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Dysregulation of Plasma Growth Factors and Chemokines in Cocaine Use Disorder: Implications for Dual Diagnosis with Schizophrenia and Antisocial Personality Disorder in an Exploratory Study

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Keywords
Growth factors · Chemokines · Cocaine · Dual diagnosis · Antisocial personality disorder · Schizophrenia · BDNF · Eotaxin-1

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Abstract
Introduction: Dual diagnosis in individuals with cocaine use disorders (CUDs) presents a mental health challenge marked by an increased susceptibility to disabling morbidities and premature mortality. Despite extensive research on depression and anxiety, other prevalent comorbidities, such as psychotic and personality disorders, have received less attention. This study explores

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inflammation-related mediators as potential biomarkers for CUD and dual diagnosis with schizophrenia (SCZ) or antisocial personality disorder (APD). ***Methods:*** This exploratory study included 95 participants, comprising 40 healthy subjects and 55 abstinent patients with CUD. Lifetime CUD was diagnosed either as single diagnosis (CUD group, $N = 25$) or as a dual diagnosis (DD group. $N = 30$) with SCZ (CUD+SCZ subgroup) or APD (CUD+APD subgroup). Participants were clinically assessed, and the plasma concentrations of growth factors (i.e., G-CSF, BDNF, and VEGF-A) and chemokines (i.e., CCL11/eotaxin-1, CCL2/MCP-1, and CXCL12/SDF-1) were determined and log(10)-transformed for analysis. ***Results:*** Growth factors and chemokines were dysregulated by CUD and psychiatric diagnoses. Specifically, patients in the CUD group exhibited significantly lower concentrations of G-CSF and CCL11/eotaxin-1 than the control group. In contrast, the DD group showed significantly higher concentrations of all analytes than both the CUD and control groups. Additionally, no differences in these analytes were observed between the CUD+SCZ and CUD+APD subgroups within the DD group. Regarding cocaine-related variables, significant associations were identified in the CUD group: an inverse correlation between the age at first cocaine use and the concentrations of BDNF and CCL2/MCP-1; and a positive correlation between the duration of the cocaine abstinence and the concentrations of BDNF and CCL11/eotaxin-1. Lastly, a logistic regression model incorporating all these analytes demonstrated high discriminatory power in distinguishing patients with CUD alone from those with dual diagnosis. ***Conclusions:*** Individuals with dual diagnosis of CUD exhibit elevated concentrations of growth factors and chemokines, distinguishing them from those with CUD alone. It is unclear whether the differences in these inflammatory mediators are specific to the presence of SCZ and APD. The study highlights potential biomarkers and associations, providing valuable insights into the intricate interplay of CUD and psychiatric disorders to enhance clinical diagnosis and therapeutics.

Introduction

Cocaine is one of the most commonly used psychoactive substances in young adults, and its use increases the incidence of severe clinical complications, including a significant loss of efficacy of current psychopharmacological interventions [1, 2]. Repeated use of cocaine can lead to cocaine use disorder (CUD), which is associated with an elevated prevalence of comorbid mental disorders [3–5]. The coexistence of substance use disorder and additional psychiatric disorders is referred to as dual diagnosis, and the most common comorbidities among individuals with CUD are mood disorders, anxiety, psychotic disorders, and personality disorders [6, 7]. In patients with dual diagnosis, clinical symptoms often overlap, posing a challenge in determining their temporal sequence (i.e., which symptoms manifest first and which emerge later). Moreover, in many cases, this temporal sequence lacks clinical relevance for diagnosis, as symptoms tend to coincide and overlap over time. This overlapping complicates both symptomatology and recovery rates, contributing to a serious problem in mental health care for individuals with CUD. Therefore, these individuals are more vulnerable to disabling morbidities and premature mortality [8, 9]. However, these adverse consequences depend on the duration of cocaine consumption, sex, age, and other environmental and biological factors [10].

Evidence indicates that cocaine blocks the reuptake of neurotransmitters (e.g., dopamine, serotonin, and norepinephrine) by binding to monoamine transporters, which increases the synaptic levels of these neurotransmitters and produces a strong psychostimulant effect [11]. Cocaine also induces changes in the expression of inflammatory mediators, which are associated with neuroinflammation and the development of neuroadaptations that are linked to cognitive impairment, depression, and psychosis [12, 13]. These immune system alterations are accompanied by dysregulation of the hypothalamic-pituitary-adrenal axis and are not limited to the central nervous system (CNS). Indeed, several prior studies in substance use disorders have concentrated on investigating peripheral inflammatory molecules, such as cytokines that can modulate and/or reflect a chronic inflammatory state and potentially serve as biomarkers [14, 15]. Within these cytokines, alterations in tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) have been observed in the plasma of individuals with cocaine dependence at baseline and in response to stress induced by imagery conditions [16]. Furthermore, plasma concentrations of IL-1β have been reported to predict the severity of CUD symptoms [17]. On the other hand, dysregulation of inflammatory and immune signaling has also been found to be associated with several psychiatric disorders. It has previously been reported that the gene expression of IL-17, IL-21, IL-23, and IL-35 was significantly higher in patients with major depression, suggesting that the expression of specific cytokines may contribute to the etiopathogenesis of these mood disorders [18].

Beyond canonical cytokines, various inflammation-related mediators, such as growth factors and chemokines, have arisen within the domain of substance use disorders as prospective biomarkers. For example, a previous meta-analysis of studies examining brain-derived neurotrophic factor (BDNF) levels in the serum and plasma of adults with substance use disorders revealed that BDNF levels are related to acute substance use and addiction severity [19]. More specifically, while serum BDNF levels have been shown to be associated with the duration of abstinence in cocaine dependence [20], decreased plasma BDNF levels have been associated with early stages of cognitive impairment in alcohol use disorder [21]. Another growth factor that appears to be dysregulated in substance use disorders is vascular endothelial growth factor A (VEGF-A), which plays a critical role in angiogenesis [22]. In this case, VEGF-A has been found to be altered in the CNS after chronic cocaine exposure in preclinical studies [23]. Furthermore, plasma VEGF-A levels are also associated with cognitive impairment in patients with alcohol use disorders [24], probably due to its ability to increase the permeability of the blood-brain barrier, facilitating the arrival of proinflammatory cytokines to the brain. Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein that modulates both cocaine-seeking behavior and cocaine self-administration in rodents [25]. Furthermore, our research group recently undertook a study involving patients with polysubstance use disorders, specifically alcohol. This research unveiled an association between differences in plasma concentrations of G-CSF and comorbid major depression, suggesting a potential contribution to the stratification of these patients seeking treatment [26].

As previously stated, chronic cocaine use is linked to modifications in the immune system that impact components distinct from cytokines, such as chemokines. These alterations may play a contributory role in the clinical progression and dual diagnosis observed in individuals with CUD. Specifically, our group has reported that plasma concentrations of CCL2 (i.e., monocyte chemoattractant protein 1 [MCP-1]) and CXCL12 (i.e., stromal cell-derived factor 1 [SDF-1]) are decreased in abstinent patients with CUD, and both CXCL12/SDF-1 and CX_3CL1 (i.e., fractalkine) are positively correlated with cocaine symptom severity, assessed by the number of criteria for CUD. In addition, CCL11 (i.e., eotaxin-1) levels have been found to be decreased in patients with CUD and have been associated with the frequency of cocaine use [17, 27]. Interestingly, all these chemokines could be used to identify cocaine users with severe CUD and a high prevalence of mood, anxiety, psychotic, and personality disorders (mainly antisocial personality disorders [APDs]) [17].

Setting aside comorbid depressive disorders, which receive considerable attention in patients with substance use disorders, the most commonly diagnosed psychotic disorder is schizophrenia (SCZ). Individuals diagnosed with SCZ who also exhibit other substance use disorders generally present more adverse outcomes in terms of physical health, life functioning, and engagement in treatment, often leading to prolonged hospitalizations and an increased prevalence of suicidal ideation [28]. Similar to other psychiatric disorders, SCZ has been linked to dysfunction of the immune system in the CNS and inflammatory abnormalities [29–31]. Evidence indicates that patients with SCZ exhibit enhanced chemokine signaling [32]. Elevated levels of specific chemokines, such as CCL2 and CCL11, are strongly correlated with cognitive impairments associated with schizophrenic symptoms [33, 34]. Regarding the APD, this condition also exhibits an elevated prevalence in CUD populations, ranging between 11% and 20%. This presence negatively impacts the prognosis of substance use disorders, heightening the risk of violent crimes and recidivism [35]. APD is also linked to substance use disorder, although there is scarce information on the neurochemical and neuroinflammatory mechanisms underlying this association [36, 37].

