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Lalousis, Paris Alexandros; Schmaal, Lianne; Wood, Stephen; Reniers, Renate; Cropley, Vanessa L; Watson, Andrew; Pantelis, Christos; Suckling, John; Barnes, Nicholas; Pariante, Carmine M.; Jones, P; Joyce, Eileen; Barnes, Thomas RE; Lawrie, SM; Husain, Nusrat; Dazzan, Paola; Deakin, Bill; Weickert, Cynthia S; Upthegrove, Rachel

DOI:
10.1016/j.bbi.2023.06.023

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Lalousis, PA, Schmaal, L, Wood, S, Reniers, R, Cropley, VL, Watson, A, Pantelis, C, Suckling, J, Barnes, N, Pariante, CM, Jones, P, Joyce, E, Barnes, TRE, Lawrie, SM, Husain, N, Dazzan, P, Deakin, B, Weickert, CS & Upthegrove, R 2023, 'Inflammatory subgroups of schizophrenia and their association with brain structure: A semi-supervised machine learning examination of heterogeneity', *Brain, Behavior, and Immunity*, vol. 113, pp. 166-175. https://doi.org/10.1016/j.bbi.2023.06.023

Brain, Behavior, and Immunity 113 (2023) 166–175

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Inflammatory subgroups of schizophrenia and their association with brain structure: A semi-supervised machine learning examination of heterogeneity

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ARTICLE INFO

Keywords:
Inflammation
Schizophrenia
MRI
Machine learning

ABSTRACT

Objective: Immune system dysfunction is hypothesised to contribute to structural brain changes through aberrant synaptic pruning in schizophrenia. However, evidence is mixed and there is a lack of evidence of inflammation and its effect on grey matter volume (GMV) in patients. We hypothesised that inflammatory subgroups can be identified and that the subgroups will show distinct neuroanatomical and neurocognitive profiles.
Methods: The total sample consisted of 1067 participants (chronic patients with schizophrenia n = 467 and healthy controls (HCs) n = 600) from the Australia Schizophrenia Research Bank (ASRB) dataset, together with 218 recent-onset patients with schizophrenia from the external Benefit of Minocycline on Negative Symptoms of Psychosis: Extent and Mechanism (BeneMin) dataset. HYDRA (HeterogeneitY through DiscRiminant Analysis) was used to separate schizophrenia from HC and define disease-related subgroups based on inflammatory

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https://doi.org/10.1016/j.bbi.2023.06.023
Received 6 October 2022; Received in revised form 15 May 2023; Accepted 26 June 2023
Available online 7 July 2023

markers. Voxel-based morphometry and inferential statistics were used to explore GMV alterations and neurocognitive deficits in these subgroups.

Results: An optimal clustering solution revealed five main schizophrenia groups separable from HC: Low Inflammation, Elevated CRP, Elevated IL-6/IL-8, Elevated IFN-γ, and Elevated IL-10 with an adjusted Rand index of 0.573. When compared with the healthy controls, the IL-6/IL-8 cluster showed the most widespread, including the anterior cingulate, GMV reduction. The IFN-γ inflammation cluster showed the least GMV reduction and impairment of cognitive performance. The CRP and the Low Inflammation clusters dominated in the younger external dataset.

Conclusions: Inflammation in schizophrenia may not be merely a case of low vs high, but rather there are pluripotent, heterogeneous mechanisms at play which could be reliably identified based on accessible, peripheral measures. This could inform the successful development of targeted interventions.

1. Introduction

Immune system dysfunction is implicated in the aetiology of schizophrenia, with circulating cytokines such as interleukin (IL)-6, IL-1β, interferon gamma (IFN)-γ, tumor necrosis factor alpha (TNF)-α, IL-12 and transforming growth factor beta (TGF)-β detected at elevated levels before the onset of psychosis and before treatment initiation (Khandaker et al., 2015; Miller et al., 2011; Noto et al., 2015; Perkins et al., 20152015; Upthegrove et al., 2014). Additionally, longitudinal studies have reported an association between higher adolescent C-reactive protein (CRP) levels and later development of schizophrenia (Metcalf et al., 2017) with a causal role of immune dysfunction suggested by genetically determined levels of IL-6 associated with schizophrenia using mendelian randomisation (Perry et al., 2021) and copy number variants in C4 associated with increased risk of schizophrenia (Sekar et al., 2016). Genome-wide association studies also show evidence of risk for schizophrenia related to the Major Histocompatibility Complex (MHC), and further evidence that the complement component 4(C4) contributes to this association (Sekar et al., 2016; Li et al., 2019; Mokhtari and Lachman, 2016).

Peripheral inflammation, as measured by cytokines and acute phase proteins, has been associated with reduced gray matter volume (GMV) in the hippocampus, orbital frontal cortex, inferior frontal gyrus, middle frontal gyrus, and cingulate cortex in people with schizophrenia as well as decreased functional connectivity in depression (Fillman et al., 2016; North et al., 2021; Wu et al., 2019; Miller et al., 2014; Felger et al., 2016). Cortical thickness may be differentially affected by immune changes, with total cortical volume negatively associated with elevated IFN-γ and IL-5 in patients with first episode of psychosis (FEP) (Laskaris et al., 2021). Furthermore, elevated CRP and pro-inflammatory cytokines have been associated with significant cognitive impairment in patients with schizophrenia (Fillman et al., 2016; North et al., 2021; Bulzacka et al., 2016; Johnsen et al., 2016; Misiak et al., 2018). Nevertheless, there is evidence suggesting different patterns with some studies having found elevated brain measures associated with inflammation (Lizano et al., 2021; Lizano et al., 2019).

