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Profiling classical neuropsychiatric biomarkers across biological fluids and following continuous lumbar puncture: A guide to sample type and time

Gallen Triana-Baltzer^{a,*}, Maarten Timmers^{b,1}, Peter De Boer^{b,2}, Manja Schoene^a, Maura Furey^a, Cathy Bleys^b, Isabeau Vrancken^b, Randy Slemmon^a, Marc Ceusters^b, Luc van Nueten^b, Hartmuth Kolb^a

^a Janssen Research & Development, LLC, San Diego, CA, USA

^b Janssen Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Belgium

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ABSTRACT

Identification of putative biomarkers for neuropsychiatric disorders has produced a diverse list of analytes involved in inflammation, hypothalamic–pituitary–adrenal axis (HPA) regulation, growth factor and metabolic pathways. However, translation of these findings to accurate and robust assays has been stalled, affecting objective diagnoses, tracking relapse/remission, and prediction/monitoring of drug affect. Two important factors to control are the sample matrix (e.g. serum, plasma, saliva, or cerebrospinal fluid) and time of sample collection. Additionally, sample collection procedures may affect analyte level. In this study, a panel of 14 core neuropsychiatric biomarkers was measured in serum, plasma, saliva, and cerebrospinal fluid (CSF), all collected from 8 healthy volunteers at the same time. In a second cohort of 7 healthy volunteers, 6 analytes were measured in serum and CSF collected at 13 timepoints over a 24-h period after catheter placement. We found that many of the analytes were quantifiable in all sample types examined, but often at quite different concentrations and without correlation between the sample types. After catheter placement, a diurnal pattern was observed for cortisol and interleukin-6 in serum, and transient spikes were observed in interleukin-1 β . In CSF, a chronic elevation of several cytokines was observed instead, perhaps due to the continuous sampling procedure. These findings enable more informed decision-making around sample type and collection time, which can be implemented in future biomarker studies.

Clinicaltrial.gov identifiers: NCT02933762, NCT02475148.

1. Introduction

Several decades of research to identify biomarkers for neuropsychiatric disorders (including major depressive disorder [MDD], treatment-resistant depression [TRD], bipolar disorder [BD], anxiety, post-traumatic stress disorder [PTSD], and schizophrenia) have repeatedly suggested that changes in inflammatory pathways, hypothalamic–pituitary–adrenal (HPA) axis regulators, growth factors, metabolic factors, and tryptophan/kynurenine metabolism correlate well to the disease state [1]. To date, no robust assay has been developed or implemented to improve diagnosis or track neuropsychiatric disorders. As such, clinical diagnosis remains the gold standard, yet it can be highly subjective based on the clinician interviewer's training and on the

patient's response. Objective analyte measurements with high specificity and sensitivity are of great importance in subtype-specific diagnoses, disease tracking, and treatment of neuropsychiatric disorders [2,3]. The attainment of this goal can be facilitated by identifying biomarkers that accurately reflect pathophysiologic processes in these disorders.

Several factors have been known to hinder translation of early neuropsychiatric biomarker findings to improve current clinical trial designs and methods: 1) evaluation in a heterogeneous study population (e.g. age, gender, ethnicity, body mass index, diet, exercise, illness, anti-inflammatory drug/hormone use, major clinical grouping), 2) use of suboptimal assays and testing methods, 3) evaluation of single biomarkers vs. aggregated analysis or pathway analysis, 4) inappropriate

* Corresponding author. Janssen Research & Development, LLC, USA.

E-mail address: gtrianab@its.jnj.com (G. Triana-Baltzer).

¹ Shared first authors.

² Deceased.

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sample collection methods, 5) inappropriate sample type, and 6) sample collection without consideration of time of day. Factors 1–3 have been discussed adequately in the literature; in this paper, we address factors 4–6 [4].

Sample collection method differences such as use of catheter vs. repeated puncture have been shown to potentially impact levels of inflammatory cytokines perhaps due to injury at the injection site, leading to local cytokine flare [5]. Additional factors that can influence apparent concentration of various analytes include: 1) anticoagulant choice for plasma, 2) presence of clotting enhancement agent for serum, 3) clotting time for serum, 4) blood contamination in saliva or CSF, and 5) processing time and temperature [6–9]. Further characterization of each of these factors for the analyte of interest will help to minimize the effect of preanalytical variables.

Identification of an appropriate sample type for biomarker evaluation is crucial because the target organ in neuropsychiatric disorders (i. e. brain) is largely inaccessible for direct biomarker level measurement in living participants. Although CSF may then be the best fluid to analyze, it is usually not collected from patients with neuropsychiatric disorders, and blood is generally the primary fluid evaluated. Saliva may in some cases be preferable due to ease of collection, leading to more frequent (and potentially at home) sampling. Numerous mechanisms have been proposed for cross talk between peripheral and central analytes; thus, measurement of peripheral fluids (e.g., saliva) remains promising as a proxy for status in the brain [10–15]. However, limited guidance is available in the literature on relative levels of neuropsychiatric biomarkers across these sample types, as well as on correlation of the levels between these fluids.

Controlling for sample collection time is highly important for measuring several neuropsychiatric biomarkers due to potential diurnal rhythm or prandial state. Cortisol and adrenocorticotropic hormone (ACTH) are well known to exhibit prominent diurnal rhythms, and leptin is also known to exhibit fluctuation after meals [16]. Beyond these analytes, little is known about time of day or food effect on core neuropsychiatric biomarkers and thus variation in collection time can add additional noise to these measures if uncontrolled.

In this paper, we report on the effects of sample type and collection time on measurements of several core neuropsychiatric biomarkers, in an effort to provide a guide to harmonize future biomarker work. We focus on 14 protein analytes that were selected based on review of the primary literature on MDD biomarkers; however, these analytes are also often reported to correlate with other neuropsychiatric disorders such as TRD, BD, anxiety, PTSD, and schizophrenia.

2. Methods

This exploratory study was performed on biomarker samples collected from healthy volunteers enrolled in two phase-1 studies (Study 1: NCT02812251, Study 2: NCT02933762). In one cohort of 8 healthy participants (Study 1), serum, plasma, and CSF bio samples were collected at 8:00 a.m. Saliva was collected at 8:00 a.m. and 4:00 p.m. Samples were analyzed for levels of interleukin (IL)-6, IL-10, tumor necrosis factor alpha (TNF α), IL-6-receptor (IL-6-R), glycoprotein-130 (GP130), C-reactive protein (CRP), cortisol, mature c (BDNF), adiponectin, and leptin.