Building on previous findings and existing knowledge gaps, the current exploratory study investigates specific growth factors and chemokines as potential biomarkers for clinical severity in patients with a lifetime CUD. Our aim was to explore circulating inflammation-related mediators in the plasma of abstinent patients with CUD alone and those with CUD and comorbid psychiatric disorders, distinct from mood and anxiety disorders, which often pose a high level of complexity in treatment. Specifically, we will focus on SCZ and APD.

Materials and Methods

Participants, Recruitment, and Eligibility Criteria

Ninety-five volunteers participated in this exploratory study, including 40 healthy subjects with no history of substance use disorders or mental disorders (control group) and 55 abstinent patients with lifetime CUD, with or without dual diagnosis. The inclusion criteria for the experimental group were as follows: ≥18 years of age (up to 65 years), a minimum of 4 weeks of abstinence from other substances distinct from cocaine (except for

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nicotine and caffeine), and a diagnosis of lifetime CUD. Lifetime CUD was diagnosed either alone (CUD group) or as dual diagnosis (DD group) with SCZ (CUD+SCZ subgroup) or APD (CUD+APD subgroup). The exclusion criteria included a personal history of chronic inflammatory diseases or cancer, infectious diseases, and pregnancy or breastfeeding. For the control group, the inclusion criteria were men and women ≥18 years of age (up to 65 years), and the exclusion criteria were personal history of chronic inflammatory diseases or cancer, infectious diseases, pregnancy or breastfeeding, the use of psychotropic medication during the last year, and diagnosis of psychiatric disorders, including substance use disorders, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria. The participants of the study were categorized into three groups: the control group ($N = 40$), CUD group ($N = 25$), and DD group ($N = 30$). Subsequently, the DD group was divided into the CUD+SCZ ($N = 9$) and CUD+APD ($N = 21$) subgroups.

Healthy controls were recruited from a multidisciplinary staff cohort of volunteers working at the Hospital Regional Universitario de Málaga (Málaga, Spain). Patients with CUD were recruited from outpatient programs for cocaine addiction at both, the *Centro Provincial de Drogodependencias* in Málaga (Spain), and the *Hospital Provincial de Castellón* (Castellón, Spain).

Clinical Assessments

All participants in the study were clinically evaluated by trained and experienced psychologists. Sociodemographic information from the entire sample was acquired during the intake interview using module I of the Spanish version of the "Psychiatric Research Interview for Substance and Mental Disorders" (PRISM). Clinical data from patients with substance use disorders were obtained through the following modules of the PRISM interview. This semistructured interview had good psychometric properties in the evaluation of substance use disorders and the common comorbid psychiatric disorders in patients with addiction [38]. The DSM-IV-TR criteria for CUD were used to gauge "cocaine trait severity" or "cocaine symptom severity" based on the unidimensionality of DSM5 diagnostic symptoms [39]. This involved combining the dependence criteria (requiring at least three or more co-occurring symptoms within a 12-month period) and abuse criteria (requiring at least one symptom in a 12-month period). A detailed description of "cocaine symptom severity" has been previously documented in the literature [40]. In addition to the PRISM for sociodemographic variables, control subjects underwent clinical evaluation using the Spanish version of the "Composite International Diagnostic Interview" (CIDI) to detect psychiatric disorders in the general population [41].

Collection of Plasma Samples

Personal health records of the participants were collected, and a nasopharyngeal swab was used for detection of SARS-CoV-2 antigen (BIOCREDIT COVID-19 Ag kit, Rapiden, Suwon, South Korea). Blood extractions were conducted under the same conditions by experienced nurses in the morning after fasting for 8–12 h. Venous blood samples were extracted into 10-mL K_2 EDTA tubes (BD, Franklin Lakes, NJ, USA), and to obtain plasma, samples were centrifuged at 2,200 *g* for 15 min (4°C). Plasma was individually assayed by rapid tests for detecting infectious diseases: HIV (Retroscreen HIV, QualPro Diagnostics-Tulip Group Ltd, Goa, India), hepatitis B (HBsAg test, Toyo Diagnostics-Turklab Inc., Izmir, Turkey), and hepatitis C (Flaviscreen HCV, QualPro Diagnostics-Tulip Group Ltd). Each plasma sample was registered and stored at −80°C until biochemical determinations.

Enzyme-Linked Immunoassay Analysis for Plasma G-CSF

The plasma G-CSF concentrations were determined using a selective enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (The human G-CSF [granulocyte colony stimulating factor 3] ELISA kit [#EH0149, Wuhan Fine Biotech Co., Wuhan, China]). The G-CSF ELISA kit had a sensitivity below 23.4 pg/mL and a standard curve range between 39.1 and 2,500 pg/mL. Two 96-well plates were used for all the samples, which were run in duplicate and appropriately diluted. Data were expressed as pg per mL of plasma, and the minimum concentration of G-CSF that could be interpolated in the standard curve was assigned to samples with an optical density lower than the limit of detection but higher than the background (zero values) ($N = 29$). The intra-assay coefficient of variability (CV) was less than 10% (i.e., 9.3) and the inter-assay was less than 12% (i.e., 11.1).

Multiplex Assay for Plasma Chemokines and Other Growth Factors

The plasma concentrations of BDNF, CCL11/eotaxin-1, CCL2/MCP-1, CXCL12/SDF-1, and VEGF-A were measured in 25 μL-plasma samples using the Luminex xMAP® technology-MAGPIX system (Thermo Fisher Scientific, Waltham, MA, USA). The measurements were conducted in the Proteomics Unit (SCAI) of the University of Malaga (Malaga, Spain) with a ProcartaPlex Human Basic Kit and five ProcartaPlex Human Simplex Kits (Thermo Fisher Scientific, Waltham, MA, USA), following manufacturer's instructions. The magnetic bead regions and standard curve ranges were as follows: 57 and 2.44–10,000 pg/mL (cat. No. EPX01A-12116-901) for BDNF; 33 and 0.61–2,500 pg/mL (cat. No. EPX01B-12120-901) for CCL11/eotaxin-1; 51 and 1.22–5,000 pg/mL (cat. No. EPX01B-10281-901) for CCL2/MCP-1; 13 and 17.09–70,000 pg/mL (cat. No. EPX01A-12138-901) for CXCL12/SDF-1; 78 and 5.86–24,000 pg/mL (cat. No. EPX01A-10277-901) for VEGF-A, respectively. The five ProcartaPlex Human Simplex Kits were combined in two 96-well multiplex plates for all the samples in duplicate and appropriately diluted. Data were expressed as pg per mL of plasma, and the minimum concentration that could be interpolated in the standard curves was assigned to samples with an optical density lower than the limit of detection but higher than the zero values. The intra-assay CV was less than 7% (i.e., 2.6, 4.6, 5.2, 3.6, 5.5, and 6.1, respectively) and the inter-assay CV was less than 10% (i.e., 6.2, 6.0, 9.0, 9.8, 7.4, and 4.0, respectively).

Statistical Analysis

Categorical variables are reported as counts and percentages (N [%]), while continuous variables are presented as the median and interquartile range. The statistical significance of categorical variables was evaluated using either χ^2 test or the Fisher's exact test. The Mann-Whitney U test was employed for non-normally distributed continuous variables comparing two (sub)groups, and the Kruskal-Wallis test was used when comparing more than two (sub)groups.

