**Supplementary Table 1. List of the 99 analytes that passed QC in the unmedicated discovery cohort**

|  |  |
| --- | --- |
| **Analytes** | **Analytes** |
| **Adiponectin** | **Interleukin-12 Subunit p40 (IL-12p40)** |
| **Alpha-1-Antitrypsin (AAT)** | **Interleukin-13 (IL-13)** |
| **Alpha-2-Macroglobulin (A2Macro)** | **Interleukin-16 (IL-16)** |
| **Alpha-Fetoprotein (AFP)** | **Interleukin-18 (IL-18)** |
| **Angiopoietin-2 (ANG-2)** | **Interleukin-3 (IL-3)** |
| **Angiotensin-Converting Enzyme (ACE)** | **Interleukin-7 (IL-7)** |
| **Angiotensinogen** | **Interleukin-8 (IL-8)** |
| **Apolipoprotein A-I (Apo A-I)** | **Leptin** |
| **Apolipoprotein C-III (Apo C-III)** | **Luteinizing Hormone (LH)** |
| **Apolipoprotein H (Apo H)** | **Macrophage Colony-Stimulating Factor 1 (M-CSF)** |
| **Apolipoprotein(a) (Apo(a))** | **Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha)** |
| **AXL Receptor Tyrosine Kinase (AXL)** | **Macrophage Inflammatory Protein-1 beta (MIP-1 beta)** |
| **B Lymphocyte Chemoattractant (BLC)** | **Macrophage Migration Inhibitory Factor (MIF)** |
| **Beta-2-Microglobulin (B2M)** | **Macrophage-Derived Chemokine (MDC)** |
| **Bone Morphogenetic Protein 6 (BMP-6)** | **Matrix Metalloproteinase-3 (MMP-3)** |
| **Brain-Derived Neurotrophic Factor (BDNF)** | **Monocyte Chemotactic Protein 1 (MCP-1)** |
| **C-Reactive Protein (CRP)** | **Myeloperoxidase (MPO)** |
| **Cancer Antigen 19-9 (CA-19-9)** | **Myoglobin** |
| **Carcinoembryonic Antigen (CEA)** | **Pancreatic Polypeptide (PPP)** |
| **CD 40 antigen (CD40)** | **Plasminogen Activator Inhibitor 1 (PAI-1)** |
| **CD40 Ligand (CD40-L)** | **Platelet-Derived Growth Factor BB (PDGF-BB)** |
| **Chemokine CC-4 (HCC-4)** | **Progesterone** |
| **Chromogranin-A (CgA)** | **Prolactin (PRL)** |
| **Complement C3 (C3)** | **Prostatic Acid Phosphatase (PAP)** |
| **Cortisol (Cortisol)** | **Pulmonary and Activation-Regulated Chemokine (PARC)** |
| **Creatine Kinase-MB (CK-MB)** | **Receptor for advanced glycosylation end products (RAGE)** |
| **EN-RAGE** | **Resistin** |
| **Eotaxin** | **Serum Amyloid P-Component (SAP)** |
| **Epidermal Growth Factor (EGF)** | **Serum Glutamic Oxaloacetic Transaminase (SGOT)** |
| **Epithelial-Derived Neutrophil-Activating Protein 78 (ENA-78)** | **Sex Hormone-Binding Globulin (SHBG)** |
| **Factor VII** | **Sortilin** |
| **Fas Ligand (FasL)** | **Stem Cell Factor (SCF)** |
| **FASLG Receptor (FAS)** | **Superoxide Dismutase 1, soluble (SOD-1)** |
| **Ferritin (FRTN)** | **T Lymphocyte-Secreted Protein I-309 (I-309)** |
| **Follicle-Stimulating Hormone (FSH)** | **T-Cell-Specific Protein RANTES (RANTES)** |
| **Glutathione S-Transferase alpha (GST-alpha)** | **Tenascin-C (TN-C)** |
| **Granulocyte Colony-Stimulating Factor (G-CSF)** | **Testosterone, Total** |
| **Growth Hormone (GH)** | **Thrombopoietin (TPO)** |
| **Growth-Regulated alpha protein (GRO-alpha)** | **Thrombospondin-1** |
| **Haptoglobin** | **Thyroid-Stimulating Hormone (TSH)** |
| **Heparin-Binding EGF-Like Growth Factor (HB-EGF)** | **Thyroxine-Binding Globulin (TBG)** |
| **Hepatocyte Growth Factor (HGF)** | **Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)** |
| **Immunoglobulin A (IgA)** | **TNF-Related Apoptosis-Inducing Ligand Receptor 3 (TRAIL-R3)** |
| **Immunoglobulin M (IgM)** | **Tumor Necrosis Factor alpha (TNF-alpha)** |
| **Insulin** | **Tumor Necrosis Factor beta (TNF-beta)** |
| **Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)** | **Tumor necrosis factor receptor 2 (TNFR2)** |
| **Intercellular Adhesion Molecule 1 (ICAM-1)** | **Vascular Cell Adhesion Molecule-1 (VCAM-1)** |
| **Interleukin-1 alpha (IL-1 alpha)** | **Vascular Endothelial Growth Factor (VEGF)** |
| **Interleukin-1 receptor antagonist (IL-1ra)** | **von Willebrand Factor (vWF)** |
| **Interleukin-10 (IL-10)** |  |