It is likely that increased inflammation may be found in only a subgroup of patients with schizophrenia. However, the current evidence is mixed; Pillinger et al. found no evidence of a bimodal variation in cytokines in first epsisode schizophrenia, proposing that alterations in the immune system may be a core feature across all psychoses (Pillinger et al., 2019). Fillman et al. demonstrated the existence of two (elevated/low pro-inflammatory cytokine transcript) inflammatory schizophrenia subgroups with the elevated cytokine group having worse verbal fluency and reduced GMV in the Broca's area but no differences in clinical symptoms (Fillman et al., 2016). Boerrigter et al. identified three (very elevated/elevated/low pro-inflammatory cytokine) inflammatory subgroups in people with chronic schizophrenia (Boerrigter et al., 2017) and Tamouza et al. reported two subgroups; (elevated/low pro-inflammatory) in patients with schizophrenia (Tamouza et al., 2021). Furthermore, a study by Hoang et al. suggested the existence of two subgroups (elevated/low) with the elevated inflammatory subgroup exhibiting increased right parahippocampal cortical thickness as well as in the caudal anterior cingulate, and banks of the superior sulcus (Hoang et al., 2022).

The inconsistency of findings may result in part from variation in patient samples, or with multiple inflammatory mechanisms operating at different stages of illness (Cropley and Pantelis, 2014), which are not currently revealed by traditional analytical approaches and/or small sample sizes that result in reporting broad quantitative differences (e.g. low vs high). The use of advanced statistical methods including semi-supervised machine learning, in larger samples is needed to identify subgroups characterised by qualitatively distinct inflammatory profiles. That is, multiple explanations might contribute to the mixed evidence in inflammatory subtypes which might be uncovered by the use of advanced statistical methods.

Most clustering studies in this field to date have employed two-step hierarchical clustering with relatively small samples and few have performed external validation (Fillman et al., 2016; Boerrigter et al., 2017; Tamouza et al., 2021). Advanced clustering analysis methods have the potential to uncover inflammation subgroups in schizophrenia which may reflect diffential pathological processes and aid the stratification of novel treatment targets (Varol et al., 2017). We used the novel semi-supervised machine learning tool, HeterogeneitY through DiscRiminant Analysis (HYDRA) (Varol et al., 2017) with the aim of identifying subgroups of patients with schizophrenia characterised by distinct inflammatory profiles, and investigating their neuroanatomical and neurocognitive correlates. In contrast to non-linear kernel classification methods which do not provide explicit information on disease subtypes, HYDRA captures multiple dimension of heterogeneity by varying numbers of hyperplanes. Each hyperplane separates healthy controls from patients and then creates between-patient clusters, providing information about the distinct disease processes that drive the heterogeneity of the data.

We hypothesized that multiple inflammatory subgroups would be identified, and that those groups with an elevated inflammatory profile would have less GMV and poorer cognitive function. We aimed to extend our clustering solution in an external data set of participants at an earlier stage in schizophrenia, to reveal potential stage-specific insights.

2. Methods

2.1. Participants

This study used data from the Australia Schizophrenia Research Bank (ASRB) comprising 1067 participants: 467 patients with chronic schizophrenia and 600 healthy controls (HCs). The exclusion criteria were organic brain disorder, brain injury with greater than 24 h post-traumatic amnesia, learning disability (IQ < 70), movement disorders, current diagnosis of substance dependence, electroconvulsive therapy received in the last 6 months and, for controls, a personal or family history of psychosis or bipolar 1 disorder. Diagnostic status was determined using the Diagnostic Interview for Psychosis (DIP) (Castle et al., 2006; Jablensky et al., 2000), which is used to establish a lifetime diagnosis of a psychotic disorder, as well as present and lifetime substance use disorder diagnoses, according to DSM-IV and ICD-10 criteria.

The DIP diagnostic algorithm also enables classification according to other systems, including DSM-IIIR and Research Diagnostic Criteria.

2.2. Blood-based biomarker data

A 40 mL whole blood sample was collected drawn under non-fasting conditions at various times and collected into Serum Separator Tubes (SST, BD Biosciences). The tubes were inverted and left at room temperature for 30 min (to allow clotting) and centrifuged at 2000g for 5 min at 4 °C. The average coefficient of variance for duplicate values (n = 160) across analytes was minimal (0.75%). The variation between plates as determined from a pooled serum sample run an internal control on all plates was 31.3% across all 17 plates in the ASRB sample. For further details please see supplementary methods (1.1). In the BeneMin dataset plasma inflammatory markers were aliquoted and frozen within 4 h. See previous publications (North et al., 2021; Loughland et al., 2010) and supplementary methods for further details and BeneMin blood-based biomarker sampling and data analysis (1.1). The average minimum detectable value across all plates was 0.11 pg/ml for IFNγ, 0.12 pg/ml for IL-10, 0.03 pg/ml for IL-12, 0.04 pg/ml for IL-1β, 0.09 pg/ml for IL-2, 0.03 pg/ml for IL-6, 0.20 pg/ml for IL-8, and 0.04 pg/ml for TNFα. Values below the range of detection were replaced by the respective minimum detectable value (entire sample: average 17 samples/cytokine = <1.5% of the total measures; imaging subset: average 3.3 samples/cytokine = 1.1% of the total measures), and values above the range of detection were replaced by the maximum detectable value (entire sample: average 7 samples/cytokine = <0.6% of the total measures; imaging subset: average 3.1 samples/cytokine = 1.0% of the total measures.

2.3. MRI imaging data acquisition, quality control, and preprocessing

Structural magnetic resonance imaging (sMRI) data were available for 296 participants: 195 patients and 101 HCs. sMRI brain scans were performed using a standard data acquisition protocol on Siemens Avanto 1.5 Tesla scanners located across Australia. See supplementary methods for full details of acquisition (1.2).

2.4. Neurocognition

Premorbid IQ was assessed using The Wechsler Test of Adult Reading (WTAR) (Kreutzer et al., 2011). The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al., 1998) and the Wechsler Adult Intelligence Scale—Third Edition (WAIS-III) Letter Number Sequencing (LNS) subtest (age-adjusted scaled scores) (Kreutzer et al., 2011) were used to examine a range of neurocognitive performance. Patients with missing data were excluded from statistical analyses, resulting in a final sample size of n = 1046.

2.5. External dataset

We used the baseline cytokine, clinical, cognitive and demographic data from 199 patients who had participated in the Benefit of Minocycline on Negative Symptoms of Psychosis: Extent and Mechanism (BeneMin) trial for exploration of our clusters in an external dataset. BeneMin is described in full in a previous publication (Deakin et al., 2018) but in brief, the participants were within 5 years of onset of first episode of schizophrenia, schizophreniform, or schizoaffective psychosis. See supplementary methods for full details (1.1).