In a second cohort of 7 healthy participants (Study 2), serum and CSF biosamples were collected at 13 timepoints over a 24-h period, beginning at 7:00 a.m. to 11:00 a.m. (depending on the participant). Serum was collected by single catheter placement and CSF by indwelling catheter at lumbar position. Cortisol, IL-6, TNF α , IL-10, IL-1 β and transforming growth factor beta (TGF- β), were measured.

Both Study 1 and 2 participant inclusion criteria (defined as healthy) were: normal serum chemistry, hematology, urinalysis, physical and neurological exam, vital signs, and lack of major medical history issues. Participants were age 55–75 with BMI 18–32 kg/m². Study 1 comprised 80 participants, but only 8 provided matching timepoint serum, plasma,

CSF, and saliva and thus make up cohort 1 used in this report. Study 2 comprised 25 participants, but only 7 were treated with placebo and therefore make up cohort 2 used in this report. Demographics of participants included from both studies are shown in [Supplementary Table 1](#).

The studies were conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki (2013) and are consistent with good clinical practices and applicable regulatory requirements. Informed consent forms and study protocols were approved by the Independent Ethics Committee or Institutional Review Board at the study center. All healthy volunteers provided written informed consent before study participation.

2.1. Biochemical analysis

All assays used in this bioanalysis have passed internal Janssen core technical validation for sensitivity, precision, accuracy, and dilution linearity; technical parameters for the assays are shown in [Supplementary Table 2](#). IL-6, IL-10, IL-1 β , TNF α , and TGF- β were quantified on a Simoa HD-1 analyzer (kits# 125, 128, 121, 143, 230, Quanterix, Lexington MA). Measurement of CRP, IL-6-R, and adiponectin were performed on MSD Sector 6000 with manufacturer recommendations (kits# K151STD, K151ALC, K151BXC, MesoScale Discovery, Rockville MD). Measurement of leptin was performed on MSD Sector 6000 with substitution of diluent 2 and 3 for manufacturer diluents (kit# K151BYC, MSD). Cortisol measurement was performed with 2 assay kits in cohort 1 due to differences in intended sample type; ALPCO kit (11-CORHU-E01 ALPCO, Salem NH ([Figs. 1D and 2](#)) and Salimetrics kit (1–3002, Salimetrics, Carlsbad CA ([Supplementary Fig. 1](#)). Mature BDNF was measured on MSD Sector 6000 with substitution of diluent 101 and 48 for manufacturer diluents (kit# N451A, MSD; [Fig. 1B and Supplementary Fig. 3](#)), and on a Simoa HD-1 analyzer (kit#237, Quanterix; [Supplementary Fig. 2](#)).

2.2. Statistical analysis

All linear correlations were performed with Pearson's model, using GraphPad Prism software v7.00 ([Fig. 1](#), [Supplementary Figs. 1 and 2](#), [Supplementary Table 3](#)). Comparison between analytes ([Fig. 1](#) data) was also analyzed using Bland Altman difference plots ([Supplementary Fig. 4](#)). Differences between Study 1 and 2 cohorts ([Supplementary Table 1](#)) were tested using Mann-Whitney (age) or chi-square (sex) tests.

3. Results

3.1. Mood biomarker levels across sample types (Cohort 1)

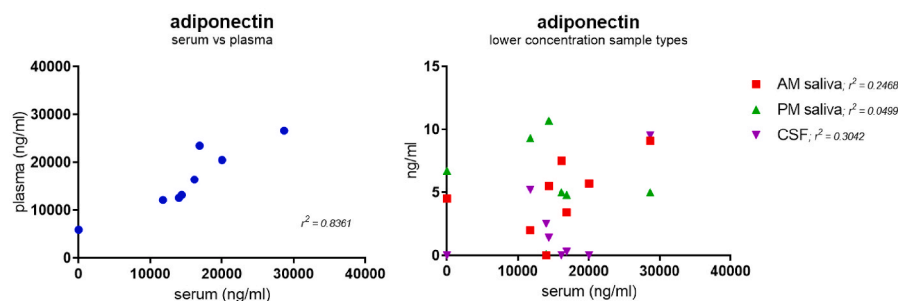
Adiponectin: Adiponectin was not quantifiable in saliva or CSF. Comparable levels were observed in serum and plasma, with good correlation ($r^2 = 0.8361$, $p = 0.0015$; [Fig. 1A](#)).

Mature BDNF: Mature BDNF was not quantifiable in saliva or CSF with standard assays. The levels were ~4x higher in serum compared with plasma ([Fig. 1B](#)). Repeat analysis with a high sensitivity BDNF assay on Simoa platform was able to quantify signal in some saliva and CSF samples; however, the levels were far below those in serum/plasma. This method reported concentrations ~10,000x lower in saliva, and ~100,000x lower in CSF, compared with serum levels ([Supplementary Fig. 2](#)). Both assays confirmed no correlation between any of the sample types.

Due to the unexpected lack of correlation between plasma and serum, the impact of clotting time on serum BDNF levels was evaluated in a cohort of 8 volunteers. Serum BDNF levels increased with clotting time up to 30 min, then were stable until 60 min, then increased further ([Supplementary Fig. 3](#)).

Leptin: Leptin was not quantifiable in AM saliva or CSF. It was quantifiable in PM saliva, suggesting elevation throughout the day and

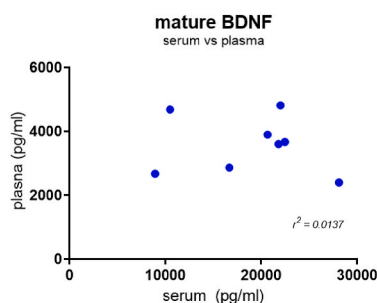
A. Adiponectin



Participants	1	2	3	4	5	6	7	8
Serum	14355.9	28648.0	16873.6	11733.7	13992.4	16146.5	20026.2	0.0
Plasma	13196.7	26612.7	23481.7	12107.4	12576.2	16369.6	20436.7	5895.4
AM saliva	5.5	9.1	3.4	2.0	ND	7.5	5.7	4.5
PM saliva	10.7	5.0	4.8	9.3	0.1	5.0	110.2	6.7
CSF	1.4	9.5	0.3	5.2	2.5	ND	ND	ND

Concentrations expressed in ng/ml. LLOQ = 120 ng/ml, ULOQ = 2,000,000 ng/ml.

