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Immune cell markers associated with early life major depressive episodes☆

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ABSTRACT

Emerging evidence indicates an association between altered immune system functioning, inflammatory response activation, and major depression (MD). This study examined the association between major depression in young people and the monocyte-to-lymphocyte (MLR) and neutrophil-to-lymphocyte ratio (NLR). Inflammation markers were estimated from DNA methylation data using an established cell-mixture deconvolution algorithm and examined in a well-characterized, antidepressant free epidemiological sample of young twins (N = 170). No statistically significant differences were observed between young persons with and without Lifetime MD for the MLR and NLR. A post-hoc analysis of time since last major depressive episode (MDE) indicated that more recent MDEs were associated with higher MLR and NLR levels compared to temporally distant MDEs. A second post-hoc analysis using the full sample determined that past year MDEs were associated with elevated MLR and NLR scores compared to persons unaffected by lifetime MD and person experiencing their last MDE over one year ago. Finally, T cell immunity was most consistently associated with recent MDEs, suggesting that levels of this adaptive immune cell were diminished. Young people experiencing a recent MDE demonstrated increased levels of inflammation with T cells exhibiting the most persistent associations with depression outcomes. Etiological and treatment implications of results are discussed.

1. Introduction

Major depression (MD) is a leading cause of disability and currently ranks second in the global burden of disease ("[24]). The disease state of MD is characterized by persistent sadness and/or markedly diminished interest/pleasure in all/most activities along with a host of symptoms (e.g., appetite disturbance, concentration difficulties, suicidal ideation) ([3]). Among young people, 16% of females and 12% of males endorse at least one major depressive episode (MDE) by age 18 [70]. An early age of onset confers increased risk of recurrence [39,69] as well as negative socioemotional outcomes [39,59] and is associated with greater symptom severity [19,26,29,74].

Accumulating evidence indicates a link between MD and systemic inflammation [11,14,35,52,53,58], suggesting that the immune system might contribute to its development. Both preclinical and clinical studies have found an association between higher levels of pro-inflammatory cytokines (such as interleukin [IL]−1β, IL-6, tumor necrosis factor-α) and C-reactive protein, and the symptoms of depression [17,25,28,32,38,66–68,73]. Moreover, this pro-inflammatory state manifests in parallel with perturbated inflammation-related processes including platelet activating factor, damage to mitochondria, and oxidative and nitrosative stress [23,4,41,43–45,54,6,61,9]. These complex mechanisms contribute to an adverse neurobiological landscape that may play a role in the pathophysiology of MD and may give rise to a pathoetiological pathway.

Two cell-based biomarkers of inflammation that may be useful for recognizing the disease state of MD are the monocyte-to-lymphocyte ratio (MLR) and neutrophil-to-lymphocyte ratio (NLR). The MLR and NLR are easily accessible blood measurements of immune status and inflammation level, reflecting the balance between innate (monocytes

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and neutrophils) and adaptive (lymphocytes) immunity. Although neutrophils and monocytes are among the first line of host defense, these cells, like other immune cells, also perform important regulatory roles in adaptive immunity. The combination of high circulating neutrophils or monocytes and low lymphocytes (i.e., elevated MLR and NLR) is associated with increased inflammation and poor health outcomes.

The MLR is associated with circulating inflammatory markers (e.g., C-reactive protein level) and inflammatory diseases (e.g., coronary artery disease severity osteoarthritis) [22]. The relationship between MLR and circulating biomarkers varies, however, and may vary depending on the pathophysiological state. For instance, the MLR may have a positive relationship with some biomarkers in healthy individuals but a negative relationship when observed in individuals with specific cancers. A few studies also suggest a relationship between the MLR and psychiatric/mood disorders such that an increased MLR is observed in individuals currently or recently affected by psychotic events [50], as well as manic episodes, schizophrenia, and bipolar disorder [51]. The results for NLR have shown more consistency and replicability compared to MLR. Elevated NLR values have been linked to negative sequelae including poor survival outcomes across many solid tumor types [10], cardiovascular diseases [16], cerebrovascular disease [5], lifetime history of schizophrenia [33] and non-affective psychosis [46]. The NLR has a positive correlation with C-reactive protein (CRP) [13,62], further supporting the notion that NLR indexes systemic inflammation. Moreover, a recent small scale meta-analysis found that a diagnosis of MD was associated with higher NLR scores compared to healthy control participants [47], suggesting that individuals with MD have increased inflammation when compared to healthy controls.

