) and healthy controls ($n = 52$). A detailed description of that study can be found elsewhere (Cheung et al. 2013); in brief, this study demonstrated the presence of neurocognitive impairment in all cognitive domains in a sample of euthymic Chinese BD-I patients. When using two or more scores below the 5th percentile as a cut-off for neurocognitive impairment, 46% of the patients with BD-I and none of the control sample scored in this range ($P < .001$). Correlational analysis among the illness variables revealed that cognitive performance was inversely correlated with the number of manic episodes and the duration of illness. According to our previous hypothesis mentioned above, we designed this study with the following objectives: (1) to compare CCC in euthymic BD-I and matched healthy control participants using fingernail specimens; (2) to assess whether differences in CCC are influenced by clinical illness variables in BD-I patients; and (3) to assess whether differences in CCC influence neurocognitive abilities in euthymic BD-I subjects and healthy controls. Odds ratios (ORs) were used for studying hypotheses (2) and (3). The concept of OR links the estimation of relative risks for cross-sectional study. We unified the term OR in the whole text.

2.1. Participants

For the current study, we included a subsample of 82 persons from Cheung et al. (2013) comprising all those who provided fingernail samples. The final numbers included were 40 individuals (14 female) with a current diagnosis of BD-I and an age- and gender-matched group of 42 controls (16 female).

3. Measures

3.1. Procedure

3.1.1. First and second visit

In the st6udy of Cheung et al. (2013), patients were diagnosed using the Chinese-bilingual version of the the SCID, patient version (SCID-I/P) (First et al. 2012). The Chinese-bilingual version of the SCID-I/P has an inter-rater reliability of 0.91 for mood disorders and inter-rater-clinician reliability of 0.84 for BD and 0.76 for depression (So et al. 2003). In that study, participants completed two visits in which all participants were rated prospectively over 4 weeks and were defined as in remission if they satisfied the criteria of the Newcastle Euthymia Protocol (Thompson et al. 2005). This protocol contains, apart from the diagnosis of BD, the clinical assessment of symptom severity during the first and second visits, using the Young Mania Rating Scale to measure the severity of manic symptoms (Young et al. 1978) and the 21-item Hamilton Rating Scale for Depression to measure the severity of depressive symptoms (Hamilton 1960), and the self-rated assessment of depressive symptom severity using the Beck Depression Inventory (Beck & Steer 1996) and manic symptom presence and severity using the Altman Mania Rating Scale (Altman et al. 1997). Both self-administered scales were administrated each week during the euthymia verification month.

During the first visit, demographic information, such as age, education, occupation and clinical illness variables (medication history, age at onset, duration of illness and euthymia, number of hospitalisations and number of depressive, manic and psychotic episodes) were also recorded, as well as IQ, which was assessed using a three-subtest short form (Similarities, Digit Span and Arithmetic) of the Chinese revised version of the Wechsler Adult Intelligence Scale (Gong 1992). This has been adapted cross-culturally and is widely used (Lui & Wang 2011; Lui et al. 2011).

The second visit was scheduled 4 weeks after the first visit. To minimise any mood, cognitive or neuroendocrine variation created by the luteal phase (Symonds 2004), female participants' visits were scheduled between day 3 and 10 of their menstrual cycle (starting from the first day of menstruation). At the second visit, participants who were verified to be in euthymia completed the Central Nervous System Vital Signs (CNSVS). The CNSVS is a computerised cognitive

assessment battery for use in clinical research in psychiatric settings. Previous studies have administered the CNSVS to patients with BD. The CNSVS is comprised of seven common neuropsychological measures, including Verbal and Visual Memory Test, Finger Tapping Test, Symbol Digit Coding Test, the Stroop Test, a Shifting Attention Test and a Continuous Performance Test (Iverson et al. 2009).

The process of translation from English to traditional Chinese was completed in 2005. The forward translation (English to Chinese) followed by a backward translation was performed by a different translation vendor. A comparison was performed to look for and correct any discrepancies.

