
# Exploring Definitions for Immuno-Metabolic Subtype of Major Depressive Disorder

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**ABSTRACT**

Objective: High treatment resistance rates for Major Depressive Disorder (MDD) have been driven by the heterogeneity of depression symptoms and biological signatures. Depression cases linked with cardio-metabolic dysfunction are particularly resistant to standard SSRI or TCA treatment. Previous NESDA teams have studied whether atypical, energy-related depression symptoms coincide with dysregulated metabolic and inflammatory signaling, proposing an immune-metabolic depression (IMD) subtype. In this study, we examine baseline and longitudinal change in bio-markers to identify whether our target inflammatory markers (hsCRP, IL-6, TNFa) and metabolic markers (cholesterols, triglycerides, glucose, leptine) are predictive of depression (IMD) severity and chronicity. Our study aims to identify the core signals and symptoms of IMD among previously linked biomarkers and depression measures.

Methods: Two waves of data (from baseline to 2-year follow-up) of the Netherlands Study of Depression and Anxiety (NESDA) were used. Our sample consisted of patients with a current MDD diagnosis within 6 months of baseline (N=675). and excluded any patients with missing sociodemographic or biological data Depression was profiled using the self-rated Inventory of Depressive Symptomatology (IDS-SR). Univariate and multivariate linear regressions were performed to study how biological measures predicted atypical, energy-related depression symptoms at baseline and over 2-year follow-up, while controlling for covariates. Logistic regression was used to analyze which measures were linked with chronic depression.

Results: We first explored overlap between top quintiles of biomarkers and symptoms. Using regression, change in atypical score was linked with inflammation marker TNFa, but not hsCRP and IL-6. Multiple metabolic measures (LDL and HDL cholesterols, triglycerides, glucose, leptin) were associated with change in IL-6, but not hsCRP. IL-6 was the only IMD biomarker predictive of chronic depression.

Conclusion: There is a weak direct link between hsCRP and atypical, energy-related symptom scale. High cholesterol/triglyceride signaling may lead to elevated IL-6 that promotes inflammation in the brain and drives chronic depression, but future analysis should clarify further the immuno-metabolic pathways that link inflammation to the emergence of IMD.

**INTRODUCTION**

The heterogeneity of depression, caused by its widely polygenic profile, range of symptoms, and variability in symptom severity, contributes to the high rate of treatment resistance in patients with Major Depressive Disorder (MDD). In the U.S., treatment-resistant depression, defined by failure to alleviate symptoms with two or more antidepressant medications (Zhdanava et al. 2021), affects 31% of all patients diagnosed with MDD: a population of 2.8 million people whose treatment failure has resulted in over 43 billion dollars lost in healthcare costs, unemployment, and loss of productivity. Treatment failure at this scale emphasizes the need for healthcare professionals to better consider symptom specificity and unique biomarker profiles when treating the various types of depression.

Previous study of depression profiles focused on common DSM-5 specifiers for depression: melancholic depression, characterized by a loss of pleasure and mood reactivity, disrupted sleep, and excessive weight loss, and atypical depression, characterized by increased weight gain and appetite, leaden paralysis, hypersomnia, and body aches (Lorenzo-Luaces et al 2021). While these specifiers were long believed to improve treatment rates for unique depression profiles, recent studies have shown that variability of depression symptoms within specifier groups has not been significantly reduced (Lorenzo-Luaces et al 2021), indicating that the DSM-V specifiers provide limited support in helping categorize and treat depression.

Atypical depression symptoms have been previously identified following dysfunction in HPA axis signaling, promoting elevated inflammation and reduced metabolic measures (Raison et al. 2006). Inflammation is a stress-induced immune response that protects the body from pathogenic infection, but dysregulation in inflammation pathways can result in cytokine-interleukin reactions that downregulate hypothalamic reward circuits. This promotes a clustered elevation of biological measures in depressed patients; meta-analyses have shown that the following markers are elevated among depressed patients: TNF-a, IL-6, HDL, VLDL, and triglycerides. The effect sizes for each of these predictors, however, is small across all patients with depression.

Based on previous NESDA and UK BioBank findings (Milaneschi et al, 2020), an immuno-metabolic subtype of depression can be identified. Milaneschi et al (2020) proposed an immuno-metabolic depression (IMD) subtype after demonstrating that symptoms of energy loss and increase in sleep and appetite/weight coincided with skewed inflammation and metabolic measures. This is further supported by the genetic signature identified by Hagenaars et al (2020) for the coincidence of MDD with cardio-metabolic disorders such as Type 2 diabetes and coronary artery disease, and cardio-metabolic traits such as increased BMI and risk of stroke.

Metabolic dysregulation and inflammation were also linked to atypical forms of depression based on latent class analysis of subgroups (Lamers et al 2013). Previous data-driven studies of IMD have identified clusters of metabolic and inflammatory markers associated with depression, but these results do not yet form a clearly defined phenotype of IMD. This study will aim to identify the most important features of IMD among previously linked metabolic and inflammatory biomarkers, in a sample of patients with current MDD within the NESDA cohort. This study will also investigate the possible effects of selected biomarkers on depression.

Our analysis will also profile IMD's incidence of treatment resistance by studying the chronicity of immuno-metabolic signals and symptoms in our patients with current depression. Since SSRI treatment has little effect on the inflammation expression driving immuno-metabolic dysfunction (Toenders et al 2021), previous studies have hypothesized that the IMD patient group coincides strongly with the treatment-resistant MDD group. In this study, we will aim to determine if inflammation and metabolic dysfunction seen in IMD predicts treatment-resistance, following a period of regular treatment with antidepressants.

**METHODS**

*NESDA Sample*

The Netherlands Study of Depression and Anxiety (NESDA) cohort consists of 2,981 participants aged 18 - 65 at the time of assessment and includes patients with a lifetime history of anxiety or depression, patients with a current diagnosis of anxiety or depression, and control subjects with no history of an anxiety or depression diagnosis (Penninx et al, 2008). Individuals with other psychiatric disorders, including psychosis, addiction, and personality disorders, were excluded from enrollment in NESDA. Biological and behavioral measures were assessed longitudinally in NESDA; the current study will look at nine years of prospective NESDA data, collected at baseline in 2004 and during follow-up wave 3 in 2006. This study was approved by the local ethics commission and all participants provided written informed consent.

