

Serum immune markers and transition to psychosis in individuals at clinical high risk

Valeria Mondelli^{a,b,1,*}, Graham Blackman^{b,c,1}, Matthew J. Kempton^{b,c}, Thomas A. Pollak^c, Conrad Iyegbe^c, Lucia R. Valmaggia^{b,d}, Paul Amminger^e, Neus Barrantes-Vidal^f, Rodrigo Bressan^g, Mark van der Gaag^{h,i}, Lieuwe de Haan^{j,k}, Marie-Odile Krebs^l, Merete Nordentoft^m, Stephan Ruhrmannⁿ, Anita Riecher-Rössler^o, Bart P.F. Rutten^{p,s}, Gabriele Sachs^q, Nikolaos Koutsouleris^{c,q,r}, EU GEI High Risk Study², Philip McGuire^{b,c}

^a King's College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Psychological Medicine, London, UK

^b National Institute for Health and Care Research (NIHR) Maudsley Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London, UK

^c King's College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Psychosis Studies, London, UK

^d King's College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Psychology, London, UK

^e Centre for Youth Mental Health, University of Melbourne, 35 Poplar Road (Locked Bag 10), Parkville, Victoria 485 3052, Australia

^f Departament de Psicologia Clínica i de la Salut (Universitat Autònoma de Barcelona), Fundació Sanitària Sant Pere Claver (Spain), Spanish Mental Health Research Network (CIBERSAM), Australia

^g LiNC – Lab Interdisciplinar Neurociências Clínicas, Depto Psiquiatria, Escola Paulista de Medicina, Universidade Federal de São Paulo – UNIFESP, Australia

^h VU University, Department of Clinical Psychology and Amsterdam Public Health Research Institute, van der Boerhorststraat 7, 1081 BT Amsterdam, The Netherlands

ⁱ Parnassia Psychiatric Institute, Department of Psychosis Research, Zoutkeetsingel 40, 2512 HN The Hague, The Netherlands

^j AMC, Academic Psychiatric Centre, Department Early Psychosis, Meibergdreef 5, 1105 AZ Amsterdam, The Netherlands

^k Arkin Amsterdam, The Netherlands

^l University Paris Descartes, Hôpital Sainte-Anne, C'JAAD, Service Hospitalo-Universitaire, Inserm U894, Institut de Psychiatrie (CNRS 3557), Paris, France

^m Mental Health Center Copenhagen and Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research, CINS, Mental Health Center Glostrup, Mental Health Services in the Capital Region of Copenhagen, University of Copenhagen, Germany

ⁿ Department of Psychiatry and Psychotherapy, Faculty of Medicine and University Hospital, University of Cologne, Cologne, Germany

^o Medical Faculty, University of Basel, Switzerland

^p Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University Medical Centre, P.O. Box 616, 6200 MD 464 Maastricht, The Netherlands

^s Max-Planck Institute of Psychiatry, Munich, Germany

^q Medical University of Vienna, Department of Psychiatry and Psychotherapy, The Netherlands

^r Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University, Munich, Germany

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ABSTRACT

Individuals at clinical high risk (CHR) for psychosis have been found to have altered cytokine levels, but whether these changes are related to clinical outcomes remains unclear. We addressed this issue by measuring serum levels of 20 immune markers in 325 participants (n = 269 CHR, n = 56 healthy controls) using multiplex immunoassays, and then followed up the CHR sample to determine their clinical outcomes. Among 269 CHR individuals, 50 (18.6 %) developed psychosis by two years. Univariate and machine learning techniques were used to compare levels of inflammatory markers in CHR subjects and healthy controls, and in CHR subjects who had (CHR-t), or had not (CHR-nt) transitioned to psychosis.

An ANCOVA identified significant group differences (CHR-t, CHR-nt and controls) and post-hoc tests indicated that VEGF levels and the IL-10/IL-6 ratio were significantly higher in CHR-t than CHR-nt, after adjusting for multiple comparisons. Using a penalised logistic regression classifier, CHR participants were distinguished from controls with an area-under the curve (AUC) of 0.82, with IL-6 and IL-4 levels the most important discriminating

* Corresponding author.

E-mail address: valeria.mondelli@kcl.ac.uk (V. Mondelli).

¹ Joint first authors.

² See Appendix for EU-GEI High Risk Study Group Author list.

features. Transition to psychosis was predicted with an AUC of 0.57, with higher VEGF level and IL-10/IL-6 ratio the most important discriminating features. These data suggest that alterations in the levels of peripheral immune markers are associated with the subsequent onset of psychosis. The association with increased VEGF levels could reflect altered blood–brain-barrier (BBB) permeability, while the link with an elevated IL-10/IL-6 ratio points to an imbalance between anti- and pro-inflammatory cytokines.

1. Introduction

The role of the immune system in the aetiology of psychotic disorders has been increasingly acknowledged over recent decades. There are associations between psychotic and autoimmune disorders, patients with psychosis have higher levels of peripheral immune markers than controls, and genetic data implicate complement factors in the aetiology of psychosis (Mondelli et al., 2015; Nettis et al., 2019; Cullen et al., 2019; Upthegrove and Khandaker, 2020; Mondelli et al., 2020; Goldsmith et al., 2016; Mondelli et al., 2011; Heurich et al., 2022; Lizano et al., 2021).

Around 20 % of people who are at clinical high risk (CHR) for psychosis will develop a psychotic disorder within 2 years (Salazar de Pablo et al., 2021). A key challenge in the clinical management of this population is that it is difficult to predict whether a given individual will go on to become psychotic. The identification of biomarkers that are associated with transition could help to address this issue and identify potential targets for prevention treatments.

A recent meta-analysis found that people at CHR for psychosis have higher levels of interleukin (IL)-6, but lower levels of IL-1 β than controls, as well as trends for higher IL-4 and lower IL-10 levels (Park and Miller, 2020). However, the extent to which alterations in inflammatory markers are linked to the risk of later psychosis is less clear. To date, three studies have reported associations with psychosis onset in CHR samples: one with higher prolactin levels (Labad et al., 2015), a second with elevated levels of IL-12/IL-23p40 (Föcking et al., 2016), and a third with the levels of 15 markers together (including IL-1 β , IL-7, IL-8, and markers of blood brain barrier integrity), but not any of these alone (Perkins et al., 2015). These mixed findings may reflect the relatively small number of CHR participants in these studies, which involved samples of $n = 39$ (Labad et al., 2015), $n = 39$ (Föcking et al., 2016), and $n = 72$ (Perkins et al., 2015), respectively, from which 10, 11, and 32 participants transitioned to psychosis.

