A Nomothetic Network Model Disclosing the Comorbidity of Depression and Unstable Angina: Effects of Atherogenicity, Insulin Resistance, Immune Activation, Antioxidants, the Endogenous Opioid System, Trace Elements, and Macrominerals

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 doi:10.20944/preprints202009.0053.v1

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 doi:10.20944/preprints202009.0053.v1

Abstract

Background. There is strong comorbidity between atherosclerosis (ATS) and depression which is attributed to increased atherogenicity, insulin resistance (IR), and immune and oxidative stress.

Aim of the study. To examine the role of the above pathways and mu opioid receptor (MOR), β-endorphin, zinc, copper, vitamin D3, calcium, and magnesium in depression due to ATS / unstable angina (UA).

Methods. Biomarkers were assayed in 58 controls and 120 ATS patients divided into those with moderate and severe depression according to the Beck Depression Inventory (BDI)-II score > 19 and > 29, respectively.

Results. Neural network and logistic regression models showed that **s**evere depression due to ATS/UA was best predicted by IL-6, UA, MOR, zinc, β-endorphin, calcium and magnesium and that moderate depression was associated with IL-6, zinc, MOR, β-endorphin, UA, atherogenicity, IR, and calcium. These neural networks yielded a significant discrimination of severe and moderate depression with an area under the ROC curve of 0.831 and 0.931, respectively. Using Partial Least Squares analysis, 66.2% of the variance in a latent vector extracted from the ATS/UA clinical features, BDI-II scores, atherogenicity, and IR could be explained by the regression on IL-6, IL-10, zinc, copper, calcium, MOR, and age. The BDI-II scores increased from controls to ATS to UA class III to UA class IV.

Conclusions. Depression due to ATS/UA is a reflective manifestation of increased atherogenicity and IR, which are modulated by immune activation, aberrations in the endogenous opioid system, antioxidants, trace elements, and macrominerals.

 doi:10.20944/preprints202009.0053.v1

Keywords: unstable angina, inflammation, neuro-immune, major depression, oxidative stress, antioxidants, atherogenicity

 doi:10.20944/preprints202009.0053.v1

Introduction

The incidence of cardiovascular disease (CVD) is strongly predicted by atherosclerosis (ATS) (Rahman and Woollard, 2017) and CVD is the top-1 cause of mortality worldwide (Mc Namara et al., 2019). As long as atherosclerotic plaques do not obstruct more than 40% of the blood vessel lumen, ATS may remain asymptomatic, but when the obstruction proceeds, myocardial ischemia may develop with or without significant myocardial necrosis and the latter condition is named unstable angina (UA) (Wiviott and Braunwald, 2004). CVD/ATS patients show an increased incidence of depressive symptoms (Hare et al., 2014) and depression is associated with increased risk to coronary heart disease (Moise et al., 2016; Raič, 2017) and UA (Lespérance et al., 2000). Moreover, depression due to ATS/UA is accompanied by an increased risk of cardiac morbidity and mortality (de Melo et al., 2017; Maes et al., 2011; Strik et al., 2001).

The main pathways causing ATS comprise increased atherogenicity, immune cell dysfunctions, increased oxidative stress, and insulin resistance (Frasca et al., 2017; Hansson, 2011). Increased oxidative stress toxicity and especially increased oxidation of low-density lipoprotein cholesterol (LDLc) with consequent IgG-mediated autoimmune responses and lowered antioxidant defenses are important pathways leading to ATS (Leopold and Loscalzo, 2009; McMurray et al., 1992; Sinha et al., 2013). There is now also evidence that major depression is a neuro-immune disorder characterized by immune activation, a chronic low-grade inflammatory state, increased oxidative stress and lowered antioxidant levels (Maes, 1995; Maes et al., 2011). Comprehensive reviews showed that shared metabolic, immune-inflammatory and nitro-oxidative pathways may explain the comorbidity between depression and ATS/CVD (de Melo et al., 2017; Maes et al., 2011).

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First, increased atherogenicity is not only a hallmark of ATS/UA, but also occurs in major depression (Bortolasci et al., 2015; Nunes et al., 2015; Vargas et al., 2014) as indicated by increased Castelli risk indices 1 and 2 and an increased atherogenic index of plasma (AIP) with lowered levels of high-density lipoprotein cholesterol (HDLc) and elevated triglyceride levels (Maes et al., 2011; Oliveira et al., 2017). Second, increased IR is not only a hallmark of ATS/UA, but also major depression is accompanied by increased IR as measured with the homeostatic model assessment of IR (HOMA-IR). Thus, a recent review and meta-analysis found a small but significant association between IR and depression, although there are also negative findings (de Melo et al., 2017; Kan et al., 2013; Landucci Bonifácio et al., 2017; Silva et al., 2012).

Third, ATS/CVD and major depression share a chronically activated immune-inflammatory response system (IRS), as indicated by increased levels of various pro-inflammatory cytokines, including interleukin (IL-6), increased production of reactive oxygen and nitrogen species (RONS), and nitro-oxidative stress toxicity (NOSTOX) as indicated by higher levels of oxidized LDLc, malondialdehyde (MDA), and autoimmunity directed against oxidative specific epitopes, and lowered levels of antioxidants with immune regulatory activities, e.g. zinc (Maes et al., 2011). As such, the onset of depression in ATS/CVD may be explained by the combined activities of IRS, RONS and NOSTOX, and depression may increase risk towards ATS/CVD via increased atherogenicity, RONS and ROSTOX (de Melo et al., 2017; Maes et al., 2011).

Recently, we found that ATS may be discriminated from the healthy state using a combination of different biomarkers in machine learning models, namely an increased HOMA-IR index, IL-6, IL-10 (a negative immune regulatory cytokine), β-endorphin, copper and magnesium, and lower zinc (Qazmooz et al., 2020). Moreover, ATS with UA was significantly discriminated from ATS without UA by increased levels of triglycerides, IR, IL-10, β-endorphin, and mu-opioid

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receptors (MOR), and lowered levels of vitamin D3, another antioxidant (Qazmooz et al., 2020). In this respect, it is interesting to note that increased IL-10 may play a protective role in ATS and in major depression (Maes and Carvalho, 2018; Mallat et al., 1999). IL-10 is a major component of the compensatory immune regulatory system (CIRS), which attenuates the IRS in immune disorders including ATS and major depression (Maes and Carvalho, 2018; Mallat et al., 1999). Lower vitamin D3 is not only detected in CVD/ATS but also in major depression and perinatal depression (Sotoudeh et al., 2020; Trujillo et al., 2018), whilst vitamin D supplementation has a protective effect in depression (Vellekkatt and Menon, 2019). Increased levels of β-endorphin and Mu opioid receptor (MOR) are not only detected in CVD/UA, but also in major depression (Al-Fadhel et al., 2019; Browne and Lucki, 2019; Callaghan et al., 2018). These endogenous opioid system (EOS) peptides have immune-regulatory effects and, therefore, may exert CIRS functions in both CVD/ATS and major depression (Al-Fadhel et al., 2019; Qazmooz et al., 2020). Finally, a recent meta-analysis showed that elevated copper levels are associated with major depression and are involved in the pathophysiology of that illness (Ni et al., 2018).

Nevertheless, no studies have examined whether UA is accompanied by increased depressive symptoms and whether the association among UA and depression may be explained by shared pathways including increased atherogenicity (as assessed with AIP and Castelli risk 1 indices) and insulin resistance (as assessed with an increased HOMA-IR index), IRS (elevated IL-6) and CIRS (elevated IL-10) activation, lowered levels of antioxidants such as zinc and vitamin D, aberrations in the EOS as indicated by increased β-endorphin and MOR levels, and increases in copper. Hence this study was conducted to examine whether increased depressive rating occur in ATS or UA and whether the above-mentioned biomarkers of ATS/UA are also associated with

 doi:10.20944/preprints202009.0053.v1

severity of depression in ATS/UA thereby suggesting that shared pathways may underpin bot ATS/UA and depression due to that condition.

Participants and Methods

Participants

This study recruited 120 ATS patients and 58 normal volunteers. All participants were admitted to the Sadr-Teaching Hospital, Najaf Governorate, Iraq from November 2019 to January 2020. We made the diagnosis of ATS employing the ICD-10 criteria (CM-170) based on a full medical history, physical examination, blood pressure estimations, echocardiography, Doppler sonography, and electrocardiogram (ECG). UA was diagnosed using the guidelines of the American College of Cardiology Foundation/American Heart Association Task Force (Anderson et al., 2013) and patients were classified according into class III (n=34) or IV (n=26) according to Canadian Cardiovascular Society (CCS) criteria (Campeau, 1976). As such, the ATS study sample was divided into three subgroups, namely ATS without UA (ATS), UA class III and UA class IV.

The European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) guidelines were used to make the diagnosis of hypertension (HT) (Mancia et al., 2013). HT patients had blood pressure measurements of > 140 / 95 mmHg using a conventional sphygmomanometer in a seated posture and with the arm in the horizontal position after fifteen minutes of quiet sitting. The World Health Organization criteria (WHO, 2006; WHO, 2011) were used to make the diagnosis of type 2 diabetes mellitus (T2DM) when fasting plasma glucose (FPG) $\geq$ 7.0 mM, and glycated hemoglobin (HbA1c) > 6.5 %.

The 58 apparently healthy controls were age and sex-matched to the ATS groups and were free from ATS/CVD/UA. Exclusion criteria for patients and controls were renal or hepatic

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diseases, myocardial infarction, stroke, cancer, (auto)immune disorders, neuroinflammatory and neurodegenerative disorders, and subjects with an albumin/creatinine ratio > 30 mg/g and serum CRP > 6 mg/dL, thereby excluding subjects with overt inflammation. We excluded subjects with psychiatric axis-I diagnosis according to DSM-5 criteria (APA, 2013), except subjects with a "mood disorder due to a general medical condition, depressed mood or diminished interest or pleasure in all or almost all activities". All patients had serum FBG <25 mmol/L and fasting insulin <400 pmol/liter to comply with the criteria of the Homeostatic Model Assessment (HOMA) calculator program and triglycerides < 4.5 mmol/L to comply with Friedewald's formula requirements. Written consent was obtained from all subjects before participating in the study, which was approved by the IRB of the University of Kufa (487/2019) in compliance with the International Guidelines for Human Research protection as required by the Declaration of Helsinki.

Methods

Depression was measured using the 1996 revision of the Beck Depression inventory (BDI-II) (Beck et al., 1996). This self-rating depression scale has high internal consistency, high test-retest reliability and shows a strong correlation with the Hamilton Depression rating Scale (Beck et al., 1996). The BDI-II score can be used as a cost-effective instrument to detect depression including in patients with medical conditions (Wang and Gorenstein, 2013). The items of the BDI-II scale cover all symptoms of the DSM-IV diagnostic criteria for major depression. We computed the total score of all items as an overall index of severity of illness. The total BDI-II score was dichotomized using two different cut-off values namely ≥19 and ≥ 29. The first threshold value delineates moderate depression (versus no + minimal + mild depression) and the second threshold

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values delineates severe depression (versus no + minimal + mild + moderate depression) (Beck et al., 1996). We also computed BDI-II subdomain scores, namely a) key depressive symptoms (sum of the items sadness, pessimism, loss of pleasure, loss of interest), b) lowered self-esteem (LSE) (sum of the items past failure, guilty feelings, punishment feelings, self-dislike, self-criticalness, worthlessness), and 3) physiosomatic symptoms (sum of the items loss of energy, changes in sleeping pattern, changes in appetite, concentration difficulties, tiredness or fatigue, loss of interest in sex).

Fasting blood samples (10 mL) were drawn in the morning from all subjects after an overnight fast. After complete clotting, the blood was separated by centrifugation at 3000 rpm for 10 minutes and then stored at -80 ^{o}C until analyzed. Total cholesterol (TC), triglycerides (TG), calcium, magnesium, glucose, albumin, urea, and creatinine, were measured spectrophotometrically by kits supplied by Biolabo® (Maizy, France). Serum high-density lipoprotein cholesterol (HDLc) was measured after precipitation of all other lipoproteins by a reagent consisting of sodium phosphotungstate and magnesium chloride and the cholesterol levels in the supernatant were measured spectrophotometrically. Low-density lipoprotein cholesterol (LDLc) was computed from Friedewald's formula: LDLc = TC − HDLc – TG/2.19. Serum CRP was measured by latex agglutination principles using a kit supplied by Spinreact® (Barcelona, Spain). Copper and zinc were measured in serum spectrophotometrically by kits supplied by Giesse Diagnostics (Rome, Italy). Commercial ELISA sandwich kits were used to measure serum IL-6 and β-endorphin (Melsin Medical Co, Jilin, China), IL-10 (Elabscience®, Hubei, China), insulin (DRG® International Inc., NJ, USA), and MOR (Mybiosource® Inc. San Diego, California, USA). The sensitivities of the kits were for IL-6: 0.1 pg/mL, IL-10: 4.69 pg/mL, β-endorphin: 0.1

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pg/mL, insulin: 12.22 pmol/L, and MOR: 7.18 pg/mL. The intra-assay CVs (precision within-assay) of all assays were less than 10%.