The main effects of independent variables (i.e., "lifetime CUD diagnosis" [cocaine groups and control group], "type of CUD diagnosis" [CUD group, DD group, and control group], and "comorbid psychiatric diagnosis" [CUD group, CUD+SCZ subgroup, and CUD+APD subgroup]) on plasma concentrations of growth factors and chemokines were assessed using one-way analyses of covariance (ANCOVA) with "age" and "sex" as confounding variables. While "age" was directly controlled in the ANCOVA models as a covariate, "sex" is a dichotomous categorical variable (men and women) that was controlled as a factor without considering its interaction with the independent variables of interest. Due to the non-normal and positively skewed distribution of growth factors and chemokine concentrations, raw data were $\log_{10}$-transformed to approximate a normal distribution and fulfill the statistical assumptions necessary for ANCOVA. The estimated marginal means and 95% confidence intervals (95% CI) of the $\log_{10}$-transformed concentrations are presented in the figures accompanying the results. Post hoc analysis for multiple comparisons was conducted using the Sidak's correction test.

Pearson correlation analyses were carried out to examine the correlations between $\log_{10}$-transformed concentrations of analytes and cocaine-related variables (i.e., DMS-IV-TR criteria for CUD, duration of cocaine abstinence, and age of first cocaine use). Lastly, a receiver operating characteristic analysis was conducted to assess the discriminative power of a binary logistic regression model, including all $\log_{10}$-transformed concentrations of analytes, through the area under the curve.

The statistical analyses were conducted using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistical Version 22 (IBM, Armonk, NY, USA). A p value less than 0.05 was considered statistically significant.

Results

Sociodemographic Characteristics

This study included 95 participants categorized into three experimental groups to investigate plasma concentrations of growth factors and chemokines: the CUD group, the DD group, and the control group. Significant differences in sociodemographic data were observed among all the groups, particularly in sex ($p < 0.01$), age ($p < 0.01$), marital status ($p < 0.05$), education ($p < 0.001$), and employment status ($p < 0.001$). These differences were statistically attributed to differences between the cocaine groups (i.e., the CUD and DD groups) and the control group. Indeed, when the DD group was subdivided into the CUD+SCZ and CUD+APD subgroups, comparisons among the CUD group and both subgroups for the same sociodemographic variables revealed no significant differences. These results are presented in Table 1.

Concerning physiological variables, while the control group was sex-balanced, the two cocaine groups were predominantly composed of men (83.6%). Additionally, the cocaine groups exhibited a significantly lower age than the control (medians of 39 years and 46 years, respectively). Unlike sex and age, BMI did not differ among the groups. These findings led us to use ANCOVAs to assess main effects on the plasma concentrations of analytes, considering the potential for sex- and age-associated biases.

Moreover, other sociodemographic variables showed significant differences. Therefore, patients in the CUD and DD groups were primarily single (56.4%), with elementary or secondary education levels (34.5% and 60.0%, respectively), and a high rate of unemployment (60.0%). In contrast, the control group comprised mainly individuals married with secondary and university education levels and a majority employed.

Clinical Characteristics Associated with Cocaine Use and Psychiatric Diagnosis

Regarding relevant variables related the diagnosis of lifetime CUD and cocaine use, we assessed the age at first cocaine use, the number of DSM criteria for CUD based on the DSM-IV-TR, and the duration of the last period of cocaine abstinence. As indicated in Table 2, these cocaine-related variables were analyzed within the cocaine groups, differentiating between the CUD+SCZ subgroup and the CUD+APD subgroup; however, no significant differences were found. Although there were no significant differences, individuals in the CUD group initiated cocaine use at a later age, exhibited fewer DSM criteria for CUD, and had a shorter period of abstinence compared to those in the DD group. Notably, the CUD+SCZ subgroup showed patients with the earliest age at first cocaine use (median of 16 years), the highest number of criteria for CUD (median of 10 criteria), and a longer duration of the last period of abstinence from cocaine (median of 90 days).

All patients in the CUD and DD groups reported a high use of psychiatric medication in the last year, primarily antidepressants (36.4%), with differences in psychiatric comorbidity detailed in online supplementary Table S1 (for all online suppl. material, see https://doi.org/10.1159/000536265). The CUD group did not receive a diagnosis of psychiatric comorbidity, while the DD group exhibited additional psychiatric disorders distinct from SCZ and APD, namely, mood disorders (40.0%) and anxiety disorders (20.0%). However, there were no differences in the prevalence of these additional psychiatric comorbidities between the CUD+SCZ and CUD+APD subgroups.

Plasma Concentrations of Growth Factors and Chemokines in the Sample

Plasma concentrations of relevant growth factors and chemokines were log-transformed and analyzed using one-way ANCOVAs. These analyses were conducted with different independent variables while controlling for sex and age.

Table 1. Sociodemographic characteristics of the sample

Variables	Control group (*N* = 40)	CUD group (*N* = 25)	DD group (*N* = 30)	*p* value	CUD group (*N* = 25)	DD Group (*N* = 30)		*p* value
						CUD+SCZ subgroup (*N* = 9)	CUD+APD subgroup (*N* = 21)	
Sex, *N* (%)								
Women	18 (45.0)	3 (12.0)	6 (20.0)	0.008[a]	3 (12.0)	1 (11.1)	5 (23.8)	ns[a]
Men	22 (55.0)	22 (88.0)	24 (80.0)		22 (88.0	8 (88.9)	16 (76.2)	
Age, years, median (IQR)	46.0 (32.3–54.0)	37.0 (30.0–45.0)	40.5 (32.5–43.0)	0.006[b]	37.0 (30.0–45.0)	37.0 (32.0–42.0)	41.0 (33.0–43.0)	ns[b]
BMI, kg/m^2, median (IQR)	25.4 (22.9–26.8)	24.5 (21.8–26.8)	24.6 (22.4–27.7)	ns[b]	24.5 (21.8–26.8)	25.1 (21.9–27.8)	24.3 (22.9–27.6)	ns[b]
Marital status, *N* (%)								
Single	15 (37.5)	11 (44.0)	20 (66.7)	0.014[a]	11 (44.0)	7 (77.8)	13 (61.9)	ns[a]
Married	18 (45.0)	9 (36.0)	2 (6.7)		9 (36.0)	1 (11.1)	1 (4.8)	
Separated	6 (15.0)	5 (20.0)	7 (23.3)		5 (20.0)	1 (11.1)	6 (28.6)	
Widowed	1 (2.5)	–	1 (3.3)		–	–	1 (4.8)	
Education, *N* (%)								
Elementary	3 (7.5)	5 (20.0)	14 (46.7)	<0.001[a]	5 (20.0)	4 (44.4)	10 (47.6)	ns[a]
Secondary	22 (55.0)	18 (72.0)	15 (50.0)		18 (72.0)	4 (44.4)	11 (52.4)	
University	15 (37.5)	2 (8.0)	1 (3.3)		2 (8.0)	1 (11.1	–	
Employment status, *N* (%)								
Employed	32 (80.0)	10 (40.0)	5 (16.7)	<0.001[a]	10 (40.0)	2 (22.2)	3 (14.3)	ns[a]
Unemployed	7 (17.5)	13 (52.0)	20 (66.7)		13 (52.0)	6 (66.7)	14 (66.7)	
Disabled	1 (2.5)	2 (8.0)	2 (6.7)		2 (8.0)	-	2 (9.5)	
Household	–	–	3 (10.0)		–	1 (11.1)	2 (9.5)	

BMI, body mass index; CUD, cocaine use disorder group; CUD+SCZ, cocaine and schizophrenia subgroup; CUD+APD, cocaine and antisocial personality disorder subgroup; DD, dual diagnosis group; IQR, interquartile range; ns, nonsignificant. [a]Data were analyzed using the χ^2 test or Fisher's exact test. [b]Data were analyzed using the Kruskal-Wallis test.

