Reporting Psychiatric Disease Characteristics in Post-Mortem- and Biological Research

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ABSTRACT: Inflammation is a prominent hypothesis in the neurobiology of depression. In our transcriptomic profiling study of microglia in chronic major depressive disorder (MDD), we revealed a distinct disease-associated microglia (DAM) transcriptomic profile exclusively found in cortical gray matter, that we have designated DepDAM. These DepDAM revealed an immune-suppressed state, with a possible upstream mechanism for microglial suppression, by upregulation of CD200 and CD47 ("don't eat me signals") located on synapses. We extensively report on disease characteristics, such as cause of death, reason for euthanasia, and psychiatric state when deceased. When excluding MDD donors in a euthymic state, the trend of lower CD45 membrane expression on white matter microglia became significant, and the difference in gray matter microglia became larger. For Western blot analysis of CD47 and CD200, both means of the definitely depressed donor groups (MDD-D) increased. This underscores the utmost importance of reporting on patient and episode characteristics, such as severity, episode traits, (type of) suicidality, mode of decease, and state of illness at death in post-mortem- and biological psychiatric research. For psychiatric post-mortem research, we suggest using well-characterized donors (eg, after "psychological autopsy") selected by an experienced clinician.

KEYWORDS: Microglia, neuroimmunology, neuropsychology

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Introduction

A major pathophysiological theory on the neurobiology of depression is dysfunctional neuroplasticity with reduced numbers of synapses.[1,2] Microglia are the main brain-resident macrophages and responsible for—among things—homeostasis of neuronal plasticity, by removing excessive proteins, dysfunctional synapses, and aberrant neurons.[3] In our transcriptomic profiling study of microglia in major depressive disorder (MDD), we revealed a distinct disease-associated microglia (DAM) transcriptomic profile exclusively found in cortical gray matter (GM), that we have designated DepDAM.[4] These DepDAM revealed an immune-suppressed state, with a lower CD45 membrane expression and differentially expressed genes downregulated in immune responses (*MK167*, SPP1, *C1QA/B/C*) and phagocytic function (*FCGR1A/C*, *FCGR3A*, *CD14*, *CD163*). We also showed that the possible upstream mechanism for microglial suppression may be neuronal interference by upregulation of CD200 and CD47 ("don't eat me signals") located on synapses. The majority of the depressed donors suffered from chronic depression, with an average age of onset of 29.5 (range 14-48) years and average duration of disease of 35.3 (range 16-54) years.

Major Depressive Disorder, a Heterogeneous Disorder

Neuropsychiatric disorders are symptom-based diagnoses, using the *Diagnostic and Statistical Manual of Mental Disorders*–5 (DSM-5) classification system, based on phenotypes within predefined disease spectra. Neuropathologic substrates for psychiatric disorders remain largely unknown and their connection with phenotypes and the heterogeneity within psychiatric diseases (especially MDD) is missing. However, from both a clinical and biological perspective, it seems evident that psychiatric neurobiology may change over time. Depression is one of the