B. Mature brain-derived neurotrophic factor (BDNF)



Participant	1	2	3	4	5	6	7	8
Serum	8908.5	22446.5	21787.5	20664.2	16664.1	21999.4	28090.9	10471.7
Plasma	2674.2	3668.8	3602.0	3897.8	2861.8	4810.0	2400.7	4682.9
AM saliva	ND	ND	ND	ND	ND	ND	ND	ND
PM saliva	ND	ND	ND	ND	ND	ND	ND	ND
CSF	ND	ND	ND	ND	ND	ND	ND	ND

Concentrations expressed in pg/ml. LLOQ = 784 pg/ml, ULOQ = 800,000 pg/ml.

Fig. 1. Comparison of analyte levels across sample types. Adiponectin, mature BDNF, leptin, cortisol, CRP, GP130, IL-6-R, IL-6, TNF α , and IL-10 (panels, A-J, respectively) were measured in matching serum, plasma, AM saliva, PM saliva, and CSF in 8 healthy volunteer donors (cohort 1). Concentrations are shown in the data tables and compared to serum levels in the graphs. Pearson's correlations (r^2) are shown in each graph. Assay linear range is noted below each table and sample measurements outside of linear range are noted in italics. ND = Not Detected (i.e. signal is below limit of detection). BDNF= brain-derived neurotrophic factor; CSF= cerebrospinal fluid; CRP=C-reactive protein; LLOQ= lower limit of quantitation; ULOQ= upper limit of quantitation; TNF α =tumor necrosis factor alpha.

after meals. Leptin levels were comparable in serum and plasma (Fig. 1C), good correlation was observed ($r^2 = 0.9983$, $p < 0.0001$). Signal in CSF was below the level of quantification (3.28 ng/ml), but it was still detectable and showed a weak correlation with serum ($r^2 = 0.5077$, $p = 0.0473$).

Cortisol: Cortisol was quantifiable in all samples (Fig. 1D). The levels were comparable in serum and plasma, with good correlation ($r^2 = 0.9081$, $p = 0.0003$). Cortisol levels were $\sim 10x$ lower in AM saliva and CSF, and $\sim 100x$ lower in PM saliva (reflecting known diurnal rhythm) compared with serum/plasma levels. Poor correlation was found amongst all other sample types (Supplementary Fig. 1). The two cortisol kits yielded similar findings.

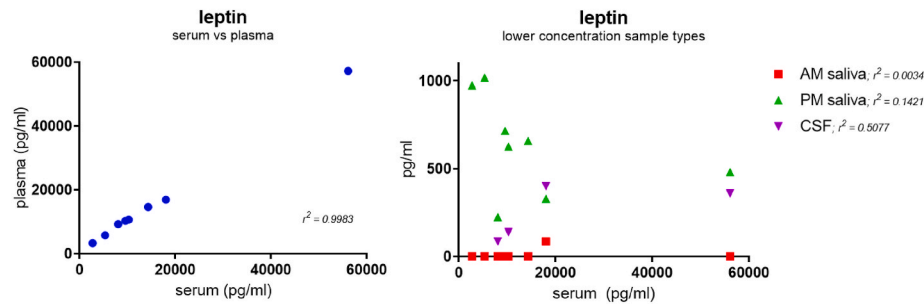
CRP: CRP levels were comparable in serum and plasma (Fig. 1E),

with good correlation ($r^2 = 0.9991$, $p < 0.0001$). Signal in saliva and CSF was below the level of quantification (13 ng/ml), but detectable. CSF showed good correlation with serum ($r^2 = 0.9834$, $p < 0.0001$).

GP130: GP130 levels were highest in serum and plasma compared with saliva or CSF levels (Fig. 1F). A weak correlation was observed between serum and plasma ($r^2 = 0.4286$, $p = 0.0781$, likely correlation inhibited by 1 outlier). Saliva and CSF levels were $\sim 5x$ lower than serum (without any correlation) and only quantifiable in some healthy volunteers.

IL-6-R: Levels were comparable in serum and plasma (Fig. 1G), with good correlation ($r^2 = 0.8899$, $p = 0.0004$). CSF levels were $\sim 10x$ lower versus serum/plasma, with a trend of correlation with serum ($r^2 = 0.4447$, $p = 0.0709$). Saliva levels $\sim 200x$ lower than serum, and only

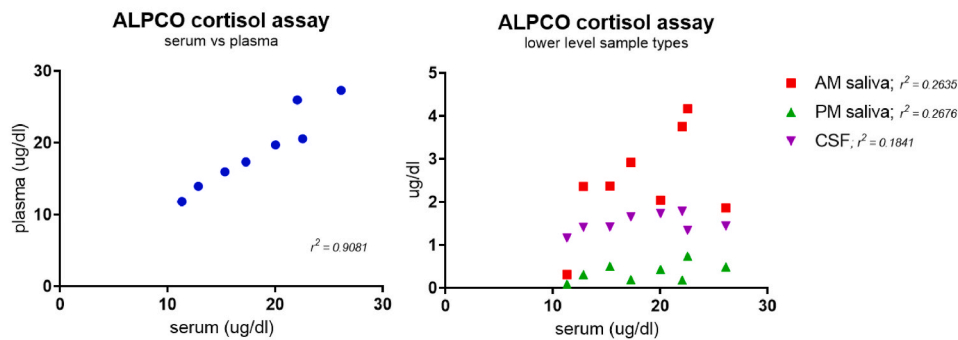
C. Leptin



Participants	1	2	3	4	5	6	7	8
Serum	14312.4	10252.4	18007.0	5315.7	9565.9	56071.7	8041.3	2716.7
Plasma	14618.6	10674.1	16917.7	5765.8	10342.9	57207.9	9271.3	3344.9
AM saliva	ND	ND	85.6	ND	ND	ND	ND	ND
PM saliva	657.5	624.5	328.7	1017.1	715.3	479.6	224.0	972.9
CSF	ND	138.9	399.9	ND	ND	360.2	85.6	ND

Concentrations expressed in pg/ml. LLOQ = 3300 pg/ml, ULOQ = 800,000 pg/ml.

D. Cortisol



Participants	1	2	3	4	5	6	7	8
Serum	22.54	15.31	12.84	17.25	22.04	11.31	20.03	26.10
Plasma	20.57	15.97	13.94	17.34	25.96	11.80	19.72	27.31
AM saliva	4.17	2.37	2.36	2.92	3.75	0.31	2.04	1.86
PM saliva	0.74	0.50	0.31	0.19	0.18	0.09	0.43	0.49
CSF	1.34	1.42	1.41	1.65	1.78	1.16	1.73	1.44

Concentrations expressed in ug/dl. LLOQ = 10 ug/dl, ULOQ = 1200 ug/dl.

Fig. 1. (continued).

quantifiable in few participants. No correlation was observed with serum.