The aim of the present study was to determine whether the levels of inflammatory markers in CHR individuals are related to the risk of later transition to psychosis in a larger sample. 270 CHR participants and 56 controls were recruited to a multi-centre study involving 10 sites. We assessed peripheral immune markers at baseline, and then followed up the CHR sample to determine their clinical outcomes. We tested two main hypotheses. First, that there would be differences in the levels of inflammatory markers between CHR and HC participants. Second, that within the CHR sample, there would be differences in the levels of inflammatory markers between those who did, and did not later transition to psychosis.

2. Methods

2.1. Participants and clinical assessments

Participants were recruited as part of the European Union Gene-Environment Interactions (EU-GEI) study (<http://www.eu-gei.eu/>) from 10 centres (London, Amsterdam, the Hague, Vienna, Basel, Melbourne, Copenhagen, Paris, Barcelona, and Sao Paulo). The study received ethical approval at each recruitment site.

CHR participants were recruited from local mental health services and were eligible if they were: 1) aged 15 to 45 years, 2) met CHR criteria according to the Comprehensive Assessment of At-Risk Mental States (Yung et al., 2005) (CAARMS) and 3) were able to provide

informed written consent. Exclusion criteria were: 1) presence of a current or past psychotic disorder, 2) symptoms relevant for inclusion could be explained by a medical disorder or drug or alcohol dependency, 3) IQ < 60. Healthy control participants were recruited from 4 of the same sites where the CHR participants were recruited (London, Amsterdam, the Hague, Melbourne). Exclusion criteria for HC were the same as those for CHR participants. In addition, they were excluded if they met criteria for CHR based on the CAARMS. Further information about recruitment strategies has been published in previous papers from the same study (Kraan et al., 2018, 2020; Tognin et al., 2020; Modinos et al., 2020).

Trained raters assessed participants using CAARMS. Inter-rater reliability was assessed with online CAARMS training videos. The final sample was composed of 326 participants, including CHR ($n = 270$) and HC ($n = 56$). Following the baseline assessments, CHR participants had repeat assessments at 12 and 24 months. When face-to-face meetings were not possible, information on outcomes was obtained from clinical records in liaison with clinical teams. Transition to psychosis was defined as the development of full threshold psychotic disorder according to CAARMS. Based on visual inspection, one patient was excluded due to being an outlier on multiple immune markers leading to a final sample for the statistical analyses of CHR ($n = 269$) and HC ($n = 56$). Based on the follow-up assessment, CHR participants were divided into those who transitioned to psychosis (CHR-t; $n = 50$) and those who did not (CHR-nt; $n = 219$).

2.2. Assessment of inflammatory markers

Serum inflammatory markers were measured blind to group status. All the samples were analysed centrally in the same laboratory. Blood samples were centrifuged at 1000–1300g for 10 min. Aliquots of 500 μ l serum were stored at -80°C until the time of analysis. Candidate cytokines were measured in duplicates using Meso Scale Discovery (MSD) V-plex immunoassays (MSD, Maryland, USA) according to the manufacturer's instructions. The standard Pro-inflammatory Panel 1 (human) kit was used to measure interferon (IFN)- γ , interleukin (IL)-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and tumour necrosis factor (TNF)- α ; the standard Cytokine Panel 1 (human) kit was used to measure granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1 α , IL-5, IL-7, IL-12/IL23p40, IL15, IL-16, IL-17A, TNF- β , vascular endothelial growth factor (VEGF). The inter-assay coefficient of variations was <10 %. To assess the balance between anti-inflammatory and pro-inflammatory cytokines, we also calculated the IL-10/IL-6 ratio. This ratio has been found to be a better marker of disease progression than individual cytokines in non-psychiatric disorders (Pochat-Cotilloux et al., 2018; Song et al., 2016).

2.3. Statistical analysis strategy

Participants comprised individuals at CHR for psychosis and HC. By follow up, a proportion of those in the CHR group had transitioned to psychosis (CHR-t), whilst others had not transitioned (CHR-nt) (see [supplementary Fig. 1](#) upper left panel). Using immune markers collected at baseline along with relevant covariables (age, body mass index (BMI), gender, ethnicity, site, smoking status), we compared CHR and HC participants. We also compared CHR participants who had developed psychosis during follow-up with those who had not.

Immune markers (including IL-10/IL-6 ratio) were log-transformed,

due to non-parametric distributions, and analysed using classical statistical approaches to characterise group differences. Separately, we applied machine learning to characterise CHR status and develop a prediction model around which CHR participants would later transition to psychosis. We used penalised logistic regression and compared this against other established classifiers. Generalisability was assessed through cross validation.

2.3.1. Characterising group differences

We tested for differences between the three groups (CHR-t, CHR-nt and controls) in the levels of immune makers through a one-way analysis of covariance (ANCOVA) with BMI, ethnicity, smoking and recruitment site included as covariates. Significant differences between the 3 groups were followed up with paired contrasts between CHR-t and controls, CHR-nt and controls, and CHR-t and CHR-nt. We adjusted for multiple comparisons using a Benjamini and Hochberg approach with a false discovery rate (FDR) set at 0.05.

2.3.2. Development and evaluation of a prediction model

We first sought to characterise an immune marker signature of CHR status, before seeking to identify an immune marker signature of CHR participants who transitioned to psychosis. We trained several classification algorithms (logistic regression, logistic regression with stepwise selection, logistic regression with penalisation and linear support vector machine) and then compared them to select the optimal classifier.

Tenfold cross validation was performed for each classifier, which was then repeated five times. Distribution of participants across cross-validation folds were identical to facilitate cross-classifier statistical comparison. The performance of algorithms was based on balanced accuracy (BAC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The importance of individual predictors in distinguishing HC and CHR, as well as CHR-t and CHR-nt, was calculated based on the absolute coefficient of each predictor in the model. The importance of each feature was based on the absolute coefficients value of the final model.

For each classification model, randomised permutation testing was performed to derive a p-value of the performance based on the BAC (Ojala and Garriga, 2010). We also compared classifiers using Quade test, which is an extension of the non-parametric Wilcoxon signed rank test (Quade, 1979). An omnibus test was performed, followed by post-hoc pairwise comparisons between classifiers based on the area under the curve (AUC) values collected across cross validation partitions. The P values for pairwise comparisons were corrected using the false discovery rate (FDR) (see [supplementary methods](#) for further details on the development and evaluation of prediction models).

