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Full Length Research Report

TITLE: Evidence for increased immune mobilization in First Episode Psychosis compared with the Prodromal stage in males.

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HIGHLIGHTS

- Increased immune function in First Episode Psychosis patients compared to prodromal subjects.
- Serum cortisol secretion and fluctuation differed between the groups only at a trend level.
- Suppression of HPA axis capacity did not differ significantly between groups.
- No significant associations among cytokines, cortisol and psychopathology.

ABSTRACT

The aim of the study was to gauge both the immune and neuroendocrine function in Ultra High Risk for psychosis (UHR) subjects and compare them with a cohort presenting with First Episode Psychosis (FEP).

We recruited two groups, the first group consisted of 12 UHR males and the second of 25 males with FEP. We measured serum cortisol levels at 08:00, 12:00, 18:00 with their Area Under Curve with respect to the ground (AUCg) and the increase (AUCi) and we measured serum cytokines levels, Interleukin-1a, IL-1a, IL-2, IL-4,IL-5,IL-6,IL-8, IL-10,IL-12, IL-17a, Tumor Necrosis Factor-a (TNF-a), Interferon-γ (IFN-γ). Dexamethasone Suppression Test (DST) was also performed .

The results suggest higher levels of both pro-inflammatory (TNF-a, IL-2, IL-12, IFN-γ) and anti-inflammatory (IL-10) cytokines in the FEP group compared with the UHR counterparts. Regarding the HPA axis function, the prodromal subjects showed a trend for higher AUCg and AUCi change/decrease cortisol levels. On the contrary, the

DST results did not differ between the groups. No significant associations were demonstrated within each group among cytokines, cortisol and psychopathology.

The findings favor a hypothesis of a relatively increased mobilization of both the pro- and anti-inflammatory cytokine networks, in FEP compared with that of UHR subjects.

Keywords: Cytokines, Cortisol, First Episode Psychosis, HPA axis, Immunity, Inflammation, Ultra High Risk.

1. Introduction

The neural diathesis-stress model (Walker and Diforio, 1997) of psychosis has been a widely used model to explain the neurobiological basis of the vulnerability the psychotic patients demonstrate to different stressors. Thus, research has focused on the Hypothalamus-Pituitary-Adrenal (HPA) axis function which is known to mediate the organism's response to stressful stimuli so as homeostasis and psychosomatic equilibrium to be maintained (Chrousos, 2000).

Recent research to elucidate the causes of stress vulnerability in psychosis, has involved the study of the HPA axis function in cohorts presented with First Episode Psychosis (FEP), as this group eliminates confounders such as medication and chronicity. The evidence from this approach favors an up regulation of basal cortisol secretion in individuals with FEP vs Healthy Controls (HC). In contrast, the reactive capacity of the HPA axis function and its relation to specific psychopathology remain vague (Karanikas et al., 2014). In addition, there is similar evidence for up regulation

of cortisol secretion even prior to the emergence of a full blown psychotic episode, that being at prodrome (Karanikas and Garyfallos, 2015). Indeed, the psychotic prodromal period is characterized by the emergence of a constellation of sub threshold psychotic-like symptoms and progressive functional decline that can precede the onset of an Axis I psychotic disorder. Preliminary evidence suggests a pattern where cortisol levels in At Genetic Risk of Psychosis cohorts tended to be numerically intermediate between the patients group (established psychosis) and the HC (Spelman et al.,2007; Yildirim et al., 2011) . Similarly to the evidence coming from FEP studies, cortisol's fluctuation and reactivity to different stressors in Ultra High Risk of psychosis (UHR) subjects compared with HC, remain vague due to the minimal literature and divergent results (Karanikas and Garyfallos, 2015).