For the current study, NESDA participants with missing relevant biological and behavioral measures across follow-up waves were excluded, leaving 1972 individuals. Of these persons we selected 675 individuals diagnosed with MDD at the baseline measure for analyses.

*CIDI and IDS*

Current diagnosis of MDD was assessed using the Composite International Diagnostic Interview (CIDI), a wide-range psychiatric assessment tool (Robins et al, 1988). Depressive symptoms were measured with the self-rated Inventory of Depressive Symptomatology (IDS-SR) survey score (Rush et al, 1996). Each IDS symptom is scored 0-3; in this study, we employed an atypical depression symptom scale, previously used in Lamers et al (2020), from a subset of IDS questions (Items 4, 11, 12, 18, and 28) pertaining to these core atypical symptoms: change in appetite, leaden paralysis, weight gain, hypersomnia, and loss of energy. Patients with severe atypical symptom expression score highly on our resulting atypical sum score, ranging from 0-15.

*Metabolic and Inflammation Biomarkers*

Biological measures included BMI, inflammatory markers (hsCRP, IL-6, TNFa), triglycerides and cholesterols, glucose, and leptin. We also included the ratio of triglycerides to HDL cholesterol (Triglyceride:HDL ratio), as this is a measure that is highly correlated with insulin sensitivity/resistance (Milaneschi et al, 2021). Triglycerides, cholesterols, and glucose were measured with routine lab assay;

leptin and inflammatory markers (hsCRP, IL-6, and TNFa) were measured with high-sensitivity assay kits (for details, see Milaneschi et al, 2021).

Sociodemographic data (age, gender, and education level) was self-reported at baseline and controlled for in the analysis. Nicotine dependence and degree of daily physical activity were covariates in our analysis of the predictive risk of inflammation on depression. Nicotine dependence and physical activity were self-reported using the Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al 1991) and the International Physical Activity Questionnaire (IPAQ; Craig et al, 2003), respectively.

*Statistical Design*

Means and frequencies are reported at baseline for sociodemographic data and IDS scores, for both the complete sample as well as the current MDD sample. Median and interquartile range (IQR) are reported for the non-normal distribution of atypical scores, as well as the non-normal distributions of all blood measures .

Quantile categorization by each measure (symptoms and biomarkers) was used to identify high-risk groups; patients with inflammation scores falling in the top quintile were compared with patients with top quintile atypical symptom scores and top quintile BMI scores to explore overlap. Additionally, high-risk groups were also identified using previously established benchmark cutoffs for atypical symptom score (>6) and BMI (>25 and >30). Atypical score was built upon the atypical, energy-related symptoms of increased appetite, increased weight, hypersomnia, leaden paralysis, and low energy identified by Lamers et al (2020) to categorize IDS scores for NESDA data. BMI cutoffs of 25 and 30 were used to categorize overweight and obesity.

Univariate linear regressions were used to assess which measures and covariates are linked to change in biomarkers over time. Multivariate linear regressions were performed measuring the effect of change in atypical score with change in each biomarker as outcome; multivariate linear regressions were also performed with multiple blood measures to predict hsCRP levels in individuals 2 years after baseline assessment, corrected for by baseline hsCRP levels . In both multivariate models, glucose, cholesterols, TNFa, IL-6, and triglyceride:HDL ratios were first assessed for their predictive strength of longitudinal change in individual simple regressions, and in the next step, age, gender, and education level were added as covariates. Smoking and degree of daily physical activity were incorporated next into the model. BMI scores were added to the model last.

To determine the associative strength of biomarkers at baseline on chronicity of depression, univariate logistic regression was applied in our sample. All patients had a current MDD diagnosis at baseline, so patients that also had a current MDD diagnosis at 2-year follow-up were considered chronically depressed.

**Table 1**

Sociodemographic and clinical data from self-assessment and blood tests of NESDA participants with a depression diagnosis at baseline

| | MDD Current Diagnosis Within 6 Months (N=675) * |
|---|---|
| **Sociodemographics** | |
| Age (years), mean ± SD | 41.5 ± 12.3 |
| min, max | 18, 64 |
| Gender (female), N (%) | 436 (64.6) |
| Education (years) mean ± SD | 11.8 ± 3.3 |
| min, max | 5.0, 18.0 |
| **Clinical Data** | |
| Atypical Symptom Score, median ± IQR | 5.0 ± 4.0 |
| min, max | 0, 14 |
| BMI, median ± IQR | 25.8 ± 5.3 |
| **Blood Markers** | |
| Glucose, median ± IQR | 5.0 ± 0.9 |
| Leptin, median ± IQR | 12.0 ± 15.8 |
| Cholesterol, median ± IQR | 4.9 ± 1.4 |
| HDL, median + IQR | 1.6 ± 0.6 |
| LDL, median + IQR | 3.1 ± 1.4 |
| Triglycerides, median ± IQR | 1.1 ± 0.8 |
| Triglyceride:HDL ratio, median + IQR | 0.7 ± 0.7 |
| hsCRP, median ± IQR | 1.4 ± 2.6 |
| IL-6, median + IQR | 0.8 ± 0.8 |
| TNFa, median + IQR | 0.8 ± 0.5 |