Most research studies examining inflammation in the context of MD have been conducted in adult populations and typically within clinically ascertained samples. To gain insight into the relationship between inflammation and MD that onsets in early life, the relationship between the MLR and NLR with measures associated with depression was tested in a healthy population-based cohort of adolescents and young adults. To our knowledge, no prior study has compared these two biomarkers in a case-control MD study of young people from the general population. Moreover, although most studies calculate cell types and their ratios using cytologic measurements, recent studies have relied on epigenome-wide epigenetic data coupled with established cell-mixture deconvolution algorithms [27,37,72]. These studies demonstrate that DNA methylation-derived immune cell estimates produce cell type proportions that are highly consistent with cytologic methods.

The current study examined the MLR and NLR indices derived from epigenome-wide DNA methylation array data in an epidemiological sample of adolescents and young adults. Based on the extant literature we hypothesized that lifetime MD experienced in young people would be characterized by increased activity of innate immunity coupled with suppressed adaptive immunity (i.e., increased MLR and NLR).

2. Methods and materials

2.1. Participants

One hundred seventy twins were drawn from a larger sample of twins (N = 860 twins) enrolled in a parent study (R01MH101518) [8], which was designed to estimate genetic and environmental contributions to Research Domain Criteria (RDoC) Negative Valence Systems (NVS) constructs using classical twin modeling (i.e., disaggregating variance into additive genetic, common shared, and unique non-shared environmental variation) [8]. Twins were selected as part of a study examining DNA methylation patterns associated with early onset MD [57]. The Mid-Atlantic Twin Registry (MATR), a population-based registry [40], recruited all twins who were raised together in the same home and were required to be free of medications with psychotropic effects at the time of study entry. The study was approved by the Virginia Commonwealth University IRB (HM15348) and all participants (parents/minor children, adults) provided written informed consent/assent. See Table 1 for sample characteristics.

Participants were excluded from the current study if they met any of the following criteria: 1) current use of psychotropic medications (e.g., antianxiety/antidepressants) or medications with psychotropic effects (e.g., beta-adrenergic blockers), b) diagnosis of an autism spectrum

Table 1
Demographic and clinical characteristics of the entire sample and by DSM-5 lifetime Major Depression status.

	Entire Sample (N = 170)	Lifetime MD			
		Unaffected (N = 120) M (SD) or n (%)	Affected (N = 50)	t/c^2	*p*
Demographic/Sample					
Sex, female	115 (67.6)	80 (66.7)	35 (70)	0.18	0.670
Age, years	17.44 (1.23)	17.47 (1.21)	17.38 (1.29)	0.41	0.683
Ethnicity, Latino/Latina	10 (5.9)	7 (5.8)	3 (6.0)	0.00	0.966
Zygosity, Monozygotic	146 (86)	106 (88.3)	40 (80)	2.02	0.160
Body Mass Index	22.34 (3.66)	22.33 (3.55)	22.35 (3.95)	0.77	0.976
Current Smoker	9 (5.3)	5 (4.2)	4 (8.0)	-0.03	0.309
AUDIT Consumption	1.32 (2.77)	1.33 (2.78)	1.30 (2.78)	0.07	0.943
Clinical Characteristics					
Panic Attacks*	17 (10.3)	9 (7.5)	8 (16.0)	3.21	0.073
Social Anxiety Disorder	23 (13.9)	11 (9.2)	12 (24.0)	7.36	0.007
Specific Phobia	18 (10.90	9 (7.5)	9 (18.0)	4.59	0.032
Generalized Anxiety Disorder	6 (3.6)	2 (1.7)	4 (8.0)	4.46	0.035
SMFQ	5.78 (4.96)	4.04 (3.67)	9.84 (5.74)	-6.68‡	< .001
MD Features					
Number of MDEs					
1			22 (44.0)		
2-3			18 (36.0)		
4 or more			10 (20.0)		
Age of Onset, years			14.41 (2.16)		
Duration of last/worst episode, weeks			11.48 (18.66)		
DSM-5 symptoms during last/worst MDE			5.77 (1.15)		
Time since last MDE, years			1.31 (1.56)		
Previous Use of Psychiatric Medication			5 (10.0)		
Psychological Service Use			15 (30.0)		