IL-6: IL-6 levels were found to be highest in saliva and CSF (Fig. 1H). Serum and plasma levels were equivalent to or up to 25x lower than saliva/CSF. Good correlation was observed between serum and plasma ($r^2 = 0.9585$, $p < 0.0001$), but not between any other sample types.

TNF α : The levels were highest in serum, plasma, and AM saliva (Fig. 1I). PM sample levels were a bit lower, and CSF much lower than other samples. Serum and plasma levels were equivalent, with good correlation ($r^2 = 0.9923$, $p < 0.0001$). No other correlation was observed between any other sample types.

IL-10: Levels of IL-10 were comparable in all sample types (Fig. 1J). Good correlation was seen between serum and plasma ($r^2 = 0.9965$, $p <$

0.0001) but not between any other sample types.

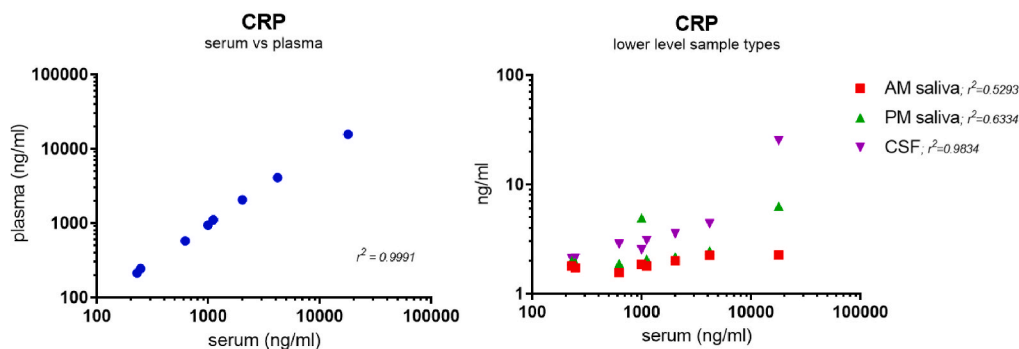
The comparison of analyte concentrations between sample types is summarized in [Supplementary Table 3](#).

3.2. Cortisol, TGF β , and cytokine level fluctuations in CSF and serum (Cohort 2)

Cortisol: Cortisol was measured only in serum and exhibited well-known diurnal rhythm with a peak at ~9:00 a.m. and trough at ~7:00 p.m. Cortisol levels fluctuated by ~2x over this period (Fig. 2A).

IL-6: As seen in cohort 1, baseline IL-6 was found at higher levels in CSF than in serum. Serum levels were found lowest in the mid-AM, then until peaking in the evening, then returned to baseline by mid-AM. IL-6

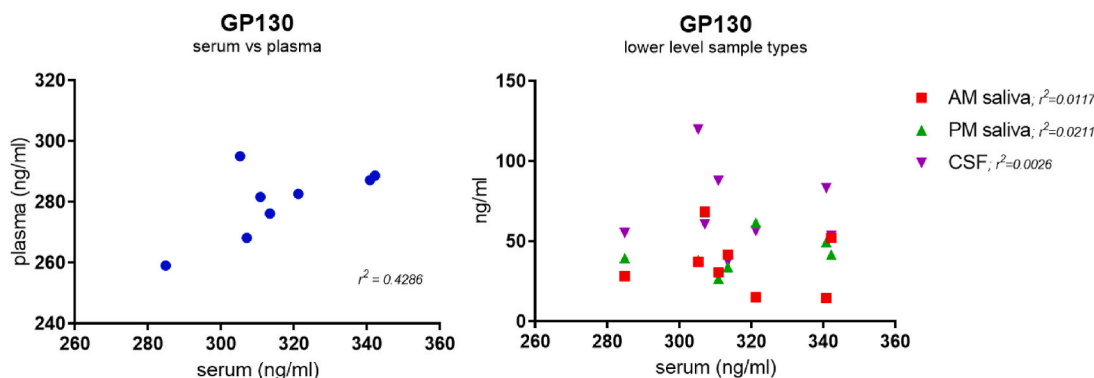
E. C-reactive protein (CRP)



Participants	1	2	3	4	5	6	7	8
Serum	2020.29	620.88	4182.92	17914.91	245.72	1105.09	993.80	228.28
Plasma	2075.86	577.62	4108.75	15720.37	244.21	1103.71	943.21	213.04
AM saliva	2.00	1.56	2.24	2.26	1.72	1.79	1.86	1.79
PM saliva	2.16	1.89	2.47	6.31	1.93	2.07	4.93	2.00
CSF	3.54	2.83	4.36	25.21	2.11	3.06	2.52	2.09

Concentrations expressed in ng/ml. LLOQ = 13 ng/ml, ULOQ = 202,000 ng/ml.

F. Glycoprotein 130 (GP130)



Participants	1	2	3	4	5	6	7	8
Serum	313.4	305.2	307	340.8	342.2	284.8	321.2	310.8
Plasma	276.2	295	268.2	287.2	288.6	259	282.6	281.6
AM saliva	41.6	37.2	68.4	14.6	52.2	28.2	15	30.6
PM saliva	33.8	38.2	68	49.4	41.6	39.4	61.4	26.6
CSF	37.4	119.8	60.6	83.2	53.4	55.2	56.4	87.8

Concentrations expressed in ng/mL. LLOQ = 25 ng/ml, ULOQ = 1600 ng/ml.

Fig. 1. (continued).

levels fluctuated by ~10x over this period (Fig. 2B). In CSF, IL-6 levels increased continuously (to ~3x of baseline), without returning to baseline, suggesting the elevation was an impact of the collection procedure (Fig. 5A).

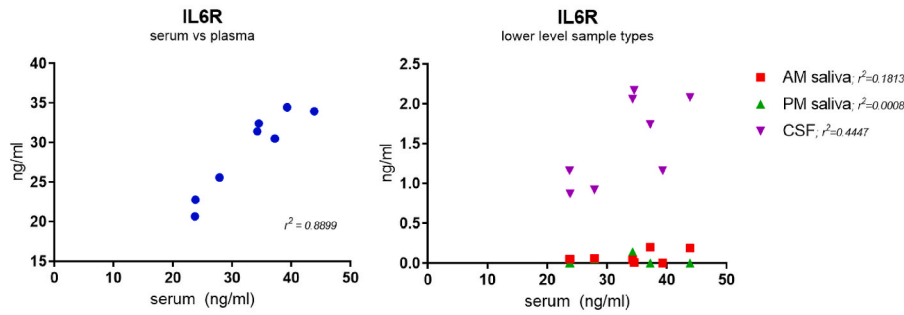
TNF α : As seen in cohort 1, baseline TNF α was found at lower levels in CSF than in serum (10% of serum levels). Serum levels did not change ($\pm 20\%$ of baseline) throughout the 24-h period (Fig. 3A). In CSF, the TNF α levels increased continuously (to ~2x of baseline), without returning to baseline, suggesting the elevation was an impact of the

collection procedure (Fig. 5B).

