



# Neuroimmune and Acute Psychotic Disorders had an Overlapping Immune-Signature in Adolescents and Young Adults; A Case Series

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## Authors' contributions

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

A misguided auto-reactive injury is responsible for diverse types of central nervous system (CNS) conditions. We suspect that, in some of these conditions, the adaptive immune system have a common cellular immune pathogenesis, driven predominantly by T cells, despite variability on the phenotypical clinical presentation.

**Aim:** the main goal of this study is to characterize a portion of the adaptive immune response (AIR) on patients presenting with clinical symptoms compatible with monophasic acute neuroimmune disorders (NID) including Psychotic Disorders (PD).

**Methodology:** flow cytometry with deep immunophenotyping of T effector (Teff) and T regulatory (Treg) cells was performed on peripheral blood obtained during the acute clinical phase and compared it to the one from an age-matched cohort group [Co].

**Results:** our preliminary findings point toward the presence of common "immunosignature" in individuals affected by NID or PD. We also found a shared dysregulation of immune related neurogenes in NID and PD that were not present in normal cohorts.

**Conclusions:** this preliminary report gives some insights into the underlying shared pathobiology. If we can improve our capacity for early accurate diagnosis and meaningful disease monitoring of pathogenic T cell subsets, we will both expedite disease detection and may serve as a guide the administration of effective immunotherapeutic agents.

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**Keywords:** NID: Neuroimmune disorders; AIR: Adaptive immune response; PD: Psychotic Disorders.

## 1. INTRODUCTION

Neurological and mental health disorders are suspected to be the product of a multifactorial imbalance involving genetics and environment factors. The adaptive immune response (AIR) has entered the fray of etiological factors that lead to chronic and acute central nervous system (CNS) debilitating diseases.

Our current understanding is that the CNS is not immune-privileged [1], the blood brain barrier does not shield the brain from the immune system [2], primary CNS events can trigger the AIR and sometimes this activation is characterized by self-reactivity [3-4]. The CNS could be the target of an AIR mediated injury with protean manifestations, from monophasic acute insults to intermittent chronic relapsing states. In these settings, the arbitrary division between neurological and psychiatric disorders is blurry, at best, when observed through immune-CNS system interactions. These aberrant interactions can lead to motor deficits, movement disorders, altered states of consciousness, as well as changes in mood, cognition and behavior [5]. Post injurious behavioral or sensory-motor compensatory mechanisms shape pathognomonic clinical manifestations pigeonholing conditions into medical subspecialties even though they share a similar pathobiology.

In selected neuroimmune disorders (NID) and some psychotic disorders (PD) a dysregulation of the AIR seem to be implicated [6-8]. Discerning peripheral immune patterns that can be utilized as "immune biomarker" of a CNS pathobiology could be adjunctive tools in the diagnostic process and surrogates heralding clinical efficacy. Uncovering Immune biomarkers, encompassing monophasic acute neuroimmune disorders (NID) and some PD can have wide reaching implications. It may constitute the foundation of novel therapeutic avenues benefiting from the cross-fertilization that occurs from the clinical experience drawn from different medical specialties.

NID tends to exhibit clinically manifestations close to the time of the injurious process, and the clinical response to therapeutic trials is relatively rapid [9]. Therefore, efficacy, or lack of it, could be ascertained shortly after therapeutic attempts.

The assessment of efficacy in PD is far more complex and does not benefit from the short temporal-initiation association.

The main goal of this study is to characterize the AIR of patients suspected to have NID, patients whose diagnoses was later found to be PD not associated with a known NID and to compare individuals whose diagnosis was Transverse Myelitis, N-methyl-D-aspartate receptor (NMDAR) encephalitis and Miller Fisher Syndrome (variant of Guillain Barre), with patients with PD and a cohort (Co) of age matched controls.

## 2. METHODOLOGY

### 2.1 Patient Enrollment

All procedures were approved by local Institutional Review Boards and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from participants (or their legal guardian in the case of minors or temporary incapacitated individuals) prior to enrollment. Patients aged from 10-26 years of age admitted or transferred to any of the participating institutions with suspicion of a NID or conditions that mimic its clinical presentation were eligible to participate.