As explained previously (Bonifácio et al., 2020; Flauzino et al., 2019) we computed different atherogenic and insulin resistance indices using z unit-weighted composite scores, namely z TC – z HDLc (zTC-zHDLc), reflecting the Castelli risk index; z TG – z HDLc (zTG–zHDLc), reflecting AIP; and z glucose + z insulin (IR), reflecting insulin resistance (IR). These composite scores are strongly correlated with the indices they are intended to reflect (Bonifácio et al., 2020; Flauzino et al., 2019)

Statistical analysis

Continuous variables are displayed as mean ± standard deviation (or standard error) and normality of distribution of continuous data was tested using the Kolmogorov-Smirnov test. Comparisons of continuous variables between study groups were performed using analysis of variance (ANOVA) and differences between nominal variables using analysis of contingency tables (using χ^2 test). Univariate generalized linear model (GLM) analysis was employed to examine the relationships among the biomarkers and diagnostic classes while controlling for covariates including age, BMI, tobacco use disorder (TUD), and sex. Effect sizes were computed using partial η^2 values, and GLM-generated estimated marginal mean (SE) values were computed. We conducted protected pairwise comparisons among treatment means and used p-correction for false discovery rate (FDR) to adjust for type 1 errors due to multiple comparisons (Benjamini and Hochberg, 1995). Binary logistic regression analysis was used to delineate the essential explanatory variables that predict depression (with no depression as reference group). We used multiple regression analysis to predict the BDI-II and subdomain scores using atherogenicity and

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IR indices, trace elements, immune and EOS biomarkers as explanatory variables while allowing for the effects of UA, age, sex, BMI and smoking.

We also conducted multilayer perceptron (MLP) Neural Network (NN) analysis to assess the prediction of increased BDI-II scores (either ≥ 19 or ≥ 29) as input variables and the biomarkers as input variables. In this study we employed an automated feedforward model and employed 2 hidden layers with up to 6 nodes and up to 250 epochs with mini-batch training and gradient descent. One consecutive step with no further decrease in the error term was used as the stopping criterion. The study sample was divided into training (46.7%), testing (20%) and holdout (33.3%) samples. Error, relative error, the area under the ROC curve, the confusion matrices, and the importance of the explanatory variables (displayed in an importance chart) were computed. Tests were 2-tailed, and a p-value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25, 2017.

Partial Least Squares (PLS) path analysis (SmartPLS) (Ringle et al., 2015) was employed to assess the causal association between biomarkers, atherogenicity, IR, and the phenome of ATS/UA including the BDI-II score. All variables were entered as single indicators or as latent vectors (LVs) extracted from their reflective manifestations. Complete PLS analysis was conducted using 5000 bootstrap samples when the outer and inner models complied with pre-specified quality data, namely a) the model fit SRMR is < 0.080, b) LVs have good composite reliability (> 0.7), Cronbach's alpha (> 0.7), and rho_A (> 0.8), with an average variance extracted (AVE) > 0.500, c) LV loadings are > 0.666 at $p<0.001$, d) Blindfolding shows adequate construct cross-validated redundancies and communalities, e) Monotrait-Heterotrait analysis indicates adequate discriminatory validity. Moreover, Confirmatory Tetrad Analysis (CTA) was used to assess whether the reflective model of the LVs is not mis-specified. Differences in the PLS model

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and pathways between men and women were assessed using permutation and Multi-Group Analysis.

Results

Socio-demographic data

Table 1 shows the socio-demographic data of ATS patients with (AST+MDD) and without depression (AST-MDD) and healthy controls. There were no significant differences in age, sex ratio, body weight, BMI, and TUD between the three study groups. There was a significantly higher incidence of UA and class IV (versus class III) according to the Canadian classification score in the ATS+MDD versus the ATS-MDD group. The systolic blood pressure was significantly different between the three diagnostic groups and increased from controls → ATS-MDD → ATS+MDD. Diastolic blood pressure was significantly higher in ATS patients than in controls. The frequency of T2DM were not significantly different between both ATS groups. This table also lists the differences in the drug state among the groups and shows that patients with AST+MDD were more frequently treated with aspirin, atorvastatin, bisoprolol, clopidogrel, isosorbide dinitrate, and trimetazidine than the AST-MDD group.

Associations depression groups and biomarkers

Table 2 shows the measurements of the biomarkers in the study groups divided according to BDI-II scores. Total cholesterol, TG, LDLc, AIP, Castelli-1, FPG, insulin, IR, IL-10, β-endorphin, and copper were significantly higher in ATS patients than in controls. Serum zinc and HDLc levels were significantly lower in the ATS patients in comparison with the control group. Total serum magnesium was not different between the three groups. Serum vitamin D3 was

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significantly lower in ATS-MDD patients than the control group. MOR and IL-6 were significantly higher in the ATS+MDD group than in the other 2 study groups. We found that p-correction did not change the significant differences of all biomarkers listed in Table 2 and covarying for the drug state did not change the results.

Best predictions of AST+MDD versus ATS-MDD

Table 3 shows the outcome of binary logistic regression analyses with AST+MDD as dependent variable (and ATS-MDD as the reference group). The first regression discriminated patients with BDI-II score ≥19 versus BDI-II < 19 and showed that 4 input variables significantly discriminated both groups (χ^2=66.306, df=4, p=<0.001), namely IL-6, MOR, age and UA (all positively associated). The Nagelkerke effect size was 0.585, and the accuracy of the classification was 82.9% with a sensitivity of 84.3% and a specificity of 80.9%. The second regression separated ATS patients with BDI-II ≥ 29 from ATS patients with BDI-II < 29 and showed that IL-6 and UA were significant (χ^2=43.02, df=2, p=<0.001) explanatory variables with a pseudo-R^2 Nagelkerke value of 0.442 and an overall accuracy of 80.3% and sensitivity=57.6% and specificity=89.3%. The forced entry of the drug state in this regression did not change the significance of these two predictors.

Table 4 shows the results of the most accurate neural network (NN#1) discriminating ATS+MDD from ATS-MDD patients (using BDI ≥ 29 as a cut-off value). This feedforward network was trained with 2 hidden layers, with 4 units in layer 1 and 3 units in layer 2 and hyperbolic tangent as the activation function in the hidden layers and identity in the output layer. The sum of squares error term was much lower in the testing than in the training sample, and the percentage of incorrect classifications was reasonably constant in the three samples, indicating that

the model learned to generalize from the trend and is not overtrained. The AUC ROC was 0.831 with a sensitivity of 71.4%, and specificity of 90.0%. **Figure 1** displays the importance chart and shows that IL-6 has the highest predictive power of the model, followed at a distance by MOR, UA, zinc, β-endorphin, and total calcium, and again at a distance by magnesium and AIP.

Table 4 also shows the network information and model summaries of NN#2 discriminating ATS+MDD from AST-MDD using BDI-II ≥ 19 as a cut-off value. This model was trained using 2 hidden layers, with 5 units in layer 1 and 4 units in layer 2, again with hyperbolic tangent and identity as activation functions in the hidden layers and output layer, respectively. The percentage of incorrect classifications was fairly constant in the three sets while the AUC ROC curve was 0.931 with a sensitivity of 88.0%, and specificity of 81.0%. **Figure 2** shows that IL-6 has the highest predictive power of the model, followed by β-endorphins, MOR, AIP, zinc, insulin resistance, total calcium, and vitamin D3.

Relationships depression scores and biomarkers

Table 5 shows the intercorrelations between the total score and subdomain scores on the BDI-II and the biomarkers. In the whole study group, the BDI-II scores were significantly correlated with most biomarkers, except albumin, magnesium, and vitamin D3. In ATS patients, the BDI-II total score was significantly correlated with total calcium ($r=0.303$, $p<0.01$), IL-6 ($r=0.530$, $p=<0.001$), IL-10 ($r=0.187$, $p=<0.01$), and MOR ($r=0.344$, $p<0.001$).

Table 6 shows the results of different multiple regression analyses with the total BDI-II score and symptom domain scores as dependent variables and the biomarkers as explanatory variables while allowing for the effects of age, sex, BMI, UA and the drug state of the subjects. We found that 53.9% of the variance in total BDI score (regression #10 , and 39.8% of the variance in BDI Key symptom score (regression #2) could be explained by age, IL-6, MOR, and UA. **Figure**

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3 shows the partial regression of the BDI-II physiosomatic score on IL-6 after adjusting for the variables listed in Table 6, regression #1. Regression #3 shows that 45.5% of the variance in self-esteem could be explained by IL-6, UA, age, atorvastatin (all positively) and male sex. We found that 42.2% of the variance in BDI-II physiosomatic sub-score (regression # 4) could be explained by IL-6, UA, and atorvastatin (all positively) and FPG (inversely).

We re-ran all multiple regression analyses but now without UA, thereby allowing for other biomarkers to enter in the models. We found that 56.2% of the variance in total BDI score (regression #5) could be explained by IL-6, IR, MOR, UA, hypertension, and age. Up to 45.0% of the variance in BDI key symptoms can be explained by IL-6, IR, MOR, triglycerides, and age (regression #6). Regression #7 found that 48.8% of the variance in self-esteem could be explained by IL-6, IR, AIP, age (all positively), zinc (inversely) and male sex. Finally, we found that 39.7% of the variance in the physiosomatic subdomain score could be explained by IL-6, IR, MOR, age, and hypertension (Regression #8).

Results of PLS analysis

Figure 4 shows a first PLS model which examined the causal paths from different biomarkers → atherogenicity and IR → phenome of ATS/UA and depression whereby each indicator may predict one or more of the downstream LVs, and the atherogenicity and IR LVs may predict the phenome LV (ATS-UA-MDD). Atherogenicity was entered as a reflective LV extracted from AIP, Caselli risk index 1 and triglyceride levels, and IR as a formative model using FPG and insulin. The phenome ATS-UA-MDD was entered as a reflective LV extracted from the total BDI-II score, its 3 subdomains, ATS, UA, and both CCS UA classes. The overall fit of the model was particularly good with SRMR=0.038. The construct reliability of the 2 reflective LVs was adequate: for ATS-UA-MDD LV a Cronbach α=0.911, rho-A=0.915, composite

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reliability=0.930, and AVE=0.656; and for the atherogenicity LV: Cronbach α=0.902, rho-A=0.905, composite reliability=0.939, and AVE=0.838. All loadings on both LVs were > 0.701 at p<0.0001. Blindfolding showed that all construct cross-validated communalities and redundancies were more than adequate. PLS analysis using 5000 bootstrap samples showed that 70.9% of the variance in the ATS-UA-MDD LV was explained by the regression on the IR and atherogenicity LVs, IL-6, IL-10, zinc, vitamin D3, MOR, calcium, and age. Up to 44.4% of the variance in the IR LV was explained by copper, IL-6, and zinc, and 39.6% of the variance is the atherogenicity LV was explained by magnesium, copper, IL-6, zinc, and MOR. There are specific indirect effects of copper, MOR, magnesium, and zinc on the ATS-UA-MDD LV which were mediated by atherogenicity, and of copper, IL-6, and zinc mediated by IR. All biomarkers showed significant total effects on the ATS-UA-MDD LV, including magnesium and copper.

Figure 5 shows a second PLS pathway analysis which considered one output LV extracted from the ATS-UA-MDD LV shown in Figure 5 and in addition, IR, AIP, and Castelli risk index 1. All biomarkers as well as age, sex, BMI, and TUD were entered as single indicators. The overall fit of this PLS model was adequate with SRMR=0.040. The construct reliability of the ATS-UA-MDD LV was excellent with Cronbach α=0.918, rho-A=0.923, composite reliability=0.932, and AVE=0.579. All outer model loadings were > 0.670 at p<0.0001 and all construct cross-validated communalities and redundancies were more than adequate. Confirmatory Tetrad Analysis showed that the model was not mis-specified as a reflective model. We found that 66.2% of the variance in the LV was explained by the regression on six biomarkers and age. Permutations and Multi-Group Analysis disclosed no significant differences between men and women in this model.

Association BDI-II scores and ATS/UA/CCS classes III and IV

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Figure 6 shows a clustered bar graphs with the summaries of the separate BDI-II and subdomain scores in 4 study groups, namely controls, ATS, UA class III, and UA class IV. Univariate GLM analysis (adjusted for age and sex) showed that the total BDI-II score ($F=86.60$, $df=3/169$, $p<0.001$), key depressive ($F=47.86$, $p<0.001$), LSE ($F=40.60$, $p<0.001$), and physiosomatic ($F=40.17$, $p<0.001$) subdomains were significantly different among the four study groups and increased from controls → AST →UA class III → UA class IV, except LSE which was not different between ATS and UA class III.

Figure 7 shows the latent variable scores computed using the first PLS analysis comprising latent scores reflecting atherogenicity, IR, ATS+UA+BDI score and a combination of these LVs (all_LS). We also added IL-6, IL-10 (as most strongly associated with the BDI-II score), and the total BDI score in this clustered bar graph. The total BDI-II, and ATS+UA+BDI and all_LS latent variable scores were significantly different between the four groups. Increased atherogenicity was a hallmark of ATS, increased IR for ATS and UA class IV, increased IL-6 for UA class IV, and IL-10 for ATS and UA class IV.