All analyses and visualisations were performed using R Statistical programming language (version 4.0.3), except for the ANCOVA, which was performed using SPSS. The threshold for statistical significance was $\alpha = 0.05$ and all tests were 2-tailed.

3. Results

The characteristics of the groups at baseline are shown in [Table 1](#). For the group-level comparisons (CHR vs HC and CHR-t vs CHR-nt) there were no differences in terms of age, gender, ethnicity, or BMI.

3.1. Group differences in inflammatory markers

An ANCOVA indicated that the three groups (CHR-t, CHR-nt and controls) differed significantly in VEGF levels and IL-10/IL-6 ratio, after adjusting for multiple comparisons (see [Table 2](#)). Post-hoc paired comparisons revealed that VEGF levels were significantly higher in the CHR-t than in the CHR-nt group ($F = 12.200$, $df = 1$, 222 , $p = 0.001$), and significantly higher in the control than in the CHR-nt group ($F = 5.293$, $df = 1$, 238 , $p = 0.006$). In addition, the IL-10/IL-6 ratio was significantly higher in the CHR-t than in the CHR-nt group ($F = 10.834$, $df = 1$,

Table 1

Socio-demographic and clinical characteristics of the groups.

	CHR-t N = 50	CHR-nt N = 219	Controls N = 56	Statistics
Age (years)	22.56 ± 0.719	22.63 $\pm$ 0.334	22.93 $\pm$ 0.541	$F = 0.103$, $df = 2$, 324 , $p = 0.9$
Gender M/F	29/21	118/101	30/26	Chi square = 0.3p = 0.9
Ethnicity (white %)	62 %	72 %	64 %	Chi square = 9.12p = 0.1
Body Mass Index (kg/m ²)	24.47 ± 0.71	24.38 $\pm$ 0.40	22.44 $\pm$ 0.455	$F = 3.345$, $df = 2$, 281 , $p = 0.37$
Smoking tobacco (yes %)	44.7 %	56.8 %	25 %	Chi square = 18.4, $p < 0.001$
Years in education	14.10 ± 0.5	14.39 $\pm$ 0.2	16.09 $\pm$ 0.401	$F = 7.858$, $df = 2$, 294 , $p < 0.001$
Baseline severity of illness (CGI)	3.69 $\pm$ 0.149	3.56 $\pm$ 0.080	—	—
Baseline GAF total score	52.47 ± 1.487	55.47 $\pm$ 0.711	—	—
Days from baseline to transition	401.5 ± 63.5	—	—	—
Number of participants exposed to antipsychotic treatment (%)	6 (12 %)	12 (5.5 %)	—	—

CGI: Clinical Global Impressions scale; GAF: Global Assessment of Functioning scale.

223, $p = 0.001$) and in the control group than CHR-nt group ($F = 4.595$, $df = 1$, 239 , $p = 0.033$). The post-hoc analyses remained significant, after adjusting for multiple comparisons.

3.2. Inflammatory marker signature of clinical high risk

We first combined immune and 6 non-immune parameters (age, sex, ethnicity, BMI, smoking, site) and applied prediction models to distinguish the entire CHR sample from HC participants (see [supplemental Figs. 1 and 2](#) for further details). Penalised logistic regression best separated the groups with an area under the curve (AUC) of 0.82 (95 % CI 0.80–0.84) (see [supplementary Table 1](#) for performance metrics for all classifiers). The final model included 18 predictors, including 14 immune variables. The most important immune variables were IL-6 and IL-4, with higher levels associated with CHR status ([Fig. 1](#)).

3.3. Inflammatory marker signature of transition to psychosis

We then used the same immune and non-immune parameters to devise prediction models to distinguish CHR individuals who later transitioned to psychosis from CHR individuals who did not. Penalised logistic regression best separated these two subgroups (AUC = 0.57; 95 % CI 0.52–0.61) (see [supplementary Table 2](#) for performance metrics for all classifiers). The final model included 6 predictors, including 3 immune variables. The most important immune variable was VEGF, followed by IL-10/IL-6; which were both higher in the CHR-t group (see [supplementary Fig. 4](#) for selected variables). The classifier was statistically significant based on random label permutation analysis (FDR adjusted $p = 0.004$). Quade's test was significant at the omnibus level ($p = 0.008$), however, paired comparisons found no significant difference between penalised logistic regression and the other classifiers. We repeated the penalised logistic regression model, this time only entering immune markers. However, the classification performance was virtually unchanged (AUC = 0.57; 95 % CI 0.53–0.61). When 4 immune variables were selected, the most important variable was VEGF, followed by IL10/IL6 ratio, as in the original analysis that included non-immune variables

Table 2

Adjusted mean $\pm$ SD of raw values of inflammatory markers in the three groups (CHR-t, CHR-nt, controls). ANCOVA analyses testing differences among the 3 groups controlling for BMI, ethnicity, smoking and site, statistics from analyses performed on logarithmic transformed values. In bold are reported the p-values which survived correction for multiple comparisons using Benjamini and Hochberg approach with a false discovery rate (FDR) set at 0.05.