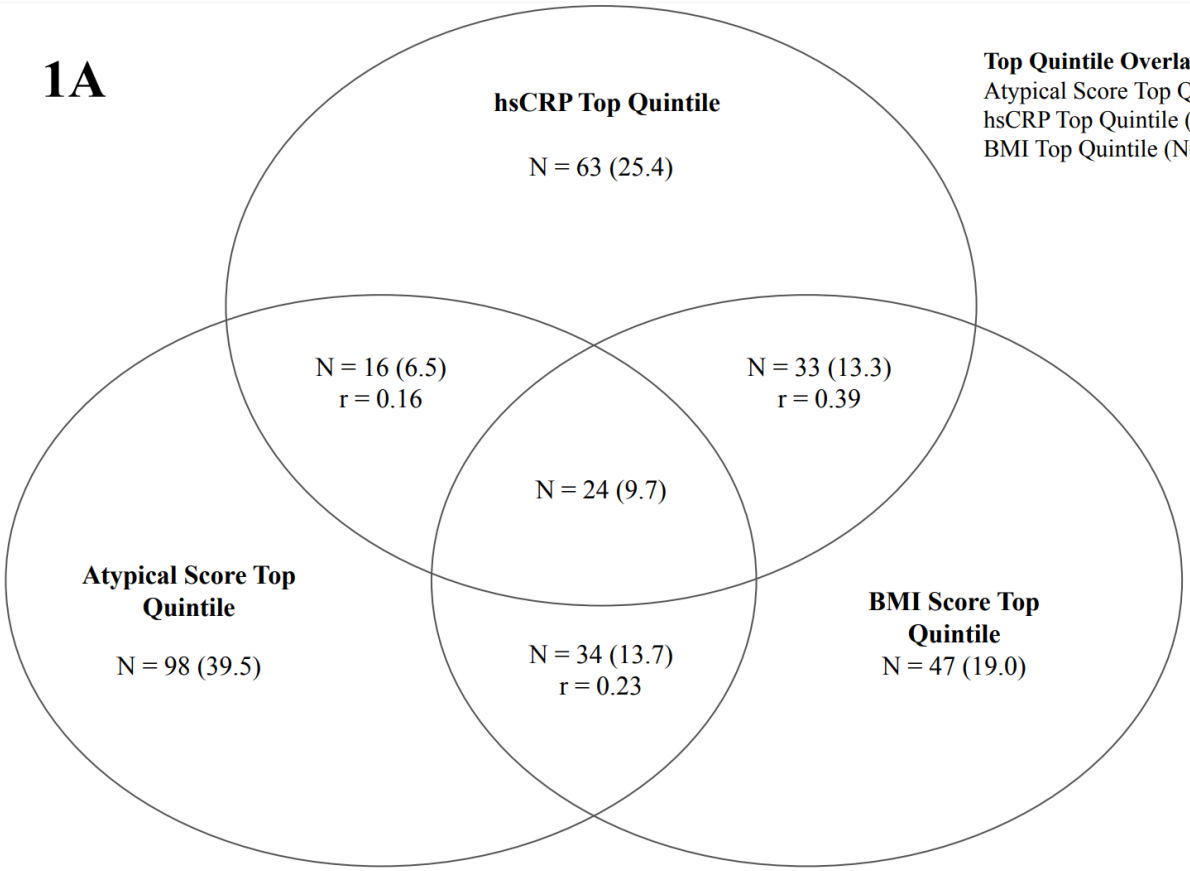
*Individuals with one or more missing values, including those who scored below zero on IDS_SR due to missing answers, were excluded from the sample

**RESULTS**

Mean and median data is reported in Table 1. The median BMI of our sample is 25.8, falling within the categorization of “overweight”. The atypical scores of our sample range widely but indicate that the average patient of our sample scores on at least two of the atypical symptoms from the IDS-SR. 42% of patients with a current MDD diagnosis at baseline were still diagnosed with depression after 2 year follow-up.

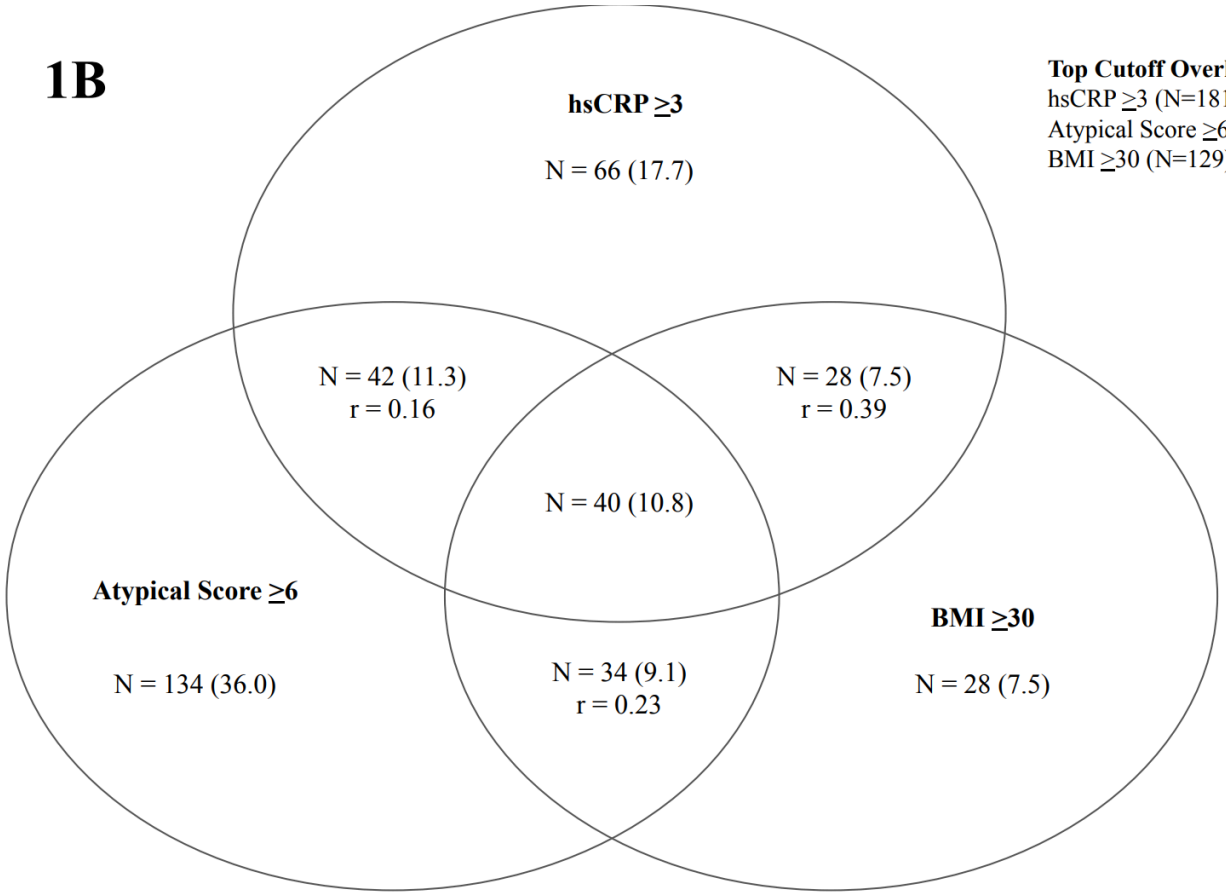
Next, we explored top risk quintiles and cutoffs, focusing on atypical score, hsCRP, and BMI as the most oft-studied components of IMD. Quintile and cutoff values are reported in supplementary tables 2 and 3. Overlap between top quintile and cutoff groups shown in Figure 1 indicates a stronger association of patients with high BMI scores and patients with severe atypical symptoms. High BMI also shares a strong overlap with high hsCRP measures. However, across top quintiles and top cutoffs the association between high hsCRP and severe atypical symptom expression is the weakest. Across different variants of risk groups (based on pre-defined cut-off, top quintile), a consistent subset of patients falls within the group of highest BMIs, atypical scores, and hsCRP measures, between 6.9% and 10.8% of categorized patients.