Table 2. Variables associated with cocaine use within the cocaine (sub)groups

Variables	CUD group (*N* = 25)	DD group (*N* = 30)	*p* value[a]	CUD group (*N* = 25)	DD group (*N* = 30)		*p* value[b]
					CUD+SCZ subgroup (*N* = 9)	CUD+APD subgroup (*N* = 21)	
Age at first cocaine use, years, median (IQR)	18.0 (16.5–20.0)	17.0 (14.5–20.5)	ns	18.0 (16.5–20.0)	16.0 (14–21.5)	17.0 (15.0–19.0)	ns
DSM criteria for CUD (1–11), median (IQR)	7 (5–9)	10 (7–11)	ns	7 (5–9)	10 (4–11)	10 (9–10)	ns
Duration of the last period of cocaine abstinence, days, median (IQR)	30 (3–90)	45 (10–580)	ns	30 (3–90)	90 (15–720)	30 (0–540)	ns

CUD, cocaine use disorder group; CUD+SCZ, cocaine and schizophrenia subgroup; CUD+APD, cocaine and antisocial personality disorder subgroup; DD, dual diagnosis group; DSM, diagnostic and statistical manual of mental disorders; IQR, interquartile range; ns, nonsignificant. [a]Data were analyzed using the Mann-Whitney U test. [b]Data were analyzed using the Kruskal-Wallis test.

Table 3. Plasma concentrations of growth factors and chemokines in the sample based on lifetime CUD diagnosis

Variables	Control group (N = 40)	Cocaine groups (CUD group and DD group) (N = 55)	p value[a]
$\log_{10}$ G-CSF (pg/mL), mean (95% CI)	3.220 (2.997–3.442)	2.824 (2.617–3.031)	0.011
$\log_{10}$ BDNF (pg/mL), mean (95% CI)	0.551 (0.382–0.719)	0.758 (0.592–0.923)	ns
$\log_{10}$ VEGF-A (pg/mL), mean (95% CI)	0.971 (0.784–1.157)	1.345 (1.186–1.504)	0.003
$\log_{10}$ CCL11/eotaxin-1 (pg/mL), mean (95% CI)	0.534 (0.454–0.615)	0.558 (0.458–0.657)	ns
$\log_{10}$ CCL2/MCP-1 (pg/mL), mean (95% CI)	0.883 (0.750–1.017)	1.163 (1.072–1.253)	<0.001
$\log_{10}$ CXCL12/SDF-1 (pg/mL), mean (95% CI)	1.612 (1.464–1.759)	1.926 (1.760–2.093)	0.007

CUD group, cocaine use disorder group; DD group, dual diagnosis group; df, degrees of freedom; ns, nonsignificant; 95% CI, 95% confidence interval. [a]Data were logarithmically transformed to ensure parametric assumptions and analyzed using one-way ANCOVA controlling for sex and age. Marginal estimated means and 95% CI are shown in the table.

Lifetime CUD Diagnosis and Plasma Concentrations of Analytes

A primary statistical analysis was conducted to assess the concentrations of these analytes based on the CUD diagnosis. As indicated in Table 3, one-way ANCOVAs demonstrated significant main effects of "lifetime CUD diagnosis" on G-CSF ($F(1, 93) = 6.673$; $p < 0.05$), VEGF-A ($F(1, 88) = 9.548$; $p < 0.01$), CCL2/MCP-1 ($F(1, 87) = 13.15$; $p < 0.001$), and CXCL12/SDF-1 ($F(1, 87) = 7.504$; $p < 0.01$) concentrations. Specifically, patients in the cocaine groups exhibited significantly lower G-CSF concentrations and higher VEGF-A, CCL2/MCP-1, and CXCL12/SDF-1 concentrations compared with the control group.

Type of CUD Diagnosis and Plasma Concentrations of Analytes

We assessed the concentrations of analytes across three groups (CUD, DD, and control groups) based on the type of CUD diagnosis. As depicted in Figure 1, one-way ANCOVAs revealed a significant main effect of "type of CUD diagnosis" on plasma concentrations of G-CSF ($F(2, 90) = 5.650$; $p < 0.010$) (Fig. 1a), BDNF ($F(2, 84) = 24.239$; $p < 0.001$) (Fig. 1b), and VEGF-A ($F(2, 85) = 23.740$; $p < 0.001$) (Fig. 1c). The *post hoc* tests indicated that the CUD group had significantly lower G-CSF concentrations ($p < 0.05$) than the control group. In contrast, the DD group exhibited significantly higher concentrations of BDNF ($p < 0.001$) and VEGF-A ($p < 0.001$) than the control group.

There was a significant main effect of "type of CUD diagnosis" on plasma concentrations of CCL11/eotaxin-1 ($F(2, 86) = 34.811$; $p < 0.001$) (Fig. 2a), CCL2/MCP-1 ($F(2, 84) = 14.054$; $p < 0.001$) (Fig. 2b), and CXCL12/SDF-1 ($F(2, 84) = 17.373$; $p < 0.001$) (Fig. 2c). The post hoc multiple comparison tests showed that the CUD group had significantly lower CCL11/eotaxin-1 concentrations ($p < 0.001$) than the control group. However, the DD group exhibited significantly higher concentrations of CCL11/eotaxin-1 ($p < 0.001$), CCL2/MCP-1 ($p < 0.001$), and CXCL12/SDF-1 ($p < 0.001$) than the control group.

Plasma Concentrations of Growth Factors and Chemokines in the Cocaine Groups

Given the significant main effects of CUD diagnosis on log-transformed concentrations of growth factors and chemokines, we investigated the potential association between these analytes and clinical variables related to cocaine use in the cocaine groups using correlations. In addition, we assessed the main effects of comorbid psychiatric diagnosis using one-way ANCOVAs while controlling for sex and age.

Cocaine-Related Variables and Plasma Concentrations of Analytes

Correlations between log-transformed concentrations of analytes and cocaine-related variables (i.e., the age at first cocaine use, the DSM criteria for CUD, and the duration of the last period of cocaine abstinence) were analyzed in patients from the cocaine (sub)groups (online suppl. Table S2). The cocaine groups exhibited a significant inverse correlation between CCL2/MCP-1 concentrations and the age at first cocaine use ($r = -0.312$; $p < 0.05$), but no additional associations were found with other analytes and cocaine-related variables. Notably, when the cocaine groups were divided into the CUD group and the DD group, significant correlations were observed only the CUD group showed (see Fig. 3). Therefore, we observed significant inverse correlations

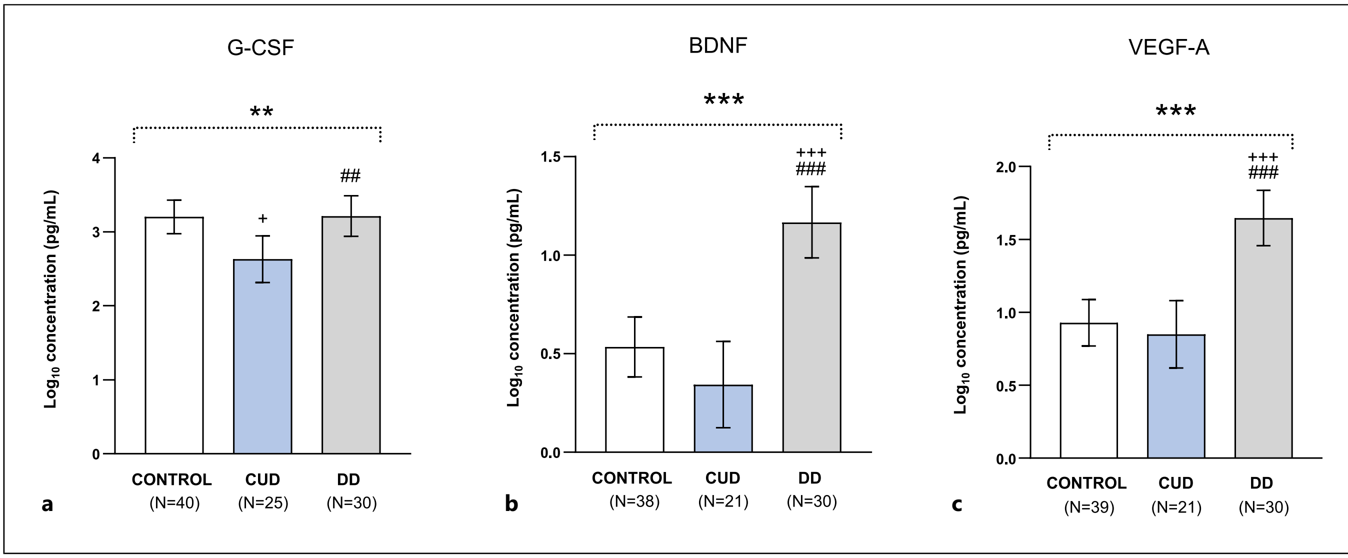

Fig. 1. Plasma concentrations of growth factors based on type of CUD diagnosis. G-CSF (**a**), BDNF (**b**), and VEGF-A (**c**). Bars are estimated marginal means and 95% CI of log(10)-transformed concentrations. Data were analyzed using one-way ANCOVA and controlling for age and sex. (***) $p < 0.001$ and (**) $p < 0.010$ denote significant main effects of the group factor. ($^{+++}$) $p < 0.001$ and ($^{+}$) $p < 0.05$ denote significant differences compared with the control group. ($^{###}$) $p < 0.001$ and ($^{#}$) $p < 0.05$ denote significant differences compared with the CUD group.