Discussion

Clinical aspects of the comorbidity of depression and ATS/UA

The first major finding of this study is that there is a strong association between depressive ratings and ATS/UA. As described in the Introduction, there is now evidence that both depression and ATS co-occur with bidirectional associations (de Melo et al., 2017; Hare et al., 2014; Maes et al., 2011; Moise et al., 2016; Raič, 2017; Strik et al., 2001). Moreover, the present study found significantly increased total and subdomain BDI-II scores in UA patients as compared with patients without UA. These findings extend those of Lesperance et al. (2000), who described an association between depression and UA (Lespérance et al., 2000). The same authors showed that

the BDI identified depression in 41.4% of UA patients and that depressed patients showed an increased risk of mortality due to nonfatal myocardial infarction or cardiac death (Lespérance et al., 2000). The Coronary Psychosocial Evaluation Studies (COPES) reported that in UA patients, a high BDI score was associated with a significantly increased mortality, 42 months later as compared with patients without depression (Whang et al., 2010). The American Heart Association, in a recent publication, states that depression predicts a poor prognosis in patients with the acute coronary syndrome (Lichtman et al., 2014). Whang et al. (2010) established that UA patients with depression have an increased risk of all-cause mortality at 42-months even after adjusting for the Charlson comorbidity index, the GRACE risk score and left ventricular ejection fraction (Whang et al., 2010). As such, comorbid depression in ATS/UA appears to increase risk towards cardiac and non-cardiac death.

Furthermore, in the present study, we found that the BDI-II score and all subdomain scores were significantly higher in UA class IV as compared with class III, indicating that along the ATS spectrum from normal controls → ATS without UA → UA class III → UA class IV there is a gradual increase in the BDI-II scores, key depressive and physiosomatic symptoms, and a gradual decrease in self-esteem. In the present study, we included only depressed patients with a mood disorder due to a general medical condition, namely ATS/UA, and as such the findings indicate that the ATS spectrum is accompanied by increased severity of "secondary" depression. In this respect, depression due to medical illness is generally accompanied by more severe symptoms and more medical costs as compared with patients without comorbid depression (Katon et al., 2007; Maes et al., 2011; Wang and Gorenstein, 2013).

Depressive symptoms, atherogenicity, and IR in ATS/UA.

We found that the BDI-II scores, the ATS features, atherogenicity and IR indices were reflective manifestations of a single latent trait, namely "severity of the atherogenicity-IR-phenome of ATS". Moreover, this new construct showed accurate "psychometric" properties, including internal consistency, predictive relevance, construct replicability, and convergent validity. As such, the latent variable scores extracted from these data constitute a replicable and reliable score reflecting the severity of ATS/UA, its atherogenic and IR-associated pathophysiology, and severity of comorbid depression as well. Moreover, these results show that the BDI-II score and its subdomains are manifestations of a same trait comprising atherogenicity and IR.

These results extend those of previous reports that major depression is accompanied by increased AIP, Castelli 1 risk index, and triglyceride levels, and lowered HDLc (Bortolasci et al., 2015; de Melo et al., 2017; Maes et al., 2011; Nunes et al., 2015; Vargas et al., 2014). Liang et al. (2014) observed that depressive symptoms are positively correlated with lipid levels including total cholesterol, triglycerides, and LDLc, and negatively with HDLc, and that these associations are partially mediated by heart disease (Liang et al., 2014). Lowered levels of HDLc in major depression are not only a consequence of activated immune-inflammatory pathways (Maes et al., 1997), but also of lowered levels of paraoxonase 1 (PON1) arylesterase activity (Maes et al., 2020; Moreira et al., 2019a; Moreira et al., 2019b). Upon secretion of the PON1 enzyme from the liver into the blood, PON1 is integrated into HDL to form an HDL-PON1 complex (Moreira et al., 2019a). The anchored PON1 protects HDL (and LDL) from oxidation and activates HDL to optimize cholesterol efflux from macrophages to the liver (Moreira et al., 2019a). As such, the lowered PON1 activity (in part determined by PON1 Q192R single nucleotide polymorphisms) in major depression may be causally related to increased oxidation of HDL and LDL and, therefore

may underpin ATS (Billecke et al., 2000; Chistiakov et al., 2017; Costa et al., 2005; Kowalska et al., 2015; Mackness et al., 2002) and the comorbidity between ATS and major depression (Moreira et al., 2019a).

A recent review and meta-analysis reported a significant association, albeit with small effect size, between major depression and IR, although some papers reported negative findings (Bonifácio et al., 2020; de Melo et al., 2017; Kan et al., 2013; Silva et al., 2012). The IR index, insulin and glucose levels are associated with major depression, which is, in turn, is closely related to an increased atherogenic potential (Di Pino and DeFronzo, 2019). All in all, our results show that increased atherogenicity and IR are pathways leading to ATS (Frasca et al., 2017; Hansson, 2011) and to depression due to ATS/UA. Moreover, it appears that depression due to ATS/UA is more strongly related to IR than major depression.

Depressive symptoms, immune and oxidative stress biomarkers, and ATS/UA.

This study found that IL-6 and MOR are important biomarkers of comorbid depression due to ATS/UA and that the BDI-II scores are significantly and positively associated with IL-6, IL-10, MOR, β-endorphins, and copper, and negatively with zinc, magnesium and vitamin D. Increased levels of IL-6 and IL-10 are now established biomarkers of major depression (Maes and Carvalho, 2018) and significant correlations between depression scores and IL-6 and IL-10 are frequently observed (Al-Fadhel et al., 2019; Wiener et al., 2019). Inflammatory cytokines, including IL-6, not only increase the risk to develop ATS via activation of macrophages leading to endothelial dysfunction and atherothrombosis (Caruso et al., 2020), but also major depression (Maes et al., 2014). Increased IL-6 is also observed in patients with depression due to multiple sclerosis (Kallaur et al., 2016). The increased levels of IL-10 indicate that activation of the CIRS is another shared

pathway among major depression (Maes and Carvalho, 2018) and depression due to ATS/UA (this study).

Moreover, lowered zinc is another shared biomarker of depression due to ATS/UA (this study) and major depression (Maes et al., 1997; Roomruangwong et al., 2017; Twayej et al., 2019). Zinc is an acute phase reactant which displays not only anti-inflammatory and antioxidant effects but also anti-atherogenic effects (Bao et al., 2010). Moreover, zinc deficiency may cause endothelial cell dysfunctions and supplementation with zinc may improve LDL oxidation, vascular endothelial cell functions, and inflammation (Bao et al., 2010).

A deficiency in vitamin D3 is associated with slow coronary flow rate, increased risk of CVD/ATS, and worse prognosis of ATS (Huang et al., 2017; Meredith and McManus, 2013; Ramadan et al., 2014; Sagarad et al., 2016) and is detected in depression due to other medical illness including chronic kidney disease, traumatic brain injury, and chronic spinal cord injury (Barbonetti et al., 2017; Jamall et al., 2016; Jhee et al., 2017). In healthy individuals, lowered vitamin D3 levels are associated with increased atherogenicity, IR, and depression (BDI) and anxiety scores (Casseb et al., 2019). Moreover, vitamin D3 supplementation may reduce angina incidents (Sagarad et al., 2016) and depressive symptoms (Vellekkatt and Menon, 2019).

Depressive symptoms, EOS, trace elements, macrominerals, and ATS/UA.

Lowered magnesium levels are not only associated with arrhythmias, ATS, UA, and heart failure (Kieboom et al., 2017; Kostov and Halacheva, 2018; Qazmooz et al., 2020; You et al., 2017; Zhang et al., 2012), but also with major depression (Al-Dujaili et al., 2019). A deficiency in magnesium may contribute to IR, hyperglycemia, atherosclerotic changes, arterial stiffness,

 doi:10.20944/preprints202009.0053.v1

vascular inflammation, and lowered levels of key antioxidants (Barbagallo et al., 2003; Belin and He, 2007; Gueux et al., 1995; Kostov and Halacheva, 2018; Qu et al., 2013).

Increased levels of MOR and β-endorphins are observed in major depression (Al-Hakeim et al., 2020) and ATS (Wilbert-Lampen et al., 2007) and also in depression due to ATS/UA (this study). Both EOS biomarkers play a role in monocytic and endothelial endothelin secretion, thereby regulating the balance between vasoactive products, which mediate vasoconstriction (Wilbert-Lampen et al., 2007). β-endorphins and/or MOR agonists may contribute to ATS, plaque instability, endothelial dysfunctions, and IR (Okano et al., 2020). Moreover, these and other EOS system peptides are secreted during immune activation and may function as part of the CIRS (Al-Fadhel et al., 2019; Al-Hakeim et al., 2020; Moustafa et al., 2020).

Nevertheless, two biomarkers of depression due to ATS/UA, namely increased copper and calcium (this study) were not always increased in major depression. For example, Narang et al. detected increased copper levels in major depression (Narang et al., 1991), whereas Twayej et al. and Styczen et al. established lower copper levels in major depression (Styczeń et al., 2016; Twayej et al., 2019). Likewise, some authors found increased serum and cerebrospinal fluid calcium/magnesium ratios in major depression (Levine et al., 1999), whereas (Al-Dujaili et al., 2019) established lowered calcium levels in major depression. Calcium mineralization of atherosclerotic arteries is associated with calcification of plaques, plaque formation, and increased blood vessel rigidity (Kalampogias et al., 2016; Małecki and Adamiec, 2005).

Limitations

The results of our study should be interpreted with regard to its limitations. First, we used a case-control design and consequently, we may not establish firm causal interpretations. Second, it would have been even more interesting if we had measured other biomarkers of oxidative stress,

including oxidized LDL and IgG responses to oxidized LDL, as well as Q192R PON1 genotypes and PON1 arylesterase activity.

Conclusions

Current views are that the functional impairments that accompany medical illnesses are a cause of depression (Wang and Gorenstein, 2013) and that depression in patients with ATS may partly be attributed to increased psychological stress (Glassman et al., 2009). Nevertheless, our results show that depression due to ATS/UA is a reflective manifestation of the atherogenicity and IR pathophysiology and the phenome of ATS/UA and that this single latent trait is strongly associated with immune activation, lowered antioxidant levels, the EOS, trace elements, and macrominerals. As such, atherogenicity, IR, immune activation, lowered zinc, and vitamin D3 as well as increased copper and calcium are new drug targets to treat depression in patients with ATS/UA. These new treatments of depression due to ATS/UA should consider a combination of anti-inflammatory agents targeting IL-6 trans-signaling, zinc, vitamin D, and magnesium, and maybe curcumin and resveratrol (de Melo et al., 2017).

Acknowledgements

The authors would like to thank the staff of the CCU unit and the internal medicine clinic in Al-Sadr Teaching Hospital-Najaf City-Iraq for their help in the collection of samples. Also, we acknowledge the highly skilled work of the staff of Asia Laboratory in measuring the biomarkers.

Declaration of interest

The authors have no financial conflict of interests.

Funding

There was no specific funding for this specific study.

Authorships.

All authors contributed significantly to the paper and approved the final version.

References

Al-Dujaili, A.H., Al-Hakeim, H.K., Twayej, A.J., Maes, M., 2019. Total and ionized calcium and magnesium are significantly lowered in drug-naïve depressed patients: effects of antidepressants and associations with immune activation. Metabolic brain disease 34, 1493-1503.
Al-Fadhel, S.Z., Al-Hakeim, H.K., Al-Dujaili, A.H., Maes, M., 2019. IL-10 is associated with increased mu-opioid receptor levels in major depressive disorder. European psychiatry : the journal of the Association of European Psychiatrists 57, 46-51.
Al-Hakeim, H.K., Zeki Al-Fadhel, S., Al-Dujaili, A.H., Maes, M., 2020. In major depression, increased kappa and mu opioid receptor levels are associated with immune activation. Acta Neuropsychiatr 32, 99-108.
Anderson, J., Adams, C., Antman, E., Bridges, C., Califf, R., Casey Jr, D., Chavey 2nd, W., Fesmire, F., Hochman, J., Levin, T., 2013. American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines 2012 ACCF/AHA focused update incorporated into the ACCF/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 127, e663-e828.
APA, A.P.A., 2013. Diagnostic and statistical manual of mental disorders, 5th ed. APA.
Bao, B., Prasad, A.S., Beck, F.W., Fitzgerald, J.T., Snell, D., Bao, G.W., Singh, T., Cardozo, L.J., 2010. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. The American journal of clinical nutrition 91, 1634-1641.
Barbagallo, M., Dominguez, L.J., Galioto, A., Ferlisi, A., Cani, C., Malfa, L., Pineo, A., Paolisso, G., 2003. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Molecular aspects of medicine 24, 39-52.
Barbonetti, A., Cavallo, F., D'Andrea, S., Muselli, M., Felzani, G., Francavilla, S., Francavilla, F., 2017. Lower vitamin D levels are associated with depression in people with chronic spinal cord injury. Archives of physical medicine and rehabilitation 98, 940-946.
Beck, A.T., Steer, R.A., Ball, R., Ranieri, W.F., 1996. Comparison of Beck Depression Inventories-IA and-II in psychiatric outpatients. Journal of personality assessment 67, 588-597.
Belin, R.J., He, K., 2007. Magnesium physiology and pathogenic mechanisms that contribute to the development of the metabolic syndrome. Magnes Res 20, 107-129.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological) 57, 289-300.
Billecke, S., Draganov, D., Counsell, R., Stetson, P., Watson, C., Hsu, C., La Du, B., 2000. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. Drug Metabolism and Disposition 28, 1335-1342.