A similar obscurity characterizes the findings in relation to the immune abnormalities in psychosis. The first attempts to review the studies evaluating circulating cytokines produced conflicting results (Drzyzga et al., 2006; Potvin et al., 2008; Miller et al., 2011). Heterogeneity appeared to be a common denominator in these studies. This heterogeneity related to differences in diagnoses, setting, medication, phase of illness, comorbidity. The hypotheses referring to the immune aetiopathological backround in psychosis involve i. the macrophage-T Lymphocyte theory (Smith and Maes, 1995), ii. the T helper type 2 shift (Müller and Schwarz, 2010), iii. the microglia activation (Monji et al., 2009), iv. the tryptophane metabolism-kynourenine pathway and astrocyte activation (Müller et al., 2011), v. the synchronous activation of both pro- and anti- inflammatory arms of immunity (Drexhage et al., 2011).

This present study constitutes a preliminary attempt to capture the immune and HPA axis function profile in 2 cohorts, whose clinical presentation lied in close proximity to the emergence of psychosis. The first group incorporated FEP patients and the other UHR subjects. The HPA axis function was investigated in a way to capture evidence not only for the total secretion of cortisol but also its fluctuation throughout time. Plus HPA axis' reactivity/suppressor capacity was gauged with the implementation of the Dexamethasone Suppression Test (DST). In addition, a group of cytokines alleged to serve different immune functions (innate vs adaptive, pro- vs-anti- inflammatory arms) were evaluated. As mentioned before, the data regarding the immune and neuroendocrine profile in the FEP and the prodromal state are minimal and to an extent contradictory.

To our knowledge this is the first study involving a direct comparison of cytokines profile and HPA axis function between the prodrome and the FEP. Given the evidence that inflammation may underlie the aetiopathogenetic substrate of psychosis, we tested the hypothesis that the FEP group (where full blown psychosis becomes clinically evident) would demonstrate increased immune mobilization as well as increased cortisol secretion and/or fluctuation and more blunted cortisol suppression on the DST compared with the UHR group (where the psychotic symptomatology is clinically demonstrated in a more attenuated fashion) .

2. Methods

2.1 Subjects

The study was conducted, from May 2012 up until May 2014, within the settings of two psychiatric clinics; the Psychiatric Department of the 424 General Military

Hospital of Thessaloniki and the 2nd Psychiatric Department of Aristotle University of Thessaloniki, located at the Psychiatric Hospital of Thessaloniki, Greece.

The recruitment of the FEP group involved 25 male patients with their first presentation of psychotic episode without affective features. We recruited participants having received a diagnosis of Brief Psychotic disorder or Schizophreniform disorder or Schizophrenia or Psychotic disorder Not Otherwise Specified according to the DSM IV-TR. Drug induced psychosis was excluded, based on the history, clinical presentation and urine drug test. Both the FEP group and the UHR group (N=12 males) consisted of military personnel who were referred to the Psychiatric Department of the 424 General Military Hospital of Thessaloniki, Greece for further evaluation of their change/deterioration in their behavior and/or function in their military duties.

2.2 Clinical assessment

2.2.1 Clinical Diagnoses

The referred subjects (military male personnel) were clinically evaluated by two psychiatrists (EK, GG) not being the principal treating doctors of the patients. Diagnoses of FEP patients were confirmed with the application of the Structured Clinical Interview (SCID)(First et al., 2002) for DSM-IV-TR. Regarding the UHR group, their recruitment was based on the PACE criteria (Yung et al., 1998) using the Comprehensive Assessment of At Risk Mental States (CAARMS) (Yung et al., 2005). According to the pre mentioned classification, UHR individuals must meet at least one of the following constellations of criteria: (a) Attenuated Psychotic Symptoms (APS); denoting the experience of sub threshold positive psychotic symptoms during

the past year; (b) Brief Limited Intermittent Psychotic Symptoms(BLIPS); the experience of episodes of frank psychotic symptoms that have not lasted longer than a week and have been self-remitting; or (c) Trait and State Risk Factor; having a first degree relative with a psychotic disorder or the identified subject has been diagnosed with Schizotypal Personality Disorder (SPD) plus a significant decrease in functioning during the previous year. No subjects were diagnosed with any other comorbid psychiatric state.