**Supplementary Table 2. List of the 171 analytes that passed QC in the NESDA cohort**

|  |  |
| --- | --- |
| **Analytes** | **Analytes** |
| **6Ckine** | **Interleukin-23 (IL-23)** |
| **Adiponectin** | **Interleukin-6 receptor (IL-6r)** |
| **Aldose Reductase** | **Interleukin-6 receptor subunit beta (IL-6R beta)** |
| **Alpha-1-Antichymotrypsin (AACT)** | **Interleukin-8 (IL-8)** |
| **Alpha-1-Antitrypsin (AAT)** | **Kallikrein 5** |
| **Alpha-1-Microglobulin (A1Micro)** | **Lactoylglutathione lyase (LGL)** |
| **Alpha-2-Macroglobulin (A2Macro)** | **Latency-Associated Peptide of Transforming Growth Factor beta 1 (LAP TGF-b1)** |
| **Angiogenin** | **Lectin-Like Oxidized LDL Receptor 1 (LOX-1)** |
| **Angiopoietin-2 (ANG-2)** | **Leptin** |
| **Angiotensin-Converting Enzyme (ACE)** | **Luteinizing Hormone (LH)** |
| **Angiotensinogen** | **Macrophage Colony-Stimulating Factor 1 (M-CSF)** |
| **Apolipoprotein A-I (Apo A-I)** | **Macrophage inflammatory protein 3 beta (MIP-3 beta)** |
| **Apolipoprotein A-II (Apo A-II)** | **Macrophage Inflammatory Protein-1 beta (MIP-1 beta)** |
| **Apolipoprotein A-IV (Apo A-IV)** | **Macrophage Migration Inhibitory Factor (MIF)** |
| **Apolipoprotein B (Apo B)** | **Macrophage-Derived Chemokine (MDC)** |
| **Apolipoprotein C-I (Apo C-I)** | **Macrophage-Stimulating Protein (MSP)** |
| **Apolipoprotein C-III (Apo C-III)** | **Matrix Metalloproteinase-1 (MMP-1)** |
| **Apolipoprotein D (Apo D)** | **Matrix Metalloproteinase-10 (MMP-10)** |
| **Apolipoprotein E (Apo E)** | **Matrix Metalloproteinase-3 (MMP-3)** |
| **Apolipoprotein H (Apo H)** | **Matrix Metalloproteinase-7 (MMP-7)** |
| **Apolipoprotein(a) (Apo(a))** | **Matrix Metalloproteinase-9, total (MMP-9, total)** |
| **AXL Receptor Tyrosine Kinase (AXL)** | **Mesothelin (MSLN)** |
| **B cell-activating factor (BAFF)** | **Monocyte Chemotactic Protein 1 (MCP-1)** |
| **Beta-2-Microglobulin (B2M)** | **Monocyte Chemotactic Protein 2 (MCP-2)** |
| **Brain-Derived Neurotrophic Factor (BDNF)** | **Monocyte Chemotactic Protein 4 (MCP-4)** |
| **C-Peptide** | **Monokine Induced by Gamma Interferon (MIG)** |
| **C-Reactive Protein (CRP)** | **Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)** |
| **Cancer Antigen 15-3 (CA-15-3)** | **Myeloperoxidase (MPO)** |
| **Carcinoembryonic Antigen (CEA)** | **Myoglobin** |
| **Cathepsin D** | **N-terminal prohormone of brain natriuretic peptide (NT proBNP)** |
| **CD 40 antigen (CD40)** | **Neuron-Specific Enolase (NSE)** |
| **CD40 Ligand (CD40-L)** | **Neuronal Cell Adhesion Molecule (Nr-CAM)** |
| **CD5 Antigen-like (CD5L)** | **Neuropilin-1** |
| **Cellular Fibronectin (cFib)** | **Neutrophil Gelatinase-Associated Lipocalin (NGAL)** |
| **Chemokine CC-4 (HCC-4)** | **Osteopontin** |
| **Chromogranin-A (CgA)** | **Osteoprotegerin (OPG)** |
| **Clusterin (CLU)** | **Pancreatic Polypeptide (PPP)** |
| **Collagen IV** | **Pepsinogen I (PGI)** |
| **Complement C3 (C3)** | **Phosphoserine Aminotransferase (PSAT)** |
| **Complement Factor H – Related Protein 1 (CFHR1)** | **Plasminogen Activator Inhibitor 1 (PAI-1)** |
| **Cortisol (Cortisol)** | **Platelet-Derived Growth Factor BB (PDGF-BB)** |
| **Creatine Kinase-MB (CK-MB)** | **Progesterone** |
| **Cystatin-C** | **Prolactin (PRL)** |
| **E-Selectin** | **Prostasin** |