	CHR-t	CHR-nt	Controls	F value (DoF)	P value
IFN- γ (pg/ml)	8.65 $\pm$ 7.40	12.96 $\pm$ 49.63	10.73 $\pm$ 20.61	0.933 (2, 278)	0.40
IL-10 (pg/ml)	0.56 $\pm$ 1.39	0.32 $\pm$ 0.23	0.57 $\pm$ 1.46	1.556 (2, 278)	0.21
IL-12p70 (pg/ml)	0.16 $\pm$ 0.18	0.30 $\pm$ 0.95	0.15 $\pm$ 0.12	1.050 (2, 206)	0.35
IL-13 (pg/ml)	0.77 $\pm$ 0.51	0.68 $\pm$ 0.67	0.70 $\pm$ 0.49	0.784 (2, 119)	0.46
IL-1 β (pg/ml)	0.09 $\pm$ 0.06	0.55 $\pm$ 2.81	0.19 $\pm$ 0.23	0.771 (2,100)	0.47
IL-2 (pg/ml)	0.22 $\pm$ 0.40	0.21 $\pm$ 0.47	0.36 $\pm$ 0.46	3.077 (2, 154)	0.049
IL-4 (pg/ml)	0.04 $\pm$ 0.04	0.03 $\pm$ 0.02	0.04 $\pm$ 0.04	1.541 (2, 216)	0.22
IL-6 (pg/ml)	0.48 $\pm$ 0.33	0.70 $\pm$ 0.91	0.44 $\pm$ 0.25	3.805 (2, 278)	0.023
IL-8 (pg/ml)	9.34 $\pm$ 3.01	11.30 $\pm$ 17.39	10.57 $\pm$ 6.24	1.233 (2, 278)	0.29
TNF- α (pg/ml)	3.04 $\pm$ 0.60	3.15 $\pm$ 1.96	2.92 $\pm$ 1.24	0.097 (2, 278)	0.91
GM-CSF (pg/ml)	0.20 $\pm$ 0.15	0.22 $\pm$ 0.23	0.20 $\pm$ 0.09	0.719 (2, 213)	0.49
IL-12/IL-23p40 (pg/ml)	125.62 $\pm$ 57.37	117.06 $\pm$ 54.78	123.84 $\pm$ 52.06	1.000 (2, 278)	0.37
IL-15 (pg/ml)	2.59 $\pm$ 0.47	2.67 $\pm$ 0.61	2.64 $\pm$ 0.46	0.271 (2, 278)	0.76
IL-16 (pg/ml)	227.14 $\pm$ 78.60	247.00 $\pm$ 98.53	225.89 $\pm$ 92.87	0.387 (2, 278)	0.68
IL-17A (pg/ml)	2.51 $\pm$ 2.54	2.20 $\pm$ 1.76	2.41 $\pm$ 3.88	0.021 (2, 278)	0.98
IL-1 α (pg/ml)	0.61 $\pm$ 1.24	0.57 $\pm$ 0.82	2.40 $\pm$ 9.57	4.850 (2, 213)	0.009
IL-5 (pg/ml)	0.94 $\pm$ 0.66	0.80 $\pm$ 0.68	0.92 $\pm$ 0.58	1.982 (2, 195)	0.14
IL-7 (pg/ml)	14.67 $\pm$ 6.07	13.46 $\pm$ 6.83	14.41 $\pm$ 6.60	2.924 (2, 278)	0.055
TNF- β (pg/ml)	0.77 $\pm$ 0.93	0.59 $\pm$ 0.27	0.58 $\pm$ 0.19	1.143 (2, 277)	0.32
VEGF (pg/ml)	139.90 $\pm$ 108.52	92.46 $\pm$ 78.56	99.51 $\pm$ 93.09	7.665 (2, 277)	0.001
IL10/IL6 ratio	1.30 $\pm$ 2.63	0.68 $\pm$ 0.57	1.27 $\pm$ 2.36	6.304 (2, 278)	0.002

(age, sex, ethnicity, BMI, smoking, site).

4. Discussion

At a group level, we found that CHR and control status were distinguishable on the basis of the levels of inflammatory, in particular higher IL-6 and IL-4 levels, and socio-demographic and anthropometric variables (AUC = 0.82). Furthermore, within the CHR sample, we found that the subsequent onset of psychosis was associated with elevated VEGF levels and with an increased IL-10/IL-6 ratio.

Our findings in the total CHR sample are consistent with previous evidence that alterations in inflammatory markers are associated with psychosis susceptibility (Park and Miller, 2020). In characterising the immune signature of CHR status, the most important cytokine markers were IL-6 and IL-4. The finding on IL-6 is in line with independent evidence from a meta-analysis that reported this marker to be the only cytokine that was significantly higher in participants at high risk for psychosis than in HC (Park and Miller, 2020). Consistent with our

findings for IL-4, the same meta-analysis also reported a trend for higher IL-4 levels in participants at high risk for psychosis than in HC (Park and Miller, 2020).

Within the CHR sample, the inflammatory marker that was most associated with transition to psychosis was VEGF. VEGF normally regulates angiogenesis and the permeability of the blood–brain barrier (BBB) (Proescholdt et al., 1999). In preclinical studies, disruption of BBB permeability is associated with increased levels of VEGF (Proescholdt et al., 1999). Our finding of elevated serum VEGF levels in the CHR-t group thus raises the possibility that increased BBB permeability could play a role in transition to psychosis. VEGF has not been identified as a predictor of transition in previous studies in CHR subjects (Labad et al., 2015; Föcking et al., 2016; Perkins et al., 2015). However, one of these studies found that transition was associated with other putative markers of BBB integrity, namely, matrix metalloproteinase-7, immunoglobulin E and coagulation factor VII (Perkins et al., 2015). In addition, VEGF levels have been linked to the onset of psychosis in individuals at familial high risk (Lizano et al., 2016). Psychotic disorders are associated with changes in peripheral inflammatory markers (Di Nicola et al., 2013; Pillinger et al., 2019; Cakici et al., 2020), as well as disruption in BBB (Pollak et al., 2018). Increased BBB permeability might therefore play a role in interactions between peripheral and central inflammatory processes in psychosis (Pollak et al., 2018; Mondelli et al., 2017). As well as effects on BBB permeability, VEGF has trophic effects on neurons and glial cells (Raab and Plate, 2007). VEGF receptors are found on neurons and glia (Lee et al., 2015), and BBB permeability may be related to the expression of VEGF-A on astrocytes (Argaw et al., 2012). The latter include effects on dopaminergic neurons (Silverman et al., 1999; Caballero et al., 2017), suggesting that VEGF could also influence psychosis risk through effects on the central dopaminergic system (Howes et al., 2011).

The second marker associated with transition to psychosis was the IL-10/IL-6 ratio. Interleukin-10 is a potent anti-inflammatory cytokine that normally plays a critical role in maintaining homeostasis. An elevated IL-10/IL-6 ratio in CHR participants who subsequently developed psychosis suggests an increase in anti-inflammatory, relative to pro-inflammatory cytokines, perhaps reflecting a compensatory response to abnormal inflammation. Interestingly, the IL-10/IL-6 ratio was similarly high in the CHR transition group and in the control group when compared to the CHR non-transition group, potentially suggesting that this anti-inflammatory/pro-inflammatory balance may only be relevant within populations already at high risk for psychosis and that a low IL-10/IL-6 ratio may be protective against transition in CHR individuals.