# 1A




**Top Quintile Overlap (N=248):**
Atypical Score Top Quintile (N=172)
hsCRP Top Quintile (N=136)
BMI Top Quintile (N=137)

# 1B




**Top Cutoff Overlap (N=372):**
hsCRP ≥3 (N=181)
Atypical Score ≥6 (N=250)
BMI ≥30 (N=129)

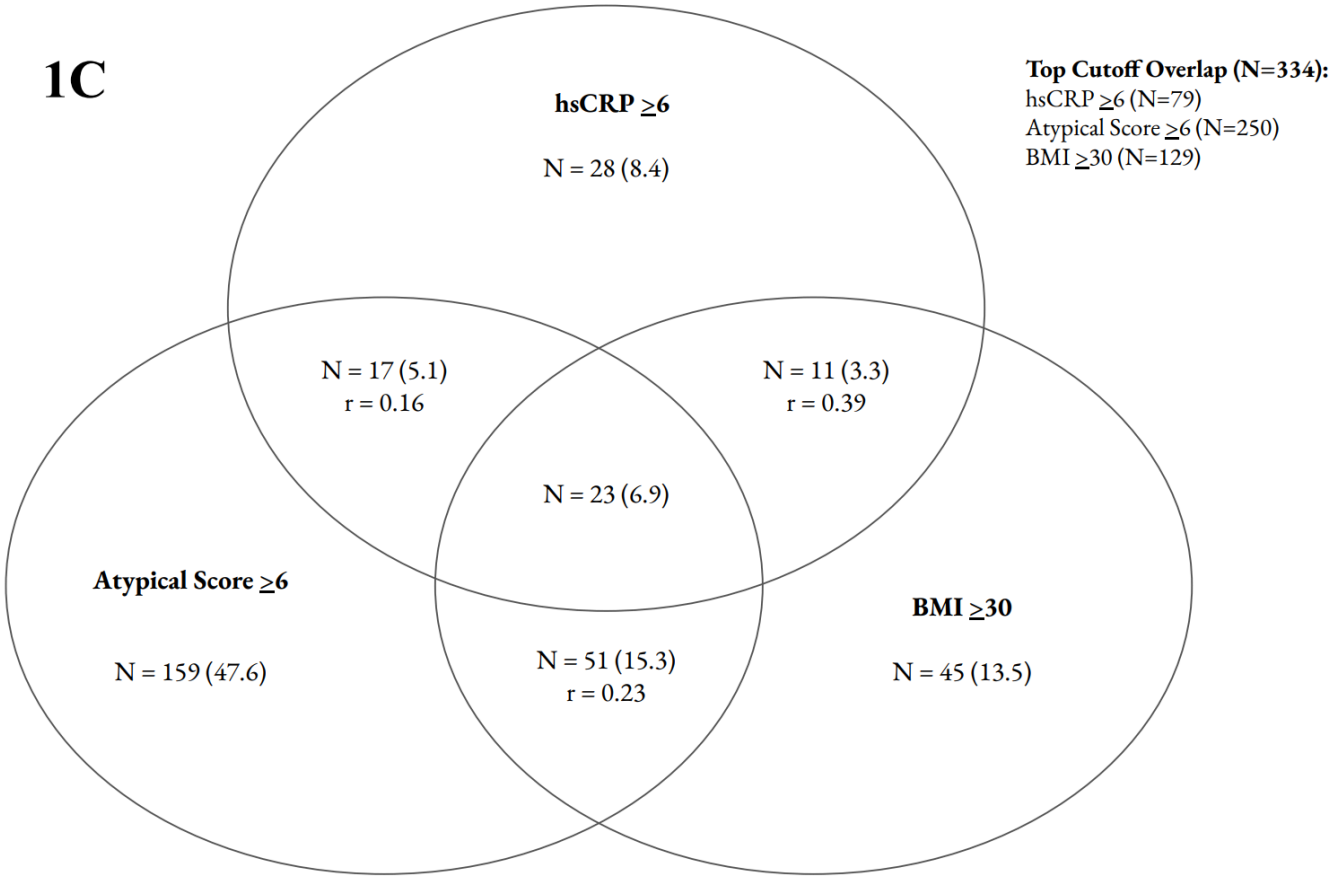



**Fig. 1.** Patients were stratified by quintile and cutoff values for hsCRP, BMI, and atypical score, and groups within the top quintile and cutoff are visualized above. Sample sizes (N) are listed in each Venn pocket, with percentages of group size compared to total number of people visualized. In 1A, top quintile groups are shown. In 1B, patient group with hsCRP ≥ 3 is shown, while in 1C, patient group with hsCRP ≥ 6 is shown.