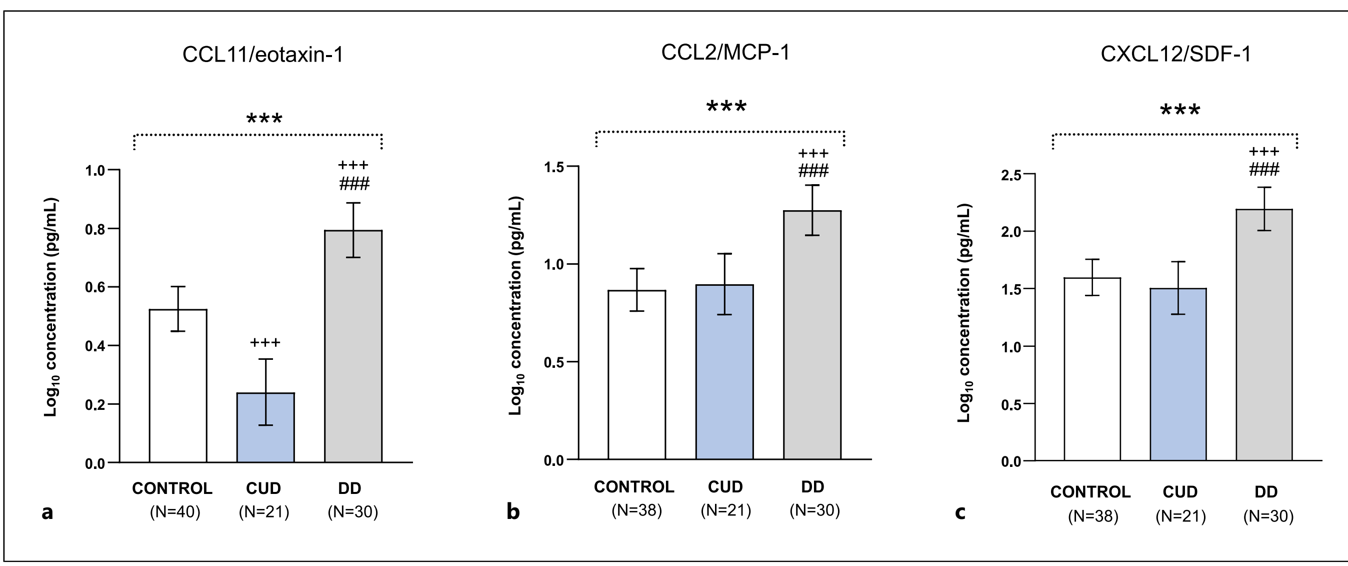

Fig. 2. Plasma concentrations of chemokines based on type of CUD diagnosis. CCL11/eotaxin-1 (**a**), CCL2/MCP-1 (**b**), and CXCL12/SDF-1 (**c**). Bars are estimated marginal means and 95% CI of log(10)-transformed concentrations. Data were analyzed using one-way ANCOVA and controlling for age and sex. (***) $p < 0.001$ denotes significant main effects of the group factor. ($^{+++}$) $p < 0.001$ denotes significant differences compared with the control group. ($^{###}$) $p < 0.001$ denotes significant differences compared with the CUD group.

between BDNF concentrations and the age at first cocaine use ($r = -0.498$, $p < 0.05$) (Fig. 3a), and between CCL2/MCP-1 concentrations and the age at first cocaine use ($r = -0.488$, $p < 0.05$) (Fig. 3b). In contrast, there were significant positive correlations between BDNF concentrations and the duration of the last period of cocaine

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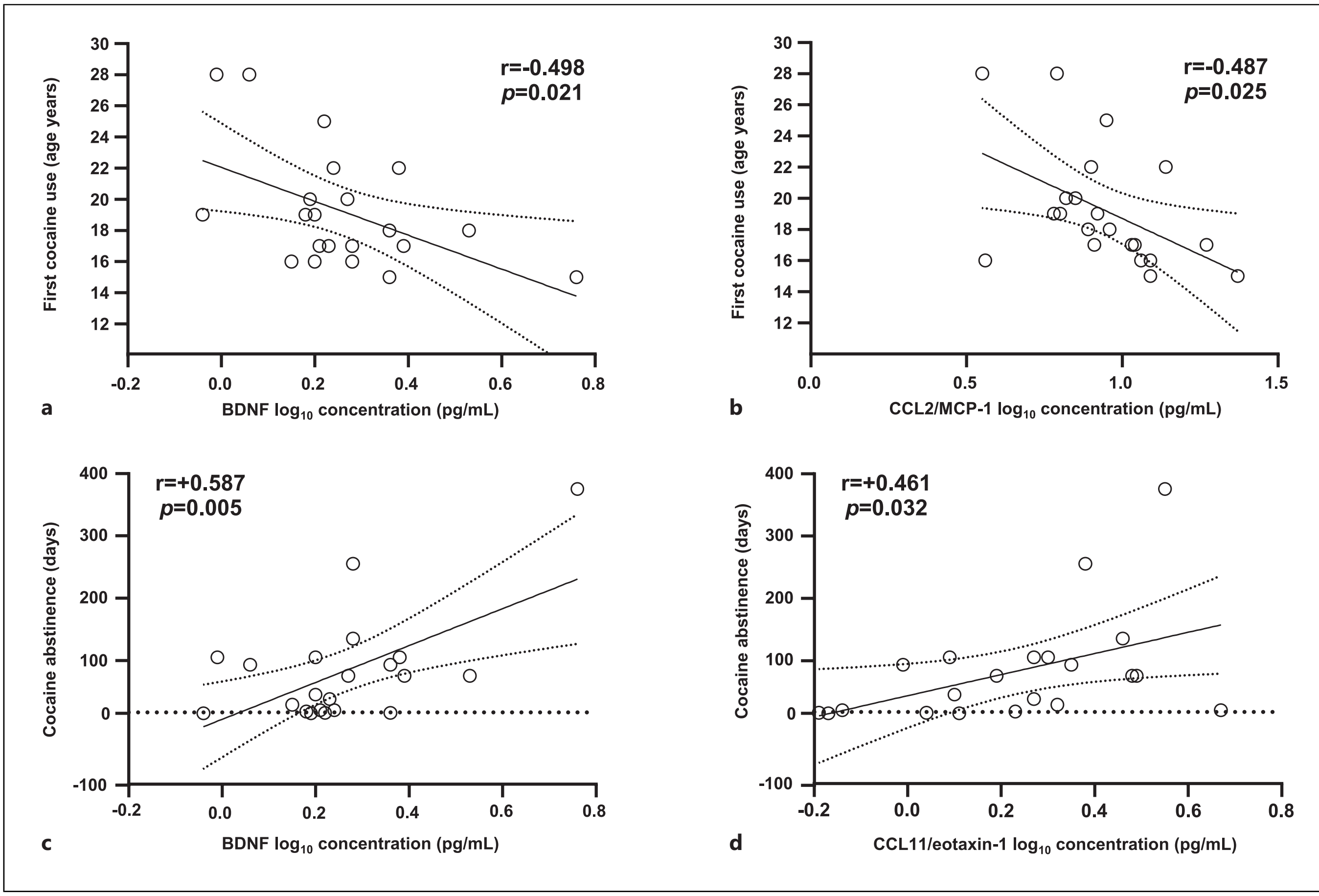

Fig. 3. Correlation between plasma concentrations of analytes and cocaine-related variables in the CUD group. **a** Age at first cocaine use and BDNF concentration. **b** Age at first cocaine use and CCL2/MCP-1 concentration. **c** Duration of cocaine abstinence and BDNF concentration. **d** Duration of cocaine abstinence and CCL11/eotaxin-1. Pearson correlation was performed using $\log_{10}$-transformed concentrations. Dots are individual values. (*r*) Pearson's correlation coefficient.

abstinence ($r = +0.589$, $p < 0.01$) (Fig. 3c) and between CCL11/eotaxin-1 and the duration of the last period of cocaine abstinence ($r = +0.468$, $p < 0.05$) (Fig. 3d). Unlike the correlations observed with these cocaine-related variables, we did not find any significant associations with the number of DSM criteria for CUD.