| **EN-RAGE** | **Pulmonary and Activation-Regulated Chemokine (PARC)** |
| **Endoglin** | **Receptor for advanced glycosylation end products (RAGE)** |
| **Endostatin** | **Receptor tyrosine-protein kinase erbB-3 (ErbB3)** |
| **Eotaxin-1** | **Resistin** |
| **Eotaxin-2** | **Serotransferrin (Transferrin)** |
| **Epidermal Growth Factor (EGF)** | **Serum Amyloid P-Component (SAP)** |
| **Epidermal Growth Factor Receptor (EGFR)** | **Sex Hormone-Binding Globulin (SHBG)** |
| **Epithelial-Derived Neutrophil-Activating Protein 78 (ENA-78)** | **Sortilin** |
| **Factor VII** | **Stem Cell Factor (SCF)** |
| **FASLG Receptor (FAS)** | **Stromal cell-derived factor-1 (SDF-1)** |
| **Fatty Acid-Binding Protein, adipocyte (FABP, adipocyte)** | **Superoxide Dismutase 1, soluble (SOD-1)** |
| **Ferritin (FRTN)** | **T-Cell-Specific Protein RANTES (RANTES)** |
| **Fetuin-A** | **Tamm-Horsfall Urinary Glycoprotein (THP)** |
| **Fibulin-1C (Fib-1C)** | **Tenascin-C (TN-C)** |
| **Follicle-Stimulating Hormone (FSH)** | **Testosterone, Total** |
| **Galectin-3** | **Tetranectin** |
| **Gelsolin** | **Thrombomodulin (TM)** |
| **Glucose-6-phosphate Isomerase (G6PI)** | **Thrombospondin-1** |
| **Glutathione S-Transferase alpha (GST-alpha)** | **Thyroglobulin (TG)** |
| **Growth Hormone (GH)** | **Thyroid-Stimulating Hormone (TSH)** |
| **Growth-Regulated alpha protein (GRO-alpha)** | **Thyroxine-Binding Globulin (TBG)** |
| **Haptoglobin** | **Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)** |
| **Hepatocyte Growth Factor (HGF)** | **Tissue type Plasminogen activator (tPA)** |
| **Hepatocyte Growth Factor receptor (HGF receptor)** | **TNF-Related Apoptosis-Inducing Ligand Receptor 3 (TRAIL-R3)** |
| **Hepsin** | **Transthyretin (TTR)** |
| **Human Epidermal Growth Factor Receptor 2 (HER-2)** | **Trefoil Factor 3 (TFF3)** |
| **Immunoglobulin A (IgA)** | **Tumor necrosis factor receptor 2 (TNFR2)** |
| **Immunoglobulin M (IgM)** | **Tumor Necrosis Factor Receptor I (TNF RI)** |
| **Insulin** | **Tyrosine kinase with Ig and EGF homology domains 2 (TIE-2)** |
| **Insulin-like Growth Factor Binding Protein 4 (IGFBP4)** | **Urokinase-type Plasminogen Activator (uPA)** |
| **Insulin-like Growth Factor Binding Protein 5 (IGFBP5)** | **Urokinase-type plasminogen activator receptor (uPAR)** |
| **Insulin-like Growth Factor Binding Protein 6 (IGFBP6)** | **Vascular Cell Adhesion Molecule-1 (VCAM-1)** |
| **Insulin-like Growth Factor-Binding Protein 1 (IGFBP-1)** | **Vascular Endothelial Growth Factor (VEGF)** |
| **Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)** | **Vascular Endothelial Growth Factor C (VEGF-C)** |
| **Insulin-like Growth Factor-Binding Protein 3 (IGFBP-3)** | **Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2)** |
| **Intercellular Adhesion Molecule 1 (ICAM-1)** | **Vascular endothelial growth factor receptor 3 (VEGFR-3)** |
| **Interferon gamma Induced Protein 10 (IP-10)** | **Vitamin D-Binding Protein (VDBP)** |
| **Interleukin-1 receptor antagonist (IL-1ra)** | **Vitamin K-Dependent Protein S (VKDPS)** |
| **Interleukin-12 Subunit p40 (IL-12p40)** | **Vitronectin** |
| **Interleukin-16 (IL-16)** | **von Willebrand Factor (vWF)** |
| **Interleukin-18 (IL-18)** | **YKL-40** |
| **Interleukin-2 receptor alpha (IL-2 receptor alpha)** |  |