2.6. Semi-supervised machine learning analysis

We used the python version of HYDRA (https://github.com/anbai106/pyHYDRA) (Varol et al., 2017) in the ASRB data to simultaneously classify patients from the HCs, and partition patients into clusters based on disease-related heterogeneity using CRP, IFN-γ, IL-10, IL-12, IL1-β, IL-2, IL-6, IL-8, and TNFα. HYDRA (Varol et al., 2017) utilizes a convex polytope formed by combination of multiple linear max-margin classifiers; i.e. multiple support vector machines (SVM). It dissects disease heterogeneity by portioning patients into groups based on the patterns or transformations between sub-populations (i.e., clusters) in the patient group *as distinct from* the reference group (i.e., healthy controls). Furthermore, HYDRA models regress out entered covariates, such as age and sex.

2.7. Model training

We used the nine aforementioned cytokines/acute phase response protein from 1067 participants (467 patients and 600 HCs) in the ASRB data set. HYDRA was trained using a repeated hold-out cross-validation strategy (i.e., 1000 repetitions with 80% of the data for training in each repetition). Age, sex, site, and time the samples spent in the freezer were included as covariates. HYDRA was run requesting 2 to 8 clustering solutions, and the Adjusted Rand Index (ARI) was used to measure cluster stability. The ARI takes values between −1 and 1. (Hubert and Arabie, 1985).

The statistical significance of clusters was assessed in two ways. First, we assessed whether the obtained subgroups were different from those which would be found if disease-related variability was not present by comparing the observed ARI to a null distribution of ARI. We created a null distribution of ARI by randomly assigning HC samples (n = 600) to an HC group and pseudo-patient group and performing a HYDRA analysis 100 times using the aforementioned repeated hold-out cross-validation strategy. We then compared the two distributions using a two-sample *t*-test. Secondly, we also assessed whether the data could be better explained by a single Gaussian distribution by employing SigClust (1000 simulations) (Huang et al., 2015) (https://github.com/pkimes/sigclust2). SigClust tests the null hypothesis that the data are from a single Gaussian distribution by using a test statistic called the cluster index (ClustI). The ClustI is defined as the sum of within-class sums of squares about the mean divided by the total sum of squares about the overall mean. The null distribution of the ClustI is approximated by simulating from a single Gaussian distribution estimated from the data.

2.8. Cluster characterisation

To aid the description of the clusters resulting from the HYDRA output we tested if inflammatory and neurocognitive profiles differed according to subgroup, and we interrogated confounds that were regressed out in the HYDRA model (age, sex, site, and time the samples spent in the freezer). Identified clusters were compared in terms of their cytokine levels, demographics, and neurocognitive performance using one-way ANOVA models corrected for multiple comparisons (Tukey's HSD).

Neuroanatomical differences were examined using voxel-based morphometry (VBM) (two sample t-tests of each cluster vs the HCs and a voxel-wise F test between identified clusters without the HCs, with age, sex, TIV, and site included as covariates, SPM12). All models were false discovery rate (FDR) corrected for multiple comparisons and a minimum cluster extent of k = 30 was applied.

2.9. Cluster solution generalizability

In order to be able to test the generalisability of the cluster solution to the BeneMin dataset, first we developed a SVM model to predict subgroup membership in the ASRB dataset. The resulting SVM model was then applied to the BeneMin dataset. Ensemble-based probability (EBP), a measure of the probability an individual belongs to a cluster, was used to test the probability of a BeneMin participant falling into one of the ASRB identified clusters. Further details about the SVM parameters can be found in the supplement (1.4). Identified clusters were compared on demographic data, length of illness and neurocognitive performance

using one-way ANOVA models.

3. Results

3.1. Demographic information

The mean age of the ASRB HC group was 43.5 years [SD 13.1] and the mean age of the patient group was 39.5 years [SD 10.9]. The HC group consisted of 240 male and 360 female participants and the patient group had 303 male and 164 female participants. A summary of socio-demographic and clinical information is provided in Table 1. The mean age of the patients in the BeneMin external datasest was 25.6 years [SD 5.2]. One hundred and forty six patients were male and 53 were female.

3.2. HYDRA semi-supervised machine learning analysis

The optimal clustering solution was eight clusters with an ARI 0.573. The ARIs of clusters 2–7 were as follows: 0.218; 0.308; 0.449; 0.483; 0.505; 0.526. Due to very small number of patients in clusters 6–8, which would prohibit any meaningful conclusions, they were omitted from further analyses. Clusters 1–5 did not differ in terms of age ($p = .611$) or site distribution ($p = .358$), but did differ in terms of time the samples spent in the freezer (Cluster 1 < Cluster 2; $p = .002$) and sex distribution, with more females in HCs ($p = .021$). Sociodemographic and full cytokine profiles of each clusters can be found in Table 1.

3.3. Cluster statistical significance

The clustering solution (ARI: 0.573) was significantly different from a clustering solution derived from HCs (ARI: 0.376) ($t(100) = -9.43$, $p < .001$) and the subgroup solution was more likely than an underlying Gaussian solution ($p < .001$). This means that the clusters identified do not exist in HCs and are specifically related to disease related inflammation and that the data distribution is not unimodal but rather that seperable clusters exist within the dataset. See supplement (1.5).

3.4. Inflammatory characteristics analysis

For clusters 1–5 we assigned labels that depicted the most notable inflammatory changes in each group (see Table 1). Cluster 1 (Low Inflammation n = 142) was characterised by statistically significant lower levels of cytokines and CRP compared with the HC group and the rest of the patient clusters. Cluster 2 (CRP cluster; n = 121) had significantly elevated levels of CRP compared with the rest of the clusters and the. Cluster 3 (Classic Inflammation cluster; n = 82) demonstrated elevated levels of IL-6 and IL-8 compared with the HCs and all the other patient clusters. Cluster 4 (IFN-γ cluster; n = 80) exhibited significantly elevated IFN-γ levels compared with the HCs and all other patient clusters. Cluster 5 (Anti-Inflammatory cluster; n = 32) exhibited elevated IL-10 levels compared with the HCs and all other patient clusters. See Table 1 and supplement 1.6 for statistical information and post-hoc comparisons.