Bonifácio, K.L., Barbosa, D.S., Moreira, E.G., Coneglian, C.F., Vargas, H.O., Nunes, S.O.V., Moraes, J.B., Maes, M., 2020. Increased nitro-oxidative stress toxicity as a major determinant of increased blood pressure in mood disorders. preprints.org, 1-25.
Bortolasci, C.C., Vargas, H.O., Nunes, S.O.V., de Melo, L.G.P., de Castro, M.R.P., Moreira, E.G., Dodd, S., Barbosa, D.S., Berk, M., Maes, M., 2015. Factors influencing insulin resistance in relation to atherogenicity in mood disorders, the metabolic syndrome and tobacco use disorder. Journal of affective disorders 179, 148-155.
Browne, C.A., Lucki, I., 2019. Targeting opioid dysregulation in depression for the development of novel therapeutics. Pharmacol Ther 201, 51-76.
Callaghan, C.K., Rouine, J., O'Mara, S.M., 2018. Potential roles for opioid receptors in motivation and major depressive disorder. Prog Brain Res 239, 89-119.
Campeau, L., 1976. Letter: Grading of angina pectoris. Circulation 54, 522-523.
Caruso, G., Fresta, C.G., Grasso, M., Santangelo, R., Lazzarino, G., Lunte, S.M., Caraci, F., 2020. Inflammation as the Common Biological Link Between Depression and Cardiovascular Diseases: Can Carnosine Exert a Protective Role? Curr Med Chem 27, 1782-1800.
Casseb, G.A., Ambrósio, G., Rodrigues, A.L.S., Kaster, M.P., 2019. Levels of 25-hydroxyvitamin D 3, biochemical parameters and symptoms of depression and anxiety in healthy individuals. Metabolic brain disease 34, 527-535.
Chistiakov, D.A., Melnichenko, A.A., Orekhov, A.N., Bobryshev, Y.V., 2017. Paraoxonase and atherosclerosis-related cardiovascular diseases. Biochimie 132, 19-27.
Costa, L.G., Cole, T.B., Furlong, C.E., 2005. Paraoxonase (PON1): from toxicology to cardiovascular medicine. Acta Biomed 76 Suppl 2, 50-57.
de Melo, L.G.P., Nunes, S.O.V., Anderson, G., Vargas, H.O., Barbosa, D.S., Galecki, P., Carvalho, A.F., Maes, M., 2017. Shared metabolic and immune-inflammatory, oxidative and nitrosative stress pathways in the metabolic syndrome and mood disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry 78, 34-50.
Di Pino, A., DeFronzo, R.A., 2019. Insulin Resistance and Atherosclerosis: Implications for Insulin-Sensitizing Agents. Endocr Rev 40, 1447-1467.
Flauzino, T., Pereira, W.L.d.C.J., Alfieri, D.F., Oliveira, S.R., Kallaur, A.P., Lozovoy, M.A.B., Kaimen-Maciel, D.R., Maes, M., Reiche, E.M.V., 2019. Disability in multiple sclerosis is associated with age and inflammatory, metabolic and oxidative/nitrosative stress biomarkers: results of multivariate and machine learning procedures. Metab. Brain Dis. 34, 1401-1413.
Frasca, D., Blomberg, B.B., Paganelli, R., 2017. Aging, Obesity, and Inflammatory Age-Related Diseases. Front. Immunol. 8, 1745.
Glassman, A.H., Bigger, J.T., Gaffney, M., 2009. Psychiatric characteristics associated with long-term mortality among 361 patients having an acute coronary syndrome and major depression: seven-year follow-up of SADHART participants. Archives of general psychiatry 66, 1022-1029.
Gueux, E., Azais-Braesco, V., Bussiére, L., Grolier, P., Mazur, A., Rayssiguier, Y., 1995. Effect of magnesium deficiency on triacylglycerol-rich lipoprotein and tissue susceptibility to peroxidation in relation to vitamin E content. British Journal of Nutrition 74, 849-856.
Hansson, G., 2011. Hermansson A. The immune system in atherosclerosis. Nat Immunol 12, 204-212.
Hare, D.L., Toukhsati, S.R., Johansson, P., Jaarsma, T., 2014. Depression and cardiovascular disease: a clinical review. European heart journal 35, 1365-1372.
Huang, J., Wang, Z., Hu, Z., Jiang, W., Li, B., 2017. Association between blood vitamin D and myocardial infarction: A meta-analysis including observational studies. Clin Chim Acta 471, 270-275.
Jamall, O.A., Feeney, C., Zaw-Linn, J., Malik, A., Niemi, M.E., Tenorio-Jimenez, C., Ham, T.E., Jilka, S.R., Jenkins, P.O., Scott, G., 2016. Prevalence and correlates of vitamin D deficiency in adults after traumatic brain injury. Clinical endocrinology 85, 636-644.

Jhee, J.H., Kim, H., Park, S., Yun, H.-R., Jung, S.-Y., Kee, Y.K., Yoon, C.-Y., Park, J.T., Han, S.H., Kang, S.-W., 2017. Vitamin D deficiency is significantly associated with depression in patients with chronic kidney disease. PLoS One 12, e0171009.
Kalampogias, A., Siasos, G., Oikonomou, E., Tsalamandris, S., Mourouzis, K., Tsigkou, V., Vavuranakis, M., Zografos, T., Deftereos, S., Stefanadis, C., Tousoulis, D., 2016. Basic Mechanisms in Atherosclerosis: The Role of Calcium. Med Chem 12, 103-113.
Kallaur, A.P., Lopes, J., Oliveira, S.R., Reiche, E.M.V., de Almeida, E.R.D., Morimoto, H.K., de Pereira, W.L.C.J., Alfieri, D.F., Borelli, S.D., Kaimen-Maciel, D.R., 2016. Immune-inflammatory and oxidative and nitrosative stress biomarkers of depression symptoms in subjects with multiple sclerosis: increased peripheral inflammation but less acute neuroinflammation. Molecular neurobiology 53, 5191-5202.
Kan, C., Silva, N., Golden, S.H., Rajala, U., Timonen, M., Stahl, D., Ismail, K., 2013. A systematic review and meta-analysis of the association between depression and insulin resistance. Diabetes care 36, 480-489.
Katon, W., Lin, E.H., Kroenke, K., 2007. The association of depression and anxiety with medical symptom burden in patients with chronic medical illness. General hospital psychiatry 29, 147-155.
Kieboom, B.C., Licher, S., Wolters, F.J., Ikram, M.K., Hoorn, E.J., Zietse, R., Stricker, B.H., Ikram, M.A., 2017. Serum magnesium is associated with the risk of dementia. Neurology 89, 1716-1722.
Kostov, K., Halacheva, L., 2018. Role of magnesium deficiency in promoting atherosclerosis, endothelial dysfunction, and arterial stiffening as risk factors for hypertension. International journal of molecular sciences 19, 1724.
Kowalska, K., Socha, E., Milnerowicz, H., 2015. Review: The role of paraoxonase in cardiovascular diseases. Ann Clin Lab Sci 45, 226-233.
Landucci Bonifácio, K., Sabbatini Barbosa, D., Gastaldello Moreira, E., de Farias, C.C., Higachi, L., Camargo, A.E.I., Favaro Soares, J., Odebrecht Vargas, H., Nunes, S.O.V., Berk, M., Dodd, S., Maes, M., 2017. Indices of insulin resistance and glucotoxicity are not associated with bipolar disorder or major depressive disorder, but are differently associated with inflammatory, oxidative and nitrosative biomarkers. Journal of Affective Disorders 222, 185-194.
Leopold, J.A., Loscalzo, J., 2009. Oxidative risk for atherothrombotic cardiovascular disease. Free Radic Biol Med 47, 1673-1706.
Lespérance, F., Frasure-Smith, N., Juneau, M., Théroux, P., 2000. Depression and 1-year prognosis in unstable angina. Archives of internal medicine 160, 1354-1360.
Levine, J., Stein, D., Rapoport, A., Kurtzman, L., 1999. High Serum and Cerebrospinal Fluid Ca/Mg Ratio in Recently Hospitalized Acutely Depressed Patients. Neuropsychobiology 39, 63-70.
Liang, Y., Yan, Z., Cai, C., Jiang, H., Song, A., Qiu, C., 2014. Association between lipid profile and depressive symptoms among Chinese older people: mediation by cardiovascular diseases? International journal of behavioral medicine 21, 590-596.
Lichtman, J.H., Froelicher, E.S., Blumenthal, J.A., Carney, R.M., Doering, L.V., Frasure-Smith, N., Freedland, K.E., Jaffe, A.S., Leifheit-Limson, E.C., Sheps, D.S., 2014. Depression as a risk factor for poor prognosis among patients with acute coronary syndrome: systematic review and recommendations: a scientific statement from the American Heart Association. Circulation 129, 1350-1369.
Mackness, M.I., Mackness, B., Durrington, P.N., 2002. Paraoxonase and coronary heart disease. Atherosclerosis Supplements 3, 49-55.
Maes, M., 1995. Evidence for an immune response in major depression: a review and hypothesis. Progress in Neuro-Psychopharmacology and Biological Psychiatry 19, 11-38.
Maes, M., Anderson, G., Kubera, M., Berk, M., 2014. Targeting classical IL-6 signalling or IL-6 trans-signalling in depression? Expert opinion on therapeutic targets 18, 495-512.
Maes, M., Carvalho, A.F., 2018. The compensatory immune-regulatory reflex system (CIRS) in depression and bipolar disorder. Molecular neurobiology 55, 8885-8903.

Maes, M., Ruckoanich, P., Chang, Y.S., Mahanonda, N., Berk, M., 2011. Multiple aberrations in shared inflammatory and oxidative & nitrosative stress (IO&NS) pathways explain the co-association of depression and cardiovascular disorder (CVD), and the increased risk for CVD and due mortality in depressed patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 769-783.
Maes, M., Sirivichayakul, S., Matsumoto, A.K., Maes, A., Michelin, A.P., de Oliveira Semeão, L., de Lima Pedrão, J.V., Moreira, E.G., Barbosa, D.S., Geffard, M., 2020. Increased Levels of Plasma Tumor Necrosis Factor-α Mediate Schizophrenia Symptom Dimensions and Neurocognitive Impairments and Are Inversely Associated with Natural IgM Directed to Malondialdehyde and Paraoxonase 1 Activity. Molecular Neurobiology, 1-13.
Maes, M., Smith, R., Christophe, A., Vandoolaeghe, E., Gastel, A.V., Neels, H., Demedts, P., Wauters, A., Meltzer, H., 1997. Lower serum high-density lipoprotein cholesterol (HDL-C) in major depression and in depressed men with serious suicidal attempts: relationship with immune-inflammatory markers. Acta psychiatrica Scandinavica 95, 212-221.
Małecki, R., Adamiec, R., 2005. [The role of calcium ions in the pathomechanism of the artery calcification accompanying atherosclerosis]. Postepy Hig Med Dosw (Online) 59, 42-47.
Mallat, Z., Besnard, S., Duriez, M., Deleuze, V., Emmanuel, F., Bureau, M.F., Soubrier, F., Esposito, B., Duez, H.l.n., Fievet, C., 1999. Protective role of interleukin-10 in atherosclerosis. Circulation research 85, e17-e24.
Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Böhm, M., Christiaens, T., Cifkova, R., De Backer, G., Dominiczak, A., 2013. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Blood pressure 22, 193-278.
Mc Namara, K., Alzubaidi, H., Jackson, J.K., 2019. Cardiovascular disease as a leading cause of death: how are pharmacists getting involved? Integrated pharmacy research & practice 8, 1-11.
McMurray, J., Ray, S., Abdullah, I., Dargie, H., Morton, J., 1992. Plasma endothelin in chronic heart failure. Circulation 85, 1374-1379.
Meredith, A.J., McManus, B.M., 2013. Vitamin D in heart failure. J Card Fail 19, 692-711.
Moise, N., Khodneva, Y., Richman, J., Shimbo, D., Kronish, I., Safford, M.M., 2016. Elucidating the Association Between Depressive Symptoms, Coronary Heart Disease, and Stroke in Black and White Adults: The REasons for Geographic And Racial Differences in Stroke (REGARDS) Study. J Am Heart Assoc 5.
Moreira, E.G., Boll, K.M., Correia, D.G., Soares, J.F., Rigobello, C., Maes, M., 2019a. Why should psychiatrists and neuroscientists worry about paraoxonase 1? Current neuropharmacology 17, 1004-1020.
Moreira, E.G., Correia, D.G., Bonifácio, K.L., Moraes, J.B.d., Cavicchioli, F.L., Nunes, C.S., Nunes, S.O.V., Vargas, H.O., Barbosa, D.S., Maes, M., 2019b. Lowered PON1 activities are strongly associated with depression and bipolar disorder, recurrence of (hypo) mania and depression, increased disability and lowered quality of life. The World Journal of Biological Psychiatry 20, 368-380.
Moustafa, S.R., Al-Rawi, K.F., Stoyanov, D., Al-Dujaili, A.H., Supasitthumrong, T., Al-Hakeim, H.K., Maes, M., 2020. The Endogenous Opioid System in Schizophrenia and Treatment Resistant Schizophrenia: Increased Plasma Endomorphin 2, and κ and μ Opioid Receptors Are Associated with Interleukin-6. Diagnostics (Basel) 10.
Narang, R.L., Gupta, K.R., Narang, A.P., Singh, R., 1991. Levels of copper and zinc in depression. Indian J Physiol Pharmacol 35, 272-274.
Ni, M., You, Y., Chen, J., Zhang, L., 2018. Copper in depressive disorder: A systematic review and meta-analysis of observational studies. Psychiatry research 267, 506-515.