Univariate linear regressions used to identify significant predictors with change in biomarker measures indicate a strong clustering of associations among the cholesterols and triglycerides. These regression scores (standardized beta coefficient) are listed in Table 2. Change in atypical symptom score is associated with baseline TNF-a ($p=0.023$), but not significantly with other biomarkers at baseline,. High BMI is strongly linked with elevated triglycerides ($p<0.001$) and inflammatory markers hsCRP ($p<0.001$) and IL-6 ($p<0.001$) over time.

To extend beyond baseline prediction of a change score, the change in measure prediction of change in measure multivariate regression models incorporated sociodemographic and health covariates. As shown in Table 3A, positive change in atypical score significantly predicted increases in LDL cholesterol ($p=0.014$) and total cholesterol between baseline and wave 3. Change in LDL, HDL, and total cholesterol, along with glucose and IL-6 was associated with change in hsCRP, as listed in 3B.

**Table 2**

Results of repeated univariate linear regressions, with baseline predictors listed in rows. In columns, change in biomarker outcomes are listed. Each cell contains the standardized beta coefficient of the univariate regression. Change in biomarker outcomes is measured from baseline to wave 3 in our sample (N=675). Significant beta values above 0.05 are bolded.

| Predictors | Total Cholesterol | | | HDL Cholesterol | | | LDL Cholesterol | | | Triglycerides | | | Triglyceride:HDL ratio | | | hsCRP | | | IL-6 | | | Glucose | | | Atypical Symptom Score | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | p | b | s.e. | p | b | s.e. | p | b | s.e. | p | b | s.e. | p | b | s.e. | p | b | s.e. | p | b | s.e. | p | b | s.e. | p | b | s.e. |
| Age | **<0.001** | **0.101** | **<0.001** | 0.273 | 0.005 | <0.001 | 0.269 | 0.065 | 0.002 | **0.002** | **0.092** | **0.001** | 0.129 | 0.039 | 0.001 | 0.687 | 0.015 | 0.016 | **<0.001** | **0.246** | **0.002** | **<0.001** | **0.159** | **<0.001** | 0.032 | 0.075 | 0.007 |
| Gender | 0.253 | 0.033 | 0.012 | **<0.001** | **0.122** | **0.022** | 0.715 | -0.010 | 0.056 | **0.002** | **-0.090** | **0.031** | **<0.001** | **-0.116** | **0.038** | 0.206 | 0.047 | 0.413 | 0.135 | -0.054 | 0.062 | **0.028** | **-0.069** | **0.009** | **0.008** | **0.094** | **0.185** |
| Education | 0.567 | -0.017 | 0.002 | 0.788 | -0.009 | -0.003 | 0.450 | -0.021 | 0.008 | 0.597 | -0.015 | 0.004 | 0.966 | -0.001 | 0.005 | 0.794 | 0.008 | 0.012 | **0.015** | **-0.089** | **0.009** | **0.028** | **-0.060** | **0.001** | **0.099** | **-0.058** | **0.027** |
| BMI | 0.467 | 0.021 | 0.001 | 0.251 | -0.028 | 0.002 | 0.511 | 0.019 | 0.005 | **<0.001** | **0.107** | **0.003** | **0.015** | **0.069** | **0.004** | **<0.001** | **0.120** | **0.008** | **<0.001** | **0.228** | **0.006** | **<0.001** | **0.155** | **<0.001** | 0.433 | 0.019 | 0.027 |
| Smoking | 0.129 | 0.044 | 0.002 | 0.788 | -0.006 | 0.004 | 0.314 | 0.028 | 0.010 | 0.600 | 0.015 | 0.005 | 0.651 | 0.012 | 0.006 | 0.502 | 0.021 | 0.014 | **0.002** | **0.114** | **0.011** | 0.277 | 0.030 | 0.002 | 0.123 | 0.054 | 0.032 |
| Total Cholesterol | ---------- | | | **0.016** | **0.056** | **0.010** | 0.691 | -0.024 | 0.056 | 0.058 | 0.059 | 0.015 | 0.211 | 0.033 | 0.017 | 0.493 | 0.021 | 0.028 | **0.042** | **0.073** | **0.029** | 0.069 | 0.050 | 0.004 | 0.853 | -0.007 | -0.087 |
| HDL Cholesterol | 0.389 | 0.051 | 0.012 | ---------- | | | 0.949 | 0.002 | 0.058 | **0.010** | **-0.080** | **0.033** | **0.019** | **-0.083** | **0.051** | 0.680 | 0.013 | 0.085 | **0.025** | **0.081** | **0.031** | **0.016** | **0.066** | **0.009** | 0.456 | -0.027 | 0.191 |
| LDL Cholesterol | 0.423 | 0.024 | 0.013 | **0.017** | **-0.056** | **0.011** | ---------- | | | **<0.001** | **0.112** | **0.016** | **0.002** | **0.084** | **0.019** | 0.669 | 0.013 | 0.040 | **0.032** | **-0.078** | **0.064** | **0.029** | **0.060** | **0.004** | 0.564 | -0.020 | 0.092 |
| Triglycerides | 0.068 | -0.058 | 0.009 | **0.003** | **-0.073** | **0.014** | 0.368 | -0.026 | 0.012 | ---------- | | | 0.119 | -0.077 | 0.045 | 0.625 | 0.019 | 0.267 | **<0.001** | **0.148** | **0.040** | **0.002** | **0.086** | **0.006** | 0.461 | 0.027 | 0.121 |
| Triglyceride:HDL ratio | **0.031** | **-0.065** | **0.001** | **0.001** | **-0.093** | **0.018** | 0.339 | -0.027 | 0.012 | 0.074 | 0.099 | 0.039 | ---------- | | | 0.951 | -0.002 | 0.058 | **<0.001** | **0.149** | **0.042** | **<0.001** | **0.098** | **0.006** | 0.289 | 0.038 | 0.129 |
| hsCRP | 0.649 | 0.003 | 0.001 | 0.986 | <0.001 | <0.001 | 0.969 | -0.001 | -0.006 | 0.269 | 0.032 | 0.003 | 0.562 | 0.015 | 0.004 | ---------- | | | **0.027** | **0.083** | **0.006** | 0.064 | 0.051 | <0.001 | 0.746 | 0.011 | 0.018 |
| IL-6 | 0.601 | 0.015 | 0.002 | 0.536 | 0.017 | 0.003 | 0.397 | 0.024 | 0.010 | 0.060 | 0.054 | 0.005 | 0.076 | 0.045 | 0.006 | 0.751 | -0.010 | 0.014 | ---------- | | | 0.343 | 0.026 | 0.002 | 0.275 | 0.039 | 0.033 |
| TNF-a | 0.203 | -0.037 | 0.004 | 0.506 | -0.018 | 0.006 | 0.403 | -0.023 | 0.018 | 0.704 | -0.011 | 0.010 | 0.941 | 0.002 | 0.012 | 0.056 | 0.071 | 0.136 | 0.072 | 0.065 | 0.020 | 0.105 | 0.044 | 0.003 | **0.023** | **0.080** | **0.061** |
| Glucose | **<0.001** | **-0.108** | **0.005** | **<0.001** | **-0.107** | **0.007** | **<0.001** | **0.105** | **0.023** | 0.485 | -0.021 | 0.013 | 0.646 | 0.012 | 0.016 | **0.001** | **0.123** | **0.174** | **<0.001** | **0.155** | **0.026** | ---------- | | | 0.494 | -0.024 | 0.079 |
| Leptin | 0.752 | -0.009 | <0.001 | 0.492 | -0.019 | 0.001 | 0.322 | -0.028 | 0.002 | 0.731 | 0.010 | 0.001 | 0.725 | -0.009 | 0.001 | **<0.001** | **0.147** | **0.003** | **<0.001** | **0.162** | **0.002** | **0.015** | **0.067** | **<0.001** | 0.065 | 0.068 | 0.007 |
| Atypical Symptom Score | 0.483 | -0.057 | 0.002 | 0.253 | -0.027 | 0.004 | 0.162 | 0.038 | 0.003 | 0.197 | -0.038 | 0.006 | 0.243 | -0.030 | 0.007 | 0.630 | 0.015 | 0.015 | **0.002** | **0.114** | **0.011** | 0.837 | -0.006 | 0.002 | ---------- | | |