**Supplementary Table 3. Analytes measured in common between the NESDA and unmedicated discovery cohorts and overlap between Bot et al and the present study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Analytes** | **Analytes measured in NESDA cohort** | **Altered in NESDA patients as reported by Bot et al** | **Analytes measured in the unmedicated discovery cohort examined in the present study** | **Analytes in the diagnostic biomarker-gender panel (present study)** |
| **EN-RAGE** | yes | yes | yes | yes |
| **Follicle-Stimulating Hormone (FSH)** | yes | yes | yes | yes |
| **Growth-Regulated alpha protein (GRO-alpha)** | yes | yes | yes | yes |
| **Interleukin-1 receptor antagonist (IL-1ra)** | yes | yes | yes | yes |
| **Interleukin-12 Subunit p40 (IL-12p40)** | yes | yes | yes | yes |
| **Macrophage Migration Inhibitory Factor (MIF)** | yes | yes | yes | yes |
| **Matrix Metalloproteinase-3 (MMP-3)** | yes | yes | yes | yes |
| **Pancreatic Polypeptide (PPP)** | yes | yes | yes | yes |
| **von Willebrand Factor (vWF)** | yes | yes | yes | yes |
| **Alpha-1-Antitrypsin (AAT)** | yes | yes | yes | --- |
| **Angiopoietin-2 (ANG-2)** | yes | yes | yes | --- |
| **Carcinoembryonic Antigen (CEA)** | yes | yes | yes | --- |
| **CD 40 antigen (CD40)** | yes | yes | yes | --- |
| **Luteinizing Hormone (LH)** | yes | yes | yes | --- |
| **Tenascin-C (TN-C)** | yes | yes | yes | --- |
| **Vascular Endothelial Growth Factor (VEGF)** | yes | yes | yes | --- |
| **Alpha-2-Macroglobulin (A2Macro)** | yes | --- | yes | yes |
| **Angiotensin-Converting Enzyme (ACE)** | yes | --- | yes | yes |
| **Angiotensinogen** | yes | --- | yes | yes |
| **AXL Receptor Tyrosine Kinase (AXL)** | yes | --- | yes | yes |
| **Cortisol (Cortisol)** | yes | --- | yes | yes |
| **Creatine Kinase-MB (CK-MB)** | yes | --- | yes | yes |
| **Factor VII** | yes | --- | yes | yes |
| **Ferritin (FRTN)** | yes | --- | yes | yes |
| **Growth Hormone (GH)** | yes | --- | yes | yes |
| **Insulin** | yes | --- | yes | yes |
| **Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)** | yes | --- | yes | yes |
| **Macrophage-Derived Chemokine (MDC)** | yes | --- | yes | yes |
| **Monocyte Chemotactic Protein 1 (MCP-1)** | yes | --- | yes | yes |
| **Myoglobin** | yes | --- | yes | yes |
| **Thyroid-Stimulating Hormone (TSH)** | yes | --- | yes | yes |
| **Thyroxine-Binding Globulin (TBG)** | yes | --- | yes | yes |
| **TNF-Related Apoptosis-Inducing Ligand Receptor 3 (TRAIL-R3)** | yes | --- | yes | yes |
| **Tumor necrosis factor receptor 2 (TNFR2)** | yes | --- | yes | yes |
| **Adiponectin** | yes | --- | yes | --- |
| **Apolipoprotein A-I (Apo A-I)** | yes | --- | yes | --- |
| **Apolipoprotein C-III (Apo C-III)** | yes | --- | yes | --- |
| **Apolipoprotein H (Apo H)** | yes | --- | yes | --- |
| **Apolipoprotein(a) (Apo(a))** | yes | --- | yes | --- |
| **Beta-2-Microglobulin (B2M)** | yes | --- | yes | --- |
| **Brain-Derived Neurotrophic Factor (BDNF)** | yes | --- | yes | --- |
| **C-Reactive Protein (CRP)** | yes | --- | yes | --- |
| **CD40 Ligand (CD40-L)** | yes | --- | yes | --- |
| **Chemokine CC-4 (HCC-4)** | yes | --- | yes | --- |
| **Chromogranin-A (CgA)** | yes | --- | yes | --- |
| **Complement C3 (C3)** | yes | --- | yes | --- |
| **Epidermal Growth Factor (EGF)** | yes | --- | yes | --- |
| **Epithelial-Derived Neutrophil-Activating Protein 78 (ENA-78)** | yes | --- | yes | --- |
| **FASLG Receptor (FAS)** | yes | --- | yes | --- |
| **Glutathione S-Transferase alpha (GST-alpha)** | yes | --- | yes | --- |
| **Haptoglobin** | yes | --- | yes | --- |
| **Hepatocyte Growth Factor (HGF)** | yes | --- | yes | --- |
| **Immunoglobulin A (IgA)** | yes | --- | yes | --- |
| **Immunoglobulin M (IgM)** | yes | --- | yes | --- |
| **Intercellular Adhesion Molecule 1 (ICAM-1)** | yes | --- | yes | --- |
| **Interleukin-16 (IL-16)** | yes | --- | yes | --- |
| **Interleukin-18 (IL-18)** | yes | --- | yes | --- |
| **Interleukin-8 (IL-8)** | yes | --- | yes | --- |
| **Leptin** | yes | --- | yes | --- |
| **Macrophage Colony-Stimulating Factor 1 (M-CSF)** | yes | --- | yes | --- |
| **Macrophage Inflammatory Protein-1 beta (MIP-1 beta)** | yes | --- | yes | --- |