Comorbid Psychiatric Disorders and Plasma Concentrations of Analytes

Expanding our analysis to include psychiatric comorbidity (i.e., CUD, CUD+SCZ, and CUD+APD), one-way ANCOVAs revealed a significant main effect of "comorbid psychiatric diagnosis" on plasma concentration of G-CSF ($F(2, 50) = 4.501$; $p = 0.016$) (Fig. 4a), BUN ($F(2, 46) = 25.316$; $p < 0.001$) (Fig. 4b), and VEGF-A ($F(2, 46) = 24.635$; $p < 0.001$) (Fig. 4c). The post hoc tests revealed that the CUD+SCZ subgroup had significantly higher plasma concentrations of BDNF ($p < 0.001$) and VEGF-A ($p < 0.001$) than the CUD group. Additionally, the CUD+APD had significantly higher plasma concentrations of G-CSF ($p < 0.05$), BDNF ($p < 0.001$), and VEGF-A ($p < 0.001$) than the CUD group. However, no differences in plasma concentrations of the growth factors were observed between the two subgroups.

Regarding chemokines, there was a significant main effect of "comorbid psychiatric diagnosis" on plasma concentration of CCL11/eotaxin-1 ($F(2, 46) = 38.871$; $p < 0.001$) (Fig. 5a), CCL2/MCP-1 ($F(2, 46) = 12.599$; $p < 0.001$) (Fig. 5b), and CXCL12/SDF-1 ($F(2, 46) = 11.812$; $p < 0.001$) (Fig. 5c). The *post hoc* tests revealed that the CUD+SCZ subgroup and the CUD+APD subgroup had significantly higher plasma concentrations of CCL11/

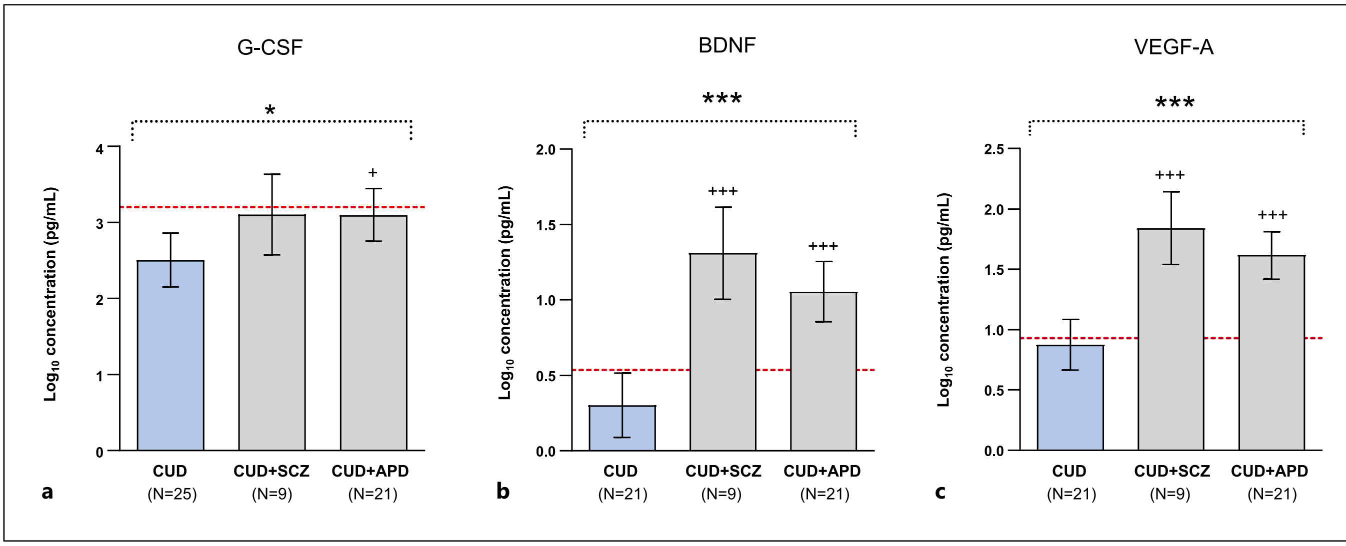

Fig. 4. Plasma concentrations of growth factors based on comorbid psychiatric diagnosis. G-CSF (**a**), BDNF (**b**), and VEGF-A (**c**). Bars are estimated marginal means and 95% CI of log(10)-transformed concentrations. Data were analyzed using one-way ANCOVA and controlling for age and sex. (***) $p < 0.001$ and (*) $p < 0.05$ denote significant main effects of the (sub)group factor. ($^{+++}$) $p < 0.001$ and ($^{+}$) $p < 0.05$ denote significant differences compared with the CUD group.

eotaxin-1 ($p < 0.001$), CCL2/MCP-1 ($p < 0.001$), and CXCL12/SDF-1 ($p < 0.001$) than the CUD group. Similar to the pattern observed with growth factors, no significant differences were found in the plasma concentrations of chemokines between the CUD+SCZ and CUD+APD subgroups.

Growth Factors and Chemokines as Predictors of CUD Alone and Dual Diagnosis with SCZ and APD

Because we found significant differences in plasma concentrations of these analytes within the cocaine (sub) groups based on cocaine-related variables and psychiatric comorbidity (i.e., SCZ and APD), we proceeded to assess the discriminatory power of growth factors and chemokines. Specifically, we aimed to distinguish patients with CUD alone from those with dual diagnosis, either CUD and SCZ, or CUD and APD.

A logistic regression model was constructed, including the log-transformed concentrations of growth factors and chemokines (Table 4). In terms of the odds ratio, the inflammatory analytes with a significant contribution to the model were BDNF ($p < 0.05$) and CCL11/eotaxin-1 ($p < 0.05$). As shown in Figure 6, the resulting probabilities of the logistic model differed significantly between patients with CUD alone and those with dual diagnosis (SCZ or APD) ($p < 0.001$). The receiver operating characteristic analysis demonstrated an excellent discriminatory power of the model with an area under the curve of 0.936 ($p < 0.001$). Representative cutoff values indicated high sensitivity and specificity [e.g., 0.448 (86% sensitivity and 84% specificity)].

