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Elevated Neutrophil to Lymphocyte Ratio in Older Adults with Cocaine Use Disorder as a Marker of Chronic Inflammation

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Objective: The neutrophil to lymphocyte ratio (NLR) is a non-specific, easy-to-obtain marker of inflammation associated with morbidity and mortality in systemic, psychiatric, and age-related inflammatory conditions. Given the growing trend of substance use disorder (SUD) in older adults, and the relationship between inflammation and SUD elevated NLR may serve as a useful inflammatory biomarker of the combined burden of aging and SUD. The present study focused on cocaine use disorder (CUD) to examine if cocaine adds further inflammatory burden among older adults, by comparing NLR values between older adults with CUD and a non-cocaine using, aged-matched, nationally representative sample.
Methods: The dataset included 107 (86% male) participants (aged 50−65 years) with cocaine use disorder. NLR was derived from complete blood count tests by dividing the absolute value of peripheral neutrophil concentration by lymphocyte concentration. For comparison, we extracted data from age-matched adults without CUD using the National Health and Nutrition Examination Survey. Individuals with immunocompromising conditions were excluded (e.g., rheumatoid arthritis and sexually transmitted infections such as HIV). A doubly-robust inverse probability-weighted regression adjustment (IPWRA) propensity score method was used to estimate group differences on NLR while controlling for potential confounding variables (age, gender, race, income, nicotine, marijuana and alcohol use).
Results: The IPWRA model revealed that the CUD sample had significantly elevated NLR in comparison to non-cocaine users, with a moderate effect size (β weight = 0.67).
Conclusion: Although non-specific, NLR represents a readily obtainable inflammatory marker for SUD research. CUD may add further inflammatory burden to aging cocaine users.

KEY WORDS: Aging; Cocaine; Inflammation; Lymphocytes; Neutrophils.

INTRODUCTION

Cocaine use disorder (CUD) is a persistent condition, often leading to health complications and emergency department visits. Cocaine use in older adults is on the rise [1-3]. Estimates suggest the prevalence of weekly cocaine use and CUD among individuals ≥ 50 increased from 2011−2015 at a rate of 236% and 271%, respectively [4]. Aging is associated with decreased health and a significant changes in immune functioning called 'immunosenescence'. An important feature of these changes is the low-grade inflammation that can be compounded by substance use and pro-inflammatory conditions such as diabetes and atherosclerosis [5-7]. As cocaine exposure is associated with elevated inflammation and altered immune functioning [8,9], the presence of CUD might exacerbate inflammatory processes in aging adults. Those with CUD are at greater risk for developing HIV and hepatitis C infection [10-12]. Further, the presence of CUD accelerates the progression of inflammatory diseases, such as atherosclerosis [13,14]. Identifying inflammatory biomarkers in aging adults with CUD can help establish important bio-

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logical mechanisms of action that could lead to better interventions and reduce the inflammatory burden on this sensitive population.

Neutrophils are first responder white blood cells that are mobilized to sites of acute endothelial damage and/or infection [15]. While neutrophils are important in acute inflammation and defense of bacterial and fungal infections, chronically elevated neutrophil activity can lead to tissue damage [16]. Lymphocytes are another type of white blood cell central to the immune response. Lymphocytes are key in the response to infection and are related to the progression of inflammatory and autoimmunity diseases [17,18]. The neutrophil to lymphocyte ratio (NLR) is obtained from complete blood counts (typically 1,000 /μl). Because neutrophil and lymphocyte counts are standard in routine blood tests, the NLR represents a cost-effective and easily available but non-specific marker of inflammation. NLR is associated with incidence, morbidity, and mortality in several systemic diseases, including cardiovascular diseases and malignancies [19-21]. More recently, elevated NLR has been associated with schizophrenia and mood disorders [22-28]. Both neutrophils and lymphocytes have also been implicated in substance use. For instance, neutrophils increase after heavy alcohol consumption [29] and higher NLR is related to risk for developing alcoholic liver disease and other alcohol-related complications [30]. Nicotine is also related to increases in neutrophils and/or lymphocytes and extant evidence suggests that smoking cessation might reverse these changes [31-35]. High NLR was also reported in subjects with heroin dependence [36,37].