2.2.2 Psychopathology severity

The symptom severity in both the FEP and UHR groups was assessed with the Greek version (Lykouras et al., 1994) of the Structured Clinical Interview for the PANSS (Kay et al., 1987) and was performed by two psychiatrists (EK, GG) on the same day or within the next two days of blood sampling. The results were calculated as Positive Factor (PANSS PF), Negative Factor (PANSS NF), General Psychopathology (PANSS GP) and Total score (PANSS TOTAL).

2.3 Other Inclusion-exclusion criteria

We included FEP subjects who were medication naïve or minimally treated; the later meaning that, depending on their clinical presentation they should not have been for more than 3 days on any type of psychotropic medication (antipsychotic, mood stabilizer, antidepressant, benzodiazepines) up until all blood sampling had been completed. In this way the least possible exposure to the effects of medication was ensured. The UHR participants needed to be drug naïve to be included in the study.

Our study groups consisted of participants between the age group of 18-40, with BMI<30. Also the design of the study required the participants to be physically healthy with no signs of active inflammation for at least 15 days prior to the study - based on the medical history, physical clinical examination and laboratory investigations.

We excluded subjects with intellectual disability, shift workers and illicit drug use based on history and urine drug tests both at their admission in hospital and prior to that at random sampling according to the practices of the Greek Armed Forces. Participants with any chronic medical state (including but not restricted to impaired thyroid function, polydipsia, asthma, diabetes, chronic fatigue, autoimmune disorders) or medication that could impair the immunological, endocrinological or neurological status were excluded.

All participants gave their informed consent after a thorough explanation was provided regarding the process. The local ethical scientific committees of the two involved hospitals, plus the scientific committee of the Headquarters of the Greek Armed Forces gave their consent for the study. The study was performed in accordance with the last edition of the Declaration of Helsinki.

2.4 Blood collection and analyses

The blood samples were collected within the first 3 days of admission for both the FEP and the UHR subjects. The day 1 of blood sampling, samples were collected at 3 separate points in time; 08:00 morning, 12:00 midday and 18:00 noon. At 23:00 of the first day, 1 mg of Dexamethasone (Dex), was given. At day 2, post/Dex, at 08:00 another blood sample was collected. All subjects were instructed to keep fasting

overnight up until the morning samples were given. The participants were also instructed to abstain from caffeine, alcohol, cigarette and physical exercise at least 30 min prior to any blood sampling. Every blood sample was centrifuged at 3000xg for 5 min and the serum was stored at deep freezer -80 C, until assayed.

2.4.1 Cortisol measurement

Cortisol was measured from the 3 samples at day 1 and the post-Dex sample at day 2. Serum cortisol levels were analyzed with radioimmunoassay using the DIA source CORTISOL-RIA-CT Kit manufactured by DIA source ImmunoAssays S.A.Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium according to the manufacturer instructions. All cortisol samples were thawed at the same day and the assays were carried out in one and the same run.

2.4.2 Cytokines measurement

The cytokines were measured from the serum samples from day 1 at 08:00 so as not be affected by Dex. The cytokine evaluation involved the principles of Fluorescent Bead Immunoassay and the FlowCytomix. The kits used were the Flowcytomix Human Th1/Th2 11plex Kit [Interferon-γ (IFN-γ), Interleukin (IL)-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, Tumor Necrosis Factor (TNF)-a, TNF-b] BMS810FF and the Flowcytomix BMS82017FF human IL-17A simplex kit of the Bender Med Systems GmbH Campus Vienna Biocenter 2, A-1030 Vienna, Austria, Europe according to the manufacturer instructions. All cytokine samples were thawed at the same day and the assays were carried out in one and the same run.

2.5 Statistical analysis

All subsequent statistical analyses were carried out using SPSS version 20. Due to marked deviations from normality all cytokine values were logarithmically transformed. Additionally, non-parametric statistics were employed to alleviate issues with any residual skewness. Intra-group correlations were assessed with the Spearman r statistic. Group differences on cytokine values were assessed by way of the Mann-Whitney test (alpha set at .05 in all cases).