3.1.2. Third visit

The third visit was scheduled 2 weeks after the second visit. On this day, participants were instructed to provide fingernail samples. Fifteen days before the second visit, participants were called and instructed to clip their fingernails and let them grow for the third visit. On this visit, participants clipped their fingernails again in front of the researcher. According to de Berker, fingernails grow 1.5 mm per 15 days (de Berker et al. 2007). In this way, we were sure that fingernail cortisol concentration represented the cortisol levels that included the time of the second visit and the neurocognitive assessment, since no participant was euthymic for less than 15 days, according to the Newcastle protocol as applied in the study of Cheung et al. (2013). Moreover, it is extremely likely that for the free nail segment, the distal part analysed represented the recent plasma cortisol concentration rather than plasma cortisol levels at the time that that nail segment was formed (i.e. 3 months previously); this is because the nail unit is analogous to a larger hair follicle (de Berker et al. 2007). This means that the whole nail unit, from its most proximal centimetre to its furthest, incorporates cortisol from the systemic blood at the same time. This has been largely demonstrated when drugs such as methamphetamine (Suzuki et al. 1984; Suzuki & Inoue 1989) or therapeutic drugs such as terbinafine and itraconazole (Doncker 1999) are incorporated both into the nail via the most distal vessels where the nail stands, and at the most proximal point of nail generation. Furthermore, there is also evidence that these drugs do not remain in the nail once treatment is stopped, so that the zone of nail plate that was created during drug treatment, will contain no drug several months later (de Berker et al. 2007). This situation, therefore, should not be different with cortisol, which is a lipophilic hormone (Mommsen et al. 1999), similar to terbinafine (Hosseini-Yeganeh & McLachlan 2001), itraconazole (Cauwenbergh 1994) and methamphetamines (Mann et al. 1997).

3.2. Cortisol analyses

Fingernails from each subject were collected at the third visit in Hong Kong and transported to London, UK. Cortisol was extracted from nail clippings using the whole nail extraction method of (Warnock et al. 2010):

- Washing (2×) of the specimens was done with 3 ml isopropanol (LC/MS grade) in glass vials and dried overnight. Isopropanol washings were discarded.
- The washed clippings (50–60 mg) were ground (Retsch ball mill mixer; 30 Hz, 5 min). There was no difference in the mean weight of fingernails extracted between groups ($P = .83$); 25.96 mg for euthymic BD-I and 25.70 mg for controls; 20–30 mg of accurately weighed specimen was used for cortisol extraction (1.5 ml LC/MS methanol; 1 h on a rotary mixer).
- After centrifugation, 1.3 ml of the methanol supernatant was transferred to a separate tube and evaporated to dryness at 60 °C under nitrogen. The residue was redissolved in 1 ml of assay buffer and stored at −30 °C until immunoassay (Mondelli et al. 2010). Cortisol analysis was conducted at the Affective Disorders Unit Laboratory, Bethlem Royal Hospital, London, UK.

3.3. Statistical analysis

The socio-demographic data, IQ scores and cognitive performance of the two groups were analysed using paired *t*-tests for continuous variables and McNemar's tests for categorical variables. Differences in cognitive performance between those BD-I subjects who provided fingernail samples and those who did not were also compared using *t*-tests. The distribution of cortisol levels in fingernails was skewed (see cortisol level distribution in Figure 1); therefore, the Wilcoxon signed-ranks test was used for comparing mean ranks.

The distribution of clinical illness variables was also skewed. A probabilistic statistical classification model (logistic regression) analysis was used to estimate the ORs and 95% confidence intervals (95% CI) of the variance in cortisol levels in fingernails for each clinical variable from a univariate predictor (BD-I subjects). For this purpose, clinical illness variables (the independent variable of interest) were transformed from a continuous to a categorical variable with three tertiles, using the first tertile as reference point. We included the