3.5. VBM analysis of neuroanatomically based clusters

Within the diagnosis of schizophrenia, Clusters 1–5 all had temporal and hippocampal GMV reduction compared with HCs. The Classic Inflammation cluster (Cluster 3) showed the greatest and most widespread GMV reduction, including the anterior cingulate. The IFN-γ cluster (Cluster 4) exhibited GMV increases in the precentral gyrus. See Figs. 1-3, supplementary Tables 1-5. When all inflammatory clusters were compared with each other, decreases in the inferior temporal gyrus, dorsolateral superior frontal gyrus, and postcentral gyrus were observed in the CRP and Classic Inflammation clusters. See supplement 1.7 for further details. See (Fig. 4).

3.6. Neurocognitive differences

Univariate ANOVAs for each neurocognitive domain (RBANS immediate memory/constructional/language/attention/delayed memory/total domains, WTAR, WASI matrix reasoning, WASI IQ equivalent, LNS respectively) showed a main effect of group in all domains ($F(5,1046) = 100.2$; $F = 22.7$; $F = 56.7$; $F = 98.6$; $F = 81.3$; $F = 138.6$; $F = 22.0F = 52.2$; $F = 50.1$; $F = 70.0$; $F = 54$, all p values < 0.001). Post-hoc analyses using the Tukey HSD post-hoc criterion ($\alpha = 0.05$) for significance showed that patients in all clusters had significantly lower scores in all domains compared with the HCs, apart from the premorbid functioning domain where differences between patients in the IFN-γ cluster and HCs showed a trend towards statistical significance ($p = 0.076$).

Repeating the univariate ANOVAs without the HCs, in order to assess differences between schizophrenia clusters on neurocognitive performance, we observed a trend towards a significant main effect of group in the following domains: premorbid functioning ($F(4,446) = 2.12$, $p = .077$) and verbal memory (LNS) $F(4,447) = 2.16$, $p = .072$.

The Low and Anti-Inflammatory clusters both had low/normative cytokine and CRP levels and could be considered to both be part of the same low cytokine group, thus were combined to increase statistical power for the purposes of comparing the neurocognitive performance tests with the inflammatory clusters 2–4. Main effects of cluster were observed in the following neurocognitive domains: language processing (RBANS language), $F(3,447) = 3.92$, $p = .009$; attention (RBANS attention), $F(3,446) = 3.73$, $p = .011$; verbal working memory (LNS), $F(3,448) = 2.87$, $p = .036$, and premorbid functioning (WTAR), $F(3,446) = 2.76$, $p = .041$. Post-hoc comparisons are given in the supplement (1.7).Overall, the IFN-γ cluster showed the least neurocognitive dysfunction compared with the rest of the clusters.

3.7. External dataset cluster exploration

Two main clusters were detected in the BeneMin dataset: Low inflammation cluster (n = 149; (mean EBP: 0.95 [SD 0.00) and the CRP cluster (n = 48; mean EBP: 0.98, [SD 0.00]. The anti-inflammatory cluster (n = 2; mean EBP: 0.97 [SD 0.02] and the IFN-γ cluster (n = 1; mean EBP: 0.99 [SD N/A] were also identified but restricted to one or two patients. The classic inflammation cluster was not detected, possibly due to lower IL-6 and IL-8 mean values in BeneMin. Patients in the CRP cluster exhibited significantly lower current IQ ($p = .028$) and premorbid IQ ($p = .005$), and worse performance on the Digit Symbol Substitution task ($p = .003$) compered with the Low Inflammation cluster. Further details can be found in the supplement (1.9).

4. Discussion

Using advanced semi-supervised machine-learning, we identified five distinct inflammatory subgroups of chronic schizophrenia. The clustering solution was statistically significant by two tests: 1) the solution was unique to disease-related variability and 2) the data could not be better explained as belonging to a continuous distribution. GMV loss in temporal and hippocampal areas was evident in all schizophrenia clusters when compared with the HCs, and the Classic Inflammation cluster, with elevations in both IL6 and IL8, showed the most widespread loss extending into the anterior cingulate. Greater GMV in the precentral gyrus was seen only in the IFN-γ cluster, the cluster which also had the lowest CRP and IL-6 values. The CRP and Low Inflammation clusters were detected in patients in BeneMin, suggesting that CRP could be a marker used to discriminate inflammatory subgroups at an earlier disease stage. This supports previous research suggesting that CRP is a potential biomarker of later disease onset (Perry et al., 2021).

Each identified cluster had an overlapping and distinct inflammatory *and* neuroanatomical profile. The CRP cluster showed increased CRP alone, and an associated pattern of reduced GMV in the inferior temporal gyrus, dorsolateral superior frontal gyrus, and postcentral gyrus. A

Table 1
Sample Sociodemographics. Sample Sizes, Participants per Study Site, Age, Sex, Freezer Time, Cytokine concentrations (F values represent main effect of group on cytokine concentrations including HCs, asterisks represent cytokines which are statistically significantly higher or lower to HCs). Abbreviations: SD = Standard Deviation, pg = picograms, ml = millilitre.