Cortisol measurement were undertaken three times during the day at 08:00, 12:00 and 18:00, prior to administration of Dex. We measured the respective values across the timeframe and assessed their total area under the curve to determine group differences. We employed two formulas in the computations, the 'area under the curve with respect to increase' (AUC_i) and 'area under the curve with respect to ground' (AUC_g) as described in Pruessner et al., (2003). With endocrinological data, it can be assumed that the use of the AUC_g will result in a measure that is more related to 'total hormonal output', whereas the use of AUC_i is more related to the sensitivity of the system, pronouncing changes over time. We have also computed the absolute difference between cortisol values pre- and post- administering dexamethasone (pre-post/Dex cort). We also compared the rate of cortisol suppressors of each group depending on whether they showed post/Dex cort values below the cutoff 5µg/dl (Meikle et al., 1975).

3. Results

3.1 Descriptive statistics

Group demographics, clinical data, cortisol and cytokine measurements are presented in Table 1. Four FEP group patients were diagnosed with Brief Psychotic

disorder, 16 with Schizophreniform disorder, 1 with Schizophrenia and 4 with Psychotic disorder Not Otherwise Specified. The mean(±SD) Duration of their Untreated Psychosis (DUP) was estimated to be 14.8(±6.3) weeks. Similarly, 8 UHR individuals presented with APS, 2 with BLIPS and 2 were diagnosed with SPD plus functional deterioration during the past year. Fourteen FEP patients (14/25; 56%) and 7 UHR (7/12; 58.3%) subjects were currently smokers ($\chi 2$=0.018, p=0.89). The groups did not differ significantly in age, education, smoking and BMI (all p>0.05). Eight (32%) out of 25 FEP patients were under treatment (risperidone, olanzapine, quetiapine, haloperidol) for a mean of 1.75±0.8 days. The mean daily Chlorpromazine equivalent dose was 555.3±388 mg. All the UHR subjects were drug naïve. Age, education, smoking and BMI did not correlate significantly with the cortisol levels nor did they with the cytokine levels (all p>0.05). Neither was the medication within the FEP group correlated significantly with either the cortisol or cytokine levels (all p>0.2). Based on the insignificant correlations of cortisol and cytokines levels with the pre-mentioned confounders, no further control analyses were carried out. Clinical assessment of the psychopathology, measured with PANSS, and the scores (PF, NF, GP and TOTAL) showed statistically significant differences between the two groups, with the FEP group, as it was expected, exhibiting higher levels of psychopathology in all measured dimensions, all p≤0.001(See Table 1).

3.2 Group comparisons for cytokine levels

Comparisons of cytokines, with the Mann-Whitney test, revealed statistically significant differences between study groups (Table 1). In particular, TNF-a(U=67.5, Z=2.846, p=0.004), IL-2(U=79, Z=2.306, p=0.021), IFN-γ(U=68, Z=2.664, p=0.008), IL-12p70(U=79, Z=2.307, p=0.021) and IL-10(U=76, Z=2.407, p=0.016) levels in the FEP

group were significantly higher compared with the UHR counterparts(Figure 1). IL-6 and IL-17A levels were below the detection limit in more than 50% of the subjects in each cohort, therefore they were omitted from further analysis.

3.3 Group comparisons for cortisol levels

The mean and SD values for AUCg, AUCi, pre-post/Dex cort for both FEP and UHR groups are shown in Table 1.

The prodromal group exhibited higher AUCg cortisol values, at a trend level (U=97.5, Z=1.70, p=0.088), in relation to the FEP group. Both groups showed negative mean AUCi values indicating that cortisol was decreasing during the day and the decrease was higher, at a trend level (U=93, Z=1.849, p=0.064), in the UHR compared with the FEP group. In regards to the pre-post/Dex cort, the 2 groups did not show significant difference (U=109, Z=1.33, p=0.183). Two subjects from the FEP group (2/25; 8%) suppressed post/Dex cort below the cutoff (5 μg/dl) as opposed to none from the UHR group ($\chi 2$=1.015, p=0.313). Interestingly, when the cutoff limit was reduced to 2 μg/dl, based on new literature suggesting that most normal individuals suppress their 8 am cortisol value to less than 2 μg/dL (Blethen and Chasalow, 1989), then 4/25 (16%) FEP patients were deemed non-suppressors whereas there was no change in the UHR group (0%); again the suppressors' rate did not differ significantly between the compared groups ($\chi 2$=2.15, p=0.142).