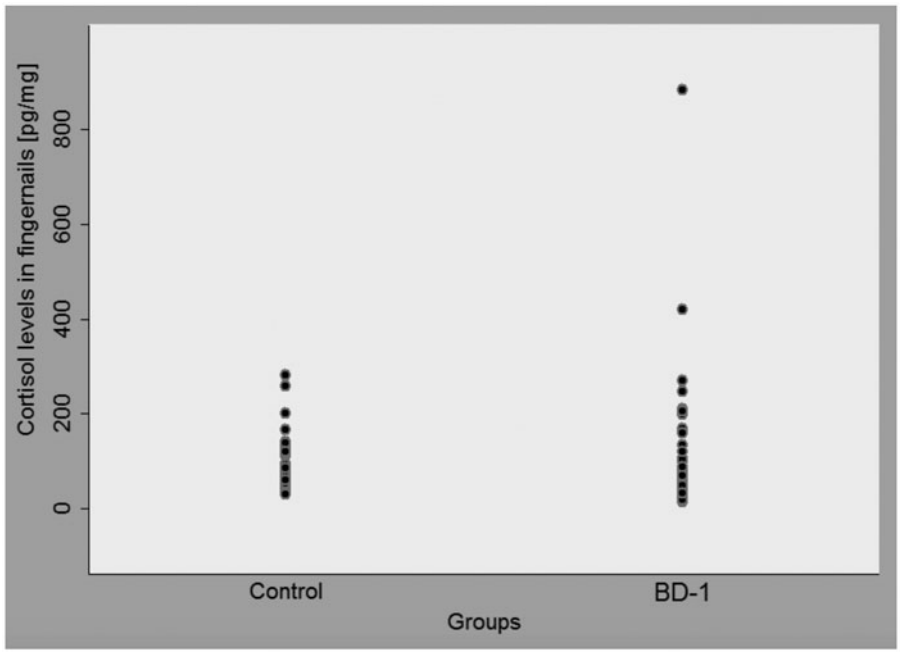

Figure 1. Scatter plot for cortisol levels in fingernails by group.

Table 1. Demographic and clinical variables results ($n = 82$).

Variable	Controls ($n = 40$)	Euthymic BD-I ($n = 42$)	P value
Age, Mean (SD)	39.5 (10.2)	38.4 (10.7)	.78
Education (years, self-reported), Mean (SD)	13.8 (0.4)	12.0 (0.4)	<.01*
Professional or technical employees, *n* (%)	28 (70)	12 (28.5)	.01*
IQ, Mean (SD)	113.2 (1.9)	105.8 (2.2)	.01*
Number of subjects taking psychiatric medication, *n* (%)	0 (0)	1 (2.5%) no medication; 12 (30%) monotherapy[a]; 26 (65%) combination of medications; 5 (12.5%) taking anticholinergic drugs	

*P value significant at <.05 level;.
IQ was assessed using the revised Wechsler Adult Intelligence Scale 3 – Subtest Short Form Estimated IQ, interquartile range. Differences in continuous variables were studied using paired *t*-tests and in categorical variables using McNemar's tests.
[a]Either sodium valproate, lithium, first- or second-generation antipsychotic, carbamazepine or lamotrigine.

confounding covariates of age and gender in a second model.

We also tested how much of the significant association observed between euthymic BD-I patients and neurocognitive impairment that had previously been found by Cheung et al. (2013) could be explained by differences in fingernail cortisol. For this purpose, conditional logistic regression was used to estimate the OR and 95%CI of the risk of having neurocognitive impairment for each cognitive domain from a binary predictor (BD-I and healthy participant). For this purpose, cortisol level (the independent variable of interest) was transformed from a continuous to a categorical variable with three tertiles, using the first tertile as reference point. We also included the confounding covariates of age and gender in a second model. Previously, this type of cortisol analysis has extensively been used in psychiatric research (Bremmer et al. 2007; Vogelzangs et al. 2007).

All tests were two-sided, and having $P < .05$ was considered statistically significant.

4. Results

4.1. Demographic characteristics

Of the 104 original participants, 82 (79%) provided fingernail samples, 40 with BD-I and 42 controls. The reasons why 12 subjects from the Cheung et al. (2013)

Table 2. Logistic regression analysis of clinical illness variables for higher cortisol levels in BD-I ($n = 42$).