IL-10: As seen in cohort 1, baseline IL-10 was found at equivalent levels in CSF and serum. Serum levels did not change ($\pm 20\%$ of baseline) throughout the 24-h period (Fig. 3B). In CSF, the IL-10 levels increased continuously (to ~4x of baseline), without returning to baseline, suggesting the elevation was an impact of the collection procedure (Fig. 5C).

IL-1 β : Baseline IL-1 β was found at equivalent levels in CSF and serum (data not shown). Serum levels fluctuated considerably throughout the

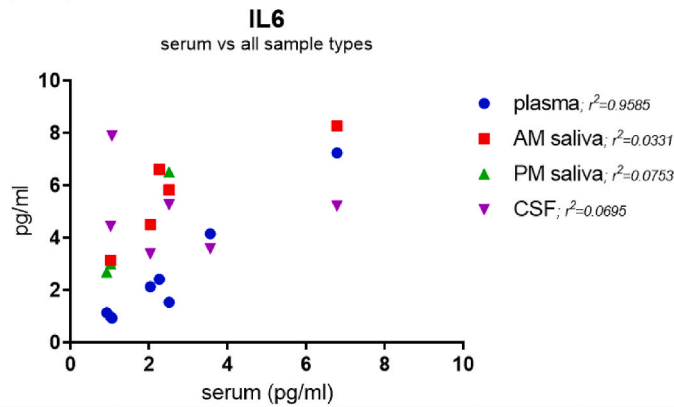
G. Interleukin-6 receptor (IL-6-R)



Participants	1	2	3	4	5	6	7	8
Serum	27.85	34.48	34.23	43.86	23.75	23.67	39.25	37.18
Plasma	25.57	32.40	31.41	33.95	22.75	20.65	34.44	30.49
AM saliva	0.06	0.01	0.04	0.19	0.05	0.05	ND	0.20
PM saliva	0.05	0.02	0.14	ND	ND	ND	ND	ND
CSF	0.92	2.17	2.06	2.08	0.87	1.16	1.16	1.74

Concentrations expressed in ng/ml. LLOQ = 0.3 ng/ml, ULOQ = 250 ng/ml.

H. Interleukin-6 (IL-6)



Participants	1	2	3	4	5	6	7	8
Serum	1.02	2.51	2.03	6.78	0.92	2.26	3.56	1.05
Plasma	0.98	1.54	2.13	7.24	1.14	2.41	4.15	0.93
AM saliva	3.14	5.83	4.50	8.27	33.15 ^a	6.60	209.76 ^a	11.07 ^a
PM saliva	3.00	6.50	28.60 ^a	19.56 ^a	2.69	31.47 ^a	109.62 ^a	25.18 ^a
CSF	4.44	5.26	3.39	5.21	25.71 ^a	35.71 ^a	3.58	7.89

Concentrations expressed in pg/ml. LLOQ = 0.13 pg/ml, ULOQ = 118 pg/ml.

^aExcluded the high saliva and CSF measurements in the graph, but included in linear correlation analysis

Fig. 1. (continued).

24-h period, as evidenced by acute elevations of ~10x concentration at variable timepoints (Fig. 4). In CSF, IL-1β levels also exhibited spikes at variable timepoints, and overall showed a continuous increase, without returning to baseline, suggesting the elevation was an impact of the collection procedure (Fig. 5D).

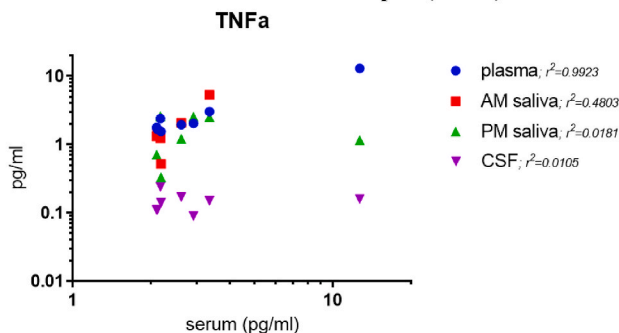
TGFβ: Baseline TGFβ was generally found in serum at levels ~100x of those in CSF (data not shown). Levels in serum and CSF were stable (±20% of baseline) throughout the 24-h period (Figs. 3C and 6).

4. Discussion

Diagnosis of neuropsychiatric disorders currently relies on subjective measures, namely on clinician’s observations of patient’s behavior, and patient’s self-reporting. In addition, even classically defined diseases, such as MDD, present with considerable heterogeneity and thus may be better represented as specific subtypes of these disorders. We propose that objective measures, such as accurate genetic and protein biomarkers, may aid in classifying these subtypes.

While widespread utility of biomarkers may have faltered due to

I. Tumor necrosis factor alpha (TNF α)

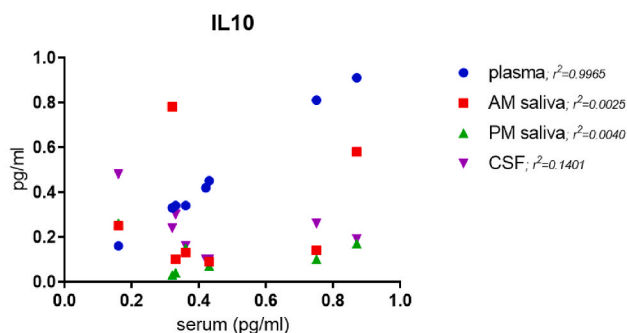


Participants	1	2	3	4	5	6	7	8
Serum	2.11	2.10	2.18	3.35	12.67	2.61	2.91	2.17
Plasma	1.67	1.78	1.55	3.04	12.96	1.95	2.04	2.41
AM saliva	1.55	1.32	0.52	5.34	52.15 ^a	2.08	51.88 ^a	1.23
PM saliva	1.73	0.71	0.33	2.51	1.15	1.21	2.55	2.57
CSF	0.11	0.11	0.14	0.15	0.16	0.17	0.09	0.24

Concentrations expressed in pg/ml. LLOQ = 0.36 pg/ml, ULOQ = 179 pg/ml.