Note: DASS=Depression, Anxiety, and Stress Scale-21;
*No lifetime cases of panic disorder; ‡ Levene's test for equality of variances $F = 25.23$, $p < .001$, *t* statistic for equality of variances not assumed reported.

disorder, c) diagnosis of an intellectual disability or IQ< 80, d) diagnosis of a spatial learning disorder, e) seizure without a clear and resolved etiology, f) current or past episodes of psychosis, g) serious, not stabilized illness (e.g., cardiovascular, endocrinologic, neurologic, immunologic, or blood disease), h) inadequate production of human growth hormone, i) sensory integration disorder, j) congenital adrenal hyperplasia, k) adrenal inefficiency, l) deaf with bicochlear implants, m) cancer (current or past diagnosis), n) pregnancy (current or lifetime) and m) age less than 15 years or older than 20 years. The age range of 15–20 was selected given this period marks the transition from mid to late adolescence, a phase characterized by significant challenges such as identity formation, increased academic and social pressures, and the onset of significant life transitions [65]. These factors can contribute to or exacerbate depressive symptoms, making this age range particularly relevant to study MD.

If only one twin from the twin pair met any of the exclusionary criteria, the whole pair was excluded from study participation. MLR values for three people and NLR values from four people were excluded from all analyses because their values were outliers (i.e., $\geq$ 3 SD above mean); two people represented outliers for both the MLR and NLR. This resulted in a final sample of 165 young people. Of these 165 individuals, 49 met DSM-5 criteria for lifetime MD (see DSM-5 Major Depression section below).

2.2. Measures

DSM-5 Major Depression. A computerized and expanded version of the CIDI-Short Form was completed by all study participants. The expanded CIDI-SF queried DSM-5 criterion A symptoms (e.g., concentration difficulties, weight changes) of a MDE as well as criterion C (i.e., symptoms caused significant distress/impairment) (for full items and algorithm see Supplement).

Zygosity. Zygosity status (monozygotic [MZ] versus dizygotic [DZ]) was confirmed using 65 SNP control probes included on the Infinium HumanMethylation450 (450 K) array to verify sample identity. The control probes target polymorphic sequences, and values for each probe cluster into three groups corresponding to genotype.

Estimating Immunomethylomic Markers. Houseman's reference-based algorithm [31] was used to deconvolute cell-type. This deconvolution algorithm takes advantage of known cell-type-specific differentially methylated regions [56] using constrained projection/quadratic programming. The algorithm models sample DNAm as a weighted combination of the individual DNAm patterns of underlying cell types [31,71] and has been independently validated in adult whole blood samples [1,36]. The Houseman algorithm estimates monocytes, granulocytes (see Supplementary Figure 1 and 2), and lymphocytes as well as lymphocyte cell subtypes including CD8T, CD4T, natural killer, and B cells.

