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Subtypes of depressive symptoms and inflammatory biomarkers: An exploratory study on a sample of HIV-positive patients

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Abstract

Depressive symptoms cause major impairment and may accelerate HIV progression despite the use of antiretroviral medication. The somatic symptoms criteria for HIV infection and depression partially overlap, which can make differential diagnosis challenging. Because of chronic inflammation caused by HIV infection, HIV-positive patients may develop somatic and affective-cognitive symptoms of depression. Inflammation-related depression is primarily characterized with severe somatic symptoms such as fatigue and sleep disturbance. This study sought to explore the patterns of somatic and cognitive-affective depressive symptoms that characterize HIV-positive patients. Our specific aims were (1) to identify subtypes of depressive symptoms in a sample of HIV-positive patients; and (2) to test the subtypes' difference on inflammatory and HIV disease progression biomarkers. HIV-positive men and women ($N = 102$) with and without depressive symptoms were randomly selected from an Italian HIV clinic. Depressive symptoms (PHQ-9), viral load (VL), CD4+, IL-6, TNF- α , and monocytes were assessed. The three subtypes formed using Latent Class Analysis (LCA) identified patients with (1) severe cognitive-affective and somatic depressive symptoms; (2) severe/moderate somatic symptoms; and (3) absent or low depressive symptoms. The subtype with severe/moderate somatic symptoms was characterized with elevated levels of IL-6 and monocytes. No difference on HIV progression biomarkers was found. The subtypes of depressive symptoms might help differentiating depressive symptoms from HIV- and inflammatory-related somatic symptoms. When present, cognitive-affective and/or somatic symptoms cause significant impairment to patients' lives and thus warrant further assessment and treatment.

Keywords

Depression; Somatic; symptoms; IL-6; Monocytes; HIV

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1. Introduction

Depression is a potentially fatal medical condition that causes major impairments and disability in the general population (Moussavi et al., 2007). People living with chronic medical illnesses such as HIV are at a greater risk of developing depressive symptoms (Simon, 2001; Rabkin, 2008), which ultimately affect the course of their physical illness (Chapman et al., 2005; Lépine and Briley, 2011; Antelman et al., 2007). Depressive symptoms are highly prevalent in HIV-positive patients (Rabkin, 2008) and are associated with immune system impairment (Kaharuza et al., 2006), poor medical care engagement and adherence to medication (Sterne et al., 2005; Horberg et al., 2008; Gonzalez et al., 2011). Untreated depression hastens the progression of HIV infection and increases mortality rates (Elliott et al., 2002; Lima et al., 2007). Routine assessment of depressive symptoms using psychometric tools would allow for early diagnosis, enlisting effective treatment to improve patients' health status (Pence et al., 2012; Schumacher et al., 2013; Kalichman et al., 2000; Ferrando et al., 1998). Assessment must consider the wide array of depressive symptoms, such as cognitive-affective (e.g., depressed mood and anhedonia) and somatic (e.g., fatigue and sleep disturbance) symptoms (Kalichman et al., 2000). While somatic symptoms are attributable to both idiopathic depression and HIV infection, cognitive-affective symptoms are more specific to clinical depression (Kalichman et al., 2000; Barroso et al., 2013; Lee et al., 2012; Low et al., 2011). Methods for distinguishing depression from HIV-related somatic symptoms should be employed when assessing HIV-positive patients (Kalichman et al., 2000). However, currently the differential diagnosis relies on subjective clinical findings without objective laboratory measurements.

The inflammatory pathway is considered as a possible contributor to the high incidence of depression in HIV-positive individuals (Zunszain et al., 2011; Lawson et al., 2011). Inflammatory biomarkers, including monocytes and pro-inflammatory cytokines, are associated with both depression and HIV progression (Raison et al., 2006; Zunszain et al., 2012; Capuron et al., 2009). Elevated levels of monocytes have been observed in medically-healthy depressed individuals (Seidel et al., 1996; Suarez et al., 2003), and are associated with HIV pathogenesis (Kedzierska and Crowe, 2002). HIV infection increases the production of pro-inflammatory cytokines including Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF- α), which promote virus replication and CD4 cells reduction (Breen et al., 1990; Fiala et al., 1997; Kedzierska et al., 2003; Thieblemont et al., 1995; Poli et al., 1990). Pro-inflammatory cytokines are associated with depressive symptoms that resemble idiopathic depression but are characterized with more severe somatic symptoms (Capuron et al., 2009). TNF- α and IL-6 produced in response to HIV infection (Poli et al., 1990; Herbein and Varin, 2010; Kuller et al., 2008) activate indoleamine 2,3-dioxygenase (IDO) enzyme, which causes tryptophan depletion (Zunszain et al., 2011; Fu et al., 2010, 2011). IDO metabolizes tryptophan into kynurenine, reducing the synthesis of serotonin (Raison et al., 2006; Zunszain et al., 2012; Fu et al., 2010, 2011; Dantzer et al., 2011). The kynurenine pathway (i.e. cytokines production and IDO activation) may be a possible mechanism for HIV comorbid depression and fatigue (Lawson et al., 2011; Martinez et al., 2014; Lee et al., 2014; Bopath et al., 2015).