3.4 Correlations within each group

In the FEP group, TNF-a was positively associated with IL-10(r=+0.786, p<0.001), whereas in the UHR this association was significant but negative(r=-0.589, p=0.044).

No significant correlations (all p>0.05) were demonstrated among the cytokines, cortisol and psychopathology within each of the 2 study groups.

4. Discussion

The results of the current study is a first report of a direct comparison of both the immune and neuroendocrine profile between FEP and UHR subjects. Thus, this study constitutes a first approach to comprehend the immune-endocrine alterations during the very initial stage of psychosis, even before the emergence of the full syndrome. The results suggest significantly higher levels of TNF-a, IL-2, IL-12, IFN-γ and IL-10 in the FEP group, compared with the UHR counterparts. Whereas the results regarding the HPA axis function, show higher mean AUCg and similarly higher AUCi cortisol change/decrease at trend level in the prodromal group, yet failing to reach statistical significance. Additionally, the cortisol suppression rate on DST did not differ between the groups. TNF-a was associated with IL-10, within each group, but with an inversive mode (positively within the FEP vs negatively in the UHR). No significant associations were demonstrated among the cytokines, cortisol and psychopathology, within each group.

4.1 Cytokines

According to the literature, an increase of TNF-a was evident in a number of studies (Fernandez-Egea et al.,2009; Kaminska et al., 2001; Kim et al., 2009) on psychosis, with varied designs, diagnoses, medication status, phase of illness. Two recent reviews (Miller et al., 2011; Kirkpatrick and Miller, 2013) suggested that TNF-a as well as IFN-γ and IL-12 may be classified as trait biomarkers in schizophrenia, as it seems that they remain increased even after medication. A recent study (Mondelli et

al., 2015) showed increased TNF-a and IFN-γ in FEP subjects compared with HC, with the later cytokine playing a predictive role in the outcome of response to treatment. Our finding of a simultaneous increase of TNF-a along with cytokines of the cell mediated immunity and specifically from the T helper type 1 immune response such as IFN-γ and IL-2, could imply an increase of magnitude of the pro-inflammatory process. This increase seems to coincide with the emergence of a full blown psychotic episode, being evident in our FEP group, as opposed to the UHR counterparts. The differences of the cytokines levels between the groups are unlikely to be attributed to demographic confounding variables such as gender, age, BMI, education, smoking since (a) the groups were matched for these parameters and (b) the correlations of the demographic parameters with the cytokines levels were insignificant. Neither could the medication factor account for the differences since its correlation with the deviated cytokines was insignificant and the exposure was minimal (mean of 1.75±0.8 days). Plus, to our knowledge there is no study of the medication effect on the cytokines levels in vivo for such a minimal exposure and even a recent review (Baumeister et al., 2016) noted many inconsistencies between the in vitro and vivo literature regarding the immunomodulatory effects of psychiatric medications.

The current findings are also in line with the macrophage-T lymphocyte theory (Smith and Maes, 1995), suggesting the implication of IL-1, IL-2, TNF-α and IFN-γ produced by chronically activated macrophages and T-lymphocytes. Our results are also in agreement with the microglial hypothesis (Monji et al., 2009), predicting activation of the Central Nervous System (CNS) microglia and subsequent release of pro-inflammatory cytokines (TNF-a, IFN-γ), thus causing abnormal neurogenesis and

neuronal degradation. A recent multisite study in UHR cohorts (Cannon et al., 2015) favored a predictive role of pro-inflammatory cytokines for conversion to psychosis. On the contrary, a recent meta-analysis (Upthegrove et al., 2014) on drug naïve FEP population showed no significant effect for IFN-γ and IL-2. Again any interpretation should be cautious due to the lack of HC group in the present study.