| **Myeloperoxidase (MPO)** | yes | --- | yes | --- |
| **Plasminogen Activator Inhibitor 1 (PAI-1)** | yes | --- | yes | --- |
| **Platelet-Derived Growth Factor BB (PDGF-BB)** | yes | --- | yes | --- |
| **Progesterone** | yes | --- | yes | --- |
| **Prolactin (PRL)** | yes | --- | yes | --- |
| **Pulmonary and Activation-Regulated Chemokine (PARC)** | yes | --- | yes | --- |
| **Receptor for advanced glycosylation end products (RAGE)** | yes | --- | yes | --- |
| **Resistin** | yes | --- | yes | --- |
| **Serum Amyloid P-Component (SAP)** | yes | --- | yes | --- |
| **Sex Hormone-Binding Globulin (SHBG)** | yes | --- | yes | --- |
| **Sortilin** | yes | --- | yes | --- |
| **Stem Cell Factor (SCF)** | yes | --- | yes | --- |
| **Superoxide Dismutase 1, soluble (SOD-1)** | yes | --- | yes | --- |
| **T-Cell-Specific Protein RANTES (RANTES)** | yes | --- | yes | --- |
| **Testosterone, Total** | yes | --- | yes | --- |
| **Thrombospondin-1** | yes | --- | yes | --- |
| **Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)** | yes | --- | yes | --- |
| **Vascular Cell Adhesion Molecule-1 (VCAM-1)** | yes | --- | yes | --- |
| **Alpha-1-Antichymotrypsin (AACT)** | yes | yes | --- | --- |
| **Angiogenin** | yes | yes | --- | --- |
| **Apolipoprotein A-IV (Apo A-IV)** | yes | yes | --- | --- |
| **Apolipoprotein D (Apo D)** | yes | yes | --- | --- |
| **Cathepsin D** | yes | yes | --- | --- |
| **Cellular Fibronectin (cFib)** | yes | yes | --- | --- |
| **Complement Factor H – Related Protein 1 (CFHR1)** | yes | yes | --- | --- |
| **Cystatin-C** | yes | yes | --- | --- |
| **Fatty Acid-Binding Protein, adipocyte (FABP, adipocyte)** | yes | yes | --- | --- |
| **Fetuin-A** | yes | yes | --- | --- |
| **Hepsin** | yes | yes | --- | --- |
| **Insulin-like Growth Factor Binding Protein 5 (IGFBP5)** | yes | yes | --- | --- |
| **Lactoylglutathione lyase (LGL)** | yes | yes | --- | --- |
| **Matrix Metalloproteinase-10 (MMP-10)** | yes | yes | --- | --- |
| **Prostasin** | yes | yes | --- | --- |
| **Receptor tyrosine-protein kinase erbB-3 (ErbB3)** | yes | yes | --- | --- |
| **Urokinase-type plasminogen activator receptor (uPAR)** | yes | yes | --- | --- |
| **6Ckine** | yes | --- | --- | --- |
| **Aldose Reductase** | yes | --- | --- | --- |
| **Alpha-1-Microglobulin (A1Micro)** | yes | --- | --- | --- |
| **Apolipoprotein A-II (Apo A-II)** | yes | --- | --- | --- |
| **Apolipoprotein B (Apo B)** | yes | --- | --- | --- |
| **Apolipoprotein C-I (Apo C-I)** | yes | --- | --- | --- |
| **Apolipoprotein E (Apo E)** | yes | --- | --- | --- |
| **B cell-activating factor (BAFF)** | yes | --- | --- | --- |
| **C-Peptide** | yes | --- | --- | --- |
| **Cancer Antigen 15-3 (CA-15-3)** | yes | --- | --- | --- |
| **CD5 Antigen-like (CD5L)** | yes | --- | --- | --- |
| **Clusterin (CLU)** | yes | --- | --- | --- |
| **Collagen IV** | yes | --- | --- | --- |
| **E-Selectin** | yes | --- | --- | --- |
| **Endoglin** | yes | --- | --- | --- |
| **Endostatin** | yes | --- | --- | --- |
| **Eotaxin-1** | yes | --- | --- | --- |
| **Eotaxin-2** | yes | --- | --- | --- |
| **Epidermal Growth Factor Receptor (EGFR)** | yes | --- | --- | --- |
| **Fibulin-1C (Fib-1C)** | yes | --- | --- | --- |
| **Galectin-3** | yes | --- | --- | --- |
| **Gelsolin** | yes | --- | --- | --- |
| **Glucose-6-phosphate Isomerase (G6PI)** | yes | --- | --- | --- |
| **Hepatocyte Growth Factor receptor (HGF receptor)** | yes | --- | --- | --- |
| **Human Epidermal Growth Factor Receptor 2 (HER-2)** | yes | --- | --- | --- |
| **Insulin-like Growth Factor Binding Protein 4 (IGFBP4)** | yes | --- | --- | --- |
| **Insulin-like Growth Factor Binding Protein 6 (IGFBP6)** | yes | --- | --- | --- |
| **Insulin-like Growth Factor-Binding Protein 1 (IGFBP-1)** | yes | --- | --- | --- |
| **Insulin-like Growth Factor-Binding Protein 3 (IGFBP-3)** | yes | --- | --- | --- |
| **Interferon gamma Induced Protein 10 (IP-10)** | yes | --- | --- | --- |
| **Interleukin-2 receptor alpha (IL-2 receptor alpha)** | yes | --- | --- | --- |
| **Interleukin-23 (IL-23)** | yes | --- | --- | --- |