The current study sought to provide a method to identify subtypes of cognitive-affective and somatic depressive symptoms, as well as their HIV-related biomarkers, in a sample of HIV-positive patients. There are no similar published studies conducted in HIV-positive patients. We adopted a two-step analytical approach. First, we used the Latent Class Analysis (LCA) to identify subtypes of depressive symptoms. LCA groups individuals, maximizing the within-group similarity and the between-groups difference in depressive symptoms patterns. Second, we tested the subtypes' difference in the clinical and inflammatory biomarkers, including viral load (VL), CD4+ cell counts, monocytes, TNF- α , and IL-6.

Based on the literature, we expected to find at least three distinct subtypes of depressive symptoms: (1) high cognitive-affective and somatic symptoms; (2) low cognitive-affective and high somatic symptoms; and (3) absent or low cognitive-affective and somatic symptoms (Kalichman et al., 2000; Bipath et al., 2015). The sub-types characterized with severe somatic symptoms (subtype 1 and 2) were expected to be associated with elevated levels of inflammatory and HIV progression biomarkers (Capuron et al., 2009; Kedzierska and Crowe, 2002; Lee et al., 2014).

2. Methods

2.1. Sample and procedures

A sample of HIV-positive patients ($N = 102$) with and without depressive symptoms was recruited at the Infectious Diseases Institute of Sant'Orsola-Malpighi Hospital (Bologna, Italy). Men represented the majority in our sample (75.49%). Participants' mean age was 38.42 years ($SD = 8.16$). Educational attainment included elementary and junior high school completion (26.53%), high school diploma (46.94%), bachelor's degree (7.14%), master's degree (9.18%), and Ph.D. or other specialization (10.21%).

A probabilistic randomized sampling method was used. The daily lists of patients attending a blood screening were used to randomly select the participants for the study. HIV-positive patients, 18 years or older, with and without depressive symptoms were recruited. Exclusion criteria included past or current co-infection (e.g., hepatitis C), diagnosis of physical illness other than HIV (i.e. cancer, Crohn's disease), and mental disorders other than depression (e.g., schizophrenia, alcohol and/or drugs abuse). All contacted patients agreed to participate in the study. Less than 10 patients were unreachable due to incorrect phone numbers or unanswered calls, and were not included in the study. Participants' written consent forms provided permission to access the clinical records for HIV progression biomarkers – CD4+ and VL – that were assessed on the day of the survey. The samples of blood drawn were shipped to an internal laboratory. Lab analyses were performed the same day to determine levels of IL-6 and TNF- α , and monocytes cell count. The study protocol was approved by the Hospital's Ethical Committee. Patients who scored 5 or more on PHQ-9 (59.8%) were referred to physicians at the clinic for further evaluation as suggested by Kroenke et al. (2001).

2.2. Psychometric instruments

Demographic information included gender (coded: 1-man or 2-woman), education and age. Depressive symptoms were evaluated through the Patient Health Questionnaire (PHQ-9) Kroenke et al., 2001, a 10-item psychometric instrument validated in Italy (Rizzo et al., 2000). Nine items assess depressive symptoms, and one item assesses perceived impairment caused by depressive symptoms (“How difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?”). Participants reported the frequency of symptoms experienced over the past two weeks. Each symptom was rated on a 4-point scale ranging from 0-Not at all to 3-Nearly every day. Clinical Biomarkers: IL-6 was assessed through chemiluminescent enzyme immunoassay, with a detection limit of 2.0 pg/mL. TNF- α was evaluated with sensitive immunoenzymatic assays with a detection limit of 4.0 pg/mL. Monocytes (%) were determined through flow cytometric method. CD4+ cell counts (cells/mm³) and viral load (VL; copies/L) were collected from patients’ clinical records. VL was recoded as follows: 1 – undetectable VL (less than 50 copies/L), 2 – greater than or equal to 50 but less than 1000 copies/L, and 3 – greater than or equal to 1000 copies/L. ART use was self-reported by the patients (1 – treated with ART, and 2 –naïve to ART).