No previous study has evaluated NLR in the context of cocaine use. While some inflammatory makers have been shown to decrease after acute administration of cocaine [38], chronic cocaine use generally leads to immune system activation. Chronic cocaine use modulates cytokine levels toward a pro-inflammatory (i.e., decreased interleukin [IL]-10 and increased tumor necrosis factor-α) profile [39-42] and activates glial cells (e.g., astrocytes and microglia) in the brain [8,43]. Cocaine administration also modulates neutrophil levels: intravenous administration of cocaine increases neutrophils within the first seven days of exposure [44,45]. Further, cocaine alters the functioning of neutrophils; both the antibacterial and tumoricidal functions of neutrophils increase after cocaine exposure [46]. Cocaine also has a broad effect on lymphocyte activity [47,48]. Beyond direct effects on immune cells, cocaine use generally enhances hypothalamic-pituitary-adrenal axis activity, which further influences immune functioning (for a more detailed review on the immunology of substance use disorders, please see [49]. Although short-term activation of the immune system can be adaptive, chronic activation due to repeated cocaine use can lead to a neuroinflammatory state that has a negative impact on central nervous system functioning [43].

While cocaine is associated with these marked changes in neutrophils and lymphocytes, NLR has not yet been evaluated as a potential maker of inflammation in aging adults with CUD. Older adults with cocaine addiction present unique issues to treatment, including complicated co-morbidities that add to the already large public heath burden of substance use disorders [50]. Aging alone is associated with low-grade inflammation [5,6]. Further, in non-drug using populations, age is positively associated with NLR [51]. The added burden of cocaine use in older populations might be expected to increase NLR. The current study examined the hypothesis that chronic cocaine use adds exacerbated inflammatory burden among older adults (aged 50−65 years old) by comparing NLR values between an aging CUD group and an age-matched nationally representative sample. If CUD adds further insult to typical immunosenescence observed in aging populations, then aging adults with CUD should have increased NLR compared with age-matched controls.

METHODS

Participants

The sample included 107 participants meeting standardized diagnostic criteria for CUD based on the Structured Clinical Interview (SCID) for the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV)/the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) and 1,309 age-matched, non-cocaine using controls. With the transition from DSM-IV to DSM-5, not all participants were evaluated under the same DSM. All participants in the CUD group endorsed 3 or more symptoms on the SCID, which resulted in 83 participants meeting DSM-IV criteria for cocaine dependence and 24 participants meeting DSM-5 criteria for CUD. SCIDs were performed by Master's level trained

clinicians or supervised graduate student trainees. The data from CUD participants were collected at the Center for Neurobehavioral Research on Addiction between 2011 and 2018. Data for non-cocaine using adults were extracted from the National Health and Nutrition Examination Survey (NHANES years 2013−2014 and 2015−2016). For the CUD group, records were included if they met the following requirements: a) CUD diagnosis, b) age 50 to 65 years, c) files were retrievable, and d) LabCorp blood lab panel data was available. Records were excluded if there was evidence of a) illicit drug use other than marijuana or cocaine, b) a current inflammatory condition not associated with natural aging (e.g., rheumatoid arthritis and sexually transmitted inflections – please refer to Fig. 1 for full exclusionary criteria), c) major psychiatric disorder previously associated with inflammation, including mood disorders and psychotic disorders, d) current medication influencing inflammatory processes or central nervous system function, or e) neurological disease. Records were not excluded if the participant had an existing pro-inflammatory condition associated with normal aging (e.g., hypertension or diabetes). The cocaine sample consort is presented in Figure 1. For the aged-matched non-cocaine using sample, records were excluded if they reported: a) age $<$ 50 or $>$ 65 years, b) illicit drug use other than marijuana, c) any inflammatory conditions not associated with normal aging, including sexually transmitted infections (see Fig. 2 for full exclusionary criteria), d) current medication influencing inflammatory processes or central nervous system function, or e) neurological disease. The NHANES consort is presented in Figure 2. A detailed explanation of inclusion/exclusion criteria is provided in the Supplementary Materials (available online). All CUD participants provided informed consent and their anonymity was persevered. All procedures involving the CUD sample were approved by the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects (no. HSC-MS-05-0322).