In the present study, the IL-10/IL-6 ratio was more strongly associated with transition than the level of IL-10 or IL-6 alone. This is consistent with the notion that the development of a normal adult brain is partly influenced by the balance between pro- and anti-inflammatory cytokine signalling, and that a disruption in this balance may lead to long-term behavioural and cognitive dysfunction, as occurs following the overexpression of IL-10 (Meyer et al., 2008). The IL-10/IL-6 ratio, as an index of the balance between anti-inflammatory and pro-inflammatory activity, has previously been identified as a predictive marker for treatment response and illness progression across a range of immune and neuroimmune disorders, with the ratio being more predictive than either IL-10 or IL-6 alone (Pochat-Cotilloux et al., 2018; Song et al., 2016; McElvaney et al., 2020). Both IL-10 and IL-6 have been previously implicated (separately) in the onset of psychosis (Di Nicola et al., 2013; Chiavetto et al., 2002), and it has been suggested that inhibition of the IL-6 signalling pathway may be helpful in the treatment of psychosis (Uptegrove and Khandaker, 2020). However, our data suggests that correcting the imbalance between anti- and pro-inflammatory cytokines could be a better approach.

Having identified differences at a group level between CHR subjects who did, or did not progress to psychosis, we used machine learning to assess whether inflammatory markers could be used to predict the subsequent onset of psychosis in an individual at CHR. Using

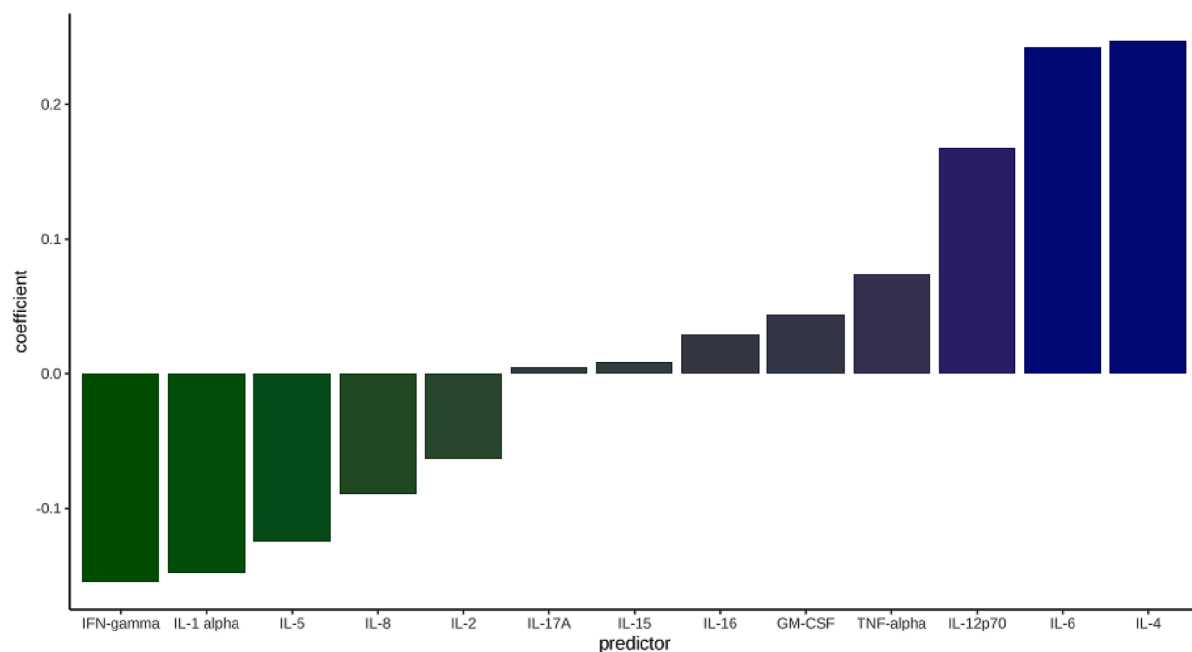


Fig. 1. Coefficients for cytokine predictors distinguishing CHR and healthy control status. Positive values indicate association with CHR status and negative values indicate association with healthy control status.

inflammatory, sociodemographic and anthropometric (i.e. BMI) measures the selected classifier had an AUC of 0.57 which is too low to be useful for outcome prediction in clinical practice (Hosmer, 2013). In a study of 72 CHR individuals, Perkins et al (Perkins et al., 2015) developed an algorithm incorporating 15 blood-based markers that predicted transition with AUC of 0.88. In a study of 76 help-seeking individuals, of whom 50 met CHR criteria, Chan et al (Chan et al., 2015) were able to predict transition with an AUC of 0.82, using 26 blood-based markers. Whilst both these studies reported larger AUCs than the present study, of note they were based on smaller samples and therefore are more prone to imprecise estimates. The onset of psychosis in CHR individuals has also been linked to the levels of proteomic (Mongan et al., 2021), lipidomic (Dickens et al., 2021) and polygenic risk (Perkins et al., 2020) markers, as well as to alterations in psychopathology, cognitive function (Brewer et al., 2006; Seidman et al., 2010), and structural, functional and neurochemical imaging measures (Howes et al., 2011; Pantelis et al., 2007; Cannon et al., 2015). It is possible that the predictive accuracy may be increased by integrating inflammatory markers with other candidate predictive biomarkers in a multi-modal machine learning approach (Koutsouleris et al., 2021).

A strength of the present study is that it is, to our knowledge, the largest study of immune markers in CHR to date. Previous studies involved CHR samples of $n = 39$ (Labad et al., 2015), $n = 39$ (Föcking et al., 2016), and $n = 72$ (Perkins et al., 2015). Because only around 20 % of CHR subjects are likely to transition to psychosis (Salazar de Pablo et al., 2021), relatively large samples are needed to ensure that the transition subgroup is large enough to detect significant associations. A further strength is that because most of the sample had not yet developed psychosis and were mostly medication-naïve, the results are unlikely to be attributable to the effects of previous illness or treatment. Although recruiting participants from 10 different centres allowed us to enrol a large CHR sample, this will have increased the heterogeneity of the sample. However, all blood samples and clinical data were collected, processed and analysed following standardised protocols, and there was no evidence from the ANCOVA that the findings were attributable to site effects. A few limitations need to be acknowledged. First, due to class imbalances between we performed SMOTE. Whilst this is a widely used oversampling technique to create synthetic data in a minority class, there are inherent limitations, including inadvertently creating unusual

patterns in the minority class. Equally, MICE is commonly used for handling missing data however can potentially introduce bias into the sample. Secondly, to maximise the number of participants included during the training phase, participants assessed in the test folds, were also included in the training folds as part of the cross-validation procedure. A disadvantage of this approach is that it increases the likelihood of overfitting. Future studies encompassing larger datasets are recommended to permit assessment of generalisability using a fully independent sample.