Nunes, S.O.V., de Melo, L.G.P., de Castro, M.R.P., Barbosa, D.S., Vargas, H.O., Berk, M., Maes, M., 2015. Atherogenic index of plasma and atherogenic coefficient are increased in major depression and bipolar disorder, especially when comorbid with tobacco use disorder. Journal of affective disorders 172, 55-62.
Okano, T., Sato, K., Shirai, R., Seki, T., Shibata, K., Yamashita, T., Koide, A., Tezuka, H., Mori, Y., Hirano, T., Watanabe, T., 2020. β-Endorphin Mediates the Development and Instability of Atherosclerotic Plaques. Int J Endocrinol 2020, 4139093.
Oliveira, S.R., Kallaur, A.P., Lopes, J., Colado Simão, A.N., Reiche, E.M., de Almeida, E.R.D., Morimoto, H.K., de Carvalho Jennings de Pereira, W.L., Alfieri, D.F., Flauzino, T., de Meleck Proença, C., Gomes, A.M., Kaimen-Maciel, D.R., Maes, M., 2017. Insulin resistance, atherogenicity, and iron metabolism in multiple sclerosis with and without depression: Associations with inflammatory and oxidative stress biomarkers and uric acid. Psychiatry research 250, 113-120.
Qazmooz, H.A., Smeism, H.N., Mousa, R.F., Al-Hakeim, H.K., Maes, M., 2020. Trace element, immune and opioid biomarkers of unstable angina, increased atherogenicity and insulin resistance: results of machine learning.
Qu, X., Jin, F., Hao, Y., Li, H., Tang, T., Wang, H., Yan, W., Dai, K., 2013. Magnesium and the risk of cardiovascular events: a meta-analysis of prospective cohort studies. PloS one 8, e57720.
Rahman, M.S., Woollard, K., 2017. Atherosclerosis. Adv Exp Med Biol 1003, 121-144.
Raič, M., 2017. Depression and heart diseases: Leading health problems. Psychiatr Danub 29, 770-777.
Ramadan, R., Vaccarino, V., Esteves, F., Sheps, D.S., Bremner, J.D., Raggi, P., Quyyumi, A.A., 2014. Association of vitamin D status with mental stress induced myocardial ischemia in patients with coronary artery disease. Psychosomatic medicine 76, 569.
Ringle, C., Da Silva, D., Bido, D., 2015. Structural equation modeling with the SmartPLS. Bido, D., da Silva, D., & Ringle, C.(2014). Structural Equation Modeling with the Smartpls. Brazilian Journal Of Marketing 13.
Roomruangwong, C., Barbosa, D.S., Matsumoto, A.K., de Souza Nogueira, A., Kanchanatawan, B., Sirivichayakul, S., Carvalho, A.F., Duleu, S., Geffard, M., Moreira, E.G., 2017. Activated neuro-oxidative and neuro-nitrosative pathways at the end of term are associated with inflammation and physio-somatic and depression symptoms, while predicting outcome characteristics in mother and baby. Journal of affective disorders 223, 49-58.
Sagarad, S.V., Sukhani, N., Machanur, B., Patil, S., 2016. Effect of Vitamin D on Anginal Episodes in Vitamin D Deficient Patients with Chronic Stable Angina on Medical Management. Journal of clinical and diagnostic research : JCDR 10, OC24-OC26.
Silva, N., Atlantis, E., Ismail, K., 2012. A review of the association between depression and insulin resistance: pitfalls of secondary analyses or a promising new approach to prevention of type 2 diabetes? Current psychiatry reports 14, 8-14.
Sinha, K., Das, J., Pal, P.B., Sil, P.C., 2013. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol 87, 1157-1180.
Sotoudeh, G., Raisi, F., Amini, M., Majdzadeh, R., Hosseinzadeh, M., Khorram Rouz, F., Khosravi, M., 2020. Vitamin D deficiency mediates the relationship between dietary patterns and depression: a case–control study. Annals of general psychiatry 19, 1-8.
Strik, J.J., Honig, A., Maes, M., 2001. Depression and myocardial infarction: relationship between heart and mind. Progress in Neuro-Psychopharmacology and Biological Psychiatry 25, 879-892.
Styczeń, K., Sowa-Kućma, M., Siwek, M., Dudek, D., Reczyński, W., Misztak, P., Szewczyk, B., Topór-Mądry, R., Opoka, W., Nowak, G., 2016. Study of the Serum Copper Levels in Patients with Major Depressive Disorder. Biological trace element research 174, 287-293.
Trujillo, J., Vieira, M.C., Lepsch, J., Rebelo, F., Poston, L., Pasupathy, D., Kac, G., 2018. A systematic review of the associations between maternal nutritional biomarkers and depression and/or anxiety during pregnancy and postpartum. Journal of affective disorders 232, 185-203.

Twayej, A.J., Al-Hakeim, H.K., Al-Dujaili, A.H., Maes, M., 2019. Lowered zinc and copper levels in drug-naïve patients with major depression: Effects of antidepressants, ketoprofen and immune activation. The World Journal of Biological Psychiatry.
Vargas, H.O., Nunes, S.O.V., Barbosa, D.S., Vargas, M.M., Cestari, A., Dodd, S., Venugopal, K., Maes, M., Berk, M., 2014. Castelli risk indexes 1 and 2 are higher in major depression but other characteristics of the metabolic syndrome are not specific to mood disorders. Life sciences 102, 65-71.
Vellekkatt, F., Menon, V., 2019. Efficacy of vitamin D supplementation in major depression: A meta-analysis of randomized controlled trials. Journal of postgraduate medicine 65, 74.
Wang, Y.-P., Gorenstein, C., 2013. Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. Brazilian Journal of Psychiatry 35, 416-431.
Whang, W., Shimbo, D., Kronish, I.M., Duvall, W.L., Julien, H., Iyer, P., Burg, M.M., Davidson, K.W., 2010. Depressive symptoms and all-cause mortality in unstable angina pectoris (from the Coronary Psychosocial Evaluation Studies [COPES]). The American journal of cardiology 106, 1104-1107.
WHO, 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation.
WHO, W.H.O., 2011. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. World Health Organization.
Wiener, C.D., Moreira, F.P., Portela, L.V., Strogulski, N.R., Lara, D.R., da Silva, R.A., Souza, L.D.M., Jansen, K., Oses, J.P., 2019. Interleukin-6 and Interleukin-10 in mood disorders: A population-based study. Psychiatry Res 273, 685-689.
Wilbert-Lampen, U., Trapp, A., Barth, S., Plasse, A., Leistner, D., 2007. Effects of beta-endorphin on endothelial/monocytic endothelin-1 and nitric oxide release mediated by mu1-opioid receptors: a potential link between stress and endothelial dysfunction? Endothelium 14, 65-71.
Wiviott, S.D., Braunwald, E., 2004. Unstable angina and non-ST-segment elevation myocardial infarction: part I. Initial evaluation and management, and hospital care. Am Fam Physician 70, 525-532.
You, S., Zhong, C., Du, H., Zhang, Y., Zheng, D., Wang, X., Qiu, C., Zhao, H., Cao, Y., Liu, C.-F., 2017. Admission low magnesium level is associated with in-hospital mortality in acute ischemic stroke patients. Cerebrovascular Diseases 44, 35-42.
Zhang, W., Iso, H., Ohira, T., Date, C., Tamakoshi, A., Group, J.S., 2012. Associations of dietary magnesium intake with mortality from cardiovascular disease: the JACC study. Atherosclerosis 221, 587-595.

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Table 1. Socio-demographic, clinical and biomarker data in healthy controls (HC), and patients with atherosclerosis (ATS) with (ATS+MDD) and without (ATS-MDD) depression

Variables	HC [A] n=58	ATS-MDD [B] n=84	ATS+MDD [C] N=33	F / χ^2	df	p
Age (years)	55.88(9.59)	54.89(8.92)	58.24(8.30)	1.63	2/172	0.199
Body mass index (Kg/m^2)	26.22(3.06)	27.05(4.71)	27.08(4.57)	0.78	2/172	0.462
Sex (F/M)	18/40	37/47	12/21	2.52	2	0.283
ATS / Unstable Angina pectoris	-	52/32 [C]	7/26 [B]	14.11	1	<0.001
Canadian Classification System (III / IV)	-	23/9 [C]	10/16 [B]	6.53	1	0.011
Systolic Blood Pressure (mmHg)	119.98(1.45) [B,C]	131.32(15.45) [A,C]	139.48(17.27) [A,B]	25.67	2/172	<0.001
Diastolic Blood Pressure (mm/Hg)	79.84(1.40) [B,C]	84.01(6.19) [A]	85.61(10.09) [A]	11.64	2/172	<0.001
Type 2 diabetes mellitus (Y/N)		30/54	15/18	0.95	1	0.330
Beck Depression Inventory (BDI-II) score*	8.95(0.68) [B,C]	17.97(0.55) [A,C]	34.94(0.88) [B,C]	231.37	2/169	<0.001
BDI Physio-somatic symptoms*	2.54(0.33) [B,C]	5.26(0.26) [A,C]	12.32(0.42) [B,C]	109.89	2/169	<0.001
BDI Key depressive symptoms*	2.25(0.30) [B,C]	4.44(0.24) [A,C]	8.13(0.38) [B,C]	86.86	2/169	<0.001
BDI low self-esteem*	2.62(0.26) [B,C]	5.10(0.21) [A,C]	6.38(0.33) [B,C]	51.37	2/169	<0.001
TUD (Y/N)	15/43	30/54	10/23	1.57	2	0.456
Glibenclamide (Y/N)		30/54	14/19	0.46	1	0.500
Aspirin (Y/N)		49/35 [C]	32/1 [B]	16.60	1	<0.001
Clopidogrel (Y/N)		32/52 [C]	23/10 [B]	9.50	1	0.002
Bisoprolol (Y/N)		15/69 [C]	12/21 [B]	4.57	1	0.033
Atenolol (Y/N)		26/58 [C]	15/18 [B]	2.19	1	0.139
Isosorbide dinitrate (Y/N)		16/68 [C]	14/19 [B]	6.79	1	0.009
Trimetazidine (Y/N)		17/67 [C]	15/18 [B]	7.58	1	0.006
Atorvastatin (Y/N)		36/48 [C]	26/7 [B]	12.28	1	<0.001

Results are shown as mean (SD). [A,B,C]: comparisons between group means (significant at p=0.05). Depression is defined as a total Beck Depression Inventory-II score ≥29. ATS: atherosclerosis, TUD: Tobacco use disorder. * These data are adjusted for sex, age, and smoking and shown as mean (SE)

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Table 2. Measurements of the biomarkers in healthy controls (HC), and patients with atherosclerosis (ATS) with (ATS+MDD) and without (ATS-MDD) depression