Analytic Approach. Linear mixed models were used for all analyses. Mixed-effects models included fixed effects for major depression and related variables and relevant covariates (see covariate selection procedure below). Twin family served as a random effect to account for within-twin pair dependence. Cohen's f^2 also was calculated as an effect size measure to assess the strength of primary independent variables on immune outcomes. Cohen's f^2 is appropriate for complex, hierarchical data structures given this estimate explains variance accounted for by different sources (e.g., fixed effects versus random effects) [60]. To estimate Cohen's f^2, three models were computed including 1) the full model, 2) the full model minus the variable being estimated for effect size, and 3) the null model. Random effects must be held constant across models so that change in variance reflects only a change in the variance due to fixed effects. Thus, the covariance dataset from the full model was read into models 2 and 3 to fix the random effect variance. According to Cohen's (1988) guidelines, small, medium, and large effects are represented by $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$, respectively. Hypothesis testing was conducted using an a= 0.05 cutoff. The SAS PROC MIXED (version 9.4) procedure was used for all analyses.

Covariates. Changes in white blood cell type heterogeneity occur as part of typical development [20] and as the result of environmental exposures (e.g., cigarette smoking) [15,21,30,55,63]. Thus, demographic factors (age, sex) and health related indicators including current smoking (past 30-day smoking), body mass index (BMI), and alcohol use (consumption score of the Alcohol Use Disorders Identification Test [AUDIT]) were univariately tested for model inclusion using a liberal p value of a$\leq$ .20 (see Supplementary Table S1 and Table S2). For MLR, sex, BMI, and current smoking status met model inclusion criteria while sex, BMI, and age met model inclusion criteria for NLR. For the individual immune cells, biological sex met model inclusion criteria for Monocytes, Neutrophils, and Lymphocytes and BMI met inclusion criteria for Neutrophils and Lymphocytes. AUDIT-C scores also were significantly associated with Neutrophils. For the lymphocyte cells, biological sex met model inclusion for all subtypes, while BMI met model inclusion for CD8T, CD4T, and natural killer cells. Age met inclusion criteria for natural killer and B cells.

3. Results

To validate the immunomethylomic markers, correlations between the MLR and NLR and cytological measurements of the MLR and NLR were estimated using data from six persons who had complete blood count (CBC) with five-part differential and DNAm-derived cell type estimates. MLR and NLR and cytologic measures were strongly correlated (R^2=.98 and R^2=.96, respectively; see Supplementary Fig. S1a and S1b). The mean absolute difference between the DNAm-derived and CBC-based measures across the six samples was 0.10 and 0.04 for MLR and NLR, respectively, suggesting minor differences between the two cell type measurements.

DSM-5 Lifetime MD. The Lifetime MD main effect was not significant for MLR ($t = -1.38$, $p < 0.170$, Cohen's f^2=.02), although current smokers (M=.39, SE=.04) had higher MLR values compared to non-smokers (M=.29, SE=.01). The Lifetime MD main effect also was not significant for NLR ($t = -1.40$, $p < 0.164$, Cohen's f^2= 0.1).

Time Since Last MDE. Given that Lifetime MD included current, recent, and past cases of MD, a post-hoc test was conducted to determine whether MLR and NLR estimates were associated with recency of MDEs, hypothesizing that more recent MDEs would be associated with greater levels of inflammation compared to more distal MDEs. To examine this hypothesis, the association between Time Since Last MDE (measured in years) and MLR/NLR estimates was tested. This analysis included only MD affected persons who provided a date for the occurrence of their last MDE (i.e., 44 of 49 MD cases). A significant main effect was observed for Time Since Last MDE with higher MLR ($t = -2.03$, $p < 0.049$, Cohen's f^2= 0.23) and NLR ($t = -3.11$, $p = 0.003$, Cohen's f^2= 0.20) values associated with more recent MDEs (see Table 2 and Figs. 1a and 1b). Effect size estimates indicated medium effects for Time Since Last MDE. A higher BMI also was associated with elevated MLR and NLR values, while current use of smoking products and older age were associated with higher MLR and NLR measures, respectively.