	HC group	Cluster 1 (Low Inflammation)	Cluster 2 (CRP Cluster)	Cluster 3 (Classic Inflammation Cluster)	Cluster 4 (IFN-γ Cluster)	Cluster 5 (Anti-Inflammatory Cluster)	t/z/ χ2/F	P Value	Cluster 6	Cluster 7	Cluster 8	t/z/ χ2/F	P Value
Sample Sizes, No.	600	142	121	82	80	32			5	3	2		
Age, Mean (SD)	43.5 (13.1)	38.3 (11.1)	40.4 (10.7)	40.1 (10.8)	39.2 (11.4)	39.7 (9.5)			42.2 (7.9)	47 (4.2)	40.3 (16.5)	F = 0.673	0.611
Sex (Male/Female)	240/360	105/37	67/54	57/25	49/31	19/13			3/2	1/1	2/1	$\chi 2$ = 11.5	0.021
Freezer Time (Days, Mean (SD))	2427.9 (352.4)	2707.6 (412.3)	2497.0 (496.2)	2596.3 (464.6)	2632.6 (466.6)	2531.8 (383.8)			2763.2 (454.5)	2748.0 (426.9)	3039 (21.2)	F = 3.8	0.005
Duration of Illness (Years, Mean (SD))	N/A	15.1 (0.878)	16.4 (0.916)	16.4 (1.23)	14.2 (1.12)	15.7 (1.70)	F = 0.793	0.530					
Site No (Percentage)													
Brisbane	193 (51.3)	44 (11.7)	49 (13.0)	42 (11.1)	34 (9.0)	11 (2.9)							
Melbourne	178 (59.9)	40 (13.4)	28 (9.4)	19 (6.3)	14 (4.7)	11 (3.7)							
Perth	97 (68.7)	17 (12.6)	12 (8.5)	6 (4.2)	7 (4.9)	2 (1.4)							
Newcastle	55 (57.2)	16 (16.6)	11 (11.4)	1 (1.0)	8 (8.3)	5 (5.2)							
Sydney	77 (49.0)	25 (15.9)	21 (13.3)	14 (8.9	17 (10.8)	3 (1.9)						$\chi 2$ = 21.2	0.168
CRP (pg/ml) (Mean (SD))	1.8 (2.3)	1.5 (1.3)	7.4 (3.0)*	2.6 (2.8)*	1.1 (1.2)	1.8 (1.2)	F = 151.8	>0.001	3.2 (3.9)	6.9 (1.5)	1.2 (0.4)		
IFN-γ (pg/ml) (Mean (SD))	29.7 (18.8)	18.4 (10.4)*	31.6 (15.3)	21.2 (12.3)	46.3 (16.9)*	36.3 (13.0)	F = 63.9	>0.001	19.3 (7.7)	15.3 (7.7)	22.4 (6.8)		
IL-10 (pg/ml) (Mean (SD))	34.3 (33.5)	26.3 (20.8)	34.5 (20.1)	31.6 (19.9)	34.2 (22.5)	89.0 (86.5)*	F = 28.7	>0.001	49.8 (24.3)	64.3 (87.2)	85.4 (36.2)		
IL-12 (pg/ml) (Mean (SD))	9.6 (6.3)	5.9 (3.3)*	10.3 (5.6)	7.2 (4.0)	14.9 (12.5)*	11.4 (3.5)	F = 27.5	>0.001	8.7 (3.0)	5.9 (2.1)	23.9 (1.2)		
IL-6 (pg/ml) (Mean (SD))	60.9 (289.4)	52.5 (185.7)	32.9 (86.6)	339.2 (547.7)*	22.7 (55.0)	40.7 (139.1)	F = 22.3	>0.001	2281.6 (79.6)	2250.6 (92.3)	1943.0 (510.4)		
IL-8 (pg/ml) (Mean (SD))	656.3 (1078.5)	529.8 (683.0)	534.0 (665.9)	3227.1 (1408.7)*	298.1 (501.2)	298.3 (355.4)	F = 193.1	>0.001	2472.9 (1601.8)	3258.0 (1008.2)	4306.0 (919.2)		
TNFα (pg/ml) (Mean (SD))	31.0 (183.6)	30.8 (55.9)	21.0 (21.3)	73.8 (100.4)	23.6 (42.2)	29.3 (49.7)	F = 11.8	>0.001	799.2 (308.4)	249.3 (77.3)	2131.8 (1038)		

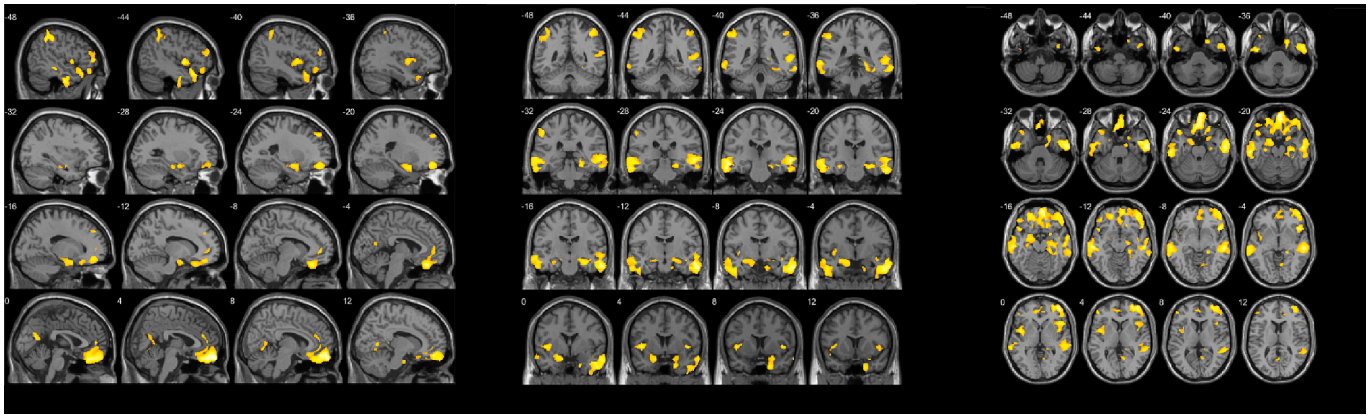

Fig. 1. Classic Inflammation Cluster GMV Reductions Compared with HCs. GMV reductions are observed in the Anterior Cingulate, Middle Frontal Gyrus, Inferior Frontal Gyrus, Superior Frontal Gyrus, Rectal Gyrus, Right Inferior frontal gyrus triangular part, Bilateral Insula, Middle Temporal Gyrus, Superior Temporal Gyrus, Inferior Temporal Gyrus, Bilateral Hippocampus, Middle Temporal Gyrus, Inferior Temporal Gyrus, Fusiform Gyrus, Superior Temporal Gyrus, Inferior Frontal Gyrus, Left Insula, Superior Frontal Gyrus, Inferior Parietal Lobule, and Right Inferior Parietal Lobe. Peak voxel MNI coordinates can be found in the supplement (Table S1).