2.3. Data analysis plan

The analyses were performed using Mplus version 7.4 (Muthén et al., 1998–2015). Descriptive analyses of the sample’s characteristics included mean and standard deviation of continuous variables, and median and mode of ordinal variables. Kurtosis and skewness were calculated to assess variables’ distribution. Latent Class Analysis (LCA; Maximum Likelihood with Robust standard error – MLR) was performed to identify subtypes of depressive symptoms. LCA approach posits that a population may be divided into mutually exclusive latent classes (Collins and Lanza, 2013). These classes are unobserved but inferred from a set of indicators (i.e. depressive symptoms). Individuals are assigned to one latent class based on the pattern of depressive symptoms. The latent classes correspond to different subtypes of depressive symptoms with characteristic patterns of symptoms. The 9 depressive symptoms assessed with the PHQ-9 were treated as ordinal variables. Because of the infrequent selection (ranging from 1.00% to 4.90%, except for fatigue, sleep and appetite disturbance ranging from 9.80% to 13.73%) the option 3-Nearly every day was collapsed into a single category with option 2-More than half the days.

When ordinal indicators are analyzed, Mplus produces item conditional probabilities, a parameter that helps to further characterize and distinguish the latent classes. Item conditional probabilities are specific to a given class and represent the probability of an individual in that class to endorse each level of the PHQ-9 items i.e., 0-Not at all, 1-Several days, 2-More than half the days/Nearly every day. Item conditional probabilities with $p < .05$ were considered statistically different from 0. Further, we conducted a formal test to compare the items conditional probability across the latent classes. Specifically, through the “model test” function in Mplus, we conducted a comparison (Wald test; 43) between each item conditional probability across the latent classes.

Mplus 7.4 provides statistical fit indices used to assess LCA models' fit and to decide on the number of classes. Particularly the most reliable indices are the Bayesian Information Criterion (BIC) and sample size adjusted BIC_N, entropy, and the parametric bootstrapped likelihood ratio test (LRT) Muthén et al., 1998–2015; Jung and Wickrama, 2008; Nylund et al., 2007. BIC and BIC_N are indices employed to compare several plausible models, where lowest values indicate the best fitting model. Entropy ranges from 0 to 1.00 and assesses whether the latent classes are well identified, where values greater than .80 suggest that the latent classes are well defined. LRT compares K classes against the $K - 1$ solution, where K denotes the number of classes. LRT with $p > .05$ would confirm a good fit of the $K - 1$ class solution. Comparatively low BIC and BIC_N, high entropy close to 1.00 (or more generally not lower than .80), and statistically significant LRT indicate good fit of the model.

The second aim of the study was to test the latent classes' difference in HIV related and inflammatory biomarkers, and perceived impairment caused by depressive symptoms. We used Lanza's method implemented in Mplus 7.4 treating the biomarkers as distal dependent variables (Muthén et al., 1998–2015). Lanza's method produces results of overall test and subgroups' pairwise comparisons. The distal outcomes analyzed were continuous (e.g., IL-6, TNF- α) or ordinal (e.g., VL, ART use). Lanza's method DCON function was used for distal continuous variables (IL-6, TNF- α , CD4+, monocytes count, and age), which also produced classes' means and SDs. DCAT function was used for distal categorical variables (ART use, VL, perceived impairment, and gender), which produced proportion for each items' response (Asparouhov and Muthén, 2013; Lanza et al., 2013). Bonferroni correction for multiple comparisons was adopted. Wald pairwise tests with p value .017 were considered statistically significant, because 3 pairwise comparisons were performed ($.05/3 = .01\bar{6}$).

Correlation between HIV progression biomarkers, pro-inflammatory cytokines, and monocytes counts was evaluated through: Pearson's r for continuous variables (e.g., Monocytes and TNF- α); polychoric coefficient for binary and/or ordinal variables (e.g., VL and ART); and point-biserial coefficient for binary and continuous variables (e.g., ART and CD4+).

3. Results

3.1. Descriptive and correlational analyses

Descriptive statistics of the clinical and inflammatory biomarkers are reported in Table 1. The majority of the participants were treated with ART, had undetectable VL, and CD4+ counts >500 cells/mm³. Mean values of IL-6, TNF- α , and monocytes are reported in Table 1.