DSM-5 MDE Recency. Given the significant Time Since Last MDE main effect, we conducted a second post-hoc test that included the full sample. In this analysis, we compared young people experiencing an MDE in the past year (MDE $\leq$ 1 year, N = 25) to persons with no history of MDEs (No MDE, N = 120) and to those experiencing an MDE over one year ago (MDE > 1 year, N = 18). For this test, MDE in the past year served as the reference group. An MDE Recency main effect emerged with the MDE $\leq$ 1 year condition exhibiting higher MLR values compared to the No MDE and MDE > 1 Year conditions ($t = -3.47$, p = 0.001; $t = -3.62$, $p < 0.001$, Cohen's f^2= 0.28, respectively). The same pattern was observed for NLR measurements with the past year MDE group demonstrating higher ratios compared to No MDE and > 1 year condition, respectively ($t = -3.31$, $p = 0.001$; $t = -3.54$, $p = 0.001$, Cohen's f^2= 0.12). Medium effect sizes also were observed

Table 2
Parameter estimates for fixed effects testing the association of Time Since Last MDE on the MLR (upper table) and NLR (lower table).

Parameter	Estimate	SE	df	t	*p*	95% CI	
						Lower Bound	Upper Bound
Sex	0.024	0.040	32.54	0.61	0.546	-0.057	0.106
BMI	0.009	0.004	42.00	2.09	0.043	0.000	0.017
Current Smoker	-0.148	0.052	15.08	-2.83	0.013	-0.259	-0.037
Time Since Last MDE	-0.023	0.012	37.27	-2.03	0.049	-0.047	0.000
Sex	0.168	0.306	38.59	0.55	0.587	-0.452	0.788
BMI	0.092	0.031	41.32	2.96	0.005	0.029	0.155
Age	0.289	0.113	38.37	2.56	0.014	0.061	0.517
Time Since Last MDE	-0.291	0.093	40.05	-3.11	0.003	-0.480	-0.102

Note: Sex: 1 =Male and 2 =Female; Current Smoker: 1 =Current non-smoker and 2=Current Smoker.

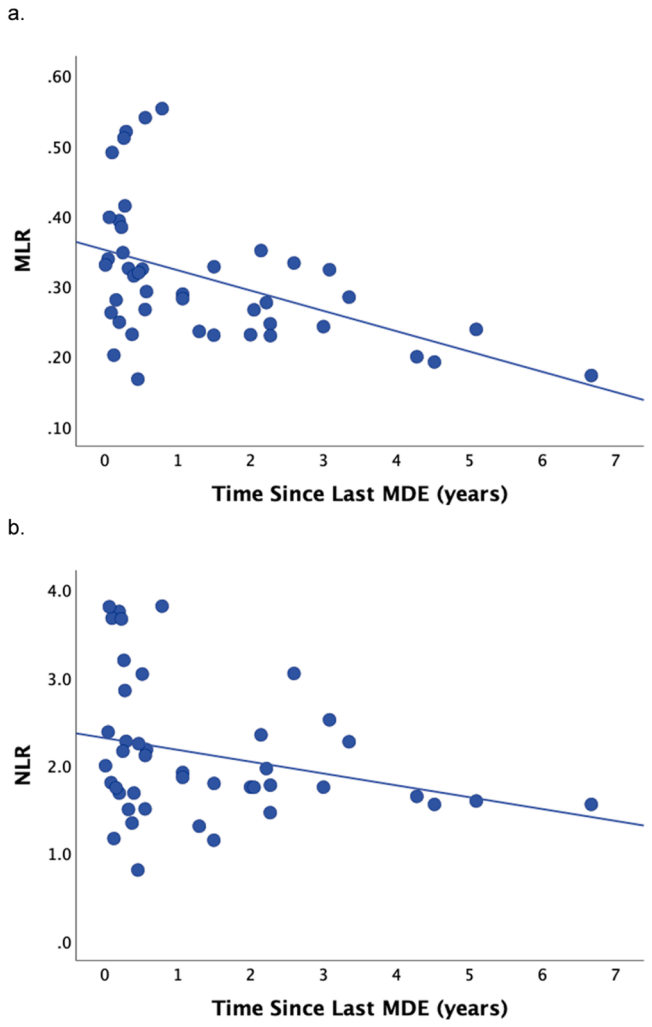

Fig. 1. a and 1b. Scatterplot depicting association between time since last MDE and MLR (upper panel) and NLR (lower panel) values.

for recency of last MDE when using the full sample. See Table 3 and Figs. 2a and 2b. A higher BMI also was associated with elevated NLR values.