^aExcluded the high saliva measurements in the graph, but included in linear analysis

J. Interleukin-10 (IL-10)



Participants	1	2	3	4	5	6	7	8
Serum	0.36	0.75	0.43	0.87	0.32	0.16	0.42	0.33
Plasma	0.34	0.81	0.45	0.91	0.33	0.16	0.42	0.34
AM saliva	0.13	0.14	0.09	0.58	0.78	0.25	12.38 ^a	0.10
PM saliva	0.15	0.10	0.07	0.17	0.03	0.26	3.01 ^a	0.04
CSF	0.16	0.26	0.10	0.19	0.24	0.48	0.10	0.30

Concentrations expressed in pg/ml. LLOQ = 0.164 pg/ml, ULOQ = 120 pg/ml.

^aExcluded the high saliva measurements in the graph, but included in linear analysis

Adiponectin, mature BDNF, leptin, cortisol, CRP, GPI30, IL-6-R, IL-6, TNF α , and IL-10 (panels, A-J, respectively) were measured in matching serum, plasma, AM saliva, PM saliva, and CSF in 8 healthy volunteer donors (cohort 1). Concentrations are shown in the data tables and compared to serum levels in the graphs. Pearson's correlations (r^2) are shown in each graph. Assay linear range is noted below each table and sample measurements outside of linear range are noted in italics.

BDNF= brain-derived neurotrophic factor; CSF= cerebrospinal fluid; CRP=C-reactive protein; LLOQ= lower limit of quantitation; ULOQ= upper limit of quantitation; ND = Not Detected; TNF α =tumor necrosis factor alpha;

Fig. 1. (continued).

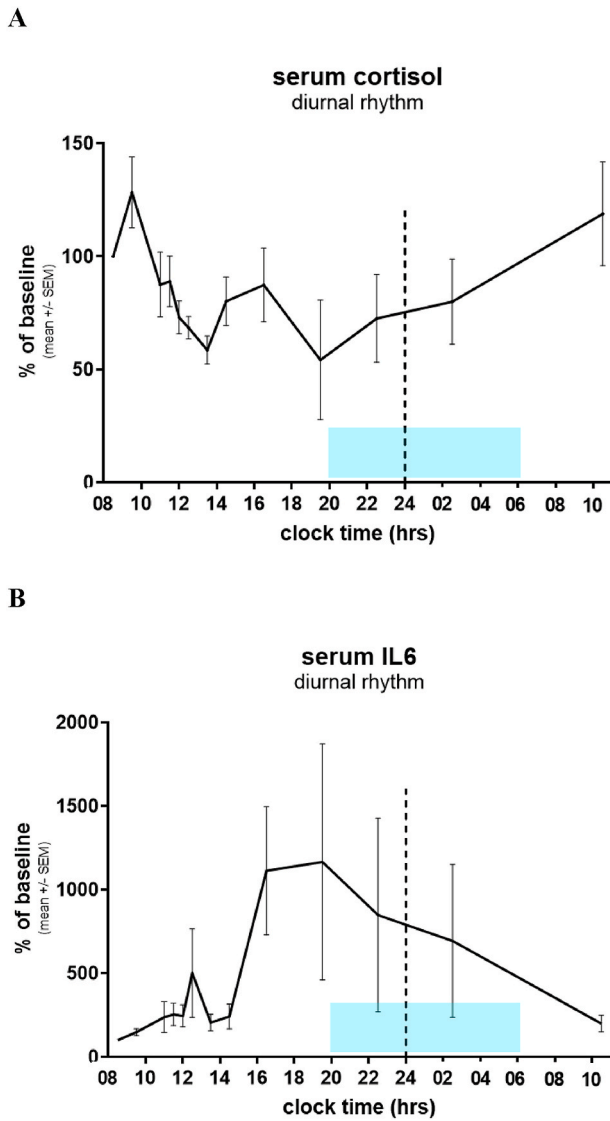


Fig. 2. Diurnal rhythm of cortisol and IL-6 in serum. Cortisol (A) and IL-6 (B) were measured in serum from 7 healthy volunteers over a 24-h period (cohort 2). Data are shown as % of baseline (mean± SEM of the 7 volunteers). Dashed line indicates midnight, blue bar indicates typical nighttime. IL- interleukin -6. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

attempts to ignore this clinical heterogeneity, most protein biomarker measurements have also been plagued by lack of standardization. Here we address variables that can impact the major neuropsychiatry biomarker measures in order to provide a guide for future studies.

Biomarker measurements can be dramatically impacted by sample type analyzed, sample collection method, and time of the day. In this study, we demonstrated that while many classical neuropsychiatric biomarkers can be analyzed in serum or plasma, the levels typically do not correlate with concentrations in CSF. The primary organ implicated in the pathology of neuropsychiatric disorders, the brain, is not in direct contact with peripheral blood, implying that neuropsychiatric biomarker measurements in blood should be interpreted with caution [17,18]. From the current results, it was observed that leptin, CRP, and IL-6-R were the only analytes that may show correlation between serum and CSF, and even then, only modest correlation. Additional studies comparing CSF concentrations to blood concentrations in patients with neuropsychiatric disorders are warranted to expand evidence on these impressions.