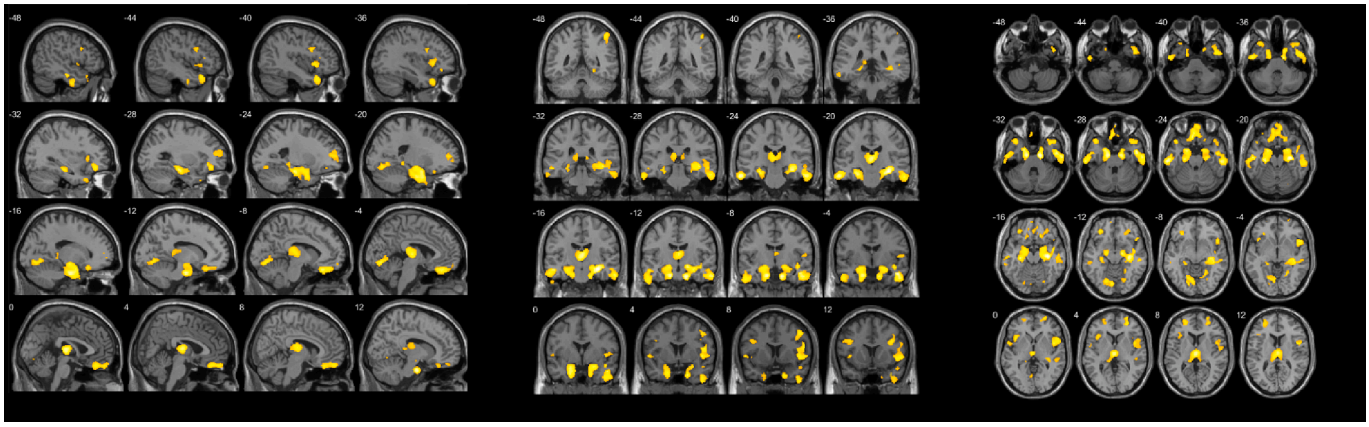

Fig. 2. CRP Cluster GMV Reductions Compared with HCs. GMV reductions are observed in the Parahippocampal Gyrus, Inferior Temporal Gyrus, Right Hippocampus, Middle Temporal Gyrus, Fusiform Gyrus, Amygdala, Bilateral Thalamus, Left Fusiform Gyrus, Inferior Frontal Gyrus, Right Insula, Left Lingual Gyrus, Superior Temporal Gyrus, Left Superior Frontal Gyrus, Left Insula, Inferior frontal gyrus opercular part Right, Right Superior Frontal Gyrus, Right Lingual Gyrus, Inferior Parietal Lobule, Angular Gyrus, Inferior frontal gyrus orbital part Left, and Inferior frontal gyrus opercular part Left. Peak voxel MNI coordinates can be found in the supplement (Table S2).

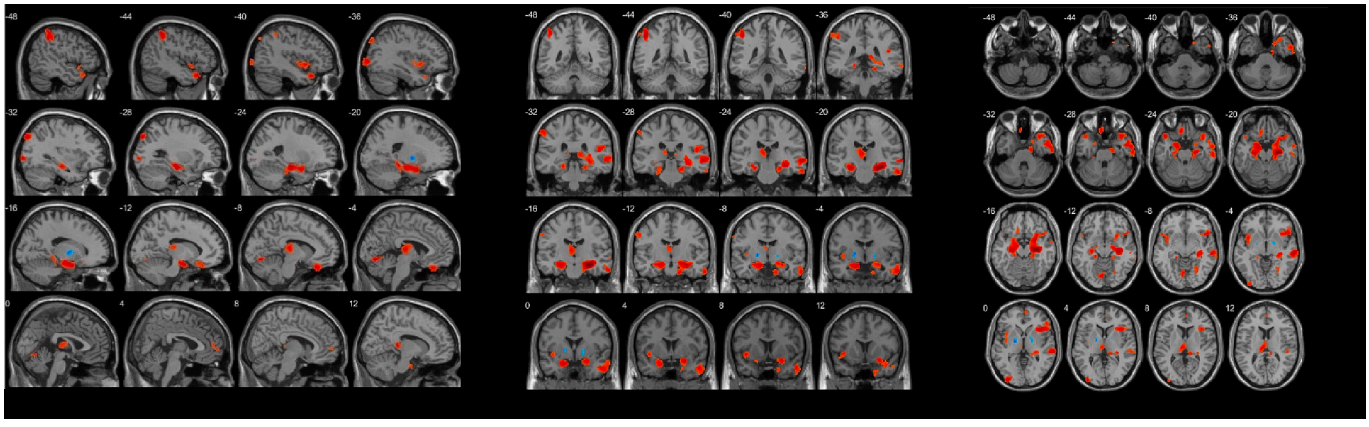

Fig. 3. Low Inflammation Cluster GMV Reductions (red) and Increases (blue) Compared with HCs. GMV reductions are observed in the Parahippocampal Gyrus, Inferior Temporal Gyrus, Right Hippocampus, Middle Temporal Gyrus, Fusiform Gyrus, Right Amygdala, Inferior Temporal Gyrus Right,Left Hippocampus, Left Amygdala, Inferior Frontal Gyrus, Left Lingual Gyrus, Middle Occipital Gyrus, Right Superior Temporal Gyrus, Precuneus, and Inferior Parietal Lobule. GMV increases are observed in the bilateral pallidum. Peak voxel MNI coordinates can be found in the supplement (Table S3).