		Unadjusted			Adjusted for age and gender		
Clinical variables in tertiles		OR	CI	*P* value	OR	CI	*P* value[a]
	Ref.	–	–			–	
Number of manic episodes	2nd	1.66	0.37, 7.38	.50	1.68	0.37, 7.53	.49
	3rd	1.24	0.28, 5.47	.76	2.13	0.26, 5.47	.80
	Ref.	–	–			–	
Number of depressive episodes	2nd	1.01	0.24, 4.17	.98	0.97	0.22, 4.09	.96
	3rd	0.99	0.22, 4.22	.98	0.95	0.21, 4.26	.94
	Ref.	–	–			–	
Number of psychotic episodes	2nd	–	–		–	–	
	3rd	1.10	0.35, 3.56	.85	1.18	0.35, 4.01	.77
	Ref.	–	–			–	
Duration of illness (years)	2nd	0.70	0.17, 1.04	.62	0.69	0.16, 2.94	.62
	3rd	0.94	0.23, 3.63	.92	0.86	0.16, 4.43	.84
	Ref.	–	–			–	
Duration of euthymia (years)	2nd	0.33	0.06, 1.59	.17	0.32	0.06, 1.66	.17
	3rd	0.2	0.04, 0.98	.04*	0.19	0.03, 0.93	.04*
	Ref	–	–			–	
Age of onset	2nd	0.75	0.19, 2.94	.68	0.77	0.18, 3.28	.73
	3rd	1.19	0.29, 4.9	.80	1.20	0.24, 5.98	.81
	Ref	–	–	–		–	–
Number of hospitalisation	2nd	0.91	0.07, 11.13	.94	0.95	0.07, 11.82	.96
	3rd	0.62	0.04, 8.16	.72	0.63	0.04, 8.33	.73
	Ref	–	–	–		–	–
Number of medications	2nd	2.18	0.11, 42.52	.60	2.07	0.10, 41.26	.63
	3rd	1.41	0.08, 24.77	.80	1.30	0.07, 24.04	.85

*Statistical significance.
[a]Holm's procedure was used for correction of the issue of multiple comparisons. OR: odds ratio; CI: 95% confidence interval. Ref., reference.

study did not provide fingernails samples were aesthetic or because they bit their fingernails. See demographic features in Table 1.

4.2. CCC in euthymic BD-I and healthy controls

Cortisol levels in fingernails were 118.6 pg/mg (interquartile range (IQR) = 25.8; 245.98) for BD-I subjects and 84.8 pg/mg (IQR = 33.5; 165.6) for healthy participants. There was no statistically significant difference in cortisol levels between these two groups using the Wilcoxon signed-rank test ($z = -1.65$; $P = .09$).

4.3. Clinical illness variables in euthymic BD-I

The mean age of onset was 25.4 years (SD = 1.2) and the mean duration of illness was 13.2 years (SD = 1.3). The mean number of depressive, manic and psychotic episodes were 4.9 (SD = 0.77), 4.8 (SD = 0.76) and 1.9 (SD = 0.47), respectively. Patients had experienced on average 4.1 hospitalisations (SD = 0.79).

4.4. The influence of clinical variables in CCC

Logistic regression analyses adjusted and for age and gender revealed that the cortisol levels in fingernails of BD-I patients was not associated with the number of manic episodes, the number of depressive episodes, the number of psychotic episodes, duration of illness, age of onset, number of hospitalisations or number of medications (Table 2). However, the OR for having high fingernail cortisol concentration (>71.2 pg/mg) for euthymic BD-I subjects within the longest tertile in relation to the duration of current euthymia (>5 years) compared to those BD-I subjects within the shortest tertile in relation to duration of current euthymia (<1.5 years) is 0.2 indicating decreased odds for having high fingernail cortisol concentration for BD-I patients with more than 5 years of current euthymia. The 95% CI of the OR (0.04, 0.98) indicates that odds of having high fingernail cortisol concentration are lower for subjects with more than 5 years of current euthymia compared to those with less than 1.5 years ($P = .04$). This result remained significant when the model was adjusted by age and gender (OR_{adj}=0.19, 95% CI = 0.03, 0.93, $P = .04$) (Table 2).