In addition, our FEP sample presented with increased levels of IL-10 compared with the UHR group, thus offering indirectly (due to the lack of HC group) grounds to the hypothesis of a simultaneous mobilization of both the pro- and anti-inflammatory cytokine networks (Drexhage et al., 2011). Interestingly, IL-10 was associated with TNF-a within each group, but with an inverse mode (positively within FEP vs negatively in UHR). If someone takes in mind the seemingly oppositional findings of an association of polymorphic haplotypes IL-10 gene with schizophrenia (Bocchio et al., 2002) on the one hand, and the IL-10's protective role against inflammation mediated degeneration of dopaminergic neurons in Substantia Nigra (Arimoto et al., 2007) on the other, then an ambiguous role for IL-10 arises. The field becomes more perplex with the publication of preliminary data from studies in the UHR suggesting a role for IL-6 (Stojanovic et al., 2014), IL-7 and IL-8 (Perkins et al., 2015). These cytokines can trigger a shift towards activation of a T helper type 2 immune response and autoimmunity (Zhang et al., 2002; Dooms, 2013), similarly to IL-10. In an attempt to incorporate the multifaceted findings of the current study along with the hitherto evidence and preliminary findings (to be published) from our group on FEP subjects, showing significantly higher IL-4 levels compared with HC, the authors tend to favor the activation of both the inner and adaptive arms of immunity as well as the simultaneous mobilization/secretion of the pro- and anti-inflammatory series of

cytokines, as a possible aetiopathogenetic hypothesis of psychosis from the immune dysregulation point of view.

Future research needs to shed more light on the exact sequence of the immune cascades as well as on the arbitrary aetiopathogenetic or neuroprotective role of IL-10.

4.2 HPA axis function

With regards to the HPA axis function, the findings were more vague, as only a trend for increase of both the mean AUCg cortisol and the mean AUCi cortisol change in the UHR was shown, compared with the FEP group. These results could imply a subtle hyper secretion of cortisol, along with a tendency for higher cortisol fluctuation/decline in the UHR group, in relation to the FEP group. According to 2 latest reviews, which suggest a tendency for up regulation of the HPA axis function in FEP state (Karanikas et al., 2014) and even prior to the emergence of full blown psychosis (Karanikas and Garyfallos, 2015), compared with the Healthy Control state, we could anticipate that both of the groups in our study might have their HPA axes up regulated. Maybe this is the reason why there was no clear difference between the 2 groups regarding the cortisol secretion.

Moreover, the results from the DST did not reveal any difference either in the pre-post/Dex cort levels or in the suppressors' rates between the compared groups. Interestingly, when the sensitivity of the non-suppression detection was increased by lowering the cutoff of post/Dex cort to 2 μg/dl, the non-suppressors FEP patients were doubled compared with the commonly used cutoff of 5μg/dl, yet not still differing significantly from the UHR counterparts. Surprisingly the detection of non-

suppressors only within the FEP group was not combined with increased secretion of cortisol in this particular group vs the UHR counterparts. Again the lack of HC group posits limits to further exploration of possible neuroendocrine alterations.

To further blur the landscape, the hitherto data regarding the application of DST in FEP cohorts are minimal and contradictory. Cesková et al.(2006), in a study without HC, demonstrated higher rates of DST (1 mg Dex, cutoff:5μg/dl) non suppression in FEP schizophrenic patients during the acute phase of the illness whereas a recent study (Phassouliotis et al., 2013) involving a variation of DST (0.25 mg Dex, cutoff:5μg/dl) on FEP patients-diagnosed with both affective and non-affective disorders of the psychotic spectrum- and HC, reported no group differences in cortisol decline as well as higher rate of hyper suppressors FEP patients in relation to HC. To the best of our knowledge, no DST studies have been conducted on UHR populations till now. Thus, more studies with larger cohorts, including an additional HC group, are needed to delineate the severity and sequence of neuroendocrine alterations both at prodrome and FEP.