Clinical and inflammatory biomarkers were significantly intercorrelated (Table 2). Patients with detectable VL, regardless of ART, were characterized with high TNF- α and low CD4+. Greater levels of TNF- α corresponded to high IL-6 expression and a lower CD4+ count. Patients naïve to ART had detectable VL, low CD4+ count, and high monocytes count. A higher monocytes count corresponded to enhanced TNF- α expression and poor HIV-related biomarkers – i.e. detectable VL and low CD4+ count. The sample's median score of cognitive-affective symptoms was 0-Not at all, whereas the median of somatic symptoms and depressed mood was 1-Several days.

3.2. Depressive symptoms profiles – LCA

Fit indexes of four competing models (1-class, 2-class, 3-class, and 4-class) were compared. The 3-class model was associated with low BICN and good entropy (Table 3). LRT test for 3-class vs. 4-class was not significant ($p > .05$), suggesting a good fit of the 3-class model. BIC value was lower in the 2-class model compared to the 3-class model, though BIC tends to underestimate the number of latent classes to form when sample sizes are small (Nylund et al., 2007).

Item conditional probabilities and p value presented in Table 4a (see also Fig. 1) correspond to the class-specific probability for different values (severity) of the PHQ-9 items. Wald test was performed to test difference between classes on each item's conditional probability (Table 4b). Symptoms were considered severe if reported 2-More than half the days/Nearly every day, moderate if rated 1-Several days, and absent if 0-Not at all category was selected on the PHQ-9.

Class 1 corresponded to severe somatic and cognitive-affective subtypes. This class grouped patients with high probability of severe sleep disturbance and fatigue; severe or moderate depressed mood and anhedonia; severe or absent symptoms of poor appetite/overeating. Patients in this class also reported guilt and impaired concentration as severe, moderate or absent with equal probability. Suicidal thoughts and psychomotor changes were moderate or absent. This subtype was labeled “severe depressive symptoms” and, according to the PHQ-9 guideline (Lee et al., 2014), might depict major depression disorder (MDD).

Class 2 corresponded to severe/moderate somatic subtypes. Cognitive-affective symptoms were moderate or absent. Fatigue seems to be the prominent feature of the class. Patients reported severe or moderate or absent poor appetite/overeating and sleep disturbance. Depressed mood and anhedonia were mostly experienced with moderate severity. The majority of the patients in this class reported absent or no symptoms of impaired concentration, psychomotor changes, guilt, and suicidal thoughts. Because of the predominant somatic component, this subtype was labeled “moderate/severe somatic symptoms”.

Class 3 corresponded to absent depressive symptoms. Nearly all patients did not experience depressive symptoms in the last two weeks.

A small, though statistically significant, proportion of patients ($<.300$) reported moderate depressed mood, sleep disturbance, fatigue, and poor appetite/overeating. This subtype was labeled absent/low depressive symptoms.

Between-group comparisons (Wald pairwise tests, Bonferroni correction, $p < .016$) were conducted to better understand the differences between the three subtypes in the 9 depressive symptoms. Results showed that only anhedonia, depressed mood, and fatigue distinguished the three subtypes. Statistically significant pairwise differences were found for the remaining depressive symptoms between the three classes. Class 1 showed more severe symptoms of guilt, impaired concentration, psychomotor changes and suicidal thoughts as compared to Class 2 and Class 3. Class 2 and Class 3 did not differ in severity ratings on these specified

symptoms. Sleep disturbances and poor appetitive symptoms were more severe in Class 1 and Class 2 than compared to Class 3. No significant difference was observed between Class 1 and Class 2 on these other symptoms.

Overall, the severe depressive symptoms subtype (Class 1) showed more severe symptoms of guilt, impaired concentration, psychomotor changes, and suicidal thought compared to the other subtypes. Severe/moderate somatic symptoms and absent/low depressive subtypes did not differ on such symptoms. Conversely, compared to absent/low depressive symptoms subtype, sleep disturbance and poor appetitive symptoms were more severe in the other two subtypes, which did not differ from one another.

3.3. Association between depressive symptoms profiles and HIV clinical biomarkers

The secondary aim of the study was to test the difference in clinical and inflammatory biomarkers, and demographic variables among the three subtypes of depressive symptoms. VL, CD4+, ART, IL-6, TNF- α , and monocytes count, controlling for age and gender, were included in the LCA model as auxiliary covariates. Lanza's method, such as DCON and DCAT functions in Mplus 7.4, for continuous and categorical/ordinal distal auxiliary variables were used.