**Table 3**

Regression results of multivariate models. In Table 3A, changes in biomarkers between baseline and wave 3 are the predictors and change in hsCRP over the same time is the repeated outcome.
In Table 3B, change in atypical score between baseline and wave 3 is the repeated predictor, with changes in biomarkers as the outcomes.

| | **3A** | Atypical Score Change on Change in Biomarker Model (Change in Biomarkers as Outcomes) | | | **3B** | Biomarker Change on Change in hsCRP Model (Change in Biomarkers as Predictors) | | |
|---|---|---|---|---|---|---|---|---|
| | | β | s.e. | p | | β | s.e. | p |
| Change in: | | | | | | | | |
| Total Cholesterol | | **0.07** | **<0.01** | **0.024** | | **0.06** | **0.05** | **0.043** |
| HDL Cholesterol | | 0.01 | <0.01 | 0.533 | | **0.57** | **0.03** | **6.31e^-7** |
| LDL Cholesterol | | **0.07** | **<0.01** | **0.014** | | **0.09** | **0.05** | **0.003** |
| Triglycerides | | 0.05 | 0.01 | 0.105 | | 0.04 | 0..04 | 0.227 |
| Triglyceride:HDL Ratio | | 0.03 | 0.01 | 0.188 | | **0.06** | **0.05** | **0.045** |
| Glucose | | 0.02 | <0.01 | 0.561 | | **0.06** | **0.04** | **0.048** |
| IL-6 | | -0.01 | 0.01 | 0.677 | | **0.19** | **0.01** | **7.74e^-10** |
| Atypical Symptom Score | | ------ | ------ | ------ | | 0.02 | 0.01 | 0.621 |
| hsCRP | | 0.02 | 0.01 | 0.621 | | ------ | ------ | ------ |

Associations with chronicity of depression, measured using multiple univariate logistic regressions, are reported in Table 4. Baseline triglyceride:HDL cholesterol ratio and IL-6 are the only significant biological measures associated with whether patients in our current MDD sample (N=675) continued to have an MDD diagnosis within 6 months of the wave 3 testing. Atypical symptom severity at baseline was also a significant reporter for chronicity of depression (p<0.002, β=0.6152, SE=0.03). A multivariate model was composed with notable reporters (age, triglyceride:HDL ratio, IL-6) and was significantly predictive of chronicity (F(3, 671)=0.207, Adjusted R^2=-0.004).

**Table 4**

Standardized beta coefficients of univariate logistic regression performed with each predictor. All individuals had depression at baseline (N=675)
Logistic regression predicts the dichotomous outcome of continuous depression (y=1) or recovery from depression (y=0).
P-value below 0.05 are bolded.

| | Univariate Models | | | Multivariate Model | | |
|---|---|---|---|---|---|---|
| Predictors | p | Odds Ratio | Odds Ratio CI 2.5% / 97.5% | p | Odds Ratio | Odds Ratio CI 2.5% / 97.5% |
| Age | **0.029** | **1.014** | **1.002 / 1.027** | 0.069 | 1.012 | 0.999 / 1.025 |
| Gender | 0.859 | 0.972 | 0.706 / 1.338 | ------ | ------ | ------ |
| Education | 0.109 | 0.962 | 0.917 / 1.008 | ------ | ------ | ------ |
| BMI | 0.203 | 1.019 | 0.990 / 1.049 | ------ | ------ | ------ |
| Smoking | 0.229 | 1.035 | 0.979 / 1.094 | ------ | ------ | ------ |
| Total Cholesterol | 0.253 | 1.091 | 0.939 / 1.268 | ------ | ------ | ------ |
| HDL Cholesterol | 0.264 | 0.828 | 0.593 / 1.149 | ------ | ------ | ------ |
| LDL Cholesterol | 0.251 | 1.097 | 0.937 / 1.284 | ------ | ------ | ------ |
| Triglycerides | 0.125 | 1.174 | 0.958 / 1.444 | ------ | ------ | ------ |
| Triglyceride:HDL ratio | **0.042** | **1.255** | **1.011 / 1.567** | 0.119 | 1.193 | 0.957 / 1.493 |
| hsCRP | 0.558 | 1.009 | 0.978 / 1.042 | ------ | ------ | ------ |
| IL-6 | **0.043** | **1.101** | **1.019 / 1.226** | 0.061 | 1.087 | 1.011 / 1.204 |
| TNF-a | 0.563 | 1.031 | 0.927 / 1.151 | ------ | ------ | ------ |
| Glucose | 0.893 | 1.009 | 0.879 / 1.155 | ------ | ------ | ------ |
| Leptin | 0.29 | 1.006 | 0.995 / 1.018 | ------ | ------ | ------ |
| Atypical Symptom Score | **< 0.001** | **1.121** | **1.058 / 1.189** | ------ | ------ | ------ |

