PROJECT

Supervisor:	Dr Raffaella Zanardi
Title:	Integrating Immune Dysregulation, Mitochondrial Dysfunction, and Oxidative Stress in Major Depressive Disorder: A Multifaceted Approach
Curriculum:	Molecular Medicine - Neurosciences and Experimental Neurology
Link to the personal page of the University or relevant hospital site website:	https://www.hsr.it/dottori/raffaella-zanardi

Description of the Project (max 3,000 characters including spaces)

<u>Background/gap of knowledge</u>

Major Depressive Disorder (MDD) is one of the leading causes of disability worldwide, and approximately 30% of patients develop treatment-resistant depression (TRD), showing limited response to conventional monoaminergic antidepressants. Despite substantial advances in the field, the biological mechanisms underlying MDD remain only partially understood.

Increasing evidence supports the hypothesis that low-grade systemic inflammation contributes to the pathophysiology of mood disorders. Elevated circulating pro-inflammatory cytokines, altered immune activation, and abnormalities in T-cell populations have consistently been described in MDD patients. Peripheral immune dysregulation appears closely associated with structural and functional brain alterations, suggesting a bidirectional interaction between the immune system and the central nervous system.

Although inflammation has been widely investigated in mood disorders, fewer studies have comprehensively integrated peripheral inflammatory markers, immune-cell phenotyping, multimodal brain MRI, and detailed clinical characterization within the same well-defined cohort. Bridging peripheral immune measures with neuroimaging and clinical phenotypes may improve our understanding of biologically distinct subtypes of MDD and contribute to the identification of clinically relevant biomarkers associated with symptom severity and treatment resistance.

<u>Rationale and hypothesis</u>

MDD is increasingly conceptualized as a systemic disorder involving reciprocal interactions between peripheral immune activation and altered brain function. However, inflammatory markers and neuroimaging measures are often investigated separately, limiting the identification of integrated signatures associated with clinical severity and treatment resistance.

The central hypothesis of the present project is that MDD patients show peripheral immune dysregulation, characterized by altered circulating inflammatory mediators and immune-cell profiles, and that these abnormalities are associated with specific structural and functional brain alterations. In particular, we hypothesize that a pro-inflammatory immune profile is linked to brain networks and regions implicated in mood regulation, cognition, and stress-related vulnerability, ultimately contributing to depressive symptomatology and treatment resistance.

Specifically, we hypothesize that:

1. MDD patients will show altered circulating inflammatory mediators and immune-cell profiles compared with expected reference patterns and/or clinically relevant subgroups;

2. Pro-inflammatory cytokine and chemokine profiles will be associated with altered T-cell populations and activation patterns;

3. Peripheral inflammatory and immune alterations will correlate with brain structural and functional MRI measures and with clinical variables, including depression severity and treatment resistance.

The integration of inflammatory, immunophenotypic, neuroimaging, and clinical variables using multivariate statistical approaches may identify biologically meaningful patterns associated with specific clinical features of MDD.

Objectives and specific aims

Specific Aims

Specific Aim 1 - Characterize peripheral inflammatory profiles in MDD

To characterize circulating inflammatory mediators in patients with MDD using available plasma samples.

Methods

- Quantification of circulating cytokines, chemokines, and growth factors using multiplex immunoassays (BioPlex);

- Assessment of inflammatory profiles in relation to clinical variables, including depression severity, treatment resistance, illness duration, BMI, smoking status, and current pharmacological treatment;

- Identification of inflammatory patterns through correlation analyses and dimensional reduction approaches.

Expected outcomes

We expect MDD patients, particularly those with more severe clinical features or treatment resistance, to show a more pronounced pro-inflammatory profile, including increased levels of selected cytokines, chemokines, and growth factors involved in immune activation and neuroimmune communication.

Specific Aim 2 - Define immune-cell profiles and their relationship with inflammatory mediators

To investigate peripheral immune-cell populations and their relationship with circulating inflammatory markers.

Methods

- Flow cytometry (FACS) analysis of immune cell populations and T-cell subpopulations, including Th1, Th17, and regulatory T cells, according to available datasets;
- Evaluation of immune activation patterns and their association with circulating inflammatory mediators;
- Correlation and regression analyses between cytokine/chemokine levels, immune-cell profiles, and clinical characteristics.

Expected outcomes

We expect pro-inflammatory cytokine and chemokine profiles to be associated with altered T-cell distribution and activation patterns, supporting the presence of peripheral immune dysregulation in clinically relevant subgroups of MDD patients.

Specific Aim 3 - Identify associations between peripheral inflammation, brain MRI measures, and clinical phenotype

To evaluate the association between peripheral inflammatory/immune alterations and multimodal MRI findings in MDD.

Methods

- Analysis of structural MRI measures, including cortical thickness, subcortical volumes, and white matter microstructure;
- Functional connectivity analyses using resting-state fMRI;
- Correlation and multivariate analyses linking MRI measures with inflammatory markers, immune-cell profiles, and clinical variables including depression severity and treatment resistance.

Expected outcomes

We expect peripheral inflammatory and immune alterations to correlate with structural and functional changes in brain regions and networks implicated in mood regulation, cognitive functioning, salience processing, and stress-related vulnerability.

Expected outcomes

We expect to elucidate the interconnection of clinical phenotypes, immune dysregulation and brain alteration in MDD, investigating how these mechanisms influence each other. Through the above mentioned analysis, the study seeks to identify potential biomarkers associated with immune dysregulation, brain alterations and clinical features. These biomarkers could serve as indicators of different clinical outcomes (e.g. disease severity, treatment response, TRD) or specific biological subtypes within the MD population, contributing to the development of personalized interventions and more targeted and effective treatment approaches.

Skills that the student should acquire (max. 600 characters including spaces):

Statistics: General and Generalized Linear Model, Structural equation modeling; Machine learning tools (PCA, K-means), Matlab, R, Python, Stata, OS: Linux;

- Cellular Analysis: Flow cytometry (FACS), Seahorse Cell Metabolism Technology, fluorescence techniques and probe- based approaches, PBMCs isolation.
- Drafting and publishing papers;
- MRI analyses: VBM, subcortical volumes and cortical thickness; Tract-Based Spatial Statistics and tractography; seed-to- voxel, ROI-to-ROI connectivity, ICA networks for fMRI.

References (max. 15)

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6. Fries, G.R., et al., Molecular pathways of major depressive disorder converge on the synapse. Mol Psychiatry, 2023. 28(1):p. 284-297.
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11. Solleiro-Villavicencio, H. and S. Rivas-Arancibia, Effect of Chronic Oxidative Stress on Neuroinflammatory Response Mediated by CD4+T Cells in Neurodegenerative Diseases. Frontiers in Cellular Neuroscience, 2018. 12.
12. Fenster, R.J. and J.L. Eisen, Checking the Brain's Immune Privilege: Evolving Theories of Brain-Immune Interactions. Biol Psychiatry, 2017. 81(2): p. e7-e9.
13. Haroon, E., A.H. Miller, and G. Sanacora, Inflammation, Glutamate, and Glia: A Trio of Trouble in Mood Disorders. Neuropsychopharmacology, 2017. 42(1): p. 193-215.