The patients in Class 2 with moderate/severe somatic symptoms showed higher level of IL-6, TNF- α , and monocytes count compared to patients in Class 1 and 3 (see Table 5 and Fig. 2). The overall test was statistically significant only for IL-6 and monocytes that were higher in patients with moderate/severe somatic symptoms (Class 2) compared to patients with severe depressive symptoms (Class 1) and absent/low symptoms (Class 3) respectively.

No statistically significant difference was found with respect to HIV progression biomarkers (VL, CD4+, and ART use), gender, and age. Finally, we tested the difference across the three subtypes on the PHQ-9 item assessing the perceived impairment caused by depressive symptoms experienced. The overall and pairwise tests ($p < .0001$) showed that all patients with severe depressive symptoms (Class 1) perceived impairment (0% 0-Not at all difficult, 76.6% 1-Somewhat difficult and 23.4% 2-Very difficult). Most of the patients showing moderate/severe somatic symptoms (Class 2) reported some impairment (25.5% 0-Not at all difficult, 71.1% 1-Somewhat difficult and 3.9% 2-Very difficult). All patients with absent or low depressive symptoms (Class 3) did not report any impairment attributed to depressive symptoms (100% 0-Not at all difficult).

4. Discussion

Depression is a severe and disabling medical condition highly prevalent in HIV-positive population (Rabkin, 2008; Chapman et al., 2005), and represents a risk factor for morbidity and mortality (Antelman et al., 2007; Kaharuza et al., 2006). Because of the overlap of symptoms, methods for distinguishing depression from HIV-related somatic symptoms are critical for differential diagnosis in the treatment of people living with HIV (Kalichman et al., 2000). Empirically derived subtypes of somatic and cognitive-affective depressive symptoms in the HIV population may help to this end, reducing the burden of lengthy diagnostic processes. The three subtypes identified in this study through Latent Class

Analysis (LCA) capture different depressive symptomatology: Class (1) severe depressive cognitive-affective and somatic symptoms; Class (2) severe or moderate somatic symptoms; and Class (3) absent or low depressive symptoms.

HIV-positive patients experiencing severe cognitive-affective and/or severe somatic symptoms (Class 1 and Class 2) perceive significant impairment on their daily lives. The first two subtypes might depict idiopathic depression and HIV-related symptoms, respectively. The subtype characterized by both severe cognitive-affective symptoms and somatic (Class 1) seems to resemble symptoms of idiopathic depression (Kroenke et al., 2001). The second subtype (Class 2) is mainly characterized by severe or moderate fatigue, with cognitive-affective symptoms endorsed only with moderate severity, and absence of guilt and suicidal thoughts. This pattern may depict inflammatory- and HIV-related depressive symptoms, in line with (Capuron et al., 2009) findings of a study conducted in patients treated with interferon (IFN)-alpha, a cytokine known to cause depressive symptoms. The majority of the patients in the third subtype report an absence or low symptom of depression. Symptoms of fatigue and sleep disturbance are quite frequently reported, although with different intensity, by patients in all subtypes. This finding is in line with previous studies that documented high rates of these symptoms among HIV-positive patients (Kalichman et al., 2000; Ferrando et al., 1998; Barroso et al., 2013; Lee et al., 2012; Low et al., 2011).

Kalichman et al. (2000) pointed out the need to employ methods to distinguish between HIV-related symptoms and depressive symptoms. Psychometric questionnaires assessing depressive symptoms, including somatic symptoms, tend to inflate scores of depression in HIV positive patients. The composite scores derived by summing individual items is certainly the most commonly used method to assess depressive symptoms severity. However, it does not distinguish between somatic and cognitive affective features, whereas empirically derived subtypes may be useful to this end. Diagnostic algorithms based on these subtypes might help distinguish between symptoms of idiopathic depression and symptoms related to HIV-infection and the associated inflammatory response.

The subtype of severe or moderate somatic depressive symptoms showed higher levels of IL-6 levels and monocytes count compared to the other two subtypes (see Table 5 and Fig. 2). This finding may support our hypothesis of an inflammatory-induced depressive symptomatology depicted by the latent Class 2 (Capuron et al., 2009). Additionally, although not statistically significant, patients in Class 2 also have elevated levels of TNF- α . Our study documents the association between somatic depressive symptoms and inflammatory biomarkers among HIV-positive individuals. These results replicate the results of previous studies conducted in HIV-negative participants demonstrating that inflammation produces depressive-like symptoms and in particular somatic symptoms such as fatigue and sleep disturbance (Lawson et al., 2011; Capuron et al., 2009; Seidel et al., 1996; Suarez et al., 2003; Fu et al., 2010, 2011; Dantzer et al., 2011).