Lymphocyte Cell Subtypes and Depression Outcomes. As a final step, we examined whether subtypes of lymphocyte cells were associated with depression outcomes (see Supplementary Table S3). Only CD8T cells were significantly associated with lifetime MD, although the effect size was small ($t = 2.06, p = 0.042$, Cohen's $f^2 = 0.02$). Time Since Last MDE was significantly, positively associated with CD8T and CD4T cells ($t = 2.99$, $p = 0.005$, Cohen's f^2=0.59; $t = 2.56$, $p = 0.014$, Cohen's f^2=0.22, respectively) such that more recent MDEs were associated with lower counts. MD Recency was significantly associated with all lymphocytes cells except natural killer cells with young people reporting their last MDE in the past year having lower numbers of CD8T, CD4T, and B cells compared to young people without a history of MD and youth experiencing their last MDE over a year ago ($t = 4.57$, $p < 0.001$, Cohen's $f^2 = 0.34$; $t = 3.67$, $p = <.001$, Cohen's f^2=0.15; $t = 2.42$, $p = 0.017$, Cohen's $f^2 = 0.07$, respectively). Natural killer cells were not associated with any depression outcome.

4. Discussion

In this study, lifetime history of MD in young people was not associated with the MLR or NLR. Given that a lifetime diagnosis captures episodes occurring at different times, two post-hoc tests were conducted with the first examining time since last MDE in persons with a positive lifetime history of MD. A strong, negative association was observed between the timing of the last MDE and immunomethylomic markers, suggesting low grade inflammation proximate to depressive episodes. A second analysis using the entire sample characterized by no history of MD versus a past year MDE versus a MDE occurring over one year corroborated the finding of increased inflammatory processes among young person's experiencing a more recent depressive episode. Findings suggest that state-based MD-related inflammation resolves during the remission/recovery phase of the disease, at least in young people. Indeed, it is possible that a lack of a sustained inflammatory state is a function of this sample's characteristics including, but not limited to, young age, number of lifetime MD episodes, and accumulated stress/adversity exposures. It is conceivable that an increasing shift in MLR and NLR values occurs as a person is affected by repeated MDEs over their lifespan. Nonetheless, current findings regarding timing of MDEs as well as composition of MD samples (i.e., lifetime versus recent versus ongoing MD) may explain, in part, differing outcomes observed across studies of MD and inflammation. Our findings also may have broader diagnostic implications, particularly in the context of bipolar disorder. It is recognized that early onset MD can be a precursor to or associated with bipolar disorder. Given this overlap, the inflammatory markers and T cell immunity changes we noted in relation to recent MDEs might also be relevant in understanding the immunological underpinnings of bipolar disorder, possibly extending our results to immunological aspects of mood disorders beyond MD.

Our study results suggest a complex relationship between immune system functioning and MDEs, particularly an increase in monocytes and neutrophils coupled with a reduction in lymphocyte proportion proximal to these episodes. Lymphocytes perform a large number of biological roles across multiple body systems, including innate and adaptive immunity and brain development [64]. These roles make lymphocytes an attractive candidate to investigate for biomarker and mechanistic studies of mood disorders, and findings from both this study and others support this speculation. For instance, a robust meta-analysis identified relative lymphopenia as a strong correlate of MD case status along with reduced lymphocyte proliferative response, neutrophilia, and increased levels of interluken-6, prostaglandin E2, and haptoglobin

Table 3
Parameter estimates for fixed effects comparing young people reporting no lifetime major depressive episodes (MDE), a past year major depressive episode, or a MDE occurring more than one year ago on the MLR (upper table) and NLR (lower table).