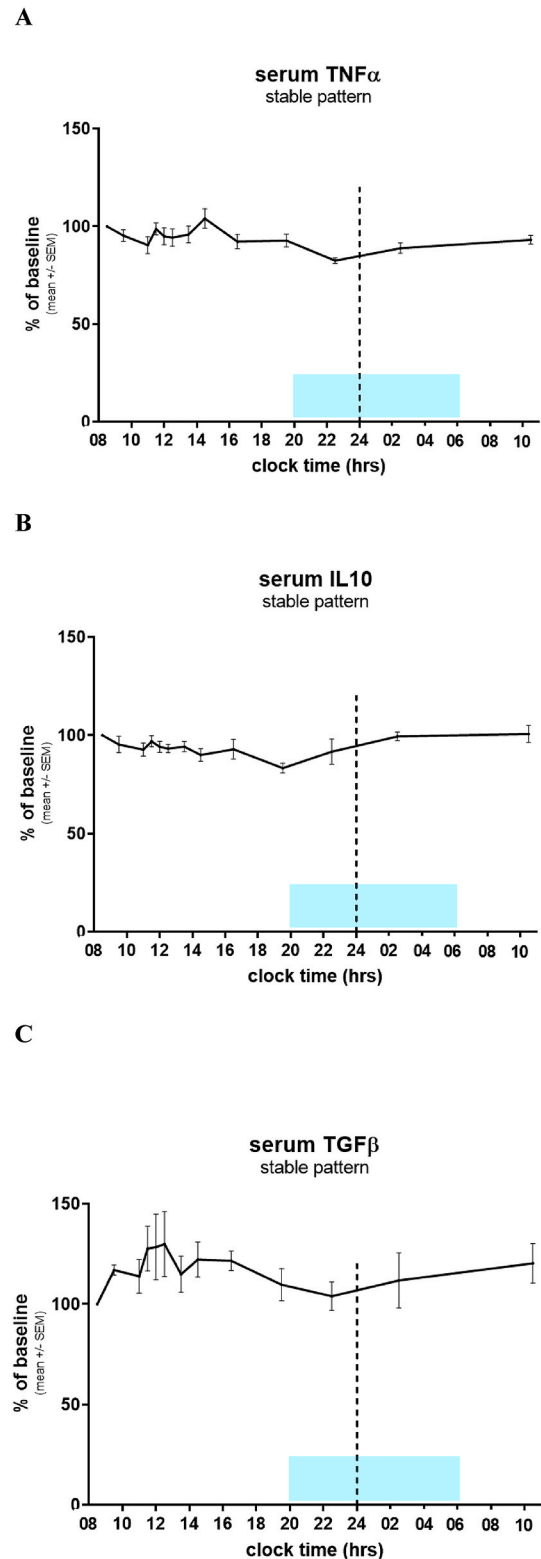


Fig. 3. Stable pattern of TNF α and TGF β in serum. TNF α (A), IL-10 (B) and TGF β (C) were measured in serum from 7 healthy volunteers over a 24-h period (cohort 2). Data are shown as % of baseline (mean± SEM of the 7 volunteers). Dashed line indicates midnight, blue bar indicates typical nighttime. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

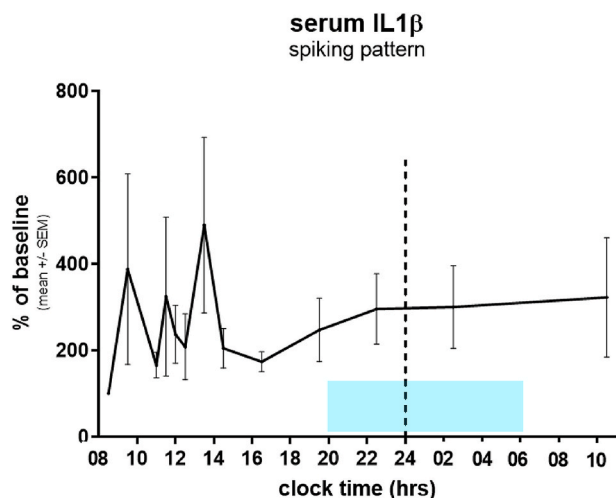


Fig. 4. Spiking levels of IL-1 β in serum. IL-1 β was measured in serum from 7 healthy volunteers over a 24-h period (cohort 2). Data are shown as % of baseline (mean \pm SEM of the 7 volunteers). Dashed line indicates midnight, blue bar indicates typical nighttime. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Levels of analyte in serum and plasma were comparable and correlated, except for GP130, IL-6-R, and mature BDNF. The latter in particular exhibited much higher levels in serum, and serum levels did not correlate with plasma levels. Higher levels in serum are thought to be due to release of sequestered BDNF from platelets upon clotting and/or enhanced processing of soluble proBDNF to mature BDNF by various proteases upon clotting [19,20]. Lack of correlation between these sample types may reflect the complexity of biological processes governing mature BDNF availability. This lack of correlation between serum and plasma BDNF may be of particular concern in translating the findings on BDNF measurements in the literature as there is no consensus on which sample type is measured [9]. Additionally, as might be expected, clotting time of serum can impact apparent levels of mature BDNF, necessitating standardization. Our data suggest serum clotting time should be within 30–60 min.

Interestingly, mature BDNF was not found at appreciable levels in CSF, even with new high sensitivity platforms that have lowered the lower limit of quantification of standard BDNF assays by nearly 3 orders of magnitude (data not shown) (Simoa HD-1 BDNF assay#102039, Quanterix). Despite the nomenclature that BDNF is “brain-derived,” BDNF is produced in high levels by peripheral vascular endothelium [21]. Given this fact and that levels are undetectable in CSF, it is suggested that measurements in blood may not be reflective of brain levels.

Of all analytes studied herein, IL-6 appears to be an exception in that levels of this analyte were found to be higher centrally vs. peripherally. Indeed, IL-6 was found at higher levels in CSF than in serum or plasma and did not exhibit the diurnal variation seen peripherally. This suggests that a significant portion of IL-6 may be produced centrally and regulated by different mechanisms than in the periphery. Regardless, peripheral IL-6 levels have been repeatedly correlated with neuropsychiatric disorders [22–25], and taken together with the current findings, IL-6 protein should be explored in future studies as a neuropsychiatric biomarker or potential therapeutic target for neuropsychiatric disorders. Utility as a biomarker should consider the diurnal rhythm in serum noted here, and by others [5,10,26]. Non-steroidal anti-inflammatory drug (NSAID) use and intense exercise have been noted to alter IL-6 levels and can be used as an exclusion criteria in IL-6 interpretation for future studies [27–31]. Finally, IL-6 levels were found at particularly high levels in saliva, higher than in serum, and higher than other cytokines. However, the biological significance of this observation is unclear. Further studies in neuropsychiatric disorders are