| **Interleukin-6 receptor (IL-6r)** | yes | --- | --- | --- |
| **Interleukin-6 receptor subunit beta (IL-6R beta)** | yes | --- | --- | --- |
| **Kallikrein 5** | yes | --- | --- | --- |
| **Latency-Associated Peptide of Transforming Growth Factor beta 1 (LAP TGF-b1)** | yes | --- | --- | --- |
| **Lectin-Like Oxidized LDL Receptor 1 (LOX-1)** | yes | --- | --- | --- |
| **Macrophage inflammatory protein 3 beta (MIP-3 beta)** | yes | --- | --- | --- |
| **Macrophage-Stimulating Protein (MSP)** | yes | --- | --- | --- |
| **Matrix Metalloproteinase-1 (MMP-1)** | yes | --- | --- | --- |
| **Matrix Metalloproteinase-7 (MMP-7)** | yes | --- | --- | --- |
| **Matrix Metalloproteinase-9, total (MMP-9, total)** | yes | --- | --- | --- |
| **Mesothelin (MSLN)** | yes | --- | --- | --- |
| **Monocyte Chemotactic Protein 2 (MCP-2)** | yes | --- | --- | --- |
| **Monocyte Chemotactic Protein 4 (MCP-4)** | yes | --- | --- | --- |
| **Monokine Induced by Gamma Interferon (MIG)** | yes | --- | --- | --- |
| **Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)** | yes | --- | --- | --- |
| **N-terminal prohormone of brain natriuretic peptide (NT proBNP)** | yes | --- | --- | --- |
| **Neuron-Specific Enolase (NSE)** | yes | --- | --- | --- |
| **Neuronal Cell Adhesion Molecule (Nr-CAM)** | yes | --- | --- | --- |
| **Neuropilin-1** | yes | --- | --- | --- |
| **Neutrophil Gelatinase-Associated Lipocalin (NGAL)** | yes | --- | --- | --- |
| **Osteopontin** | yes | --- | --- | --- |
| **Osteoprotegerin (OPG)** | yes | --- | --- | --- |
| **Pepsinogen I (PGI)** | yes | --- | --- | --- |
| **Phosphoserine Aminotransferase (PSAT)** | yes | --- | --- | --- |
| **Serotransferrin (Transferrin)** | yes | --- | --- | --- |
| **Stromal cell-derived factor-1 (SDF-1)** | yes | --- | --- | --- |
| **Tamm-Horsfall Urinary Glycoprotein (THP)** | yes | --- | --- | --- |
| **Tetranectin** | yes | --- | --- | --- |
| **Thrombomodulin (TM)** | yes | --- | --- | --- |
| **Thyroglobulin (TG)** | yes | --- | --- | --- |
| **Tissue type Plasminogen activator (tPA)** | yes | --- | --- | --- |
| **Transthyretin (TTR)** | yes | --- | --- | --- |
| **Trefoil Factor 3 (TFF3)** | yes | --- | --- | --- |
| **Tumor Necrosis Factor Receptor I (TNF RI)** | yes | --- | --- | --- |
| **Tyrosine kinase with Ig and EGF homology domains 2 (TIE-2)** | yes | --- | --- | --- |
| **Urokinase-type Plasminogen Activator (uPA)** | yes | --- | --- | --- |
| **Vascular Endothelial Growth Factor C (VEGF-C)** | yes | --- | --- | --- |
| **Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2)** | yes | --- | --- | --- |
| **Vascular endothelial growth factor receptor 3 (VEGFR-3)** | yes | --- | --- | --- |
| **Vitamin D-Binding Protein (VDBP)** | yes | --- | --- | --- |
| **Vitamin K-Dependent Protein S (VKDPS)** | yes | --- | --- | --- |
| **Vitronectin** | yes | --- | --- | --- |
| **YKL-40** | yes | --- | --- | --- |
| **Cancer Antigen 19-9 (CA-19-9)** | --- | --- | yes | yes |
| **Granulocyte Colony-Stimulating Factor (G-CSF)** | --- | --- | yes | yes |
| **Interleukin-1 alpha (IL-1 alpha)** | --- | --- | yes | yes |
| **Interleukin-13 (IL-13)** | --- | --- | yes | yes |
| **Interleukin-3 (IL-3)** | --- | --- | yes | yes |
| **Serum Glutamic Oxaloacetic Transaminase (SGOT)** | --- | --- | yes | yes |
| **Alpha-Fetoprotein (AFP)** | --- | --- | yes | --- |
| **B Lymphocyte Chemoattractant (BLC)** | --- | --- | yes | --- |
| **Bone Morphogenetic Protein 6 (BMP-6)** | --- | --- | yes | --- |
| **Eotaxin** | --- | --- | yes | --- |
| **Fas Ligand (FasL)** | --- | --- | yes | --- |
| **Heparin-Binding EGF-Like Growth Factor (HB-EGF)** | --- | --- | yes | --- |
| **Interleukin-10 (IL-10)** | --- | --- | yes | --- |
| **Interleukin-7 (IL-7)** | --- | --- | yes | --- |
| **Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha)** | --- | --- | yes | --- |
| **Prostatic Acid Phosphatase (PAP)** | --- | --- | yes | --- |
| **T Lymphocyte-Secreted Protein I-309 (I-309)** | --- | --- | yes | --- |
| **Thrombopoietin (TPO)** | --- | --- | yes | --- |
| **Tumor Necrosis Factor alpha (TNF-alpha)** | --- | --- | yes | --- |
| **Tumor Necrosis Factor beta (TNF-beta)** | --- | --- | yes | --- |