Parameter	Estimate	SE	df	t	*p*	95% CI	
						Lower Bound	**Upper Bound**
Sex	-0.026	0.021	139.05	-1.21	0.229	-0.068	0.016
BMI	0.005	0.003	152.98	1.91	0.058	0.000	0.010
Current Smoker	-0.067	0.034	134.86	-1.96	0.052	-0.135	0.001
MD Recency‡							
No MDE	-0.079	0.023	138.23	-3.47	0.001	-0.124	-0.034
MDE > 1 Year	-0.113	0.031	140.53	-3.62	0.000	-0.176	-0.051
Sex	-0.276	0.155	134.27	-1.78	0.078	-0.583	0.032
BMI	0.040	0.019	148.04	2.12	0.035	0.003	0.078
Age	0.070	0.065	84.78	1.09	0.281	-0.058	0.199
MD Recency‡							
No MDE	-0.553	0.167	142.69	-3.31	0.001	-0.884	-0.222
MDE > 1 Year	-0.814	0.230	145.11	-3.54	0.001	-1.268	-0.359

Note: Sex: 1 =Male and 2 =Female; Current Smoker: 1 =Current non-smoker and 2=Current Smoker; ‡MDE ≤ 1 Year = reference group.

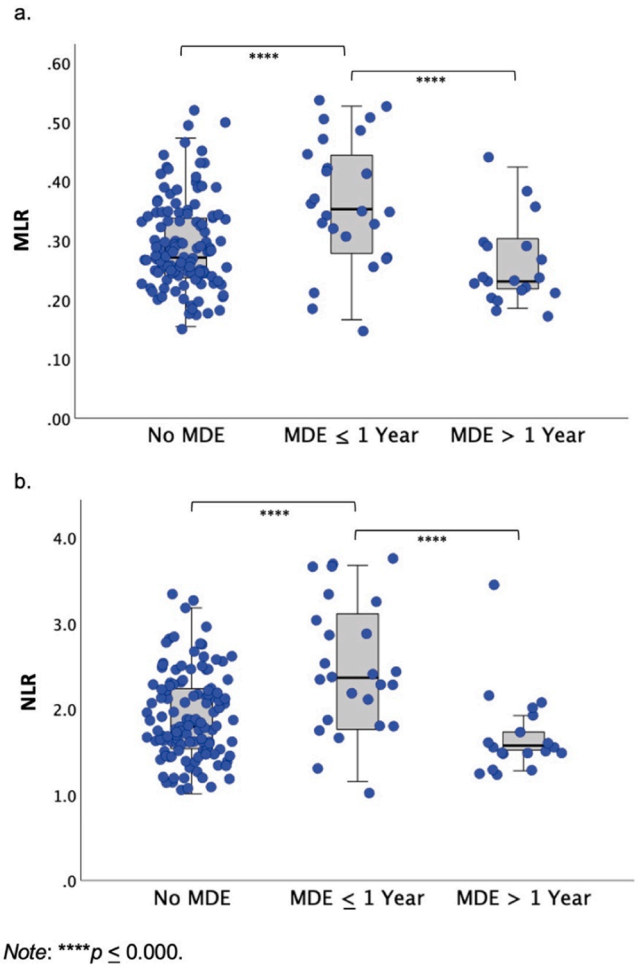

Fig. 2. a and 2b. Box and Whiskers plot of MLR (upper panel) and NLR (lower panel) by Major Depressive Episode (MDE) status including No MDEs, MDE occurring within the past year (≤ 1 year), and MDE occurring more than one year (> 1 year).