**DISCUSSION**

Heterogenous depression profiles have complicated the possibility of blanket treatment, so considering the unique metabolic and inflammatory biomarker profile of IMD will be instrumental in improving treatment efficacy. In this study the profile of IMD is characterized by biomarker influence on severity of atypical depression symptoms and chronicity of depression. There is moderate convergence in our sample between three core features of IMD: BMI, inflammation, and atypical symptoms; yet this overlap does not capture beyond 20% of the current MDD population. Longitudinal models express stronger associations between metabolic biomarkers and CRP inflammation, suggesting the importance of studying the temporal profile of IMD. These inflammatory and metabolic markers likely do not coincide directly with one another but may drive feedback loops downstream, steadily worsening the condition of IMD.

There is strong overlap between the patient group with top quintile BMI and the patient group with top quintile hsCRP; the link is even stronger between top quintile BMI and top quintile atypical symptom scores. In the cutoff diagrams, there is stronger overlap between top cutoff hsCRP patient groups and the top cutoff atypical symptom score group, rather than the BMI group . Instead, the overlap between hsCRP and BMI groups grows weaker and the overlap between BMI and atypical symptom score strengthens as the hsCRP cutoff increases, suggesting that the signal between BMI and hsCRP is elevated in the range of hsCRP $\geq$ 3 while the signal between BMI and atypical symptom severity is sharply elevated among patients with hsCRP $\geq$ 6.

Across each quintile and cutoff overlay, though, there is a sector of patients who fall within all top categories of BMI, atypical score, and hsCRP measures, comprising approximately 7-11% of the categorized patients. This overlap between high hsCRP and high atypical symptom severity is minimal across the sample, perhaps suggesting that the highest hsCRP measures are not necessarily indicative of IMD. Elevated atypical symptom severity over a period of two years is linked only to elevated total cholesterol, driven primarily by an increase in LDL cholesterol over the same period of time.

Our results have shown there to be a weak direct link between hsCRP and the atypical symptom scale, but both may be potentially influenced upstream by a number of biomarkers, primarily LDL cholesterol and triglycerides. As suggested in Lamers et al (2013), high glucose, triglyceride, and cholesterol intake may drive downstream factors that elevate inflammation factors in the blood and promote brain inflammation, leading to a higher risk of expression of atypical depression.

Elevation in triglyceride and hsCRP measures over time is strongly predicted by BMI. Each health and demographic covariate used, save for education level, was linked to multiple biomarkers, but regression output of BMI as a predictor demonstrated that it was most strongly linked to a sharp increase in triglyceride, hsCRP, and glucose measures over a period of two years.

Elevation of total cholesterol among patients with high inflammation seems to be primarily driven by an elevation of LDL cholesterol rather than HDL cholesterol. This association can be partially explained by the inflammation response evoked by accumulation of LDL cholesterols in blood vessels. High cholesterol

and triglyceride signaling is also linked with an elevation of glucose between baseline and 2-year follow-up, while higher glucose and leptin signaling is linked with positive change in hsCRP measures.

Chronic depression over a period of two years was linked with elevated measures of triglycerides and IL-6, rather than hsCRP. These predictors are stronger in individual univariate models rather than an aggregate multivariate model, suggesting strong collinearity between these measures. Elevated atypical scores were also linked strongly with chronicity of depression; patients with worsening atypical symptoms seem to fall within treatment-resistant categorization, but further study is needed to investigate if patients with atypical depression are more treatment-resistant. Treatment-resistance has been previously linked to elevated CRP within the NESDA population (Chamberlain et al 2019); in our sample, however, there was a weak signal between elevated hsCRP and chronicity of depression. It is important to remember that chronic depression and treatment-resistant depression are not synonymous; chronic depression by definition captures a larger pool of MDD cases.

Our study benefits from having a large, well-phenotyped sample with extensive biological measures. The distributions of these measures violate normality, but have been log-transformed and adjusted with Bonferroni corrections, increasing the power of our tests. Many previous depression studies have also been cross-sectional (Buch and Liston, 2020), but our study benefits from analyzing the longitudinal change of biomarkers and depression. There are also some limitations to our analysis; IL-6, but not hsCRP, seems to be the inflammation marker that drives chronic depression, but this measure may be flawed as its testing kits were switched between baseline and wave 3. Additionally, childhood trauma is associated with higher IL-6 expression in adulthood (Lamers et al, 2020), which may confound this association with atypical depression. The atypical symptom scale used in this study and previous NESDA studies poses potential flaws in our data analysis because of its self-reported nature. As a discrete scale, the atypical sum score is also limited in its comparison with continuous biological measures.

Future NESDA analysis should further clarify the mediating factors of inflammation markers with the emergence of depression, since the results of this study demonstrate a weak direct link between the two. This study has yet to demonstrate whether inflammation is elevated downstream of depression markers or whether both inflammation and depression are simultaneously mediated by metabolic markers, but our results indicate that change in LDL cholesterol signaling is predictive of immuno-metabolic change and inflammation is predictive of chronic depression. There is minimal evidence from our analysis, however, that immuno-metabolic dysregulation coincides strongly enough with treatment-resistant chronic depression to allow for a clear definition of IMD at this time.