In contrast, the 2 compared groups in our study, exhibited clearer differences in their immune profile when being compared in between them. This preponderance of the evidence for immunological alteration, as opposed to the more subtle endocrine differentiation in the FEP state vs the UHR state, may potentiate the principally inflammatory aetiopathogenetic hypothesis in psychosis. Thus, the presumption (Altamura et al., 1999; Mondelli and Howes, 2014) that inflammatory cytokinaemia precedes and triggers the HPA axis in the context of the psychotic pathophysiological process, could be justified. Conversely, it could be assumed that a deficient HPA axis,

with regard to the cortisol secretion and/or fluctuation/reactivity, may not efficiently offer anti-inflammatory protection, thus rendering the organism vulnerable to disinhibited inflammatory immune reactions (Baumeister et al., 2014). The finding that the HPA axis function, in the FEP group of our study, showed a trend for less reactivity, compared with the UHR, may explain the significantly increased circulating pro-inflammatory cytokines in this group as opposed to the prodromal counterparts.

4.3 Strengths and Limitations

Collectively, this study represents a first attempt to compare the immune state of FEP with the prodrome. Additionally, the comprehensive investigation of the HPA axis function, through the estimation of cortisol's secretion, fluctuation and suppressive capacity as well as the evaluation of the psychopathology, all add to the strengths of the study. A lack of a HC group, the small cohorts and the restriction of the cohorts to only male gender could be considered as drawbacks. Also the lack of measurement of both the serum Dex levels and the post/Dex cort in the afternoon need to be included in the limitations. Lastly, the cross sectional design of the study eliminate the capacity for exploration of causal relationships within the psycho-immuno-endocrine context.

In conclusion, the results strengthen the evidence for increased inflammation playing a role in the aetiopathogenesis of psychosis. The exact sequence among the immune, endocrine and psychopathology mechanisms needs to be further clarified. Longitudinal studies proximal to the psychotic conversion are likely to be further enlightening.

The Authors declare no conflict of interest.

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Figure 1. Cytokine mean scores in the two research groups (logarithmically transformed values, bars represent 95% confidence intervals) UHR: Ultra High Risk for Psychosis, FEP:First Episode Psychosis.