Discussion

Despite the elevated prevalence of psychotic and personality disorders, the predominant focus of studies within the context of cocaine has been on mood disorders, specifically major depression [5, 42]. In particular, dual diagnosis with SCZ and APD exacerbates the severity of these disorders, such as difficulties in decision-making, and compromises the daily functioning of these patients, and complicates the course and treatment of CUD [43]. Hence, the identification of biomarkers in dual diagnosis is pertinent as a potential therapeutic tool and contributes to the enhanced stratification of these patients in diverse treatment programs, given the complexity of the diagnosis. Among the various molecular signaling systems susceptible to being associated with substance use disorders and psychiatric comorbidity, inflammatory mediators have garnered increasing attention in recent years [17, 18, 30]. In addition to archetypal cytokines, other molecules related to inflammation have emerged as new candidates for biomarkers [17, 44]. The present

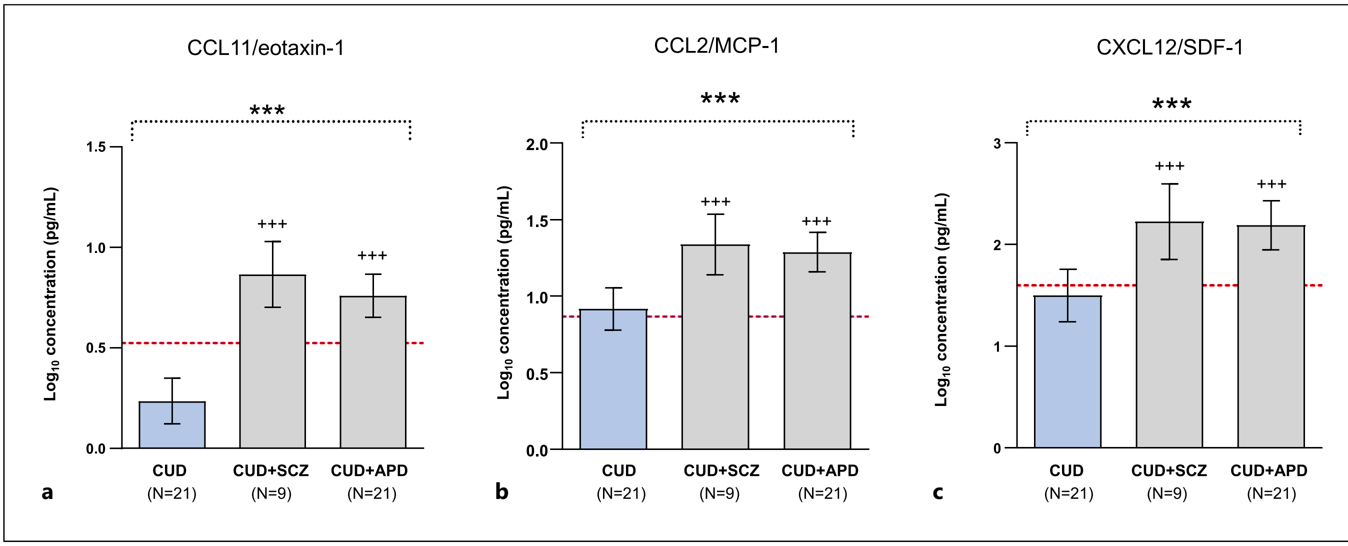

Fig. 5. Plasma concentrations of chemokines based on comorbid psychiatric diagnosis. CCL11/eotaxin-1 (**a**), CCL2/MCP-1 (**b**), and CXCL12/SDF-1 (**c**). Bars are estimated marginal means and 95% CI of log(10)-transformed concentrations. Data were analyzed using one-way ANCOVA and controlling for age and sex. (***) $p < 0.001$ denotes significant main effects of the (sub)group factor. ($^{+++}$) $p < 0.001$ denotes significant differences compared with the CUD group.

Table 4. Binary logistic regression analysis for discriminating patients with CUD alone from those with dual diagnosis (i.e., SCZ and APD)

Variables	B	Odd ratio	95% CI for odd ratio		*p* value
			lower	upper	
log_{10} G-CSF (pg/mL)	0.001	1.001	1.000	1.002	0104
log_{10} BDNF (pg/mL)	−0.105	0.900	0.829	0.977	0.012
log_{10} VEGF-A (pg/mL)	0.120	1.127	0.977	1.301	0.101
log_{10} CCL11/eotaxin-1 (pg/mL)	1.100	3.004	1.246	7.246	0.014
log_{10} CCL2/MCP-1 (pg/mL)	0.018	1.018	0.837	1.018	0.837
log_{10} CXCL12/SDF-1 (pg/mL)	−0.001	0.999	0.996	1.001	0.215
Constant	−2.628	0.011	–	–	0.138

APD, antisocial personality disorder; CUD, cocaine use disorder group; DD, dual diagnosis group; SCZ, schizophrenia; 95% CI, 95% confidence interval.

exploratory study examined plasma concentrations of growth factors and chemokines in a cohort of patients diagnosed with CUD and a specific subset of comorbid psychiatric disorders. The analysis aimed to understand their relationship with both clinical variables associated with CUD and psychiatric comorbidity, specifically focusing on SCZ and APD.

The main findings of the study were as follows: (1) plasma concentrations of growth factors and chemokines were significantly associated with the type of CUD diagnosis (i.e., CUD alone, DD, and control). Specifically, patients with dual diagnosis exhibited the highest concentrations of analytes. (2) Significant correlations were observed between CUD-related variables (i.e., age at first cocaine use and duration of the last period of cocaine abstinence) and certain inflammatory analytes (i.e., BDNF, CCL2/MCP-1, and CCL11/eotaxin-1 concentrations) in the CUD group. In contrast, these associations were not observed in the DD group, despite patients with dual diagnosis showing higher DSM criteria for CUD than those with CUD alone. (3) The higher concentrations of analytes observed in patients

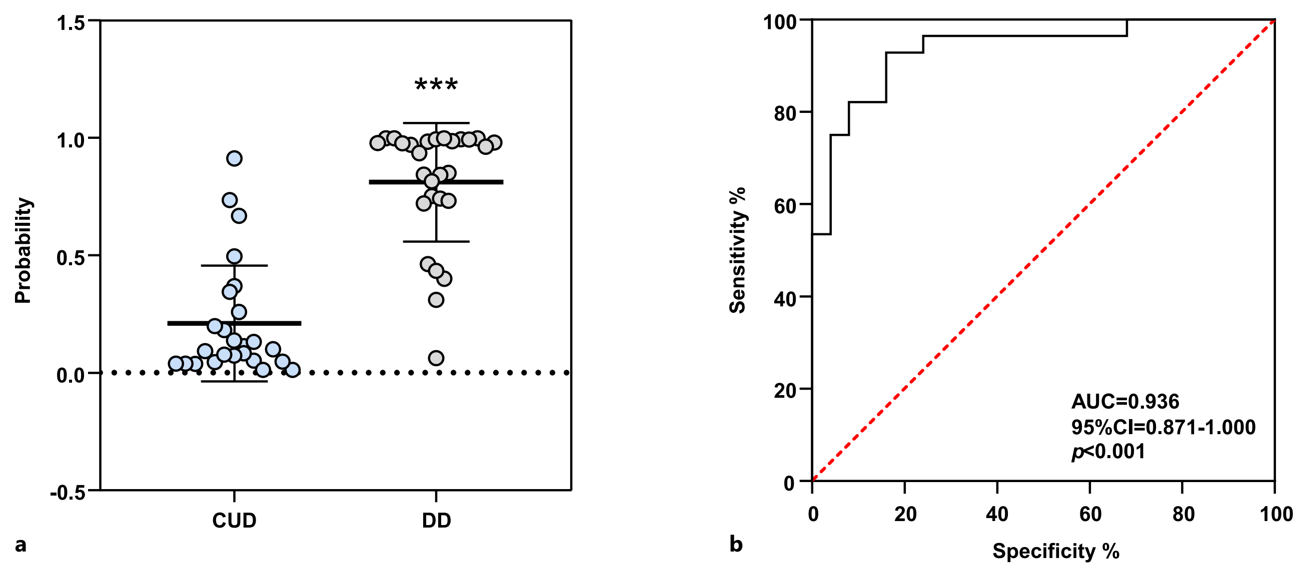

Fig. 6. Receiver operating characteristic analysis for a logistic regression model to distinguish patients with CUD alone from those with dual diagnosis (CUD and SCZ or APD). **a** Scatter plot of the predictive probabilities. **b** Receiver operating characteristic curve generated with probability values from a binary logistic model including log(10)-transformed concentrations of G-CSF, BDNF, VEGF-A, CCL11/eotaxin-1, CCL2/MCP-1, and CXCL12/SDF-1. Lines on the scatter plot are median and IQR. (***) denotes significant differences using the Mann-Whitney U test.

with dual diagnosis were maintained when the DD group was divided into the CUD+SCZ and CUD+APD subgroups. Both subgroups had significantly higher concentrations of analytes than the CUD group, with no differences observed between the DD subgroups. (4) A logistic regression model, including all growth factors and chemokines, demonstrated a high discriminatory power to distinguish patients with CUD alone from those with CUD and SCZ or APD. BDNF and CCL11/eotaxin-1 were significant predictive variables in the model.