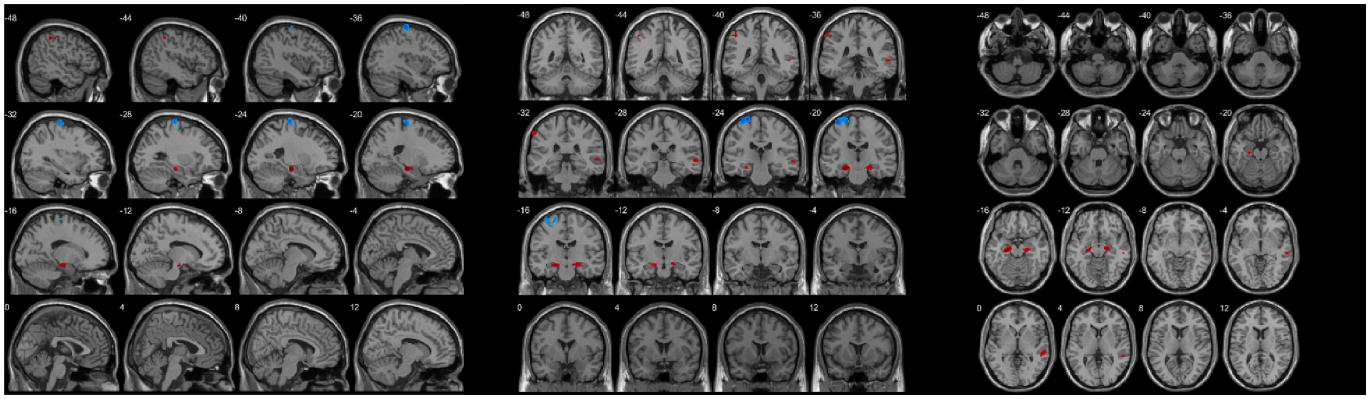

Fig. 4. IFN-γ Cluster GMV Reductions (red) and Increases (blue) Compared with HCs. GMV reductions are observed in the Parahippocampal Gyrus, Left Hippocampus, Parahippocampal Gyrus, Right Hippocampus, Middle Temporal Gyrus, Inferior Parietal Lobule GMV increases are observed in the precentral gyrus. Peak voxel MNI coordinates can be found in the supplement (Table S4).

potential mechanism for the effects of CRP on GMV could be that increased CRP levels (indicative of systemic low-grade inflammation, but not necessarily simultaneous with elevations in cytokines- as CRP is a more stable marker than cytokines) (Jacomb et al., 2018) might result in an increase of blood–brain barrier permeability and subsequent pro-inflammatory cytokine entrance to the central nervous system. However, it seems unlikely that mild elevations in CRP would be a sufficient proximal cause of the GMV loss. More plausibly, raised CRP and reduced GMV could independently reflect an earlier developmental or ongoing neuroimmune abnormality such as maternal immune activation (MIA) or be a sign of more chronic inflammation. The relationship of higher CRP and altered neurodevelopment is supported by findings of a separate analysis of BeneMin participants which found that a subgroup of patients with low premorbid and low current IQ had higher levels of CRP and smaller MRI-derived total brain and intracranial volumes when compared with subgroups with a normal premorbid IQ (Watson et al., 2023). As these MRI findings are indicative of smaller premorbid brain size, this was suggestive of a neurodevelopmental subtype of schizophrenia additionally characterised by impaired premorbid cognitive function and low-grade inflammation (Watson et al., 2023). Nevertheless, research in rodents has shown that MIA does not always alter peripheral cytokines in male or female offspring (Brown et al., 2022).

Patients in the Classic Inflammation cluster had higher IL-6 and IL-8 levels compared with all other clusters and HCs. In the brain, IL-6 and IL-8 transcripts are extremely elevated in the frontal cortex in about 40% of those with schizophrenia (Fillman et al., 2016; Fillman et al., 2013; Zhang et al., 2016). Peripheral IL-6 is the most consistently increased cytokine in all phases of schizophrenia (Fond et al., 2020) and the most consistenly elevated cytokine found in the brain of people with schizophrenia, with increased levels found in the dorsolateral prefontal, orbital frontal, midbrain and subependymal zone (Fillman et al., 2016; North et al., 2021; Fillman et al., 2013; Zhang et al., 2016; Purves-Tyson et al., 2020). Patients in the Classic Inflammation cluster exhibited the most widespread GMV loss compared with HCs and other clusters, with specific reduction in anterior cingulate cortex. Anterior cingulate GMV may be an endophenotypic biomarker resulting from reductions in neuronal, synaptic, and dendritic density (Fornito et al., 2009). Our finding of anterior cingulate GMV reduction in the Classic Inflammation cluster may suggest the potential for IL-6 function as a a key mediator of GMV loss with relevance to schizophrenia. Recent evidence from Mendelian Randomisation also demonstrated potential causality of circulating levels of IL6/IL6R and GMV loss relevant to neuropsychiatric disorders including schizophrenia (Williams et al., 2022). Inhibition of IL-6 function in targeted groups of patients with schizophrenia holds significant promise not only for the alleviation of positive symptoms but also ameliorating cognitive impairment (Borovcanin et al., 2017), and is a mechanism that is being explored in experimental medicine studies (https://gtr.ukri.org/projects?ref = MR%2FS037675%2F1).

The IFN-γ cluster was comprised of patients with markedly elevated levels of IFN-γ compared with HC and all the other clusters, but it also had the lowest CRP levels of all groups and comparatively low levels of most other cytokines in our sample. Studies in patients with schizophrenia have shown that IFN-γ levels have an inverse relationship with whole-brain GMV and also left middle frontal gyrus thickness, potentially through alteration of the permeability of the blood brain barrier and activation of inflammatory T-cells and antigen-presenting cells along with the regulation of MHC class 2 genes (Abbott et al., 2006; Lesh et al., 2018). In contrast to previous studies, the patients with schizophrenia in our INF-γ cluster showed the least GMV reduction compared with HCs and least neurocognitive deficits in language, attention, and verbal memory tasks. It also suggests that elevated INF-γ may be beneficial in patients with schizophrenia, perhaps via helping to suppress viruses that can damage the brain (Dickerson et al., 2019; Warre-Cornish et al., 2020). Furthermore, the beneficial effect of natural killer cells (the main source of IFN-γ) has previously been reported in various studies in psychiatric settings (Tarantino et al., 2021; Furlan et al., 2019). Altogether, our results point towards a potential preserved neuroanatomical and neurocognitive profile which is supported by recent studies reporting a positive effect of IFN-γ on symptoms (Vetlugina et al., 2016). Furthermore, the fact that these patients still had hippocampal GMV loss suggests that this loss is central to schizophrenia even in patients who are functionally more preserved, as proposed by others (Velakoulis et al., 1999). The fact that hippocampal GMV loss was present in this preserved group points to a modulatory hippocampal effect on the pathophysiology of schizophrenia (with patients showing more pronounced GMV alterations exhibiting a worse neurocognitive profile) (Grace, 2012; Van Rheenen et al., 2018). Similarly, previous evidence also suggests higher levels of IL-10, as seen in in the Anti-Inflammatory cluster, may be protective against severe symptoms (Kay et al., 1987), also reflecting the relative sparing of GMV reduction seen in this cluster.