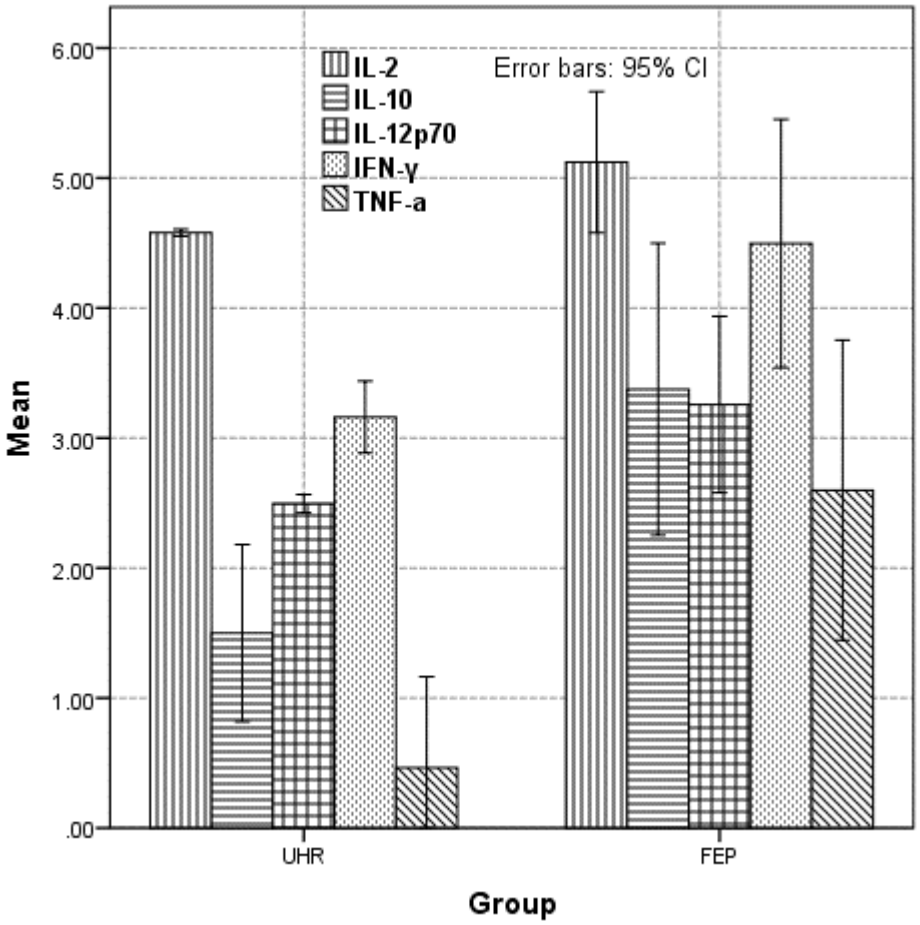

TABLE 1

Group demographics, clinical data, cytokine levels and cortisol measurements

	ACRP (N=12)	FEP (N=25)			
Demographics	Mean (SD)	Mean (SD)	Mann Whitney U	Z	p
Age	24.5 (3.1)	25.48 (5.4)	148.5	0.049	0.961
Education	13.16 (2.1)	12.44 (1.8)	126	0.864	0.388
BMI	24.15 (1.9)	24.8 (4.2)	141	0.292	0.77
Clinical Assesment	Mean (SD)	Mean (SD)	Mann Whitney U	Z	p
PANSS PF	4.92 (9.06)	18.64 (4.7)	43.5	3,488	<0.001

PANSS NF	5.33 (9.78)	18.4 (4.2)	52	3.21	0.001
PANSS GP	8.92 (16.27)	35.36 (5.37)	41.5	3,551	<0.001
PANSS TOTAL	19.17 (34.75)	72.8 (11.2)	50	3,269	0.001
Cytokines	Median (25th,75th)	Median (25th,75th)	Mann Whitney U	Z	p
IL-1b	64 (40,67)	51 (39,117)	128	0.714	0.491
IL-2	95 (94,97)	99 (96,121)	79	2,306	0.021
IL-4	71(70,71)	71 (69,76)	148	0.065	0.948
IL-5	0.4 (0.4,3.8)	11.5 (0.4, 53)	103	1,662	0.097
IL-8	331 (0.2,1028)	218 (35,694)	141	0.294	0.769
IL-10	6 (1,7)	19 (4,55)	76	2,407	0.016
IL-12p70	11 (10,12)	14 (11,23)	79	2,307	0.021
IFN-γ	20 (17,27)	39 (23,122)	68	2,664	0.008
TNF-a	0.8(0.8,0.8)	9(0.8,69.4)	67.5	2,846	0.004
TNF-b	0.6(0.6,0.6)	0.6 (0.6,50)	129	0.871	0.384
Cortisol	Mean (SD)	Mean (SD)	Mann Whitney U	Z	p
AUCg	206.4(72.85)	170.44(61.91)	97.5	1.70	0.088
AUCi	80.63(56.45)	42.37(58.88)	93	1,849	0.064
pre-post/Dexcort	12.80(3.49)	10.45(4.44)	109	1.33	0.183

UHR: Ultra High Risk for Psychosis, FEP: First Episode Psychosis

Age, Education in years; BMI: Body Mass Index

PANSS: Positive and Negative Syndrome Scale; [PF: Positive Factor, NF: Negative Factor,
GP: General Psychopathology, TOTAL: Total score], IL: Interleukin, IFN: Interferon, TNF: Tumor Necrosis Factor
AUCg:Area Under Curve ground, AUCi: Area Under Curve increase,
pre-post/Dex cort: cortisol levels prior Dex minus cortisol post Dex, Dex: Dexamethasone
Cytokine levels presented as median, 25th,75th percentile in pg/ml
AUCg-AUGi: absolute values in μg/dl/min, pre-post Dex cort in μg/dl

p: Between -group differences with the Mann-Whitney test, p set at 0.05