4.5. Comparison of cognitive performance

The neurocognitive performance of the BD-I subjects who provided fingernail samples in our study ($n = 40$) did not differ in any of the nine cognitive domains from the BD-I subjects ($n = 12$) who did not provide fingernail samples in the original study (Cheung et al. 2013) (all $P > .05$).

4.6. CCC and neurocognitive impairment

Conditional logistic regression analyses showed that there was no association between cortisol levels and

Table 3. Effect of chronic cortisol levels in fingernail in the relationship between neurocognitive impairment in in BD-I and controls ($n = 82$).

Neurocognitive Domain	Tertiles of cortisol	Unadjusted OR	Unadjusted 95%CI	*P* value*	Adjusted for age and gender OR	Adjusted for age and gender 95%CI	Adjusted for age and gender *P* value[a]
Executive function	Ref	–	–		–	–	
	2nd	1.52	0.24–9.38	.65	1.10	0.12–9.85	.93
	3rd	1.41	0.15–12.97	.75	1.25	0.12–12.10	.84
Processing speed	Ref	–	–		–	–	
	2nd	–[b]	–	–	–[b]	–	–
	3rd	–[b]	–	–	–[b]	–	–
Visual Memory	Ref	–	–		–	–	
	2nd	0.85	0.03–22.63	.92	0.85	0.03–22.63	.92
	3rd	1.36	0.04–43.59	.85	1.36	0.04–43.59	.85
Reaction time	Ref	–	–		–	–	
	2nd	2	0.18–22.05	.57	2.17	0.11–40.27	.60
	3rd	0.66	0.02–18.05	.81	0.70	0.02–22.25	.84
Cognitive Flexibility	Ref	–	–		–	–	
	2nd	1.52	0.24–9.38	.65	1.10	0.12–9.85	.93
	3rd	1.41	0.15–12.97	.75	1.25	0.12–12.10	.84
Psychomotor Speed	Ref	–	–		–	–	
	2nd	1	0.06–15.98	1	0.85	0.03–22.63	.92
	3rd	1.5	0.05– 40.63	.81	1.36	0.04–43.59	.85
Complex Attention	Ref	–	–		–	–	
	2nd	1.52	0.24–9.38	.65	1.10	0.12–9.85	.93
	3rd	1.41	0.15–12.97	.75	1.25	0.12–12.10	.84
Verbal Memory	Ref	–	–		–	–	
	2nd	0.85	0.03–22.63	.92	0.85	0.03–22.63	.92
	3rd	1.36	0.04–43.59	.85	1.36	0.04–43.59	.85
Composite Memory	Ref	–	–		–	–	
	2nd	–[b]	–	–	–[b]	–	–
	3rd	–[b]	–	–	–[b]	–	–

*Statistical significance.
[a]Holm's procedure was used for correction of the issue of multiple comparisons. OR, odds ratio; CI, 95% confidence interval. Ref., reference.
[b]OR were not estimated for convergence problems.

cognitive impairment in any domain before and after adjustment for age and sex (Table 3). Categorised cortisol levels did not significantly explain cognitive impairment in any domain before and after adjustments.

5. Discussion

5.1. CCC in euthymic BD-I

In this study, we found no evidence that CCC is altered in euthymic BD. There is a limited amount of previous data that suggests there may be neurobiological disturbances in the euthymic stage of BD, with some claiming that these disturbances may be a possible stage biomarker. Vieta & Gasto (1997), for example, showed that euthymic patients had a significantly higher peak of corticotropin in comparison to healthy participants using blood specimens. This finding was later replicated by the same research group (Vieta et al. 1999). However, neither of these studies found concomitant alterations in cortisol levels in euthymic patients. This negative finding has been the norm (e.g. Cervantes et al. 2001). The results from the current study are not, therefore, an exception. We found no statistically significant differences in CCC between healthy participants and BD-I patients using fingernail samples, suggesting that this hormone is not a trait biomarker in euthymic BD-I. It is true that a trend for high fingernail cortisol concentration was found in euthymic BD-I subject in comparison to healthy subjects ($P = .09$). It might also be possible that a larger sample size would be needed to see if this difference was just too small to be measured by the power of this study.