In line with prior studies demonstrating that cocaine induces cellular damage and exacerbates proinflammatory immune responses [45, 46], our results revealed differences in plasma concentrations of G-CSF, BDNF, VEGF-A, CCL11/eotaxin-1, CCL2/MCP-1, and CXCL12/SDF-1 among abstinent patients diagnosed with CUD. However, notable differences emerged when lifetime CUD was diagnosed alone or as a dual diagnosis (i.e., CUD and SCZ or APD). Specifically, patients with CUD alone exhibited significant decreases in G-CSF and CCL11/eotaxin-1 concentrations compared with the control group. In contrast, patients with dual diagnosis demonstrated a different profile with significant increases in plasma concentrations of growth factors and chemokines (except for G-CSF), potentially indicative of a systemic inflammatory state. These clear differences have allowed the development of a logistic regression model with excellent discriminatory power to distinguish patients with CUD alone from those with this dual diagnosis.

The dysregulation in G-CSF plasma concentration in the CUD group aligns with a previous study conducted in a cohort of patients diagnosed with substance use disorders and a high prevalence of comorbid depression [26]. A preclinical investigation has reported an association between G-CSF expression and the seeking and consumption of cocaine [25], which could explain the reduced concentrations observed during abstinence from cocaine in the CUD group. Regarding CCL11/eotaxin-1, the decreased concentrations in patients with CUD alone were positively associated with the duration of cocaine abstinence, consistent with another study demonstrating a negative correlation between elevated concentrations of CCL11/eotaxin-1 and the frequency of cocaine use [27]. Although we observed no differences in BDNF concentrations in patients with CUD alone from the CUD group, there was a positive correlation between concentrations of this growth factor and the duration of cocaine abstinence. Accordingly, other studies conducted in cocaine-dependent

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patients have reported that decreased serum BDNF concentrations are associated with early abstinence [47] and cocaine relapse outcomes [48].

Regarding the high concentrations of these inflammatory-related molecules in patients with dual diagnosis, different studies have been conducted investigating circulating mediators in the context of CUD, SCZ, and APD. Previous studies on proinflammatory mediators have reported significant decreases in CCL2/MCP-1 and CXCL12/SDF-1 concentrations in individuals seeking treatment for cocaine use, compared with a control group [17, 49]. However, the concentrations of these chemokines were positively associated with cocaine symptom severity. Although these prior findings may appear contradictory to the present results, there are important distinctions among the studies. The samples in the previous studies comprised patients with CUD who were also diagnosed with additional substance use disorders (primarily alcohol and cannabis) and exhibited a high prevalence of mood and anxiety disorders. Here, we focused on patients with CUD without other substance use disorders, and those with dual diagnosis were specifically associated with SCZ or APD. Furthermore, patients with dual diagnosis in our study exhibited high cocaine symptom severity (i.e., 10 of 11 DSM criteria for CUD), consistent with the findings in these prior studies. Therefore, the coexistence of substance use disorders and the type of dual diagnosis may have a clear impact on the circulating levels of these inflammatory-related molecules, as well as on both neuroplasticity and neuroinflammation processes. It is also important to note that these molecules may increase in the acute or severe phases of these psychiatric conditions and return to normal values or decrease drastically when the organic deterioration is already complete [24]. Lastly, commonly used psychiatric medications have been shown to influence the expression of inflammatory molecules [50]. Therefore, differences in the use of mood stabilizers and antipsychotics in patients with dual diagnosis can potentially mask alterations in these circulating mediators linked to the psychiatric conditions and their symptomatology.

Previously, alterations in certain inflammatory mediators have been linked to SCZ diagnosis. Elevated concentrations of CCL11/eotaxin-1 are associated with negative symptoms and psychosis, such as hallucinations and delusions, in patients with SCZ [51]. Furthermore, CCL11/eotaxin-1 concentrations have been correlated with the duration of the episodes and the severity of negative symptoms [34]. Interestingly, another study reported that CCL11/eotaxin-1 is related to the progression of severity in major psychiatric disorders [52]. In the case of CCL2/MCP-1 concentrations, these have been also studied in severe mental disorders, and high concentrations were linked to the aberrant response of the immune system in patients with SCZ [53]. Consistent with our findings, CCL2/MCP-1 is also found to be increased in patients with SCZ compared with a control group [44, 52].

Additionally, other chemokines have been associated with the diagnosis of personality disorders. For example, plasma concentrations of CCL2/MCP-1 and CXCL12/SDF-1 have been observed to be increased among individuals with various types of personality disorders, including APD, with existing sex differences [54].

Limitations

The CUD and DD groups in this study were predominantly composed of men, aligning with the sex proportions of individuals actively seeking treatment for CUD [55] and reflecting the prevalence rates of cocaine use in Spanish population surveys, specifically 16.1% men and 5.1% women [56]. However, we acknowledge significant limitations in this study: (1) achieving a balance between women and men, along with comparable age ranges in the sample groups, will be crucial to explore the potential effects of these physiological variables on the expression of growth factors and chemokines (i.e., heightened inflammatory tone in females compared with males and increased inflammation associated with aging). (2) The presence of other confounding variables related to sociodemographic and clinical variables that remain unknown could influence the results (e.g., economic status, diet, physical activity, and medication use). (3) Our findings and the predictive model need replication in a larger sample to confirm their clinical relevance. (4) The exploratory and cross-sectional nature of the study preclude the establishment of causality between these differences in analytes and the patients' diagnoses. Instead, our focus is on determining the presence or absence of associations that may allow for the formulation of working hypotheses. (5) Consequently, we believe that longitudinal studies are essential in the future to monitor changes in these inflammatory mediators during extended abstinence periods and active cocaine use, providing a better understanding.

Conclusions

In the context of cocaine, these results are consistent with the understanding that patients with dual diagnosis exhibit significantly altered inflammatory responses compared with abstinent patients diagnosed with CUD alone. It is crucial to consider that dysregulations in plasma concentrations of growth factors and chemokines in patients with lifetime CUD, when compared with healthy subjects, may overlap and mask those alterations associated with comorbid psychiatric disorders, including the use of psychiatric medications. Whether these alterations are linked to the severity and type of the diagnosis or contribute to worse outcomes among patients with CUD remains unclear. The co-occurrence of CUD and other psychiatric disorders complicates the therapeutic approach to dual diagnosis in mental health services, given overlapping symptoms, deteriorated substance treatment, and complicated follow-up [57]. Our results reveal the importance of studying and characterizing valid biological markers to enhance clinical treatment. Further research is needed to fully elucidate the role of these inflammatory mediators in dual diagnosis.

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Statement of Ethics

Written informed consent was obtained from each participant after a complete description of the study. All the participants had the opportunity to discuss any questions or issues. The study and protocols for recruitment were approved by the Local Ethics Committee (PEIBA) (PND 2020/048) and by the Research Ethical Committee from the *Hospital Provincial of Castellón* (11/23/2015) in accordance with the Ethical Principles for Medical Research Involving Human Subjects adopted in the Declaration of Helsinki by the World Medical Association (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the Regulation (EU) 2016/679 of the European Parliament and of the Council, 27 April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, repealing Directive 95/46/EC (General Data Protection Regulation). All collected data were given code numbers to maintain privacy and confidentiality.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

María Flores-López, Nerea Requena-Ocaña, Pedro Araos, Jesús Herrera-Imbroda, and Nuria García-Marchena recruited participants, performed clinical evaluations, and created the clinical database. Enrique Ochoa and Antonia Serrano obtained and processed blood samples to obtain plasma and tested plasma for detecting infectious diseases, supervised data of quantification of growth factor and chemokines. Nuria García-Marchena and Francisco Javier Pavón-Morón conducted statistical analyses. Sandra Torres-Galván, María Flores-López, and Enrique Ochoa wrote the manuscript. Roberto Muga and Antonia Serrano provided critical revision for important intellectual content. Fernando Rodríguez de Fonseca, Francisco Javier Pavón-Morón, and Gonzalo Haro conceived and designed the study. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are not publicly available due to ethical requirements regarding pertinent clinical data. Further inquiries can be directed to the corresponding authors.

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