Variables	HC [A] n=58	ATS-MDD [B] n=84	ATS+MDD [C] N=33	F	df	p
Total Cholesterol mmol/L	4.56 (0.14) [B,C]	5.96 (0.11) [A]	5.79 (0.18) [A]	34.90	2/168	<0.001
Triglycerides mmol/L	1.18 (0.14) [B,C]	3.14 (0.09) [A]	3.23 (0.14) [A]	44.16	2/167	<0.001
HDL cholesterol mmol/L	1.18 (0.027) [B,C]	0.95 (0.02) [A]	0.96 (0.03) [A]	27.26	2/168	<0.001
LDL cholesterol mmol/L	2.50 (0.12) [B,C]	3.56 (0.10) [A]	3.35 (0.16) [A]	25.04	2/168	<0.001
AIP (z score)	-0.966 (0.103) [B,C]	0.493 (0.082) [A]	0.506 (0.132) [A]	75.21	2/168	<0.001
Castelli-risk index 1 (z score)	-0.933 (0.103) [B,C]	0.553 (0.082) [A]	0.424 (0.132) [A]	73.86	2/167	<0.001
FBS mmol/L	5.27 (0.23) [B,C]	6.98 (0.18) [A]	7.238(0.290) [A]	22.31	2/167	<0.001
Insulin pmol/L	93.9 (9.8) [B,C]	150.6 (7.3) [A]	143.8(10.0) [A]	23.21	2/167	<0.001
Insulin resistance (z score)	-1.038 (0.102) [B,C]	0.429 (0.082) [A]	0.509 (0.131) [A]	79.00	2/166	<0.001
Vitamin D3 nmol/L	61.4(5.4) [B]	52.1(3.8) [A]	57.2(5.5)	3.47	2/168	0.033
Interleukin-6 pg/ml*	11.1(2.7) [C]	12.2(1.9) [C]	26.4(2.8) [A,B]	22.32	2/168	<0.001
Interleukin-10 pg/ml*	9.4(1.9) [B,C]	18.5(1.3) [A]	21.5(1.9) [A]	73.15	2/168	<0.001
β-Endorphin pg/ml*	19.7(2.7) [B, C]	26.8(1.9) [A]	27.2(2.8) [A]	14.35	2/168	<0.001
Mu opioid receptor pg/ml*	5.79(0.93)[C]	5.54(0.65) [C]	7.77 (0.94) [A, B]	14.26	2/168	<0.001
Zinc mg/l	0.76(0.03) [B,C]	0.58(0.02) [A]	0.55(0.03) [A]	24.73	2/168	<0.001
Copper mg/l	0.72(0.04) [B,C]	1.06(0.03) [A]	1.04(0.04) [A]	47.61	2/168	<0.001
Total Calcium mmol/L	2.46(0.04)	2.43(0.03) [C]	2.53(0.04) [B]	3.69	2/168	0.027
Total Magnesium mmol/L	0.70(0.04)	0.74(0.03)	0.74(0.04)	0.45	2/168	0.637

Results are shown as model-generated estimated marginal mean values after covarying for the effects of age, sex, smoking, and body mass index. Depression is defined as a total Beck Depression Inventory-II score ≥29. *These data were processed in Ln transformation. Results are shown as mean (SD). [A,B,C]: comparisons between group means (significant at $p<0.05$). ATS: atherosclerosis, TUD: Tobacco use disorder, FBS: fasting blood sugar, AIP: atherogenic index of plasma, LDL: low-density lipoprotein, HDL: high-density lipoprotein, Insulin resistance: homeostatic model assessment-insulin resistance index. * These data are adjusted for sex, age, and smoking and shown as mean (SE).

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Table 3. Results of binary logistic regression analysis with atherosclerosis (ATS with depression (ATS+MDD) as a dependent variable and ATS without depression (ATS-MDD) as a reference group.

Dichotomies	Explanatory variables	B	SE	Wald	p	OR	95% CI
#1. ATS+MDD vs ATS-MDD (BDI-II ≥ 19)	Interleukin-6	1.838	0.414	19.70	<0.001	6.28	2.79-14.14
	Age	0.101	0.033	9.25	0.002	1.11	1.04-1.18
	Mu opioid receptor	0.602	0.298	4.08	0.043	1.83	1.02-3.27
	Unstable Angina	1.743	0.650	7.18	0.007	5.72	1.60-0.45
#2. ATS+MDD vs ATS-MDD (BDI ≥ 29)	Interleukin-6	1.405	0.318	19.55	<0.001	4.10	2.19-7.60
	Unstable angina	2.134	0.574	13.84	<0.001	8.45	2.75-25.99

OR: Odds ratio; 95% CI: 95% confidence intervals

#1: depression is defined as patients with a Beck-Depression Inventory (BDI-II) score ≥19.

#2: depression is defined as patients with a Beck-Depression Inventory (BDI-II) score ≥29.

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Table 4. Results of neural networks with atherosclerosis (ATS) with depression (ATS+MDD) versus ATS without depression (ATS-MDD) as output variables and biomarkers as input variables.

	Models	**NN#1 ATS+MDD vs. ATS-MDD (BDI>29)**	**NN#2 ATS+MDD vs. ATS-MDD (BDI>19)**
Input Layer	Number of units	14	13
	Rescaling method	Normalized	Normalized
Hidden layers	Number of hidden layers	2	2
	Number of units in hidden layer 1	4	5
	Number of units in hidden layer 2	3	4
	Activation Function	Hyperbolic tangent	Hyperbolic tangent
Output layer	Dependent variables	ATS+MDD vs. ATS-MDD	ATS+MDD vs. ATS-MDD
	Number of units	2	2
	Activation function	Identity	Identity
	Error function	Sum of squares	Sum of squares
Training	Sum of squares error term	13.528	11.904
	% incorrect or relative error	22.5%	16.5%
	Prediction (sens, spec)	77.4%, 77.1%	88.2%, 80.7%
Testing	Sum of Squares error	5.975	6.562
	%incorrect or relative error	23.5%	17.3%
	Prediction (sens spec)	83.3%, 68.6%	92.0%, 74.1%
	AUC ROC	0.831	0.931
Holdout	%incorrect or relative error	21.8%	14.1%
	Prediction (sens, spec)	71.4%, 90.0%	88.0%, 81.0%

AUC ROC: area under curve of receiver operating curve, BDI-II: Beck Depression Inventory-II, NN: neural network.

Table 5. Partial correlations matrix between the total score on the Beck Depression Inventory-II (BDI-II), total score and sub-domains, and biomarkers

Variables	Total	Key symptoms	Self-esteem	Physiosomatic symptoms
Total BDI-II score	-	0.834**	0.653**	0.903**
BDI-II key symptoms	0.834**	-	0.407**	0.653**
BDI-II Self-esteem	0.653**	0.407**	-	0.450**
BDI-II Physiosomatic	0.903**	0.653**	0.450**	-
Total Cholesterol	0.356**	0.351**	0.263**	0.287**
Triglycerides	0.462**	0.475**	0.340**	0.341**
HDL cholesterol	-0.341**	-0.255*	-0.357**	-0.265**
LDL cholesterol	0.287**	0.256*	0.236*	0.243*
Atherogenic index of plasma	0.501**	0.455**	0.436**	0.378**
Castelli risk index 1	0.460**	0.399**	0.411**	0.364**
Zinc	-0.389**	-0.352**	-0.363**	-0.278**
Copper	0.379**	0.322**	0.402**	0.252*
Fasting Plasma Glucose	0.362**	0.313**	0.354**	0.245*
Insulin	0.329**	0.291**	0.261*	0.287**
Insulin Resistance	0.517**	0.452**	0.462**	0.398**
Albumin	-0.107	-0.140	-0.015	-0.130
Calcium	0.185*	0.099	0.175*	0.164*
Magnesium	0.018	0.019	0.018	0.005
Vitamin D3	-0.133	-0.132	-0.007	-0.156*
Interleukin-6	0.513**	0.357**	0.369**	0.470**
Interleukin-10	0.551**	0.542**	0.454**	0.458**
β-endorphins	0.289**	0.293**	0.248*	0.244*
Mu Opioid Receptor	0.296**	0.322**	0.134	0.251*

Shown are partial correlations after adjusting for effects of age, sex, body mass index and smoking. *p<0.01; **p<0.001. HDLc: High-density lipoprotein cholesterol, LDLc: Low-density lipoprotein cholesterol, Castelli risk index 1: computed as z score of total cholesterol – z score of HDL cholesterol.

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Table 6. Results of multiple regression analysis with the total Beck Depression Inventory-II (BDI-II) score and BDI-II subdomain scores as dependent variables.

Dependent Variables BDI-II scores	Explanatory Variables	β	T	p	F model	df	p	R^2
#1. Total	**Model**				32.678	4/112	<0.001	0.539
	Interleukin	0.475	7.348	<0.001				
	Unstable Angina	0.306	3.936	<0.001				
	Age	0.329	5.060	<0.001				
	Mu opioid Receptor	0.170	2.197	0.030				
#2. Key symptoms	**Model**				18.531	4/112	<0.001	0.398
	Unstable angina	0.270	3.046	0.003				
	Age	0.388	5.213	<0.001				
	Interleukin-6	0.258	3.498	0.001				
	Mu Opioid Receptor	0.199	2.248	0.027				
#3. Self-esteem	**Model**				18.547	5/111	<0.001	0.455
	Age	0.516	7.325	0.000				
	Interleukin-6	0.235	3.300	0.001				
	Sex	-0.226	-3.144	0.002				
	Unstable Angina	0.175	2.464	0.015				
	Atorvastatin	0.168	2.334	0.021				
#4. Physiosomatic	**Model**				20.437	4/112	<0.001	0.422
	Interleukin-6	0.484	6.495	0.000				
	Unstable Angina	0.353	4.865	0.000				
	Fasting Plasma Glucose	-0.211	-2.823	0.006				
	Atorvastatin	0.162	2.188	0.031				
Without UA								
#5. Total	**Model**				35.944	6/168	<0.001	0.562
	Insulin resistance	0.241	3.930	<0.000				
	Interleukin-6	0.336	5.970	<0.000				
	Mu Opioid Receptor	0.216	4.076	<0.000				

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	Age	0.274	5.311	<0.000				
	Atherogenic index of plasma	0.161	2.408	<0.017				
	Hypertension	0.137	2.214	<0.028				
#6. Key symptoms	**Model**				27.633	5/169	<0.001	0.450
	Insulin resistance	0.216	3.135	0.002				
	Age	0.314	5.452	<0.001				
	Mu Opioid Receptor	0.229	3.852	<0.001				
	Interleukin-6	0.229	3.799	<0.001				
	Triglycerides	0.246	3.589	<0.00				
#7. Self-esteem	**Model**				26.723	6/168	<0.001	0.488
	Age	0.420	7.478	<0.001				
	Atherogenic index of plasma	0.193	2.924	0.004				
	Interleukin-6	0.189	3.224	0.002				
	Insulin resistance	0.197	2.874	0.005				
	Sex	-0.173	-3.086	0.002				
	Zinc	-0.138	-2.240	0.026				
#8. Physiosomatic	**Model**				22.298	5/169	<0.001	0.397
	IL-6	0.345	5.329	<0.001				
	Insulin resistance	0.209	3.164	0.002				
	Mu Opioid Receptor	0.217	3.579	<0.001				
	Age	0.184	3.060	0.003				
	Hypertension	0.169	2.546	0.012				

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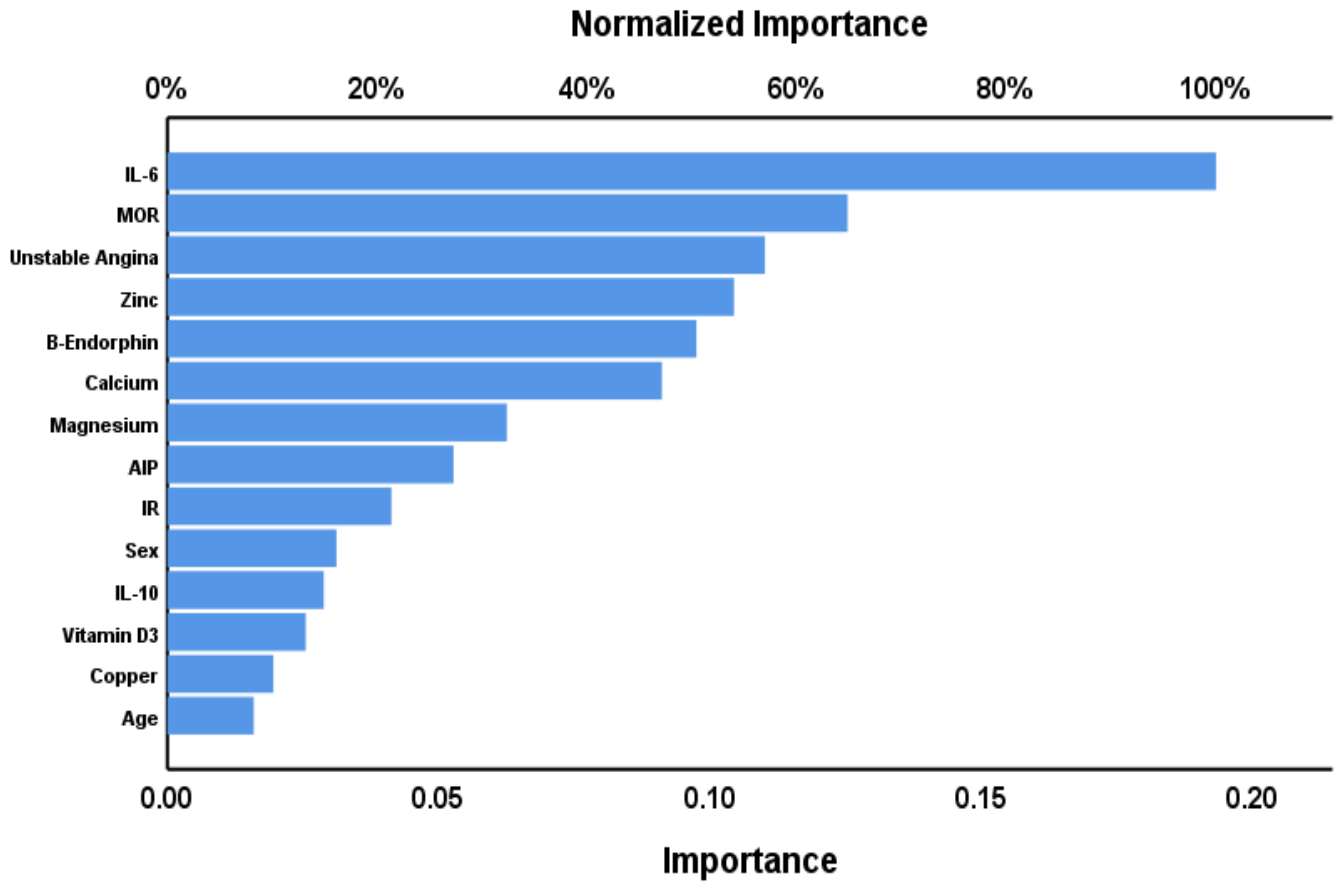

Figure 1. Results of neural network (importance chart) with depression due to atherosclerosis (using Beck Depression Inventory-II scores ≥ 29 as a cut-off value) as output variables and biomarkers (in z-scores) as input variables. IL: interleukin; MOR: mu opioid receptor; AIP: atherogenic index of plasma; IR: insulin resistance.