In conclusion, the CHR state is associated with alterations in the levels of peripheral immune markers. Among people at CHR for psychosis, the progression to illness may be associated with increased level VEGF and of the IL-10/IL-6 ratio suggesting a potential role for immune dysfunction in the onset of psychosis. However, the ability of machine learning models using immune markers to predict transition to psychosis at an individual level was low. Further studies are needed to clarify whether these markers may be better predictors of transition to psychosis when used in the context of specific clinical presentations/risk factors or in combination with other biological markers.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: VM has received research funding from Johnson & Johnson as part of a research program on depression and inflammation, but this is unrelated to the work presented in this study. The remaining authors report no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbi.2023.03.014>.

References

- Argaw, A.T., Asp, L., Zhang, J., Navrazhina, K., Pham, T., Mariani, J.N., Mahase, S., Dutta, D.J., Seto, J., Kramer, E.G., Ferrara, N., Sofroniew, M.V., John, G.R., 2012. Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *J. Clin. Invest.* 122 (7), 2454–2468.
- Brewer, W.J., Wood, S.J., Phillips, L.J., Francey, S.M., Pantelis, C., Yung, A.R., Cornblatt, B., McGorry, P.D., 2006. Generalized and specific cognitive performance in clinical high-risk cohorts: a review highlighting potential vulnerability markers for psychosis. *Schizophr. Bull.* 32 (3), 538–555.
- Caballero, B., Sherman, S.J., Falk, T., 2017. Insights into the mechanisms involved in protective effects of VEGF-B in dopaminergic neurons. *Parkinsons Dis* 2017, 1–13.
- Cakici, N., Sutterland, A.L., Penninx, B., Dalm, V.A., de Haan, L., van Beveren, N.J.M., 2020. Altered peripheral blood compounds in drug-naïve first-episode patients with either schizophrenia or major depressive disorder: a meta-analysis. *Brain Behav. Immun.* 88, 547–558.
- Cannon, T.D., Chung, Y., He, G., Sun, D., Jacobson, A., van Erp, T.G.M., McEwen, S., Addington, J., Bearden, C.E., Cadenhead, K., Cornblatt, B., Mathalon, D.H., McGlashan, T., Perkins, D., Jeffries, C., Seidman, L.J., Tsuang, M., Walker, E., Woods, S.W., Heinssen, R., 2015. Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. *Biol. Psychiatry* 77 (2), 147–157.
- Chan MK, Krebs MO, Cox D, Guest PC, Yolken RH, Rahmoune H, et al. Development of a blood-based molecular biomarker test for identification of schizophrenia before disease onset. *Transl Psychiatry*. 2015;5:e601.
- Chiavetto, L.B., Boin, F., Zanardini, R., Popoli, M., Michelato, A., Bignotti, S., Tura, G.B., Gennarelli, M., 2002. Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. *Biol. Psychiatry* 51 (6), 480–484.
- Cullen, A.E., Holmes, S., Pollak, T.A., Blackman, G., Joyce, D.W., Kempton, M.J., et al., 2019. Associations between non-neurological autoimmune disorders and psychosis: A meta-analysis. *Biol. Psychiatry* 85 (1), 35–48.
- Di Nicola, M., Cattaneo, A., Hepgul, N., Di Forti, M., Aitchison, K.J., Janiri, L., Murray, R.M., Dazzan, P., Pariente, C.M., Mondelli, V., 2013. Serum and gene expression profile of cytokines in first-episode psychosis. *Brain Behav. Immun.* 31, 90–95.
- Dickens, A.M., Sen, P., Kempton, M.J., Barrantes-Vidal, N., Iyegbe, C., Nordentoft, M., Pollak, T., Riecher-Rössler, A., Ruhrmann, S., Sachs, G., Bressan, R., Krebs, M.-O., Amminger, G.P., de Haan, L., van der Gaag, M., Valmaggia, L., Hyötyläinen, T., Oresic, M., McGuire, P., McGuire, P., Valmaggia, L.R., Kempton, M.J., Calem, M., Tognin, S., Modinos, G., de Haan, L., van der Gaag, M., Velthorst, E., Kraan, T.C., van Dam, D.S., Burger, N., Nelson, B., McGorry, P., Amminger, G.P., Pantelis, C., Politis, A., Goodall, J., Riecher-Rössler, A., Borgwardt, S., Rapp, C., Ittig, S., Studerus, E., Smieskova, R., Bressan, R., Gadelha, A., Brietzke, E., Asevedo, G., Asevedo, E., Zugman, A., Barrantes-Vidal, N., Domínguez-Martínez, T., Racciopi, A., Kwapił, T.R., Monsonet, M., Rosa, A., Frajerman, A., Chaumette, B., Bourgin, J., Kebir, O., Jantac, C., Krebs, M.-O., Nordholm, D., Randers, L., Krakauer, K., Glenthøj, L., Glenthøj, B., Nordentoft, M., Ruhrmann, S., Gebhard, D., Arnhold, J., Klosterkötter, J., Sachs, G., Lasser, I., Winklbaur, B., Delespaul, P.A., Rutten, B.P., van Os, J., 2021. Dysregulated lipid metabolism precedes onset of psychosis. *Biol. Psychiatry* 89 (3), 288–297.
- Föcking, M., Dicker, P., Lopez, L.M., Cannon, M., Schäfer, M.R., McGorry, P.D., Smešny, S., Cotter, D.R., Amminger, G.P., 2016. Differential expression of the inflammation marker IL12p40 in the at-risk mental state for psychosis: a predictor of transition to psychotic disorder? *BMC Psychiatry* 16 (1).
- Goldsmith, D.R., Rapaport, M.H., Miller, B.J., 2016. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol. Psychiatry* 21 (12), 1696–1709.
- Heurich, M., Föcking, M., Mongan, D., Cagney, G., Cotter, D.R., 2022. Dysregulation of complement and coagulation pathways: emerging mechanisms in the development of psychosis. *Mol. Psychiatry* 27 (1), 127–140.
- Hosmer Jr DW, Lemeshow S, Sturdivant RX. *Applied logistic regression*: John Wiley & Sons; 2013.
- Howes, O.D., Bose, S.K., Turkheimer, F., Valli, I., Egerton, A., Valmaggia, L.R., Murray, R.M., McGuire, P., 2011. Dopamine synthesis capacity before onset of psychosis: a prospective [18F]-DOPA PET imaging study. *Am. J. Psychiatry* 168 (12), 1311–1317.