Our study adds further to the state of knowledge in relation to cortisol and BD. One of the uncertainties in understanding the possible role of cortisol changes has arisen from the different sampling methods used to date. Previous specimens, i.e. blood, saliva and urine, have been able only to assess cross-sectional levels of cortisol. Due to cortisol being a highly reactive hormone (Kudielka & Kirschbaum 2003), the results obtained from a cross-sectional assessment point with these specimens may not necessarily reflect the underlying longitudinal pattern. Some experimenters have attempted to use different techniques using the same specimens in order to gain a CCC approximation (Pruessner & Kirschbaum 2003). However, there is no consensus on their real utility. For instance, the area under the curve (AUCg) is a measure able to quantify the total diurnal salivary cortisol output. This measure is composed of data from several samples during the day, so as well as being time-consuming, ensuring full

compliance can be difficult to achieve. Moreover, salivary cortisol does not exactly represent the systemic values, as there is an inactive fraction which is bound to proteins that cannot be measured by this sampling method (Kirschbaum & Hellhammer 1994). In addition, the reactivity of cortisol means that several factors such as exercise, food, smoking and drinking alcohol may alter its acute levels (Kudielka et al. 2012); variation in these factors may obscure the underlying habitual CCC. Similar considerations apply to methods using repeated measures of urine and blood collection.

Recently, hair has been introduced and validated as a new specimen to measure CCC more accurately (van Holland et al. 2012). Unfortunately, there remain some uncertainties as to the best way in which to analyse these specimens (Pragst & Balikova 2006). Furthermore, many strands are needed for hair sample analysis, which may limit patient acceptability. In addition, some individuals do not have sufficient hair, while hair self-sampling may be difficult for some participants (Izawa et al. 2015). In this sense, fingernail specimens have some potential advantages. Both hair and fingernail specimens have the ability to reflect an integrated long-term systemic cortisol secretion value, although hair is potentially able to cover a longer time frame than nails. The similarity of the measures has been demonstrated by Izawa et al. (2015), who found in two experiments a significant correlation between the cortisol level in hair and fingernails and between cortisol levels in saliva and in the fingernail samples.

The current study is, to our knowledge, the first study conducted which utilises fingernail samples in BD. Manenschijn et al. (2012) previously studied hair cortisol levels in a population of BD in-patients (type I, type II and not otherwise specified) at different phases of the illness. They found that there were no significant differences between bipolar patients and controls in terms of cortisol levels in hair. Furthermore, in the BD patients there were also no significant differences in hair cortisol levels between bipolar patients in different illness phases including euthymic patients (43% of the sample) (Manenschijn et al. 2012), consistent with previous results with other samples. There remain no studies of CCC in fingernails in different illness phases; we suggest that this may be a target of future studies attempting to define a possible stage biomarker role of cortisol.

5.2. The influence of clinical illness variable in CCC

We also looked for any regression equation between different clinical illness variables and cortisol levels in fingernails in order to find any relevant associations (Table 2). Previous studies have shown a positive association between number of previous mood episodes and mean cortisol levels in saliva samples (Havermans et al. 2011). However, the study of Manenschijn et al. (2012) did not find any associations between type of medication, duration of the illness, and age of onset (age at which the first episode of (hypo)mania or depression presented) on cortisol levels in hair samples. It should also be noted that the sample characteristics in the current study are different to the study of Manenschijn et al. (2012); while we studied a pure sample of euthymic BD-I subjects, they combined subjects with different subtypes of BD and in different illness phases, therefore limiting the comparability with this study. We found that BD-I subjects with longer periods of euthymia had decreased odds (OR = 0.2) for having higher fingernail cortisol concentration in comparison to those BD-I subjects with shorter period of euthymia, suggesting that affective episodes in BD-I subjects, either depressive, manic or hypomanic, may be associated with long-term HPA axis disturbances. We must, however, acknowledge that this finding was only significant at $P = 0.04$ and would not survive a correction for multiple testing. On the other hand, because the analysis is based on what is a pilot study (the first time the link between fingernail cortisol and cognition in BD has been investigated) by definition the study has only limited statistical power, and would be very vulnerable to any method of multiple-comparison correction. Because we undertook only a small number of parallel tests ($n = 8$), there is not a large problem of multiple testing to be addressed; nevertheless, the result must remain tentative pending replication and/or larger samples with greater statistical power.