Despite our novel findings, the results of cross-sectional studies have limited generalizability and do not allow us to infer the directionality of the associations found. Therefore, we cautiously emphasize the role of pro-inflammatory in promoting depressive symptoms

among HIV positive patients. Our results are to be considered exploratory and preliminary. Furthermore, additional inflammatory biomarkers should be investigated to identify biological criteria to distinguish between idiopathic depression and HIV-related symptoms. In addition to IL-6, TNF- α , and monocytes, future studies should include Interleukin(IL)-1, Interleukin(IL)-2, high-mobility group protein 1 (HMGB1), C-reactive protein (CRP), cortisol as well as gene expression (Zunszain et al., 2011; Lawson et al., 2011; Valkanova et al., 2013). Similarly, the role of kynurenine in mediating the association between pro-inflammatory cytokines and depressive symptoms among HIV positive population needs further investigation. Pro-inflammatory cytokines promote tryptophan depletion by activating IDO enzyme; this causes a significant decrease in the bioavailability of serotonin (Raison et al., 2006; Zunszain et al., 2012; Fu et al., 2010, 2011; Dantzer et al., 2011; Fujigaki et al., 2006). The kynurenine pathway has been hypothesized as the mechanism underlying the high rates of depression, cognitive impairment and somatic symptoms among HIV positive population (Lawson et al., 2011). However, until now there were no published findings supporting this hypothesis.

The inflammatory biomarkers that characterize the severe somatic symptoms subtype correlate with HIV-progression biomarkers including VL, ART use, and CD4+ (Breen et al., 1990; Poli et al., 1990; Herbein and Varin, 2010; Kuller et al., 2008; Navikas et al., 1995). Our results show that patients with detectable VL and naïve to ART have high levels of TNF- α and monocytes (Poli et al., 1990; Herbein and Varin, 2010; Kuller et al., 2008), whereas IL-6 correlates only with TNF- α . We did not find significant associations between HIV-progression biomarkers and the three depressive symptoms subtypes, likely because of the relatively small sample size.

Compared to patients with no or low depressive symptoms, those who report somatic and/or cognitive-affective symptoms also reported perceived impairment caused by these symptoms. This means that even moderate cognitive-affective symptoms and severe or moderate somatic symptoms negatively impact on patients' lives. Our findings confirm the pressing need of routine screening and psychiatric and psychological support in HIV clinics (Ferrando et al., 1998; Wainberg et al., 2014). PHQ-9 scoring procedures provide significant clinical information regarding the severity of depressive symptoms, though they cannot discriminate between different subtypes of depressive symptoms and differential diagnosis between idiopathic depression and HIV-related symptoms.

The subtypes identified in the current study may help clinicians distinguishing HIV-related symptoms and idiopathic depression. Because of the cross-sectional nature of the data analyzed, we cannot clearly disentangle psychosocial causes of depression (e.g., depression caused by HIV diagnosis) from the inflammatory causes. We also believe that multiple causes concur in promoting depression among HIV, and that psychosocial and inflammatory factors may act conjunctively.

5. Conclusion

For the first time, and to the best of our knowledge, we have identified subtypes of cognitive-affective and somatic depressive symptoms, and their inflammatory and HIV-

infection biological correlates among HIV-positive patients. Our results confirm previous work that recommends screening and follow-up of depressive symptoms using a validated instrument like the PHQ-9 as invaluable practice when providing primary care to HIV infected individuals (Kalichman et al., 2000; Crane et al., 2005; Shacham et al., 2009; Lawrence et al., 2010). Based on our depressive symptoms subtypes, diagnostic algorithms may be developed to help in the differential diagnosis between idiopathic depression and HIV-related symptoms and target appropriate treatments. Further understanding of the inflammatory factors underlying the different subtypes of depressive symptoms in HIV-positive individuals will help to identify new approaches targeting inflammation to employ as adjuvant to anti-depressants or stimulants (Udina et al., 2014; Breitbart et al., 2001; Hill and Lee, 2013) in association with psychotherapy (Crane et al., 2005; Kennard et al., 2014) among HIV positive depressed patients. In addition to IL-6, TNF- α , and monocytes, future longitudinal studies should be conducted to test the effect of IL-1, IL-2, HMGB1, CRP, cortisol, kynurenine, and genes expression on depressive symptoms CD4+ levels and virus replication (Zunszain et al., 2011, 2012; Valkanova et al., 2013; Boasso et al., 2007). Lastly, future research should also test the effect of psychosocial stress on depressive symptoms and inflammatory biomarkers since negative emotions and social stressors including racial discrimination may increase inflammatory biomarkers (Ratner et al., 2013; Dickerson et al., 2004; Kemeny, 2009).