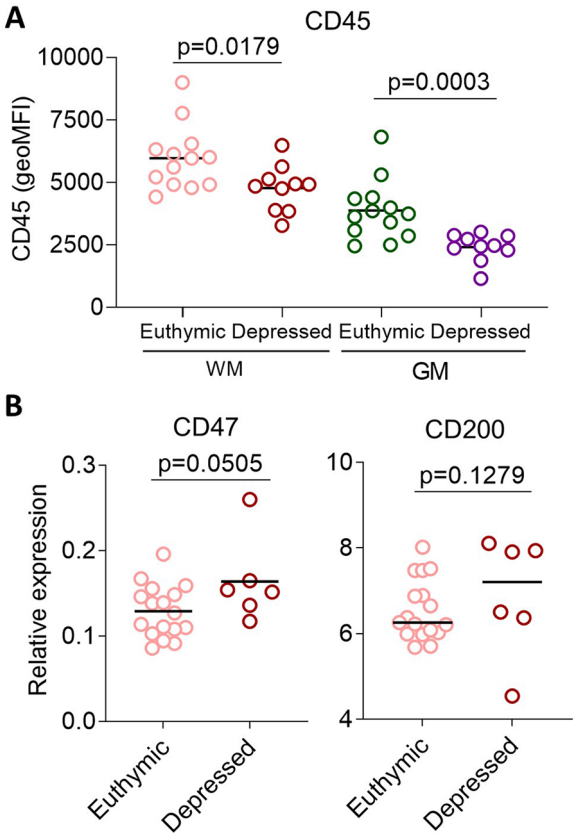

Figure 1. Membrane CD45 expression on microglia and CD45/CD200 expression in synaptosomes outcomes when excluding the MDD donors in a euthymic state. (A) CD45 membrane expression on GM and WM microglia was detected by flow cytometry directly after isolation. (B) CD47 and CD200 expression were analyzed by Western blots in cortical synaptosomes. See Scheepstra et al[4] for technical details. Statistics used are one-tailed Mann-Whitney *U* tests.

most heterogeneous disorders, with a large interindividual variability along the phenotypical spectrum, and a fluctuating course of disease. Some patients report primarily behavioral inhibition, such as psychomotor retardation and hypersomnia, while others report the exact opposite with symptoms, such as insomnia, agitation, and anxiety. Both, however, are classified as MDD according to the DSM-5. Also, neuroimmunology and -plasticity are among the most fluctuating and dynamic pathways of the brain. As in other diseases of the brain, 1 neuroplastic and microglial state that reflects the neurobiology of all MDD patients is unlikely. One of the core characteristics of microglia is to interact with- and adapt to the cellular environment and their (patho)physiological conditions, resulting in many different cell states and distinct disease-associated microglia.[5,6] Postmortem studies on multiple sclerosis also show a strong correlation between clinical characteristics (such as disease severity) with pathological findings and microglial state.[7] Collectively, the large phenotypical variability and accumulating biological evidence suspects multiple biotypes and states in MDD. Nevertheless, the state of disease, mode of decease (eg, euthanasia), severity and episode traits (including suicidality) remain largely underreported in human molecular psychiatric research, especially in post-mortem studies.

Disease Characteristics as Confounding Factors

In our paper, we extensively report on disease characteristics, such as cause of death, reason for euthanasia, and psychiatric state when deceased. The selection of donors was done by an experienced psychiatrist, performing a "psychological autopsy" and selecting those with a definite diagnosis of severe and recurrent MDD, without comorbidities. As reported in the original manuscript, the most significant findings in our CD163 immunoblots were found in patients with a definite depressed state when deceased. This prompted us to explore the effects of a depressed state on our other outcomes, such as the CD45 membrane expression on microglia and CD47/CD200 expression on synapses. Originally we reported a significantly lower CD45 expression on GM microglia and a trend towards lower expression on WM microglia, detected by flow cytometry directly after microglia isolation. When adding donors in a euthymic state to the control group, this trend in WM microglia became significant and the difference in GM microglia became even larger (Figure 1A). For Western blot analysis of CD47 and CD200, both means of the definite depressed donor groups increased (Figure 1B), however lacking statistical power to make a definite conclusion. Nevertheless, these data may illustrate the impact of severity and state of psychiatric disease on biological findings.

This is in line with other studies, such as Holmes et al. (2019) that used a new positron emission tomography (PET) radioligand ([^{11}C] USB-J) for synaptic vesicle 2A (SV2A) protein to quantify synapse density in MDD and posttraumatic stress disorder (PTSD)[2]. The most significant decrease in synapse density was found in patients with a high severity. They also showed that only patients with a "synaptic deficiency" showed reversal after esketamine treatment, suggesting a distinct neuroplastic biotype in MDD.[8] Another disease characteristic that has shown distinct molecular findings in post-mortem research is suicidality. Suicide brains have shown distinct molecular alterations compared to depressed non-suicidal brains. It is suggested that suicidality is involved in distinct pathways in the brain, but it could also be explained due to depression severity in suicidal MDD patients or the definite depressed state at death.[9] Suicidality is a complex phenomenon with different types of suicidality, co-dependent on other episode characteristics, such as psychotic traits, anxiety, episode duration, and severity.[10]

To rule out effects related to the mode of decease (euthanasia vs. natural death), we analyzed CD45 expression for euthanized donors only in control versus MDD patients (n = 4 vs 9). Euthanasia did not influence CD45 membrane expression, as CD45 remains significantly lower in GM microglia despite the low number of donors ($P = .0378$). Also age did not have an effect on CD45 expression ($P = .925$).