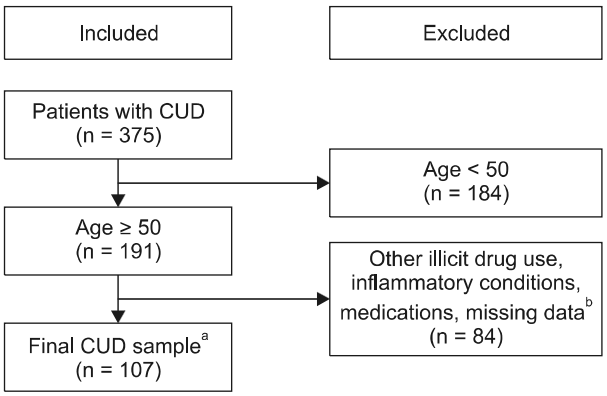

Fig. 1. Cocaine use disorder (CUD) sample consort.
[a]Participants with the following existing pro-inflammatory conditions associated with normal aging were included in the sample: hypertension, elevated serum glucose, diabetes, and hyperlipidemia.
[b]Participants were excluded if they reported any illicit drug use other than cocaine and marijuana. Participants were excluded if they had irretrievable files or missing LabCorp data (n = 56). Participants were also excluded if they were on anti-inflammatory medications or had inflammatory medical conditions (n = 28) including hepatitis C, HIV, rheumatoid arthritis, syphilis, and trichomoniasis.

Measures

Neutrophil to lymphocyte ratio

As part of standard medical screening for participation in CUD research studies at the Center for Neurobehavioral Research on Addiction, participants provided a blood sample. Peripheral blood was collected from fasting par-

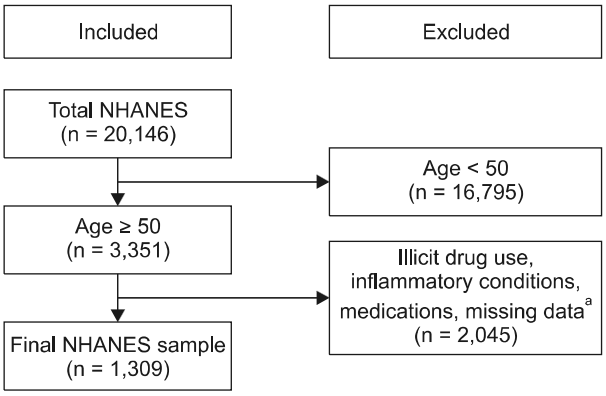

Fig. 2. National Health and Nutrition Examination Survey (NHANES) sample consort.
[a]Exclusionary criteria (other than age) were as follows. Participants were excluded if they reported using the following illicit drugs: cocaine, heroin, methamphetamine, or anabolic steroids. Participants were also excluded if they reported any of the following inflammatory diseases or conditions: rheumatoid arthritis/psoriatic arthritis, gout, liver disease, cancer, preventative aspirin use, or asthma attack within the past year. The following sexually transmitted infections were also exclusionary: hepatitis B, hepatitis C, HIV or chlamydia. Participants were excluded if they had any of the following conditions that may affect inflammation within the past 30 days: flu, pneumonia, ear infection, common cold, or stomach/intestinal illness. Participants reporting medications potentially influencing the central nervous system or inflammatory processes were excluded. For example: anti-fungals, anti-infectives, penicillin, analgesics, hormone modifiers, steroids, immunosuppressive agents, and antipsychotics.

ticipants in the morning (between 8 AM−12 PM) by venipuncture into heparin-containing vacutainers. Samples were sent on the same day of blood draw to LabCorp for standard blood laboratory panel analysis, including comprehensive metabolic panel, complete blood count, and thyroid panel. The NHANES data, blood draw, and analysis procedures are described in detail online (https://www.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?BeginYear=2013). For both the CUD and NHANES datasets, NLR was calculated by dividing the absolute value of peripheral neutrophil concentration (1,000 cells /μl) by lymphocyte concentration (1,000 cells /μl) based on standard laboratory panel data.

Variable Selection and Harmonization

Selection of exclusionary criteria and key covariates for inclusion in the propensity scoring model was based on a literature search related to potential sources of inflammation followed by group discussion and consensus among the authors. Based on this selection process, the following variables were included as covariates in the final statistical model (detailed below): age, gender, race, estimated income, nicotine use, marijuana use, and alcohol use (note that current use of other illicit substances was exclusionary in both groups). The final exclusion and matching covariates required harmonization between datasets. While the NLR calculation was necessarily derived from equivalent standardized laboratory methods (neutrophil and lymphocyte counts 1,000 cells /μl), several of the covariates were measured differently between the CUD and NHANES datasets. Specifics of the variable selection and harmonization process are enumerated in the Supplementary Material (available online); to summarize, for the formal data analysis the outcome measure NLR and the covariate age were treated as continuous variables. The covariate income level was treated as an ordinal categorical variable. The covariates race, gender, alcohol, nicotine, and marijuana use were treated as nominal categorical variables.