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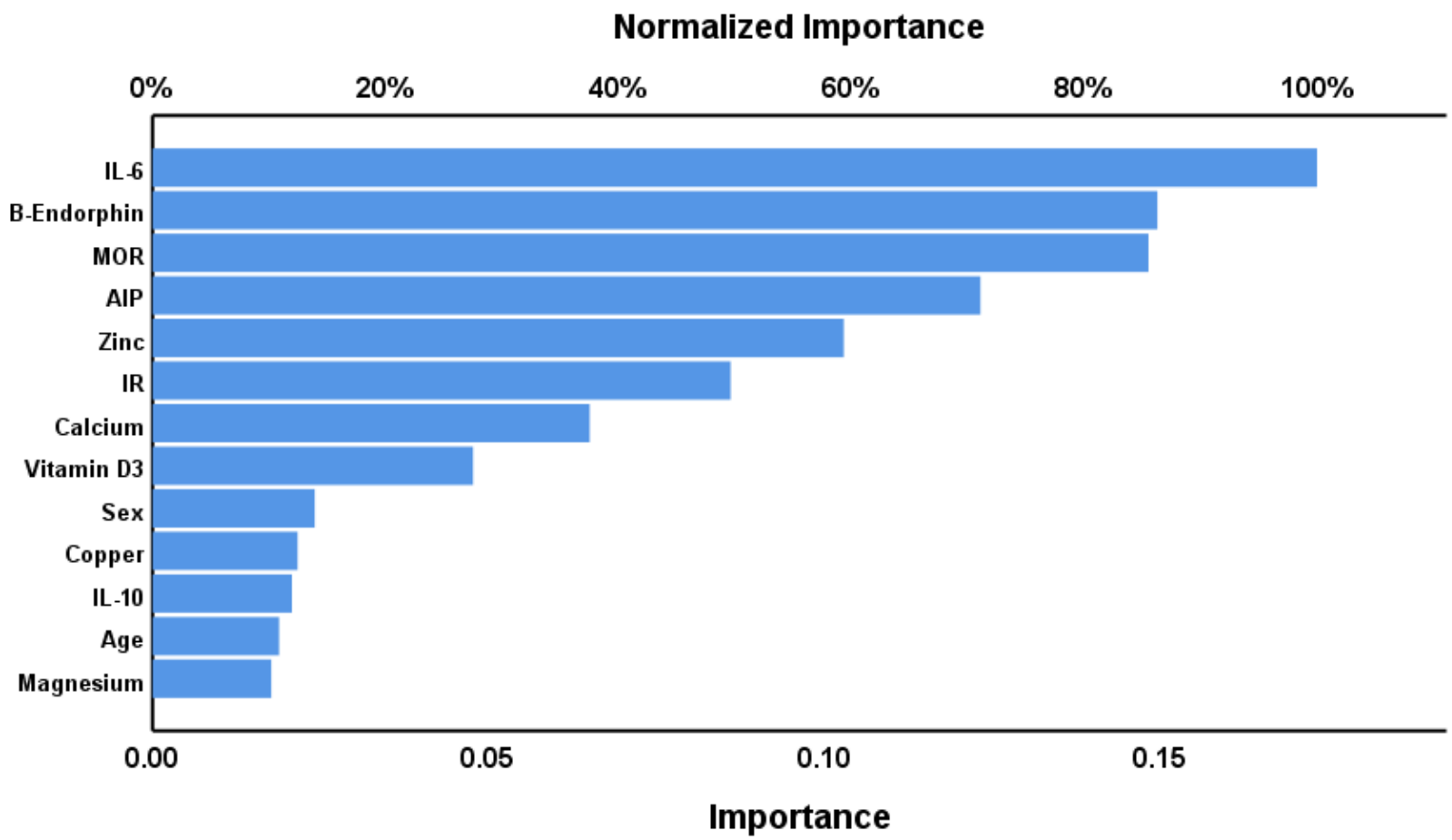

Figure 2. Results of neural network (importance chart) with depression due to atherosclerosis (using a Beck Depression Inventory-II score ≥ 19 as a cut-off value) as output variables and biomarkers (in z-scores) as input variables. IL: interleukin; AIP: atherogenic index of plasma; IR: insulin resistance; MOR: mu opioid receptor.

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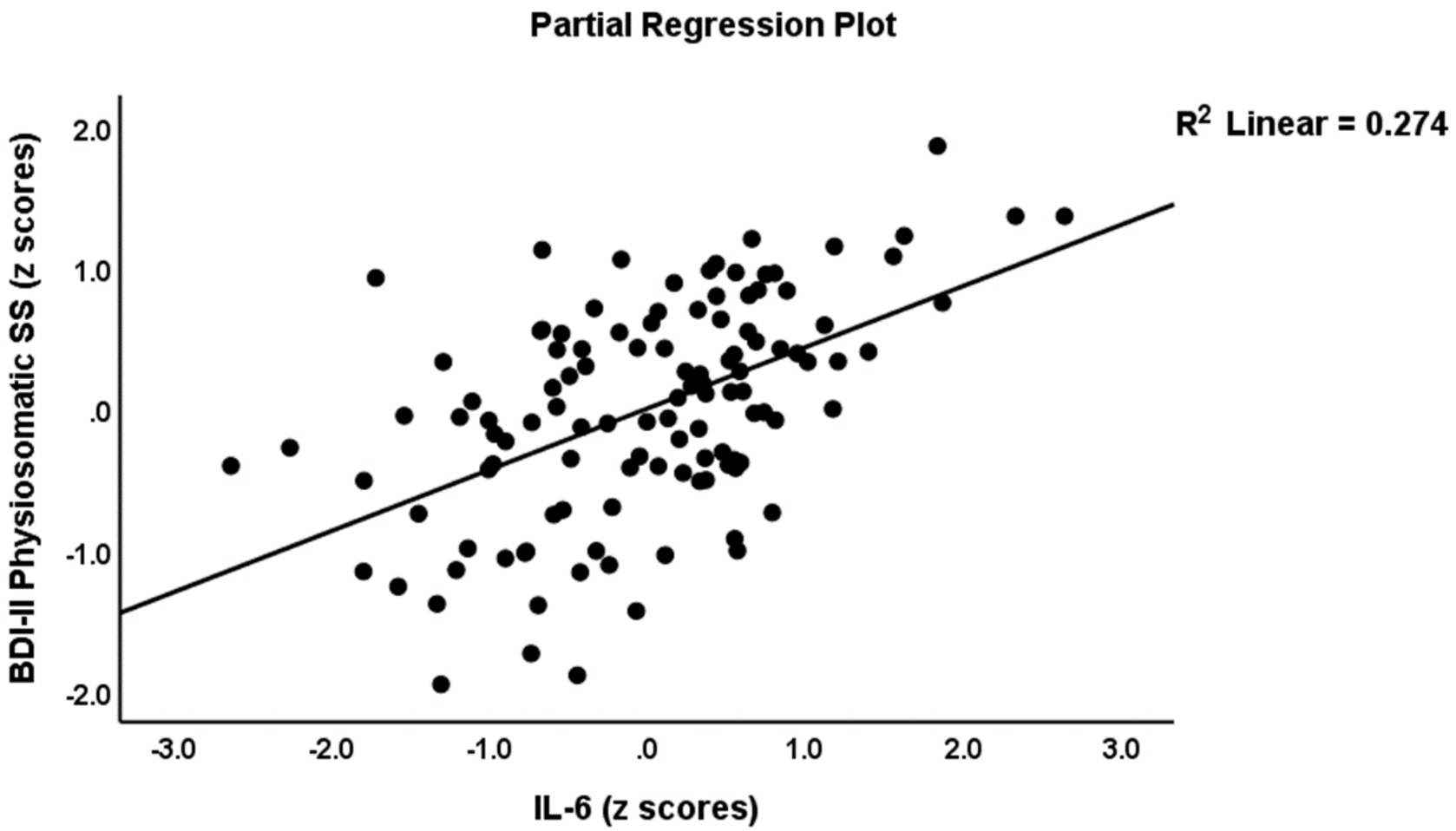

Figure 3. Partial regression of the Beck Depression Inventory-II physiosomatic score on interleukin-6 (IL-6)

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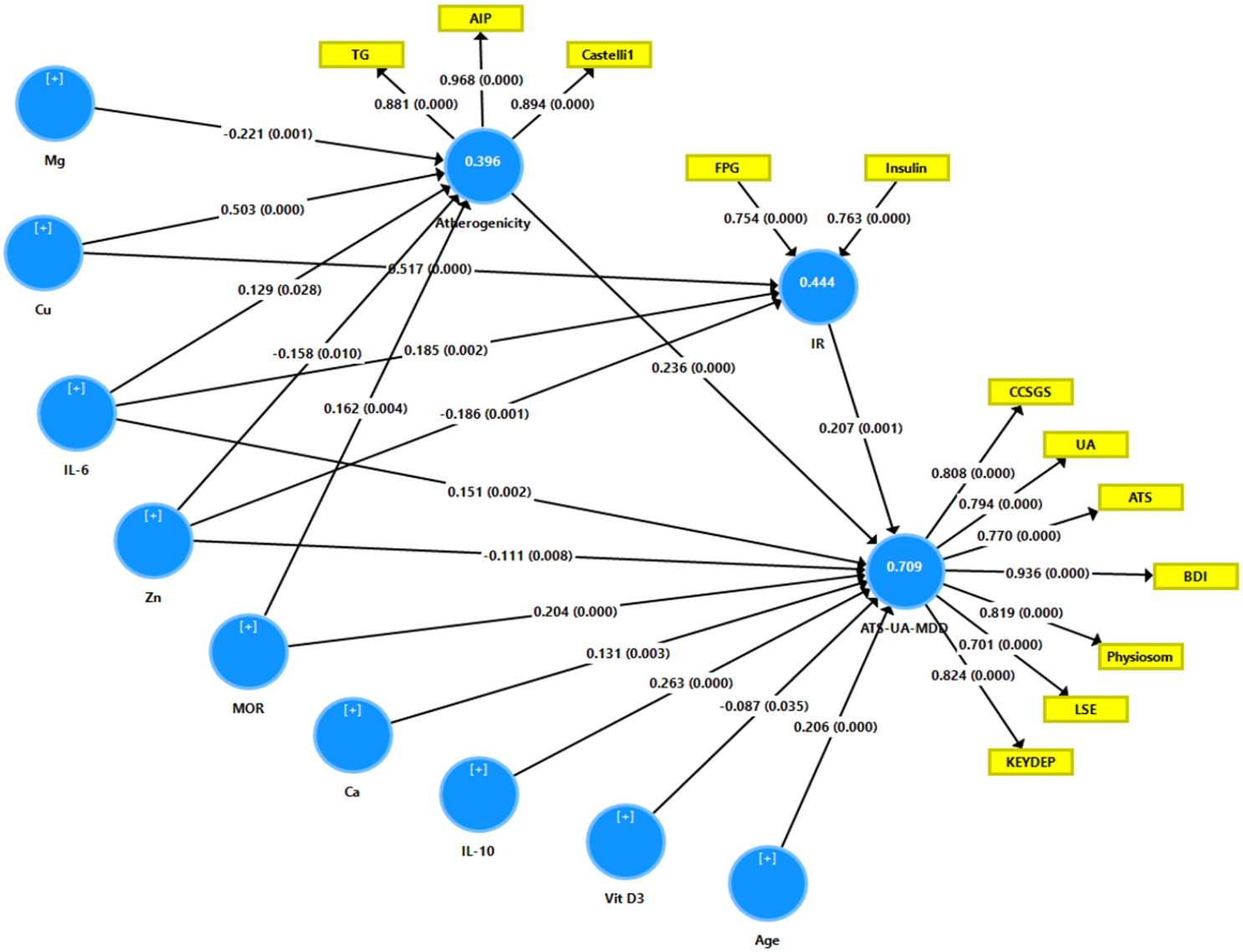

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Figure 4. Results of a multistep Partial Least Squares (PLS) pathway analysis.
Atherogenicity and insulin resistance (IR) mediate the effects of different biomarkers on a latent vector (ATS-UA-MDD) extracted from atherosclerosis (ATS), unstable angina (UA), the Canadian Cardiovascular Society Grading Scale (CCSGS), the Beck Depression Inventory-II total (BDI) and subdomain scores, namely physiosomatic (physiosom), LSE (low self-esteem), and key depressive (KEYDEP) symptoms.
Mg: magnesium; Cu: copper; IL: interleukin; Zn: zinc; MOR: mu opioid receptor; Ca: calcium; Vit D3: vitamin D3.
The white figures in the blue dots denote the explained variance.