**Supplementary Table 4. Test performance achieved using the diagnostic biomarker-gender panel for differentiating depression patients and controls from the NESDA cohort**

Performance was evaluated using accuracy, sensitivity, specificity, predictive values, ROC curves and AUC [AUC (1) = perfect; AUC (≥0.8) = good; 0.6<AUC<0.8 = moderate; AUC (<0.6) = poor]. Optimal trade-offs between sensitivity and specificity were determined by maximising the Youden's index (J; calculated by J=sensitivity + specificity – 1) [1].

**A) NESDA patients and controls unfiltered for chronic non-psychiatric diseases or medications**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **AUC (95% CI)** | **\*p-value** (*AUC difference*: *panel vs panel with covariates*) | **FP** | **TP** | **TN** | **FN** | **Sens (%)** | **Spec (%)** | **FPR (%)** | **Acc** **(%)** |
| **MDD patients (468) against controls (305)** |
| MDD biomarker panel alone | 0.69 (0.65-0.73) | 3.496E-10 | 112 | 307 | 193 | 161 | 66 | 63 | 37 | 65 |
| MDD biomarker panel with covariates1 | **0.80 (0.77-0.83)** | 86 | 349 | 219 | 119 | 75 | 72 | 28 | 73 |
| Covariates alone1 | 0.74 (0.71-0.78) | ---- | 125 | 370 | 180 | 98 | 79 | 59 | 41 | 71 |
| **First episode MDD patients (220) against controls (305)**  |
| MDD biomarker panel alone | 0.71 (0.66-0.75) | 5.772E-09 | 96 | 140 | 209 | 80 | 64 | 69 | 31 | 66 |
| MDD biomarker panel with covariates2 | **0.82 (0.78-0.86)** | 37 | 134 | 268 | 86 | 61 | 88 | 12 | 77 |
| Covariates alone2 | 0.75 (0.71-0.79) | ---- | 139 | 181 | 166 | 39 | 82 | 54 | 46 | 66 |
| **First episode antidepressant free MDD patients (109) against controls (305)** |
| MDD biomarker panel alone | 0.73 (0.67-0.78) | 1.000E-04 | 86 | 69 | 219 | 40 | 63 | 72 | 28 | 70 |
| MDD biomarker panel with covariates3 | **0.80 (0.75-0.85)** | 78 | 80 | 227 | 29 | 73 | 74 | 26 | 74 |
| Covariates alone3 | 0.71 (0.65-0.76) | ---- | 71 | 58 | 234 | 51 | 53 | 77 | 23 | 71 |
| **MDD patients without anxiety comorbidity (166) against controls (305)** |
| MDD biomarker panel alone | 0.69 (0.64-0.74) | 0.001 | 122 | 121 | 183 | 45 | 73 | 60 | 40 | 65 |
| MDD biomarker panel with covariates4 | 0.76 (0.71-0.80) | 92 | 118 | 213 | 48 | 71 | 70 | 30 | 70 |
| Covariates alone4 | 0.67 (0.61-0.72) | ---- | 88 | 93 | 217 | 73 | 56 | 71 | 29 | 66 |
| **First episode MDD patients without anxiety comorbidity (73) against controls (305)** |
| MDD biomarker panel alone | 0.72 (0.65-0.78) | 4.000E-04 | 94 | 49 | 211 | 24 | 67 | 69 | 31 | 69 |
| MDD biomarker panel with covariates5 | **0.81 (0.75-0.87)** | 44 | 47 | 261 | 26 | 64 | 86 | 14 | 81 |
| Covariates alone5 | 0.71 (0.64-0.78) | ---- | 71 | 41 | 234 | 32 | 56 | 77 | 23 | 73 |
| **First episode antidepressant free MDD patients without anxiety comorbidity (43) against controls (305)** |
| MDD biomarker panel alone | 0.73 (0.65 0.82) | 0.021 | 49 | 24 | 256 | 19 | 56 | 84 | 16 | 80 |
| MDD biomarker panel with covariates6 | **0.80 (0.72 0.88)** | 89 | 34 | 216 | 9 | 79 | 71 | 29 | 72 |
| Covariates alone6 | 0.68 (0.59 0.77) | ---- | 125 | 30 | 180 | 13 | 70 | 59 | 41 | 60 |
| **MDD patients with anxiety comorbidity (302) against controls (305)**  |
| MDD biomarker panel alone | 0.71 (0.67-0.75) | 5.633E-10 | 88 | 188 | 217 | 114 | 62 | 71 | 29 | 67 |
| MDD biomarker panel with covariates7 | **0.83 (0.80-0.86)** | 38 | 190 | 267 | 112 | 63 | 88 | 12 | 75 |
| Covariates alone7 | 0.76 (0.73-0.80) | ---- | 68 | 189 | 237 | 113 | 63 | 78 | 22 | 70 |
| **First episode MDD patients with anxiety comorbidity (147) against controls (305)** |
| MDD biomarker panel alone | 0.74 (0.70-0.79) | 5.243E-07 | 120 | 114 | 185 | 33 | 78 | 61 | 39 | 66 |
| MDD biomarker panel with covariates8 | **0.85 (0.82-0.89)** | 52 | 105 | 253 | 42 | 71 | 83 | 17 | 79 |
| Covariates alone8 | 0.79 (0.74-0.83) | ---- | 101 | 116 | 204 | 31 | 79 | 67 | 33 | 71 |
| **First episode antidepressant free MDD patients with anxiety comorbidity (66) against controls (305)** |
| MDD biomarker panel alone | 0.77 (0.71 0.83) | 0.009 | 83 | 48 | 222 | 18 | 73 | 73 | 27 | 73 |
| MDD biomarker panel with covariates9 | **0.84 (0.79 0.89)** | 28 | 42 | 277 | 24 | 64 | 91 | 9 | 86 |
| Covariates alone9 | 0.75 (0.68 0.82) | ---- | 73 | 46 | 232 | 20 | 70 | 76 | 24 | 75 |