[75]. Stressful life events, which are well-known risk factors for MD, also correlate with spikes in pro-inflammatory markers, some of which (e.g., TNF-alpha) [18] can downregulate lymphocyte proliferation by suppressing key cytokines like interleukin-2 [7]. It is possible that chronic and acute stressors give rise to a mixed immune response that includes an upregulation in inflammation and a concomitant drop in lymphocyte activity and responsiveness [7]. This suggests a potential mixed immune response in MD, characterized by increased inflammation and reduced lymphocyte activity, which could predispose individuals to MDEs and offer new pharmacotherapeutic targets. Indeed, two placebo-controlled double-blind studies found that combining an anti-inflammatory medication (celecoxib) with either an selective serotonin reuptake inhibitor (fluoxetine) or a norepinephrine reuptake inhibitor (reboxetine) significantly improved depression symptom load compared with reboxetine alone [2,48] and these results may be tied to restoring immune regulation.

The reduction of lymphocytes appeared to be driven primarily by T cell suppression in this sample. Although the mechanism(s) of T cell alterations in human stress and depression have not been firmly established, evidence for several pathways have been identified including inhibition of T cell function by glucocorticoids, increased T cell apoptosis, and disruption of T cell function by inflammatory cytokines (Miller, 2010). However, it is crucial to consider the mixed outcomes of clinical studies targeting specific inflammatory mechanisms, such as TNF-alpha and IL-6, in treating major depression. While some studies have shown promising results, others have been inconclusive or unsuccessful [12,42]. This variability might be attributed to the heterogeneity of MD and the complexity of immune system interactions. The reduction of lymphocytes, primarily driven by T cell suppression in our sample, and the evidence for pathways including glucocorticoid-induced inhibition of T cell function, increased T cell apoptosis, and disruption by inflammatory cytokines (Miller, 2010), further underscore the intricate interplay between the immune system and depression. This complexity underscores the ongoing challenge of translating these immunological insights into effective treatments for mood disorders.

This study benefited from many favorable cohort characteristics including reliance on DSM-5 MD criterion versus measurement of depressive symptoms that may or may not index the complexity and severity of MD [34]. This study also included valuable measurements of lifestyle factors in a psychotropic medication naïve sample. Additionally, the participants' young and narrow age range limited heterogeneity. Nonetheless, the current study does possess several limitations, most notably lack of demographic diversity and a relatively modest sample size of MD affected young persons. Moreover, we did not account for the effects of menstrual phase on cell counts. Data suggest that neutrophils increase in number around ovulation while lymphocytes and mixed cell counts (including monocytes, eosinophils, basophils) remain stable throughout the menstrual cycle [49]. That said, the NLR estimate may be somewhat affected by menstrual cycle phase. Finally, although substantial correlations between cell proportion estimates derived from DNAm and complete blood count replicated those observed by Koestler and colleagues [37], these measures of association are based on a very small subset of participants. Thus, future studies should explore the generalizability and robustness of DNAm derived

estimates of cell type heterogeneity across ages, clinical phenotypes, and demographic variables. More information also is needed to understand what biological role, if any, immune cells play in MD etiology and pathophysiology. Thus, longitudinal studies are needed to determine if shifts in blood cell composition precede, follow, or occur concurrent with MDE onset. Characterizing the trajectories of blood cell composition remodeling will be an essential part of determining the utility of MLR, NLR, and possibly lymphocyte counts to serve as biomarkers for clinical care management of persons suffering with MD. Moreover, the MLR and NLR markers should be considered as one of many potential markers that might help in understanding the complex interplay between the immune system, inflammation, and psychiatric conditions. Nonetheless, this study contributes to mounting evidence of immune disruption among young people suffering with MD, suggesting that pharmacological and psychosocial interventions designed to regulate these systems or prevent their dysregulation may improve the life course of MD.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Roxann Roberson-Nay reports financial support was provided by National Institute of Mental Health. Roxann Roberson-Nay reports financial support was provided by Brain and Behavior Research Foundation. Timothy P. York reports financial support was provided by Brain and Behavior Research Foundation. Dana Lapato reports financial support was provided by National Institute of Mental Health. Amanda Gentry reports financial support was provided by National Institute of Mental Health. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.xjmad.2024.100049.

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