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Fig. 1. PHQ-9 item conditional probabilities – histograms

Conditional probabilities: class-specific probability of each PHQ-9 item's answer (i.e. 0-Not at all, 1-Several days, 2-More than half the days/Nearly every day) to be endorsed.

Fig. 2.
Levels of inflammatory biomarkers by depressive symptoms subtypes (latent classes).

Table 1

Descriptive statistics overall sample.

	Overall sample (N = 102)
<i>Gender, %</i>	
Men	75.49
Women	24.51
Age, mean (SD)	38.42 (SD = 8.16)
<i>ART use, %</i>	
Treated	70.59
Naïve	29.41
<i>Education attainment, %</i>	
Elementary/junior high school	26.53
High school diploma	46.94
University degree	7.14
Master's degree	9.18
Ph.D. Or other specialization	10.21
<i>Depressive symptoms – PHQ-9, median</i>	
1 – Anhedonia	0 – Not at all
2 – Depressed mood	1 – Several days
3 – Sleep disturbance	1 – Several days
4 – Fatigue	1 – Several days
5 – Poor appetite overeating	1 – Several days
6 – Guilt	0 – Not at all
7 – Impaired concentration	0 – Not at all
8 – Psychomotor changes	0 – Not at all
9 – Suicidal thoughts	0 – Not at all
10 – Functional impairment	0 – Somewhat difficult
CD4+, mean (SD)	666.36 (319.10)
<i>Viral load (VL), %</i>	
1– <50 copies/L	57.43
2 – 50 and <1000 copies/L	21.78
3 – 1000 copies/L	20.79
Il-6 pg/mL, mean (SD)	3.01 (2.24)
TNF-α pg/mL, mean (SD)	14.44 (10.13)
Monocytes %, mean (SD)	6.42 (1.58)

Table 2

Clinical biomarkers (cytokines, monocytes, CD4+ %, viral load [VL] and ART use) correlation.

	1	2	3	4	5
1 IL-6	–				
2 TNF-α	.24***	–			
3 Monocytes (%)	.10	.21*	–		
4 CD4+ (%)	-.19	-.11**	-.29***	–	
5 VL	.08	.33***	.27*	-.39***	–
6 ART	.09	.31***	.40***	-.26*	.82***

Point-biserial coefficient (binary with continuous variables); polychoric correlation (ordinal with ordinal/binary variables); Pearson's r correlation (continuous with continuous variables); VL = viral load.

* p .05.

** p .01.

*** p .001.

Table 3

Fit indices for LCA models with 1–4 classes.

	BIC	BIC_N	LRT	Entropy
1-class	1683.35	1626.50		1.00
2-class	1572.97	1456.10	$p < .001$	.87
3-class	1604.05	1427.16	$p < .001$	.90
4-class	1651.63	1414.74	$p = .07$	.90

Latent Class Analysis (LCA); Bayesian Information Criterion (BIC); sample size adjusted Bayesian Information Criterion (BICN); parametric bootstrapped likelihood ratio test (LRT).

Table 4a

Latent classes' conditional probabilities PHQ-9 items and p value.