- **Inclusion clinical criteria:** rapidly progressive changes in consciousness or motor deficits, dyskinesia, or acute psychiatric disturbance.
- **Exclusion criteria:** patients with known autoimmune disorders or CNS degenerative, vascular or bacterial/fungal infectious condition or with likely adverse effects from recreational drugs or therapeutic agents; individuals that may have received, previous to the acute clinical presentation, agents that are directed to modify the immunological response.
- **Conclusive diagnosis:** was reached utilizing CSF and serological results, MRI and or nerve conduction studies combined with medical trajectory and opinions from critical care, neurology and psychiatry consultants as well as 6 month follow up.

After the data was collected, prior to data analysis and subsequent to hospital discharge patients were allocated into groups by conclusive

diagnosis: Group 1) acute psychosis (AP) not associated with an oncologic process or autoimmune condition; Group 2) known NID conditions and; Group 3) a cohort of age-matched healthy controls (Co) to Groups 1 and 2. Enrolled patients with inconclusive diagnosis or non-NID conditions were excluded from this report.

Peripheral blood was drawn upon clinical presentation. We performed a comprehensive flow cytometric immunophenotyping in peripheral T effector (Teff) and T regulatory (Treg) cells. Direct immunofluorescence surface staining of whole blood samples with 6 antibody panels was performed. Data were acquired on an LSRFortessa (BD Biosciences) and analyzed with FlowJo software (v9; Tree Star). Our analysis focused on peripheral Teff and Treg cells based on the pertinent psychiatric and neuroimmune literature.

## 2.2 Statistical Analysis

Immunophenotyping: Unpaired and paired t-tests between groups was utilized for continuous variables when appropriate. This was utilized to develop an algorithm of cellular lines, the *Neuro Immune Disorders Adaptive Immunity* score (NIDAI-score), taking in to consideration the regulatory and counterregulatory cell subsets Teff, Treg and Tconv [10] Statistical significance was established at  $p < 0.05$ .

## 3. RESULTS

Ten patients were enrolled, seven with NID and three with PD as final diagnosis, the participant's vital statistics and diagnosis are described in Table 1.

Patients with NID or PD had a similar immunosignature when compared among them characterized by an altered of selected CD4+/CD8+ ratio.

The Tcell as % of lymphocytes, CD4+ % of the Tcell population and CD4/CD8 ratio were not dissimilar between NID/PD and Co (Table 2).

However, the CD4<sup>+</sup> Teff subsets associated with autoreactive cell lineage underwent an expansion in NID/PD (Table 2). This clonal expansion was "uncontrolled", as the ratio between autoreactive cells and regulatory cells in the CD4+ group was affected. Most strikingly, RO+ Treg cells in NID/PD had a significant decrease in the mfi CD25 receptor than the cohort most likely due to shedding of the

receptor. Moreover, the expression CCR4 in RO+ as % Tconv cells also underwent a clone expansion. The CD8+ group as % T cell population, was increased in the setting of self-injurious milieu, with a significant decrease in the Tem lineage as % of CD8+ (Table 2).

When we apply this interrelated CD4/CD8 sub-lineage changes in the NIDAI-score algorithm the groups can be distinguished Fig. 1 as we have reported previously in an abridged NIDAI score [10].

## 4. DISCUSSION

In the present report, we describe a case series of young individuals with an overlapping, pathological AIR when affected by PD or selective NID (Transverse Myelitis, NMDAr encephalitis and Fisher Miller Syndrome). This is tantamount to a common pathobiological mechanism involving a selective immune dysregulation with the potential to be the foundation for treatments based on precision medicine. It is tantalizing to conceptualize that these entities, while having widely different clinical manifestation, shared a similar pathobiology.