Statistical Analyses

A doubly robust propensity scoring method, inverse-probability-weighted regression adjustment (IPWRA), was employed via the teffects command in STATA [52,53] to estimate group differences (CUD vs. NHANES) on NLR while controlling for predetermined covariates: age, gender, race, income, nicotine use, marijuana use, and alcohol use. IPWRA combines augmented inverse probability weighting of covariates (treatment model) and regression variable adjustment (outcome model) and concurrently implements both models to estimate the outcome parameters. This combined approach is robust to misspecification, thus optimizing "treatment" effects estimates (i.e., cocaine use) in observational datasets like those used presently. By (1) sampling from a population of diagnostically-defined individuals with CUD and a nationally representative age-matched sample of the population, (2) eliminating as many inflammation-related factors as possible in both datasets and (3) including potential covariates in the model, we aimed to meet the IPWRA assumptions of covariate balance, overlap, and conditional mean independence of outcomes [53].

RESULTS

The CUD sample (n = 107) was 86% male and 14% female. The race distribution was heavily African American (Table 1). This distribution is representative of the sample of metropolitan-area Houston cocaine users typically in enrolled in Center for Neurobehavioral Research on Addiction studies. As commonly observed in CUD, some participants met criteria for other substance use disorders. Fifteen participants met for current alcohol use disorder, while seven participants met for current cannabis use disorder. Cocaine was the primary substance of abuse for all CUD participants. The NHANES sample was 50% male. The NHANES categories were collapsed to African American, White, and Other to match the CUD dataset (Table 1). Standardized measures of substance use

Table 1. Sample characteristics

Variable		CUD (n = 107)	NHANES (n = 1,309)
Age (yr)	Mean	54.4	57.4
Sex (n)	Male	92	701
	Female	15	608
Race (n)	African American	90	329
	White	9	571
	Other	8	510
Income (USD)	Monthly median	800	1,250
Education (yr)		12.6	12.7

CUD, Cocaine use disorder; NHANES, National Health and Nutrition Examination Survey; USD, United States dollar.

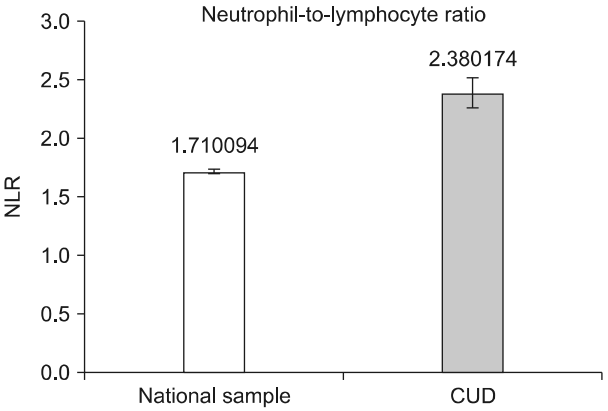

Fig. 3. Neutrophil to lymphocyte ratio values from the propensity score matching model. Error bars represent standard deviations. CUD, cocaine use disorder; NLR, neutrophil to lymphocyte ratio.

disorder were not obtained in the NHANES dataset. Note that the demographic imbalance between the CUD and NHANES samples underscores the utility of the IPWRA methodology.

Shown in Figure 3, the NLR means and standard errors for the groups were CUD = 2.38 (± 0.13); controls = 1.71 (± 0.02). The propensity score model revealed a statistically reliable difference in NLR between the groups, β = 0.67, robust standard error = 0.14, $p < 0.001$. The β weight of 0.67 indicates a moderate effect size, confirming the initial hypothesis of greater inflammation in CUD subjects aged 50− 65 versus an age matched nationally representative sample. To examine if the model adequately balanced the covariates, the overidentification test was applied via the STATA tebalance overid command [53]. The overidentification test result was $\chi^2(10) = 3.09$, $p = 0.97$, thus failing to reject the null hypothesis that the model-adjusted means of the covariates were equivalent across groups.