- Koutsouleris, N., Dwyer, D.B., Degenhardt, F., Maj, C., Urquijo-Castro, M.F., Sanfelici, R., Popovic, D., Oeztuerk, O., Haas, S.S., Weiske, J., Ruef, A., Kambeitz-Ilanovic, L., Antonucci, L.A., Neufang, S., Schmidt-Kraepelin, C., Ruhrmann, S., Penzel, N., Kambeitz, J., Haidl, T.K., Rosen, M., Chisholm, K., Riecher-Rössler, A., Egloff, L., Schmidt, A., Andreou, C., Hietala, J., Schirmer, T., Romer, G., Walger, P., Francini, M., Traber-Walker, N., Schimmelmann, B.G., Flückiger, R., Michel, C., Rössler, W., Borisov, O., Krawitz, P.M., Heekeren, K., Buechler, R., Pantelis, C., Falkai, P., Salokangas, R.K.R., Lencer, R., Bertolino, A., Borgwardt, S., Noethen, M., Brambilla, P., Wood, S.J., Upthegrove, R., Schultze-Lutter, F., Theodoridou, A., Meisenzahl, E., 2021. Multimodal machine learning workflows for prediction of psychosis in patients with clinical high-risk syndromes and recent-onset depression. *JAMA Psychiatr.* 78 (2), 195.
- Kraan TC, Velthorst E, Themmen M, Valmaggia L, Kempton MJ, McGuire P, et al. Child Maltreatment and Clinical Outcome in Individuals at Ultra-High Risk for Psychosis in the EU-GEI High Risk Study. *Schizophr Bull.* 2018;44(3):584-92.
- Labad, J., Stojanovic-Pérez, A., Montalvo, I., Solé, M., Cabezas, Á., Ortega, L., Moreno, I., Vilella, E., Martorell, L., Reynolds, R.M., Gutiérrez-Zotes, A., 2015. Stress biomarkers as predictors of transition to psychosis in at-risk mental states: roles for cortisol, prolactin and albumin. *J. Psychiatr. Res.* 60, 163–169.
- Lee, B.-H., Hong, J.-P., Hwang, J.-A., Ham, B.-J., Na, K.-S., Kim, W.-J., Trigo, J., Kim, Y.-K., 2015. Alterations in plasma vascular endothelial growth factor levels in patients with schizophrenia before and after treatment. *Psychiatry Res.* 228 (1), 95–99.
- Lizano, P.L., Keshavan, M.S., Tandon, N., Mathew, I.T., Mothi, S.S., Montrose, D.M., Yao, J.K., 2016. Angiogenic and immune signatures in plasma of young relatives at familial high-risk for psychosis and first-episode patients: A preliminary study. *Schizophr. Res.* 170 (1), 115–122.
- Lizano, P., Lutz, O., Xu, Y., Rubin, L.H., Paskowitz, L., Lee, A.M., Eum, S., Keedy, S.K., Hill, S.K., Reilly, J.L., Wu, B., Tamminga, C.A., Clementz, B.A., Pearson, G.D., Gershon, E.S., Keshavan, M.S., Sweeney, J.A., Bishop, J.R., 2021. Multivariate relationships between peripheral inflammatory marker subtypes and cognitive and brain structural measures in psychosis. *Mol. Psychiatry* 26 (7), 3430–3443.
- McElvaney OJ, Hobbs BD, Qiao D, McElvaney OF, Moll M, McEvoy NL, et al. A linear prognostic score based on the ratio of interleukin-6 to interleukin-10 predicts outcomes in COVID-19. *EBioMedicine*. 2020;61:103026.
- Meyer, U., Murray, P.J., Urwyler, A., Yee, B.K., Schedlowski, M., Feldon, J., 2008. Adult behavioral and pharmacological dysfunctions highlighting disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. *Mol. Psychiatry* 13 (2), 208–221.
- Modinos, G., Kempton, M.J., Tognin, S., Calem, M., Porffy, L., Antoniadis, M., Mason, A., Azis, M., Allen, P., Nelson, B., McGorry, P., Pantelis, C., Riecher-Rössler, A., Borgwardt, S., Bressan, R., Barrantes-Vidal, N., Krebs, M.-O., Nordentoft, M., Glenthøj, B., Ruhrmann, S., Sachs, G., Rutten, B., van Os, J., de Haan, L., Velthorst, E., van der Gaag, M., Valmaggia, L.R., McGuire, P., 2020. Association of adverse outcomes with emotion processing and its neural substrate in individuals at clinical high risk for psychosis. *J. Am. Med. Assoc. Psychiatry* 77 (2), 190.
- Mondelli, V., Cattaneo, A., Murri, M.B., Di Forti, M., Handley, R., Hepgul, N., Miorrelli, A., Navari, S., Papadopoulos, A.S., Aitchison, K.J., Morgan, C., Murray, R.M., Dazzan, P., Pariente, C.M., 2011. Stress and inflammation reduce brain-derived neurotrophic factor expression in first-episode psychosis: a pathway to smaller hippocampal volume. *J. Clin. Psychiatry* 72 (12), 1677–1684.
- Mondelli, V., Ciufolini, S., Belvederi Murri, M., Bonaccorso, S., Di Forti, M., Giordano, A., Marques, T.R., Zunszain, P.A., Morgan, C., Murray, R.M., Pariente, C.M., Dazzan, P., 2015. Cortisol and inflammatory biomarkers predict poor treatment response in first episode psychosis. *Schizophr. Bull.* 41 (5), 1162–1170.
- Mondelli, V., Vernon, A.C., Turkheimer, F., Dazzan, P., Pariente, C.M., 2017. Brain microglia in psychiatric disorders. *Lancet Psychiatry* 4 (7), 563–572.
- Mondelli, V., Di Forti, M., Morgan, B.P., Murray, R.M., Pariente, C.M., Dazzan, P., 2020. Baseline high levels of complement component 4 predict worse clinical outcome at 1-year follow-up in first-episode psychosis. *Brain Behav. Immun.* 88, 913–915.
- Mongan, D., Föcking, M., Healy, C., Susai, S.R., Heurich, M., Wynne, K., Nelson, B., McGorry, P.D., Amminger, G.P., Nordentoft, M., Krebs, M.-O., Riecher-Rössler, A., Bressan, R.A., Barrantes-Vidal, N., Borgwardt, S., Ruhrmann, S., Sachs, G., Pantelis, C., van der Gaag, M., de Haan, L., Valmaggia, L., Pollak, T.A., Kempton, M.J., Rutten, B.P.F., Whelan, R., Cannon, M., Zammit, S., Cagney, G., Cotter, D.R., McGuire, P., 2021. Development of proteomic prediction models for transition to psychotic disorder in the clinical high-risk state and psychotic experiences in adolescence. *JAMA Psychiatr.* 78 (1), 77.
- Nettis, M.A., Pergola, G., Koliakou, A., O'Connor, J., Bonaccorso, S., David, A., Gaughran, F., Di Forti, M., Murray, R.M., Marques, T.R., Blasi, G., Bertolino, A., Pariente, C.M., Dazzan, P., Mondelli, V., 2019. Metabolic-inflammatory status as predictor of clinical outcome at 1-year follow-up in patients with first episode psychosis. *Psychoneuroendocrinology* 99, 145–153.
- Ojala, M., Garriga, G.C., 2010. Permutation tests for studying classifier performance. *J. Mach. Learn. Res.* 11, 1833–1863.
- Pantelis, C., Velakoulis, D., Wood, S.J., Yücel, M., Yung, A.R., Phillips, L.J., Sun, D.-Q., McGorry, P.D., 2007. Neuroimaging and emerging psychotic disorders: the Melbourne ultra-high risk studies. *Int. Rev. Psychiatry* 19 (4), 371–379.
- Park, S., Miller, B.J., 2020. Meta-analysis of cytokine and C-reactive protein levels in high-risk psychosis. *Schizophr. Res.* 226, 5–12.
- Perkins DO, Jeffries CD, Addington J, Bearden CE, Cadenhead KS, Cannon TD, et al. Towards a psychosis risk blood diagnostic for persons experiencing high-risk