		Class 1 (n = 17)	Class 2 (n = 42)	Class 3 (n = 43)
		Severe symptoms Prob.	Moderate symptoms Prob.	Low/no symptoms Prob.
Anhedonia	Not at all	.00 ^{NS}	.38 ^{***}	.96 ^{***}
	Several days	.42 ^{***}	.48 ^{***}	.04 ^{NS}
	More than half/Nearly every day	.58 ^{***}	.14 ^{**}	.00 ^{NS}
Depressed mood	Not at all	.00 ^{NS}	.39 ^{***}	.78 ^{***}
	Several days	.30 ^{**}	.61 ^{***}	.22 ^{**}
	More than half/Nearly every day	.70 ^{***}	.00 ^{NS}	.00 ^{NS}
Sleep disturbance	Not at all	.13 ^{NS}	.29 ^{***}	.76 ^{***}
	Several days	.00 ^{NS}	.46 ^{***}	.24 ^{***}
	More than half/Nearly every day	.87 ^{***}	.25 ^{***}	.00 ^{NS}
Fatigue	Not at all	.00 ^{NS}	.04 ^{NS}	.69 ^{***}
	Several days	.12 ^{NS}	.56 ^{***}	.31 ^{***}
	More than half/Nearly every day	.88 ^{***}	.41 ^{***}	.00 ^{NS}
Poor appetite overeating	Not at all	.23 [*]	.31 ^{***}	.80 ^{***}
	Several days	.13 ^{NS}	.39 ^{***}	.16 [*]
	More than half/Nearly every day	.64 ^{***}	.30 ^{***}	.04 ^{NS}
Guilt	Not at all	.35 ^{**}	.70 ^{***}	.88 ^{***}
	Several days	.29 ^{**}	.16 ^{**}	.12 ^{NS}
	More than half/Nearly every day	.35 ^{**}	.14 ^{**}	.00 ^{NS}
Impaired concentration	Not at all	.23 [*]	.69 ^{***}	.92 ^{***}
	Several days	.47 ^{***}	.24 ^{**}	.08 ^{NS}
	More than half/Nearly every day	.30 ^{**}	.07 ^{NS}	.00 ^{NS}
Psychomotor changes	Not at all	.31 ^{**}	.86 ^{***}	.96 ^{***}
	Several days	.52 ^{***}	.08 ^{NS}	.04 ^{NS}
	More than half/Nearly every day	.18 ^{\$}	.07 ^{NS}	.00 ^{NS}
Suicidal thoughts	Not at all	.53 ^{***}	.88 ^{***}	.96 ^{***}
	Several days	.29 ^{**}	.08 ^{NS}	.04 ^{NS}
	More than half/Nearly every day	.18 ^{\$}	.04 ^{NS}	.00 ^{NS}

PHQ-9 depressive symptoms; Prob. is the conditional items probabilities (range from 0 to 1.000).

*
p < .05.

**
 $p < .01$.

 $p < .001$.

^s
 $p < .06$ and $> .05$ (approaching the statistical significance).

NS
 $p > .06$.

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Table 4b

Latent classes' conditional probabilities comparison – Wald pairwise test.

	C1 vs. C2	C2 vs. C3	C1 vs. C3
Anhedonia	***	***	***
Depressed mood	***	**	**
Sleep disturbance	NS	***	***
Fatigue	***	**	***
Poor appetite Overeating	NS	***	***
Guilt	**	NS	**
Impaired concentration	**	NS	***
Psychomotor changes	***	NS	***
Suicidal thoughts	**	NS	**

Wald pairwise tests (Bonferroni correction).

**
 $p < .01$ ***
 $p < .001$.NS
 $p > .06$.

Table 5

Latent classes' demographics and clinical biomarkers difference – Lanza's method.

	Class 1	Class 2	Class 3	Overall	C1 vs. C2	C2 vs. C3	C1 vs. C3
	Severe symptoms	Moderate symptoms	Low/no symptoms	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>
IL-6 [‡] , Mean (SE)	2.32 (.16)	3.43 (.43)	2.85 (.31)	*	NS	NS	NS
TNF-α [‡] , Mean (SE)	12.82 (1.30)	14.89 (1.72)	13.92 (1.87)	NS	NS	NS	NS
Monocytes (%), Mean (SE)	6.50 (.44)	6.82 (.24)	5.89 (.23)	*	NS	**	NS
CD4+ (%), Mean (SE)	629.13 (231.42)	669.24 (258.82)	677.40 (391.37)	NS	NS	NS	NS
<i>Viral load</i> [§] , <i>Prob. (SE)</i>							
<50	.56 (.13)	.56 (.08)	.59 (.08)	NS	NS	NS	NS
50, <1000	.12 (.08)	.27 (.07)	.20 (.07)				
1000	.32 (.12)	.17 (.07)	.20 (.07)				
<i>ART use, Prob. (SE)</i>							
Treated	.65 (.12)	.69 (.10)	.75 (.11)	NS	NS	NS	NS
Naïve	.36 (.12)	.31 (.10)	.25 (.11)				
Age, Mean (SE)	38.56 (1.89)	37.03 (1.24)	39.78 (1.28)	NS	NS	NS	NS
<i>Gender, Prob. (SE)</i>							
Male	.88 (.08)	.71 (.07)	.75 (.07)	NS	NS	NS	NS
Female	.12 (.08)	.29 (.07)	.25 (.07)				

Bonferroni correction for the multiple pairwise tests; Prob., conditional probability.

NS
p > .05.

*
p < .05.

**
p < .01.

[‡]
pg/ml.

[§]
copies/L.