This result makes it unlikely that the trend found ($P = .09$) for having higher fingernail cortisol concentration in euthymic BD-I would reach significance if the sample size were increased, since the logistic regression model results showed an association between low fingernail cortisol concentration and duration of the current period of euthymia. Our results are supported by the previous findings of Colla et al. (2007) who found that euthymic bipolar subjects on long-term treatment with lithium had associated decreased cortisol levels relative to healthy controls, but they were using acute cortisol levels. Future studies could investigate chronic cortisol levels using fingernail samples in different illness phases of BD. In that sense, Dettenborn et al. (2012) already found that subjects with major depressive disorder exhibited high CCC in comparison to healthy subjects when they are

suffering from depression, but using hair specimens, the other validated specimen for measuring CCC.

5.3. The influence of CCC in neurocognitive performance

Finally, conditional logistic regression analyses were run to analyse the putative effect of cortisol in the relationship between cognitive functions in healthy and euthymic BD-I participants. However, no association between CCC and cognitive variables were found in any domain before and after adjustment for age and sex. Regarding the validity of the results, the present study population seems representative as we did not find any significant difference in the neurocognitive performance in any of the domains assessed here between our BD-I sample and those BD-I subjects who did not provide fingernails from the original full study (Cheung et al. 2013). Alterations in cortisol levels, mainly hypercortisolaemia, have been widely postulated as a possible cause to explain neurocognitive deterioration in affective disorders, especially in memory (Buchanan et al. 2006; Buchanan & Tranel 2008; Wolf 2009). This theory has been supported by at least two main findings. One of these comes from Sapolsky (1985), who analysed the possible role that hypercortisolaemia could play in hippocampal damage which, in turn, could affect cognitive performance in bipolar patients (Altshuler 1993). Further support comes from the demonstration that the chronic administration of 20 mg of hydrocortisone twice daily for 10 days can cause selective and reversible deficits in neuropsychological function in normal male volunteers (Young et al. 1999). Nevertheless, there are two key differences which may explain this apparent inconsistency with our findings. Firstly, the mean level of urinary cortisol reached by participants in the study of Young et al. (1999) after the prescription of hydrocortisone was 530 μg/24 h using urine samples. This is more than five times higher than the urinary cortisol mean (104 μg/24 h) found in a sample of bipolar depressive subjects using the same samples (Carroll et al. 1976). Additionally, in Young et al. (1999), participants had elevated cortisol for 10 days, whereas Angst and Sellaro (2000) reported that the average bipolar episodes, and thus the presumed duration of any associated increases in cortisol levels, last for 3–6 months. Thus, the cognitive decline found by Young and colleagues may have been the result of the high cortisol doses the participants received, but which may not adequately reflect the true cortisol levels and time of exposure experienced by bipolar patients. Furthermore, hypercortisolaemia is not a constant finding in BD. Markopoulou (2013), for instance, found that subjects with treatment-resistant bipolar depression exhibited a lower salivary cortisol than controls, remitted patients and patients with treatment-resistant unipolar depression. In fact, low cortisol levels have been also associated with neurocognitive impairment (Rimmele et al. 2010). Rimmele and Besedovsky (2013) described an inverted U-shaped relationship between cortisol levels and memory retrieval.

Futhermore, Rimmele and Besedovsky (2013) found that the neurocognitive impairment is glucocorticoid receptor specific. Other studies have provided some corroboration that this specific receptor relationship underlies the cognitive impairment effects of cortisol (Watson et al. 2012; Otte & Wingenfeld 2015). However, these studies were conducted in patients with ongoing depression. This is a clear confounder because cognitive symptoms are common in depression, and indeed impaired concentration forms part of the diagnostic criteria. Additionally, these previous studies investigated fewer cognitive domains than the current study, and the domains studies may not be fully comparable.