The CNS is not immune privileged. The existence of a "glymphatic system" in the CNS has been well established, being the efferent arm for debris from the CNS to expose antigen to the adaptive immune system. [8,11] Indeed, Traka, et al. described the presence of material from oligodendrocyte apoptosis in peripheral lymph nodes and this was sufficient to trigger autoimmune reactions leading to demyelination. [12]

Autoimmune mechanisms are well established in NID and strongly suspected in PD disorders. NID have now become the subject of intense scientific scrutiny as new pathogenic auto-antibodies are being discovered at precipitous rates [13]. Dalmau, et al. described the association of altered consciousness, movement disorders and psychosis with the presence of N-methyl-D-aspartate receptor antibodies (NMDAr) associated with teratomas. [14] Patients whose symptoms until then had been classified as idiopathic and psychiatric in nature, were found to have an autoimmune etiology which responded to treatment with immune-modulating drugs. Once the immunosignature for NMDAr was available it impacted discoveries in an area associated with infectious disease. Herpes

encephalitis can trigger an AIR producing a secondary self-mediated CNS insults, via NMDAR antibodies, weeks after the initial infection [4].

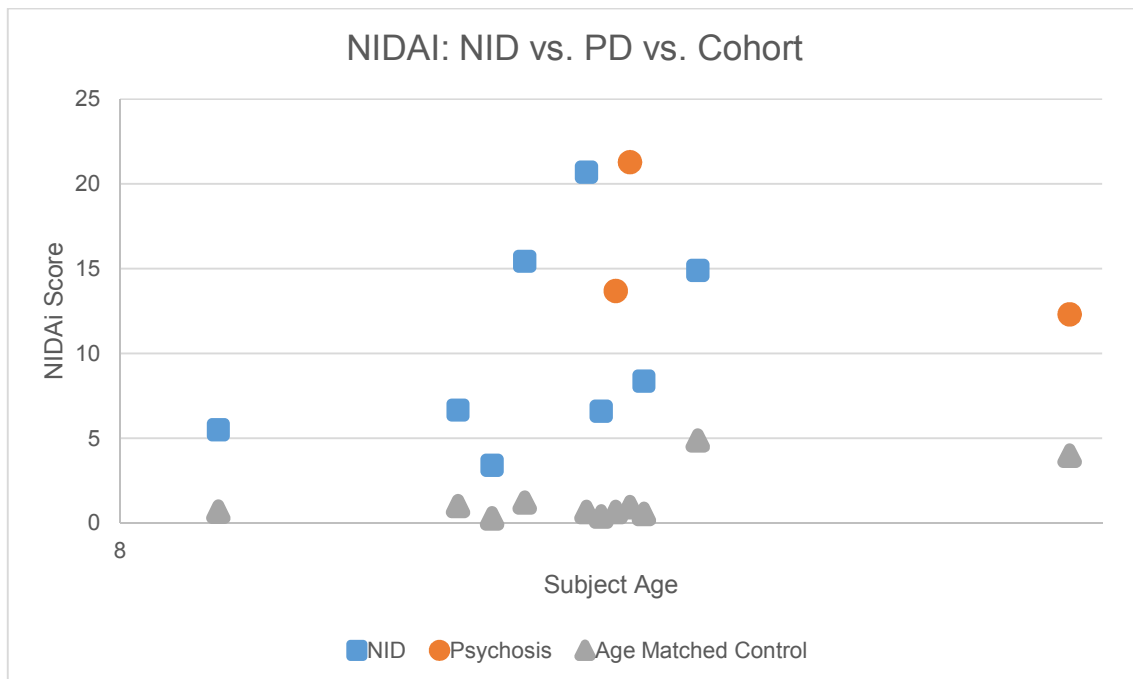
The immune-psychiatry/neurologic literature is revealing a shared pathology while uncovering new associations. Patients with acute psychosis were found to have basal ganglia encephalitis on neuro-imaging while testing positive for dopamine receptor 2 [D2R] auto-antibodies. [12,15] In a case-control study of 43 patients with new onset psychosis, 9 had detectable antibodies to D2R and NMDAR. [16] A 2013

meta-analysis reported increased numbers of cells positive for CD56 [a marker of natural killer cells and activated T cells], and an increased CD4/CD8 T cell ratio in schizophrenia. [17] We have a similar finding but with selected cells from CD4+ and CD8+ lineage. In a genome wide association study, 108 genetic loci, expressed in neural tissue and in cells involved with adaptive immunity, were found to have an association with schizophrenia. [18] It has been reported a significant genetic overlap in regions coding for the MHC in subjects with schizophrenia and multiple sclerosis. [19]