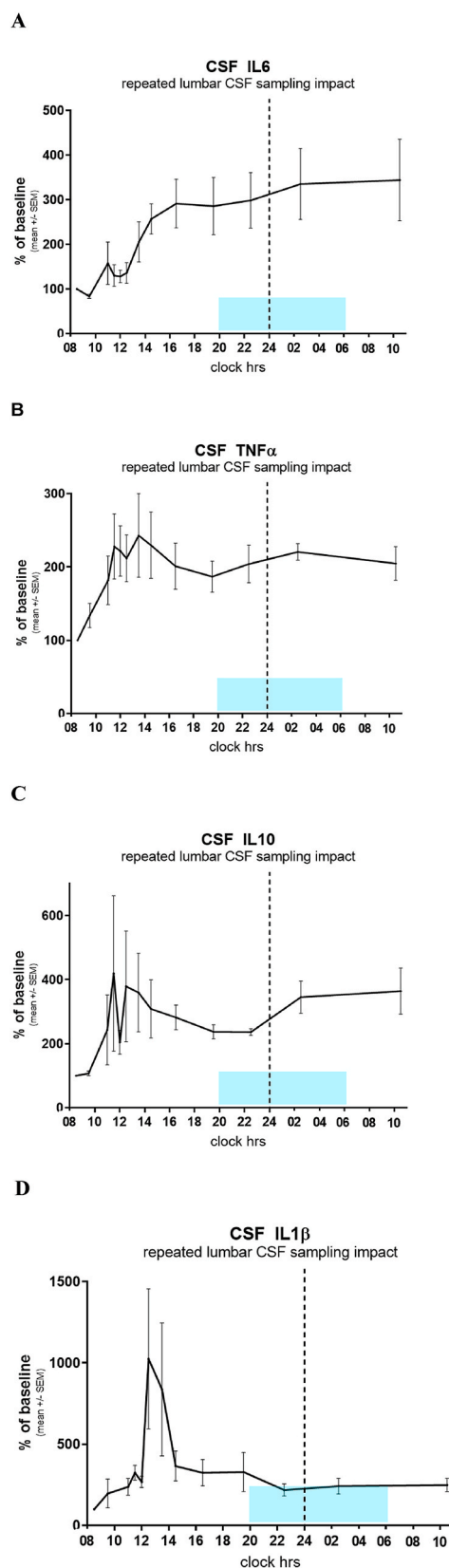
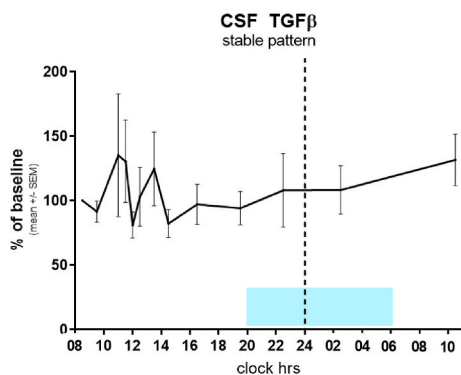


Fig. 5. Lumbar collection impact on levels of IL-6, TNF α , IL-10, and IL-1 β in CSF. IL-6 (A), TNF α (B) and IL-10 (C), and IL-1 β were measured in CSF from 7 healthy volunteers over a 24-h period (cohort 2). Data are shown as % of baseline (mean \pm SEM of the 7 volunteers). Dashed line indicates midnight, blue bar indicates typical nighttime. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



TGF β was measured in CSF from 7 healthy volunteers over a 24-hour period (cohort 2). Data are shown as % of baseline (mean \pm SEM of the 7 volunteers). Dashed line indicates midnight, blue bar indicates typical nighttime.

CSF=cerebrospinal fluid; TGF β -tumor growth factor beta

Fig. 6. Stable pattern of TGF β in CSF. TGF β was measured in CSF from 7 healthy volunteers over a 24-h period (cohort 2). Data are shown as % of baseline (mean \pm SEM of the 7 volunteers). Dashed line indicates midnight, blue bar indicates typical nighttime. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

necessary to confirm these data.

IL-1 β is a highly potent proinflammatory cytokine; however, circulating levels peripherally and in CSF are usually too low to quantify in traditional ELISAs. Even with the high sensitivity platform used in current study, many samples were below the quantification limit. Further, transient spikes of IL-1 β were observed in many participants, which can strongly confound interpretation of IL-1 β measurements across a population. These spikes were up to 10x of baseline level, seen in both CSF and serum, and did not follow any consistent timing. Therefore, analysis of circulating IL-1 β levels should be interpreted with caution. Conversely, measurement of stimulated IL-1 β release in blood culture and other *ex vivo* methods has been shown to be quite robust [32,33], so perhaps translation of IL-1 β findings would be most fruitful in the context of release capacity rather than circulating levels.

Finally, many early stage clinical trials for drug candidates targeting the central nervous system perform continuous CSF collection to obtain central pharmacokinetics data as well as secondary measurement of potential pharmacodynamic markers related to the drug target. We observed that many cytokines in CSF are elevated in a slow chronic manner after lumbar puncture, as has been reported previously for amyloid beta 1–42 peptide (A β 42) [34,35]. This is distinct from a diurnal rhythm since the levels did not return to baseline at 24 h after initial collection. This suggests that artifacts of frequent or continuous CSF sampling procedure can impact biomarker measurement, particularly for cytokines, and appropriate controls are critical in these studies.

Additional studies evaluating saliva in patients with neuropsychiatric disorders are also warranted given that many classical neuropsychiatric biomarkers can be quantified in this easily accessible fluid. However, there was lack of strong correlation with serum or CSF in most cases; thus, the results should be interpreted with caution.

Limitations of this study include the small number of participants ($n = 8$ in cohort 1 [study 1] and $n = 7$ in cohort 2 [study 2]); the study population comprising healthy controls, not patients with neuropsychiatric disorder; and the limited number of analytes and assays evaluated. Future work in confirming the findings reported herein is warranted.

In conclusion, we observed that many of the measured analytes were quantifiable in all sample types examined, yet often at quite different concentrations and without correlation between the sample types. Longitudinal assessment in serum over a 24-h period identified a) a diurnal rhythm for cortisol and IL-6, b) a stable pattern for TNF α , IL-10 and TGF β , and c) transient spikes at variable timepoints for IL-1 β . In

contrast, longitudinal assessment in CSF over a 24-h period identified a continued increase for IL-6, TNF α , IL-1 β , and IL-10, and a stable pattern for TGF β . Since these patterns do not return to baseline, they may be due simply to the method of CSF collection.

These findings support the need for more informed decision making around sample type and collection time for future neuropsychiatric biomarker studies. As general guidance, when measuring these analytes at a single timepoint, serum IL-6 or cortisol should be measured at a consistent timepoint each day, while all other analytes/matrices here can be measured at any 24 h timepoint. When measuring these analytes repeatedly in a 24-h period, serum IL-1 β and CSF IL-1 β , IL-6, TNF α , and IL-10 are not recommended due to the drifting/spiking nature of the data with this sample collection scheme.

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Author contributions

Gallen Triana-Baltzer, Maarten Timmers, Peter De Boer contributed to the study design. All authors contributed to data collection, data analysis, interpretation, and critically reviewed the manuscript. All authors meet ICMJE criteria and all those who fulfilled those criteria are listed as authors. All authors had full access to study data and final responsibility for the decision to submit for publication.

Declaration of competing interest

GTB, MT, MS, CB, IV, RS, and KB are employees of Janssen Research & Development, LLC or Janssen Research and Development, a division of Janssen Pharmaceutica NV, and hold stock/stock options in the company (Johnson & Johnson). MF, MC, LvN were employees of Janssen at the time the study was conducted. LvN is retired; MC is currently employed by The Marc Ceusters Company, and MF is currently employed by Neurocrine Biosciences. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in the subject matter or materials discussed in the manuscript apart from those disclosed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cpnec.2022.100116>.

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