In light of the above findings, future studies should investigate other possible factors in the association between neurocognitive impairment and BD. In that sense, it is quite possible that other clinical variables are involved in the relationship with neurocognitive impairment in euthymic BD-I patients. For instance, Cheung et al. (2013) corroborated findings in previous studies reporting an association between the duration of the illness and number of manic episodes and neurocognitive impairment. In that study, they also found the number of hospitalisations to be involved in this association.

5.4 Limitations and future directions

Several methodological issues may be found in our study. First of all, we did not compare fingernail samples with any other previously validated samples. A recent study designed two experiments; one comparing fingernails with hair and another one comparing fingernails with saliva, finding a moderate positive correlation between CCC in fingernails and both hair and saliva samples (Izawa et al. 2015). However, that study has several other methodological problems: the inclusion criteria in terms of age and gender in both experiments were different, limiting the comparability between them; several exclusion criteria were not taken into account; and the time frame that the authors chose to compare fingernails with hair and saliva samples did not match. Additionally, their results

were restricted to healthy population which are not necessarily representative of the current euthymic BD-I sample. Therefore, future validation studies with an appropriate study design may be conducted not only in a healthy population but also in people with affective disorders.

We did not find differences in fingernail length between groups. However, this observation was done by eyesight. We should have used a more accurate tool for measuring their length. However, our strict well-designed protocol minimised any potential significant differences in the length of fingernails. Moreover, we did not find differences in the mean weight of the extracted fingernails samples, confirming that these samples very likely represented the same time frame in both groups.

A total of 97.5% of our sample was taking psychotropic medication at the time of assessment. Medication use has been found to be involved in the association between neurocognitive impairment and BD (López-Jaramillo 2010), and may also effect cortisol levels (Holsboer & Barden 1996). While hard to recruit, definitive exclusion of medication effects would require the use of unmediated patients; however, there is a risk that this may then skew he sample studies in other ways, such as towards a milder form of illness. While we were able to investigate the potential role of several clinical variables that could be involved in that association between BD, cortisol and cognition, we did not include all possible such variables. Thus, we did not have information on previous rapid cycling, severity of episodes (Martínez-Arán et al. 2004) and functional outcome (Rosa et al. 2009).

We also included controls from the hospital staff. This may introduce bias. It has been said that these workers do not represent the general population, since they tend to be healthier (Li & Sung 1999). However, we estimate that, even considering this bias effect, the extremely large difference found in the study by Cheung et al. (2013) for neurocognitive impairment in euthymic BD-I patients in comparison to controls would have remain significant. Moreover, several previous studies have also supported this association (Winokur et al. 1969; Johnstone et al. 1985; Gitlin & Swendsen 1995; Martinez-Aran et al. 2002).

Caregivers who are also hospital staff have also been associated with increased CCC (Stalder et al. 2014). However, this result was found in hair specimens and we did not include these workers in our sample of controls.

Finally, several covariates that have not been studied so far, may affect cortisol levels in this novel specimen. For instance, the nail growth rate varies with seasonal changes, sex, different finger digits, age, clipping frequency, nail filing and nail-biting habits (Gupta et al. 2005) or cosmetic products. However, a modulatory role of a common cosmetic product, such as nail varnish, on fingernail cortisol has already been ruled out (Ben Khelil et al. 2011).

5. Conclusions

Our results showed the presence of an association between affective episodes and long-term effects on the HPA axis; those subjects who had experienced longer periods of euthymia had decreased odds for having higher CCC in comparison to those with shorter periods. However, we found no evidence of alterations in chronic cortisol levels in euthymic patients with BD-I overall, and no evidence that changes in cortisol levels underlie the cognitive impairments seen in BD between episodes. Fingernail samples may be a potentially useful specimen to investigate chronic levels of cortisol in different illness phases in future studies in BD.

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Statement of interest

AHY has given paid lectures and sits on advisory boards for all major pharmaceutical companies with drugs used in affective and related disorders. AJC has in the last three years received honoraria for speaking from Astra Zeneca (AZ) honoraria for consulting from Allergan and Livanova and

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