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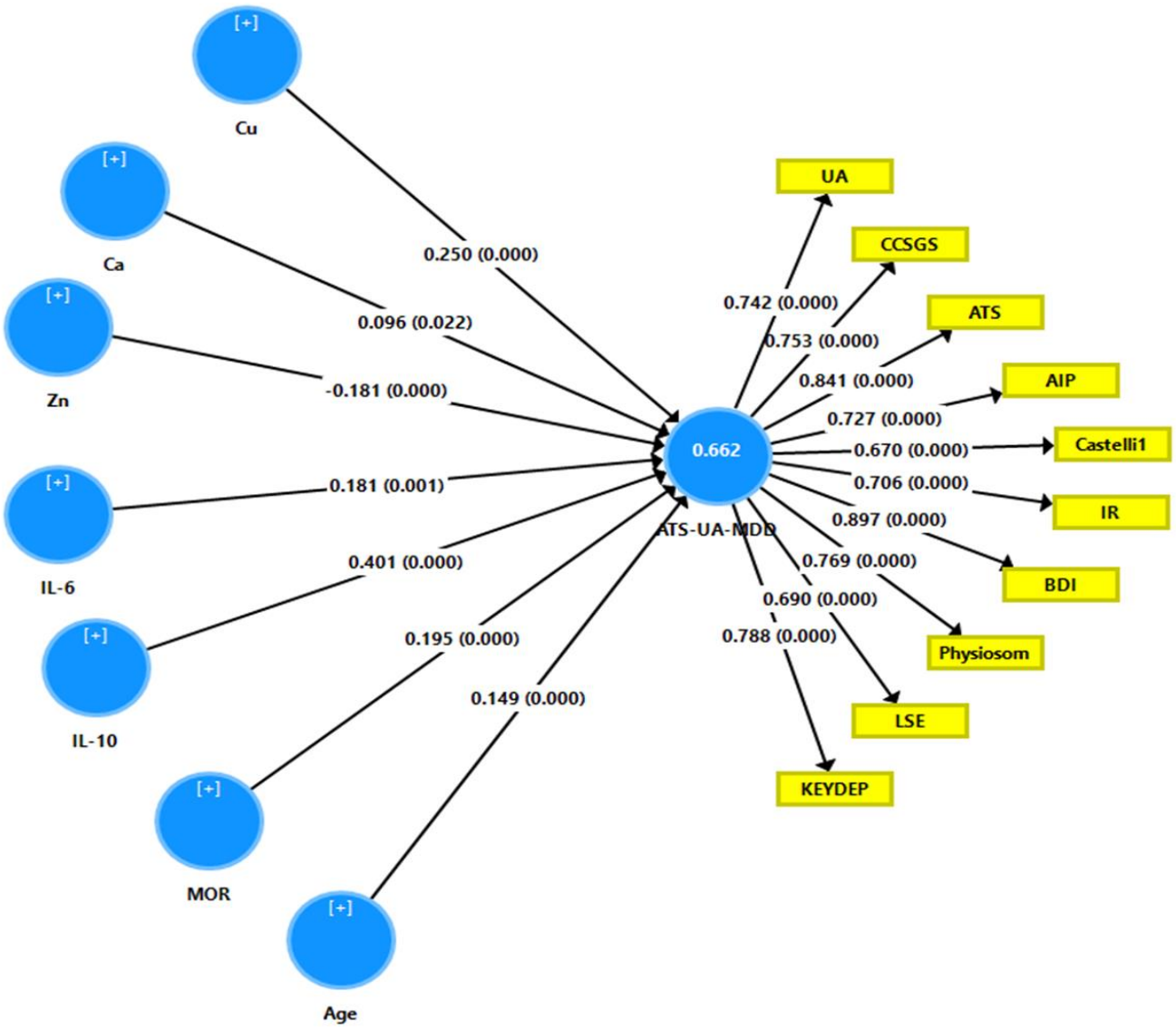

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Figure 5. Results of a Partial Least Squares (PLS) pathway analysis.
Different biomarkers explain 66.2% of the variance in a latent vector (ATS-UA-MDD) extracted from atherosclerosis (ATS), unstable angina (UA), the Canadian Cardiovascular Society Grading Scale (CCSGS), indices reflecting the atherogenic index of plasma (AIP), Castelli risk index 1, insulin resistance (IR), the Beck Depression Inventory-II total (BDI) and subdomain scores, namely physiosomatic (physiosom), LSE (low self-esteem), and key depressive (KEYDEP) symptoms.
Cu: copper; Ca: calcium, Zn: zinc; IL: interleukin; and MOR: mu opioid receptor.

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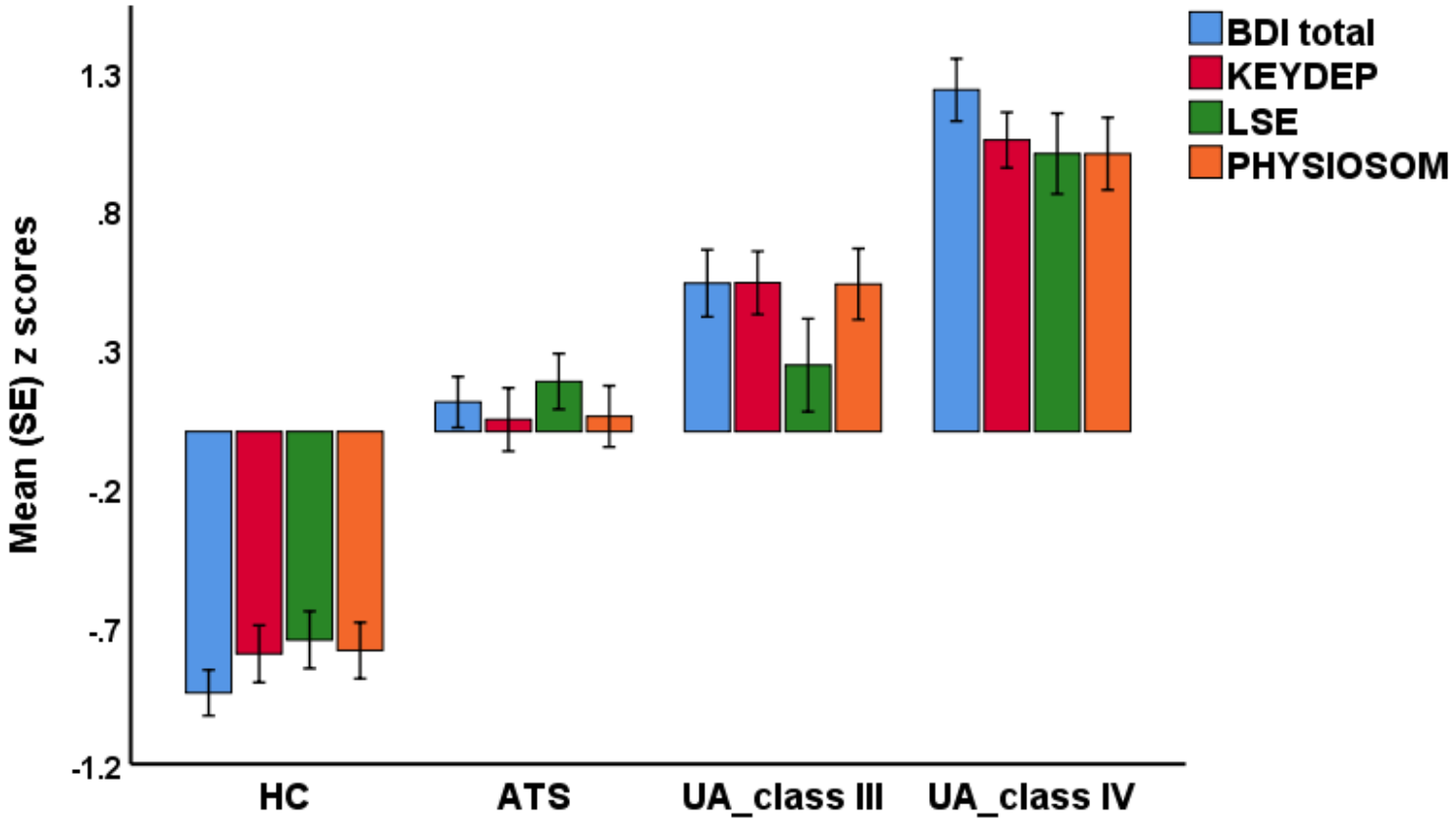

Figure 6. Bar chart (mean ±SE) of the z scores of the total Beck' depression inventory (BDI) score and subdomain scores, namely key depression symptoms (KEYDEP), low self-esteem (LSE), and physiosomatic symptoms (PHYSIOSOM) in healthy controls (HC), atherosclerosis (AST), unstable angina class III (UA_class III), and unstable angina class IV (UA_class IV).

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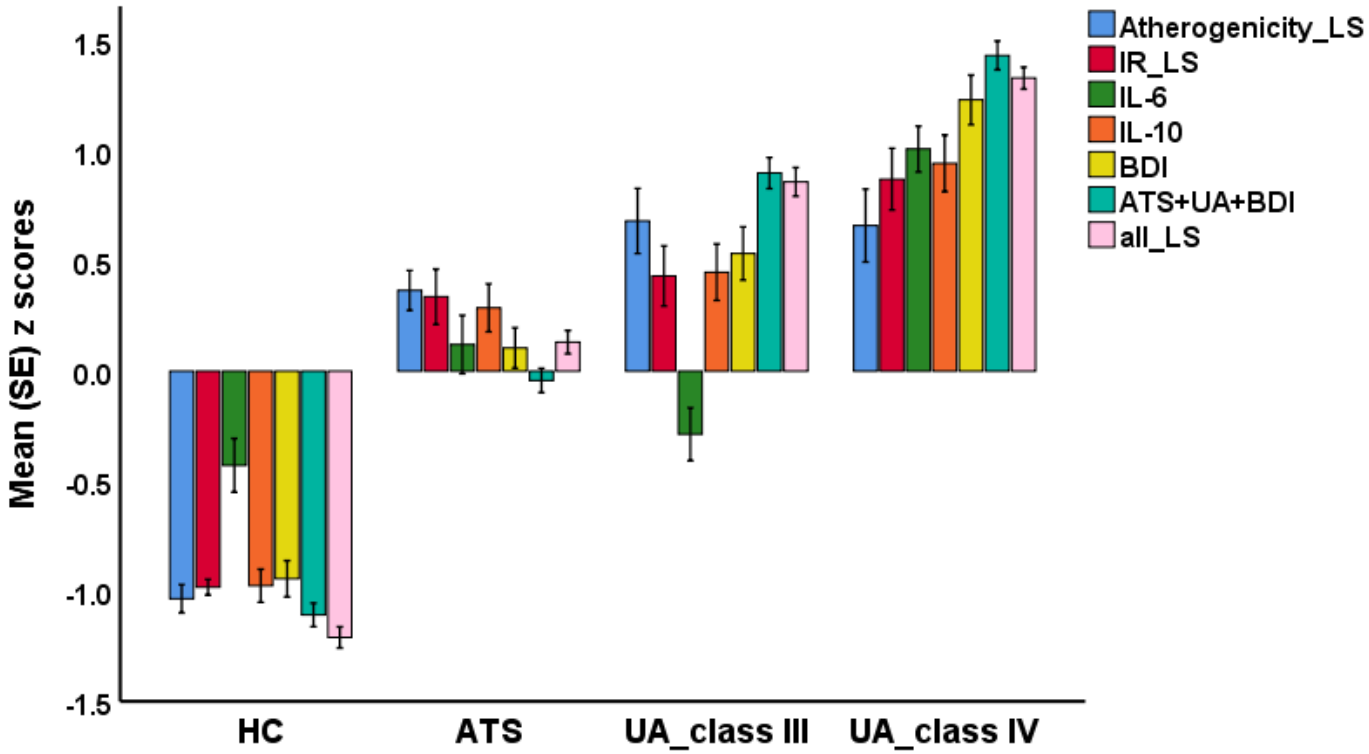

Figure 7. Bar chart (mean ±SE) of the z scores of the latent variable scores (LS) reflecting atherogenicity and insulin resistance (IR), and the LS extracted from atherosclerosis, unstable angina and depression (ATS+UA+BDI) data, and the LS extracted from the latter and the atherogenic index of plasma, Castelli risk index 1, and the IR index (ALL_LS). In this graph we also show the measurements

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of interleukin-6 (IL-6) and IL-10, and the total Beck Depression Inventory-II (BDI) score in healthy controls (HC), and patients with atherosclerosis (AST), unstable angina class III (UA-class III), and unstable angina class IV (UA-class IV).