DISCUSSION

The current study used NLR—a non-specific biomarker of inflammation—to test if CUD adds further inflammatory burden in aging adults. We used a doubly robust propensity score method to account for potential confounding factors including age, gender, race, income, and other drug use. After harmonization on these important variables, comparison of NLR values between the two groups revealed that NLR was increased in older adults with CUD in comparison to the aged-matched, control group without CUD. To the best of our knowledge, this is the first study that compared NLR values in a CUD sample to a nationally representative dataset, and also the first to focus specifically on cocaine use and aging.

The biochemical pathway that exists between inflammation/NLR, cocaine, and aging has not yet been clearly elucidated. As discussed above, cocaine can affect immune functioning, leading to a state of low-grade chronic inflammation. Aging itself is marked by several changes in the immune system, including the development of a chronic pro-inflammatory state frequently called 'inflammaging' [54]. Immunosenescence changes include involution of the thymus [55], decline in T and B cell functioning [56], decrease in IL-2 production [57] and alterations of neutrophil phagocytic capability [58]. Human aging is also associated with elevated activation of the sympathetic nervous system and stress response [59], primarily through decreased norepinephrine reuptake [60,61]. For instance, as a robust modulator of monoamine transmission, cocaine also alters both central and peripheral norepinephrine function [13,62,63]. Cocaine is also a sigma-1 receptor agonist, acting on various leukocyte populations [64]. Exposure to cocaine leads to reduced proliferation of T cells and decreased IL-2 levels [65]. Therefore, both aging and cocaine use might lead to increased NLR possibly through reduced number of lymphocytes due to decreased IL-2 levels among other factors and/or increased number of neutrophils through increased stress response. It is worth mentioning that neither aging nor chronic cocaine use induce leukocyte changes outside the normal range count. Moreover, because of the relatively wide variation of leukocyte count within the normal range, the cutoff for NLR is debated [27,66]. Accordingly, most studies with NLR rely on a reference population, due to challenge translating observed group differences into individual cases.

There are several notable limitations to this study. First, the dataset lacked the size and distributional properties to examine the synergistic effect of cocaine dose-response (i.e., lifetime use) on inflammation and age. As we did not have a comparable younger CUD population, we cannot fully interpret the inflammatory effects related uniquely to cocaine use versus those related directly to age. However, our findings suggest a combined impact of cocaine and aging, such that presence of CUD adds further inflammatory burden to the increase in inflammation associated

with aging. Second, the manner in which the CUD dataset and the NHANES dataset were collected did not allow for perfect harmonization, which likely added noise and decreased the precision of the estimated effect. Third, the relationship between peripheral inflammatory markers and neuroinflammation is still being characterized and is in the nascent stages of understanding, but has been the source of contention in the scientific literature [49,67]. To further complicate this limitation, NLR is non-specific inflammatory marker, leaving restricted insight regarding the mechanisms by which cocaine and aging interact to increase inflammation. Subsequent work will require more focused investigation using specific immune markers, neuroimaging, and their relative relationships to NLR.

In conclusion, elevated NLR values were observed in older patients with CUD when compared to age-matched non-CUD controls. These findings support the role of NLR as a readily available blood-based biomarker with potential scientific utility. Given that NLR is readily obtainable from standard laboratory panels, it may be particularly valuable in the examination of large, representative cohort or longitudinal datasets of SUD and other disease processes. Future studies investigating the relation between elevated NLR and clinical outcomes in patients with CUD are needed. If inflammatory mechanisms underlying anhedonia, stress, and impaired cognitive function in CUD are associated with higher NLR values, this would have implications for treatment [41,68]. Currently there is a growing trend toward testing anti-inflammatory agents as pharmacological tools in addiction treatment [41,69,70]. Further investigation of NLR as an indicator of inflammatory response would aid in these medication development efforts. Further, although beyond the scope of the present project, development of a cutoff value for NLR that optimally discriminates between healthy controls and individuals with cocaine use disorder may provide unique value to the literature.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Conception and design of the study: Scott D. Lane, Charles E. Green, and Joy M. Schmitz. Acquisition and analysis of data: Amber M. Berumen, Kira E. Gomez, Jessica Vincent, Heather E. Soder, Scott D. Lane, Robert Suchting, Charles E. Green. Original draft and figures: Heather E. Soder. Final editing of manuscript: Heather E. Soder, Scott D. Lane, Charles E. Green, Joy M. Schmitz, Margaret C. Wardle, Amber M. Berumen, Kira E. Gomez, Jessica Vincent, Robert Suchting, Antonio L. Teixeira.

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