**Table 1. Vital statistics of subjects**

| Demographic                          | N = |
|--------------------------------------|-----|
| Age:                                 |     |
| NID 9-12                             | 3   |
| NID 13-16                            | 4   |
| PD 13-25                             | 3   |
| Sex:                                 |     |
| Male                                 | 6   |
| Female                               | 4   |
| Diagnosis                            |     |
| Transverse myelitis                  | 4   |
| NMDAr                                | 2   |
| Miller Fisher variant Guillain Barre | 1   |
| PD                                   | 3   |

Abbreviations: NID Neuroimmune disorders; PD Psychotic Disorders; NMDAr N-methyl-D-aspartate receptor



**Fig. 1. Comparison of NID and PD with the age matched cohort group utilizing the NIDAI-score**  
Abbreviations: NID Neuroimmune Disorders; PD Psychotic Disorders; Co Cohort group

**Table 2. T lymphocytes Cell Lines**

| Cell lineage              | Mean/SD                            | p value  |
|---------------------------|------------------------------------|----------|
| Tcell as % of lymphocytes | NID/PD: 46±22<br>Co: 60±6          | NS       |
| CD4/CD8                   | NID/PD: 1.4± 0.6<br>Co: 1.9±0.5    | NS       |
| Th1 + Th17 % CD4          | NID/PD: 48±11<br>Co: 33±8          | NS       |
| Th1+Th17/ Treg %CD4       | NID/PD: 10.8±5<br>Co: 5.6±1.4      | p < 0.05 |
| Treg mfi CD25             | NID/PD: 672± 242<br>Co: 1155± 285  | p < 0.05 |
| CCR4+ receptor as % Tconv | NID/PD: 27.6±8.1<br>Co: 19.7±7.5   | p < 0.05 |
| CD8+ group as % T cell    | NID/PD: 37.5± 7.2<br>Co: 29.9± 4.8 | p < 0.05 |
| Tem lineage as % of CD8+  | NID/PD: 16± 5.3<br>Co: 35.4± 11.2  | p < 0.05 |

Abbreviations: NID/PD Neuroimmune/ Psychotic Disorders; Co Cohort group

In NID, therapies could be “time-sensitive”, as delay in their introduction of efficacious immunomodulatory regimens lead to resistance to treatments or sorter episodes between relapses. [20] Could PD conditions also exhibit a similar “time-sensitive efficacy”? If the adaptive immune system is involved in AP, treatments addressing the underlying pathobiology in a timely manner are most likely warranted.

Khandaker et al call attention to the need for randomized trials of immunotherapy as an adjunct of standard antipsychotic treatment. [21] Accessible immune biomarkers for such trials to take place are needed. Clinical efficacy could be slow to detect in psychiatric disorders. While the immune dysregulation may have subsided with adequate immunoregulatory therapy, the “aberrant” behavioral compensation still need to be de-escalated or be de-constructed.

We focused on a potential biological marker that could be scalable and have generalizability for Neuroimmune disorders. Cellular phenotypes associated with NID have been described on the past. [22] In autoimmune conditions, including those affecting the CNS, the Tcell lineage of CD4+ effector cells Th1 and Th17 are strongly associated with self-directed reactivity. [23] The clonal expansion of Th1 and Th17 cells are under the tight regulation of the Treg cells, in particular those from these lineage that co-express CD25+. In a variety of autoimmune conditions, including those affecting the CNS, a reduction in the number or function of suppressor T cell has been described [24-28]; a similar

pathophysiology may be involved in areas pertaining to mental health. [29,30]

We propose that a clinical AIR score, composed of selected cell Tell subsets, could serve as potential biomarker for NID and PD disorders.

Limitations: translating our findings into biological insight possess challenges due to the limited number of observations. However, our pilot observations remain thought provoking and may serve as bases for future studies.

## 5. CONCLUSIONS

The potential ramifications of our findings are that shared alterations of immune pathways seem to play a role in the pathobiology of both NID and some PD disorders. The overlapped nature of the neuroimmune dysregulation gives us a broader understanding of these conditions irrespective of the outward clinical manifestations and may provide with a biological insights as to how to correct them.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## SIGNIFICANCE OF THE STUDY

This study uses flow cytometry to characterize the adaptive immune response in pediatric patients with neuro immune and psychotic disorders, to develop a score with potential diagnostic implications

## CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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