Patients in the Low Inflammation cluster revealed minimal inflammation compared with HCs and the rest of the clusters, which could be indicative of a less severe disease status or potentially successful treatment. Research has shown that the therapeutic efficacy of antipsychotic medication may be mediated by its effects on the basal ganglia with medicated patients exhibiting pallidal GMV increases compared with a placebo group (Chopra et al., 2021). Our GMV results show that patients in the Low Inflammation cluster exhibited GMV increases in the bilateral pallidum, consistent with a good treatment response. However, they still had temporal lobe volume loss. There is also a suggestion that acute relapse of illness may be associated with cortical swelling and ventricular reduction, with remission associated with the opposite. This effect has been observed in hippocampal volumes and ventricles in Clinically High-Risk patients (Garver et al., 2000; Velakoulis et al., 2006; Berger

et al., 2017). Interestingly, antipsychotic medication such as olanzapine, risperidone, aripiprazole, quetiapine, and haloperidol has been found to modulate the immune system and have anti-inflammatory effects through several mechanisms, including inhibition of pro-inflammatory cytokines, modulation of the hypothalamic–pituitary–adrenal axis, and activation of anti-inflammatory pathways (Marcinowicz et al., 2021). This could further explain our findings in the Low Inflammation cluster.

4.1. Strengths and limitations

This study has several strengths including a large dataset, significance testing of the clustering solutions and exploration in an external dataset. Furthermore, advanced semi-supervised machine-learning (HYDRA) addresses challenges associated with conventional clustering methods by capturing multiple dimension of heterogeneity with varying numbers of hyperplanes. We demonstrated distinct brain structure patterns and neurocognive performance (when the Low and Anti-Inflammatory clusters were combined) associated with subgroups. However results should be cautiously interpreted due to certain limitations. First, the algorithm detected 8 clusters, three of these clusters had a very small number of patients, therefore rendering any further investigation or their structural or functional differences not feasible. Those clusters may have been driven by unmeasured factors (e.g. infection) given that inflammatory levels were extremely high. Secondly, in the ASRB dataset, detailed positive symptom measures are not readily available, and thus the relation of inflammation to positive symptom severity is not captured. Furthermore, no Body Mass Index (BMI) data were available. We also combined two clusters (Low Inflammation and Anti-Inflammatory) to enhance statistical power in our neurocognitive comparisons. This does lead to loss of information and introduces bias but the choice was made based on the mechanistic similaties of the two clusters. The BeneMin sample may not have had sufficient size and cytokine measurement sensitivity to identify all clusters observed in the ASRB dataset. Despite the fact that having an external dataset is a strength it should also be acknowledged that an external dataset that is comprised of patients with similar illness characteristics may be have been more optimal. Finally, the role of cytokines belonging to the IL-17 pathway in the development of schizophrenia is important and while not investigated in the current study, future work should attempt to include IL-17 as one of the cytokines measured.

5. Conclusions

Identification of valid, reproducible subgroups of patients with schizophrenia based on inflammatory and neuroanatomical profile can aid in understanding putative and seperable mechanisms of illness and pathogenic processes (Comer et al., 2020). This is the first study to date to show that inflammation in schizophrenia is not merely a case of low vs high, but rather there are pluripotent, heterogeneous mechanisms at play. This could contribute to the identification of subgroup candidates for targeted novel anti-inflammatory treatments in schizophenia. Evidence for anti-inflammatory treatment in schizophrenia is mixed possibly due to the lack of immune subgrouo identification for targeted treatment(Deakin et al., 2018). Our results show that it may be possible to identify reliable subgroups of schizophrenia patients based on accessible, peripheral measures of inflammation in large sample sizes using advanced clustering techniques. These results need to be validated in international datasets and explored in longitudinal designs to further establish their stability.

6. Data sharing

Requests for sharing the anonymized database should be addressed to the lead authors.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Paris Alexandros Lalousis has received honoraria for talks presented at educational meetings organised by Boehringer-Ingelheim. Rachel Upthegrove reports grants from Medical Research Council, grants from National Institute for Health Research: Health Technology Assessment, grants from European Commission - Research: The Seventh Framework Programme, and personal speaker fees from Sunovion, outside the submitted work. This study was supported by grant UKRI MR/S037675/1 from the MRC Psychosis Immune Mechanism Stratified Medicine Study (PIMS). It was also supported by the NIHR Oxford Health Biomedical Research Centre. The views expressed are those of the author (s) and not necessarily those of the NIHR or the Department of Health and Social Care. Christos Pantelis was supported by a National Health and Medical Research Council (NHMRC) Senior Principal Research Fellowship (1105825), an NHMRC L3 Investigator Grant (1196508), and NHMRC-EU grant (1075379); he has participated on Advisory Boards for Janssen-Cilag, Astra-Zeneca, Lundbeck, and Servier; and has received honoraria for talks presented at educational meetings organised by Astra-Zeneca, Janssen-Cilag, Eli-Lilly, Pfizer, Lundbeck and Shire. Nusrat Husain is a past Trustee of Manchester Global Foundation (MGF), The Pakistan Institute of Living and Learning (PILL), Abaseen Foundation UK and Lancashire Mind UK. He is executive member of the Academic Faculty of the Royal College of Psychiatrists, London. He is a NIHR Senior Investigator. In the last five years he has attended educational events organized by various pharmaceutical industries.

Data availability

Data will be made available on request.

Acknowledgements

We thank Roxanne Cadiz and Dr. Hayley North in for excellent research assistance and expertise in preforming the cytokine and hsCRP assays.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.06.023.

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