Temporal Effects of Depression on Microglia

Our donors had a long mean disease duration, with often chronic depression and a chronic form of suicidality, as a large proportion of donors were euthanized due to refractory depression (6 out of 13 in the RNA sequencing group). Also in pre-clinical models for depression, there is evidence that depression can be associated with the presence of distinct and contrasting phenotypes of microglia. These microglial phenotypes may depend on disease characteristics, and were coined activated or suppressed depressive disorder–associated microglia (ActDepDAM or SupDepDAM, respectively) by Yirmiya (2023).[11] One possibility for contrasting phenotypes is a temporal effect of depression in microglia, with primarily ActDepDAM in the acute phase and SupDepDAM in a chronic phase of depression. This temporal connection is substantiated by research conducted on a mouse model of depression. In these experiments, repeated exposure to stressors led to initial activation of microglia within the first few days, followed by reduced expression of activation markers as well as dystrophic morphology at a later time point[12]. Additionally, in mice exhibiting depressive-like symptoms, treatment with immunostimulatory drugs or electroconvulsive therapy (ECT, which is usually given in a more chronic phase with non-response to initial antidepressants) restored normal microglia morphology and demonstrated microglia-stimulating effects.[12,13]

To conclude, MDD is a heterogeneous disorder, with a fluctuating course of disease and a broad phenotypical spectrum. There is increasing evidence that molecular findings may depend on the state and course of disease. It is therefore of utmost importance that studies in humans extensively report on patient- and episode characteristics, such as severity, episode traits, (type of) suicidality, and state of illness at death. For psychiatric postmortem research, we suggest using well-characterized donors (eg, after "psychological autopsy") selected by an experienced clinician.

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Author Contributions

KS and IH drafted the manuscript. MM, DW, CH, LZ, DS and JH critically revised the manuscript.

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REFERENCES

1. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med*. 2016;22:238-249.
2. Holmes SE, Scheinost D, Finnema SJ, et al. Lower synaptic density is associated with depression severity and network alterations. *Nat Commun*. 2019;10:1529.
3. Hammond BP, Manek R, Kerr BJ, Macaule MS, Plemel JR. Regulation of microglia population dynamics throughout development, health, and disease. *GLIA*. 2021;69:2771-2797.
4. Scheepstra KWF, Mizee MR, van Scheppingen J, et al. Microglia transcriptional profiling in major depressive disorder shows inhibition of cortical gray matter microglia. *Biol Psychiatry*. 2023;94:619-629.
5. Böttcher C, Schlickeiser S, Sneeboer MAM, et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci*. 2019;22:78-90.
6. Paolicelli RC, Sierra A, Stevens B, et al. Microglia states and nomenclature: a field at its crossroads. *Neuron*. 2022;110:3458-3483.
7. Luchetti S, Fransen NL, van Eden CG, Ramaglia V, Mason M, Huitinga I. Progressive multiple sclerosis patients show substantial lesion activity that correlates with clinical disease severity and sex: a retrospective autopsy cohort analysis. *Acta Neuropathol*. 2018;135:511-528.
8. Holmes SE, Finnema SJ, Naganawa M, et al. Imaging the effect of ketamine on synaptic density (SV2A) in the living brain. *Mol Psychiatry*. 2022;27:2273-2281.
9. Zhao J, Lucassen PJ, Swaab DF. Suicide is a confounder in postmortem studies on depression. *Biol Psychiatry*. 2019;86:e37-e40.
10. Díaz-Oliván I, Porras-Segovia A, Barrigón ML, Jiménez-Muñoz L, Baca-García E. Theoretical models of suicidal behaviour: a systematic review and narrative synthesis. *Eur J Psychiatry*. 2021;35:181-192.
11. Yirmiya R. Depressive disorder–associated microglia as a target for a personalized antidepressant approach. *Biol Psychiatry*. 2023;94:602-604.
12. Kreisel T, Frank MG, Licht T, et al. Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol Psychiatry*. 201419:699-709.
13. Rimmerman N, Verdiger H, Goldenberg H, et al. Microglia and their LAG3 checkpoint underlie the antidepressant and neurogenesis-enhancing effects of electroconvulsive stimulation. *Mol Psychiatry*. 2022;27:1120-1135.