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**Supplementary Table 1:**

Quintile and quartile ranges for current MDD sample (N=675). Above 80%, patients fall within the top quintile for the respective biomarker or depression measure.

**Stratified Quintiles of Current MDD Sample**

| | IDS | Atypical Score | BMI | Glucose | hsCRP | IL-6 | TNFa | Cholesterol | HDL | LDL | Triglycerides | Triglyceride:HDL ratio | Leptin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0%** | 1 | 0 | 14.7 | 3.2 | 0.1 | 0.001 | 0.1 | 2.3 | 0.7 | 0.65 | 0.29 | 0.12 | 0.5 |
| **20%** | 22 | 3 | 21.3 | 4.5 | 0.48 | 0.46 | 0.5 | 4.2 | 1.22 | 2.3 | 0.72 | 0.41 | 5.1 |
| **40%** | 30 | 4 | 23.67 | 4.9 | 0.96 | 0.67 | 0.7 | 4.76 | 1.45 | 2.8 | 0.98 | 0.59 | 9.8 |
| **60%** | 36 | 5 | 26.26 | 5.2 | 1.95 | 0.95 | 0.9 | 5.21 | 1.67 | 3.27 | 1.23 | 0.82 | 14.86 |
| **80%** | 43 | 7 | 29.76 | 5.6 | 4.04 | 1.53 | 1.2 | 5.9 | 1.95 | 3.96 | 1.7 | 1.27 | 25.98 |
| **100%** | 69 | 14 | 55.83 | 18.8 | 52.3 | 55 | 19.7 | 8.84 | 4.2 | 7.14 | 7.18 | 5.52 | 99.7 |

**Stratified Quartiles of Current MDD Sample**

| | IDS | Atypical Score | BMI | Glucose | hsCRP | IL-6 | TNFa | Cholesterol | HDL | LDL | Triglycerides | Triglyceride:HDL ratio | Leptin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0%** | 1 | 0 | 14.7 | 3.2 | 0.1 | 0.001 | 0.1 | 2.3 | 0.7 | 0.65 | 0.29 | 0.12 | 0.5 |
| **25%** | 24 | 3 | 21.97 | 4.6 | 0.56 | 0.51 | 0.6 | 4.4 | 1.29 | 2.4 | 1.8 | 0.44 | 6.2 |
| **50%** | 33 | 5 | 24.79 | 5 | 1.33 | 0.8 | 0.8 | 5 | 1.55 | 3.02 | 1.1 | 0.7 | 12 |
| **75%** | 41 | 7 | 28.73 | 5.5 | 3.35 | 1.32 | 1.1 | 5.74 | 1.88 | 3.7 | 1.58 | 1.11 | 22.5 |
| **100%** | 69 | 14 | 55.83 | 18.8 | 52.3 | 55 | 19.7 | 8.84 | 4.2 | 7.14 | 2.18 | 5.52 | 99.7 |

**Supplementary Table 2:**

Mean and median data for cutoff groups of current MDD sample (N=675). Cutoffs for hsCRP based on previous literature (see Lamers et al 2013) are set at 1.00, 3.00, and 6.00.
BMI cutoffs based on medical classifications are set at ~25 and ~30, for overweight and obese diagnosis.

| | | N | IDS (mean ± SD) | Atypical Score (median ± IQR) | Glucose (median ± IQR) | hsCRP (median + IQR) | IL-6 (median + IQR) | TNFa (median + IQR) |
|---|---|---|---|---|---|---|---|---|
| **hsCRP** | >1 | 401 (59.4%) | 33.3 ± 12.2 | 5.0 ± 4.0 | 5.0 ± 0.8 | 4.6 ± 3.6 | 1.0 ± 0.9 | 0.8 ± 0.6 |
| | >3 | 182 (27.0%) | 33.5 ± 17.8 | 5.0 ± 3.8 | 5.0 ± 0.8 | 5.5 ± 4.6 | 1.1 ± 1.2 | 0.9 ± 0.6 |
| | >6 | 72 (10.7%) | 34.0 ± 15.0 | 5.5 ± 3.0 | 5.0 ± 0.8 | 9.4 ± 5.8 | 1.5 ± 1.4 | 1.0 ± 0.7 |
| **BMI** | >24.99 | 367 (54.4%) | 35.0 ± 17.5 | 5.0 ± 4.0 | 5.2 ± 0.8 | 2.3 ± 3.9 | 1.0 ± 0.9 | 0.8 ± 0.6 |
| | >29.99 | 117 (17.3%) | 38.0 ± 17.0 | 6.0 ± 4.0 | 5.3 ± 0.9 | 3.6 ± 5.2 | 1.2 ± 1.0 | 0.9 ± 0.6 |

| | | N | Cholesterol (medi | HDL (median + IQR) | LDL (median + IQR) | Triglycerides (median ± | Triglyceride:HDL ra | BMI (median + IQR) |
|---|---|---|---|---|---|---|---|---|
| **hsCRP** | >1 | 401 (59.4%) | 5.1 ± 1.5 | 1.5 + 0.6 | 3.1 ± 1.3 | 1.2 ± 0.8 | 0.8 ± 0.8 | 26.5 ± 7.5 |
| | >3 | 182 (27.0%) | 5.1 ± 1.3 | 1.4 + 0.5 | 3.2 ± 1.4 | 1.3 ± 0.8 | 0.9 ± 1.0 | 27.9 ± 8.2 |
| | >6 | 72 (10.7%) | 4.9 ± 1.3 | 1.4 + 0.5 | 3.0 ± 1.3 | 1.5 ± 1.0 | 1.0 ± 1.2 | 29.2 ± 9.8 |
| **BMI** | >24.99 | 367 (54.4%) | 5.2 ± 1.4 | 1.4 + 0.5 | 3.2 ± 1.3 | 1.3 ± 0.9 | 0.9 ± 0.9 | 28.9 ± 5.2 |
| | >29.99 | 117 (17.3%) | 5.2 ± 1.1 | 1.4 + 0.4 | 3.2 ± 1.1 | 1.5 ± 1.0 | 1.1 ± 1.0 | 32.7 ± 5.0 |

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