- symptoms: preliminary results from the NAPLS project. *Schizophr Bull.* 2015;41(2): 419–28.
- Perkins, D.O., Olde Loohuis, L., Barbee, J., Ford, J., Jeffries, C.D., Addington, J., Bearden, C.E., Cadenhead, K.S., Cannon, T.D., Cornblatt, B.A., Mathalon, D.H., McGlashan, T.H., Seidman, L.J., Tsuang, M., Walker, E.F., Woods, S.W., 2020. Polygenic risk score contribution to psychosis prediction in a target population of persons at clinical high risk. *Am. J. Psychiatry* 177 (2), 155–163.
- Pillinger, T., Osimo, E.F., Brugger, S., Mondelli, V., McCutcheon, R.A., Howes, O.D., 2019. A meta-analysis of immune parameters, variability, and assessment of modal distribution in psychosis and test of the immune subgroup hypothesis. *Schizophr. Bull.* 45 (5), 1120–1133.
- Pochat-Cotilloux, C., Bienvenu, J., Nguyen, A.-M., Ohanessian, R., Ghesquière, H., Sève, P., Garnier, L., Kodjikian, L., 2018. Use of a threshold of interleukin-10 and IL-10/IL-6 ratio in ocular samples for the screening of vitreoretinal lymphoma. *Retina* 38 (4), 773–781.
- Pollak, T.A., Drndarski, S., Stone, J.M., David, A.S., McGuire, P., Abbott, N.J., 2018. The blood-brain barrier in psychosis. *Lancet Psychiatry* 5 (1), 79–92.
- Proescholdt, M.A., Heiss, J.D., Walbridge, S., Mühlhauser, J., Capogrossi, M.C., Oldfield, E.H., Merrill, M.J., 1999. Vascular endothelial growth factor (VEGF) modulates vascular permeability and inflammation in rat brain. *J. Neuropathol. Exp. Neurol.* 58 (6), 613–627.
- Quade, D., 1979. Using weighted rankings in the analysis of complete blocks with additive block effects. *J. Am. Stat. Assoc.* 74 (367), 680–683.
- Raab, S., Plate, K.H., 2007. Different networks, common growth factors: shared growth factors and receptors of the vascular and the nervous system. *Acta Neuropathol.* 113 (6), 607–626.
- Salazar de Pablo, G., Radua, J., Pereira, J., Bonoldi, I., Arienti, V., Besana, F., Soardo, L., Cabras, A., Fortea, L., Catalan, A., Vaquerizo-Serrano, J., Coronelli, F., Kaur, S., Da Silva, J., Shin, J.I., Solmi, M., Brondino, N., Politi, P., McGuire, P., Fusar-Poli, P., 2021. Probability of transition to psychosis in individuals at clinical high risk: An updated meta-analysis. *JAMA Psychiatry* 78 (9), 970.
- Seidman, L.J., Giuliano, A.J., Meyer, E.C., Addington, J., Cadenhead, K.S., Cannon, T.D., et al., 2010. Neuropsychology of the prodrome to psychosis in the NAPLS consortium: relationship to family history and conversion to psychosis. *Arch. Gen. Psychiatry* 67 (6), 578–588.
- Silverman, W.F., Krum, J.M., Mani, N., Rosenstein, J.M., 1999. Vascular, glial and neuronal effects of vascular endothelial growth factor in mesencephalic explant cultures. *Neuroscience* 90 (4), 1529–1541.
- Song, Y., Zhang, W., Zhang, L., Wu, W., Zhang, Y., Han, X., Yang, C., Zhang, L.u., Zhou, D., 2016. Cerebrospinal fluid IL-10 and IL-10/IL-6 as accurate diagnostic biomarkers for primary central nervous system large B-cell lymphoma. *Sci. Rep.* 6 (1).
- Tognin S, Catalan A, Modinos G, Kempton MJ, Bilbao A, Nelson B, et al. Emotion Recognition and Adverse Childhood Experiences in Individuals at Clinical High Risk of Psychosis. *Schizophr Bull.* 2020.
- Upthegrove, R., Khandaker, G.M., 2020. Cytokines, oxidative stress and cellular markers of inflammation in schizophrenia. *Curr. Top. Behav. Neurosci.* 44, 49–66.
- Yung, A.R., Yung, A.R., Pan Yuen, H., McGorry, P.D., Phillips, L.J., Kelly, D., Dell'olio, M., Francey, S.M., Cosgrave, E.M., Killackey, E., Stanford, C., Godfrey, K., Buckley, J., 2005. Mapping the onset of psychosis: the comprehensive assessment of at-risk mental states. *Aust. N. Z. J. Psychiatry* 39 (11-12), 964–971.