**The full list of covariates subjected to forward and backward stepwise logistic regression included** smoking status, alcohol dependence or abuse, gender, physical activity, chronic non-psychiatric diseases, education, anti-Inflammatory drug, heart medication, hormonal status, family history, BMI and age (**full model:** diagnosis (patient/control status) ~ covariate 1 + covariate 2 + ...... + covariate 12). The null model included just the intercept.

**The covariates selected in each stepwise regression model were:**

1covariates: alcohol dependence or abuse, chronic non-psychiatric diseases, education, family history and age.

2covariates: alcohol dependence or abuse,chronic non-psychiatric diseases, education and family history.

3,5covariates: alcohol dependence or abuse,chronic non-psychiatric diseases and education.

4covariates: alcohol dependence or abuse,chronic non-psychiatric diseases, family history and anti-Inflammatory drug.

6covariates: alcohol dependence or abuse andchronic non-psychiatric diseases

7,8covariates: alcohol dependence or abuse,chronic non-psychiatric diseases, education, family history and BMI.

9covariates: alcohol dependence or abuse,education, family history and age.

**B) NESDA** **patients and controls free of any chronic non-psychiatric diseases or medications**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **AUC (95% CI)** | **\*p-value** (*AUC difference*: *panel vs panel with covariates*) | **FP** | **TP** | **TN** | **FN** | **Sens (%)** | **Spec (%)** | **FPR (%)** | **Acc****(%)** |
| **MDD patients (215) against controls (193)** |
| MDD biomarker-gender panel alone | 0.71 (0.66-0.76) | 4.000E-04 | 71 | 149 | 122 | 66 | 69 | 63 | 37 | 66 |
| MDD biomarker panel with covariates8 | 0.78 (0.74-0.82) | 54 | 157 | 139 | 58 | 73 | 72 | 28 | 73 |
| Covariates alone8 | 0.69 (0.64-0.74) | ---- | 43 | 118 | 150 | 97 | 55 | 78 | 22 | 66 |
| **First episode MDD patients (96) against controls (193)** |
| MDD biomarker-gender panel alone | 0.74 (0.69-0.80) | 4.000E-04 | 93 | 81 | 100 | 15 | 84 | 52 | 48 | 63 |
| MDD biomarker panel with covariates9 | **0.82 (0.77-0.87)** | 37 | 67 | 156 | 29 | 70 | 81 | 19 | 77 |
| Covariates alone9 | 0.73 (0.66-0.79) | ---- | 77 | 76 | 116 | 20 | 79 | 60 | 40 | 66 |
| **First episode antidepressant free MDD patients (55) against controls (193)** |
| MDD biomarker-gender panel alone | 0.78 (0.71-0.84) | 0.007 | 50 | 39 | 143 | 16 | 71 | 74 | 26 | 73 |
| MDD biomarker panel with covariates10 | **0.85 (0.79-0.90)** | 66 | 47 | 127 | 8 | 85 | 66 | 34 | 70 |
| Covariates alone10 | 0.74 (0.66-0.82) | ----- | 72 | 44 | 121 | 11 | 80 | 63 | 37 | 67 |
| **MDD patients without comorbid anxiety (76) against controls (193)** |
| MDD biomarker panel alone | 0.73 (0.66-0.79) | ---- | 57 | 48 | 136 | 28 | 63 | 70 | 30 | 68 |
| **First episode MDD patients without comorbid anxiety (31) against controls (193)** |
| MDD biomarker-gender panel alone | 0.79 (0.70-0.88) | ---- | 55 | 23 | 138 | 8 | 74 | 72 | 28 | 72 |
| **First episode antidepressant free MDD patients without comorbid anxiety (21) against controls (193)** |
| MDD biomarker-gender panel alone | **0.85 (0.75-0.96)** | 0.542 | 16 | 16 | 177 | 5 | 76 | 92 | 8 | 90 |
| MDD biomarker panel with covariates11 | **0.87 (0.78-0.96)** | 9 | 14 | 184 | 7 | 67 | 95 | 5 | 93 |
| Covariates alone11 | 0.62 (0.51-0.73) | ---- | 36 | 9 | 157 | 12 | 43 | 81 | 19 | 78 |
| **MDD patients with comorbid anxiety (139) against controls (193)** |
| MDD biomarker-gender panel alone | 0.75 (0.70-0.80) | 7.000E-04 | 91 | 119 | 102 | 20 | 86 | 53 | 47 | 67 |
| MDD biomarker panel with covariates12 | **0.82 (0.77-0.86)** | 40 | 100 | 153 | 39 | 72 | 79 | 21 | 76 |
| Covariates alone12 | 0.73 (0.68-0.79) | ---- | 68 | 100 | 125 | 39 | 72 | 65 | 35 | 68 |
| **First episode MDD patients with comorbid anxiety (65) against controls (193)** |
| MDD biomarker-gender panel alone | **0.81 (0.75-0.86)** | 0.002 | 62 | 55 | 131 | 10 | 85 | 68 | 32 | 72 |
| MDD biomarker panel with covariates13 | **0.88 (0.83-0.92)** | 41 | 55 | 152 | 10 | 85 | 79 | 21 | 80 |
| Covariates alone13 | 0.77 (0.71-0.84) | ---- | 29 | 41 | 164 | 24 | 63 | 85 | 15 | 79 |
| **First episode antidepressant free MDD patients with comorbid anxiety (34) against controls (193)** |
| MDD biomarker-gender panel alone | **0.86 (0.80-0.92)** | 0.020 | 57 | 30 | 136 | 4 | 88 | 70 | 30 | 73 |
| MDD biomarker panel with covariates14 | **0.92 (0.87 0.96)** | 50 | 32 | 143 | 2 | 94 | 74 | 26 | 77 |
| Covariates alone14 | **0.80 (0.70 0.89)** | ---- | 29 | 24 | 164 | 10 | 71 | 85 | 15 | 83 |

**The full list of covariates subjected to forward and backward stepwise logistic regression included** smoking status, alcohol dependence or abuse, gender, physical activity, education, hormonal status, family history, BMI and age (**full model:** diagnosis (patient/control status) ~ covariate 1 + covariate 2 + ...... + covariate 9). The null model included just the intercept.

**The covariates selected in each stepwise regression model were:**

8,12covariates: alcohol dependence or abuse, education, family history and age.

9,10covariates: alcohol dependence or abuse, education and age.

11covariates: alcohol dependence or abuse.

13,14covariates: education, family history and age

**Key:** **AUC =** area under curve; **CI =** confidence interval; **\*p-value (*AUC difference*: *panel vs panel with covariates*) =** AUC of panel compared to the AUC of panel with covariates; **FPR =** false positive rate; **FP =** number of false positives; **TP =** number of true positives; **TN =** number of true negatives; **FN =** number of false negatives; **Sens =** sensitivity; **Spec =** specificity; **Acc** = Accuracy. **Bold =** AUC>0.8.

**Table 3 reference**

1 Fluss R, Faraggi D, Reiser B: Estimation of the youden index and its associated cutoff point. Biom J 2005;47:458-472.