# The association between simvastatin augmentation and anti-inflammatory properties in SSD patients

Tim van Brouwershaven S2997738
Examiner: I.E.C. Sommer
Supervisor: S.S. Gangadin

# ABSTRACT

A subset of Schizophrenia Spectrum Disorders (SSD) patients show increased levels of inflammation which may contribute to the development of symptoms. Drug administration that lower this pro-inflammatory status may be beneficial. Simvastatin can cross the blood-brain barrier and has anti-inflammatory properties. In this study, we investigated the effect of 12 months of simvastatin administration versus placebo on the inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6). Furthermore, high inflammation cluster analysis was performed and used to evaluate symptom severity as measured with the Positive and Negative Syndrome Scale (PANSS). Recent-onset SSD patients were randomized 1:1 to simvastatin 40 mg (N=61) or placebo (N=58). Primary outcomes were IL-6 and CRP serum levels. Analyses were performed using linear mixed models. Compared to placebo, simvastatin augmentation led to a reduction of CRP ($X(1) = 5,33$, $P=0.021$). This treatment effect did not interact with time. IL-6 did not show a significant treatment effect or an interaction between treatment and time ($P>0.05$). Cluster analysis on baseline inflammatory biomarkers (IL-6, IL-8, IL-1RA and CRP) failed to create clusters with enough discriminative power and were therefore not further analysed. Elevated levels of CRP at baseline (>3mg, N=25) normalized after simvastatin treatment ($X(7)=28,284$, $P= 0.0002$). However, simvastatin had no effect on symptom severity in the high CRP subgroup ($P>0.05$). These findings suggest that simvastatin normalizes peripheral CRP levels, but fails to improve symptom severity in an elevated CRP subgroup.

# TABLE OF CONTENTS

# INTRODUCTION

Schizophrenia Spectrum Disorders (SSD) are characterized by positive (e.g. hallucinations and delusions) and negative (e.g. social or emotional withdrawal, blunted affect and poverty of speech) symptoms[1]. Although conventional treatment by antipsychotics has improved schizophrenia prognosis since the 1950s, the treatment and the response rate still have numerous weaknesses. First, a longer duration of untreated psychosis is associated with an impaired response to treatment[2]. Second, antipsychotics show little effect on negative symptoms[3,4]. These symptoms, roughly experienced by 15-20% of schizophrenia patients, are considered to be a major burden[5]. Thirdly, antipsychotics can lead to severe neurobiological and metabolic side effects and may eventually lead to sexual dysfunction or agranulocytosis[6]. Finally, compared to healthy peers, mortality is significant higher in schizophrenia patients[7]. Although suicide partially accounts for this increased mortality, a large contribution can be found in cardiovascular deaths due to a high prevalence of metabolic syndrome[7].

Mounting evidence suggests that chronical low-grade inflammation is a potential contributor in the pathogenesis of schizophrenia, at least in a subgroup of patients[8–10]. Several studies reported elevated levels of various inflammatory biomarkers in blood, including C-reactive protein (CRP)[11], IL-6[12], IL-1β[12], TNF-α[12], IL-8[13], IL-10[14] and Il-1RA[15] in patients with schizophrenia. Interestingly, elevated levels of CRP and IL-6 are associated with more severe negative symptoms[16]. Additionally, two individual studies suggested an association between increased CRP levels and elevated risk of SSD[17,18]. Furthermore, a meta-analysis about the efficacy of anti-inflammatory agents in these patients yielded promising results, arising the question whether beneficial effects on symptom severity could be mediated by anti-inflammatory mechanisms[19].

A possible approach can be found in the administration of statins, cholesterol lowering agents that have pleiotropic effects[20]. Next to lipid lowering, statins have a neuroprotective effect by affecting cellular signalling, hereby resulting in reduced oxidative damage, vascular function improvement and immune response reduction[21]. Evidence exists that statins induce clinically relevant anti-inflammatory effects independent of the lipid lowering effects[22]. A recent meta-analysis found several statins to be efficient in reducing CRP levels in patients with different types of cardiovascular diseases[23]. When considering both the cardioprotective and neuroprotective properties, statins are thought to potentially have a benefit for at least a subset of schizophrenia patients. Specifically simvastatin, due to its capacity to cross the blood-brain-barrier, cholesterol lowering effect and excellent safety profile[24].

At present, only a few trials of simvastatin administration in SSD patients exist. All of the studies provided 40 mg simvastatin a day for a period varying between 6 weeks and 12 months[25–28]. However, all of the studies failed to show significance on clinical endpoints. This was supported by a review on statins as adjuvant therapy, which reported a lack of solid evidence[29]. A recent RCT tested the effect of simvastatin addition on symptom severity and cognition in recent-onset SSD patients[28]. No significant changes were found on cognition, yet symptom severity was only significant after 6 months of the 12 month treatment. This arises the question to what extent simvastatin reduces inflammation and if there is an association between anti-inflammation and improvement of symptoms.

The aim of this study was to explore the effect of simvastatin administration on inflammatory markers in SSD patients, as additional analyses to a previous RCT[28]. We analysed CRP and IL-6 over time in recent-onset patients in both simvastatin and placebo groups. Furthermore, since previous studies show that a subset of SSD patients show chronic low-grade inflammation, we clustered high-inflammation groups and investigated the effects of simvastatin on symptom severity in these groups. We hypothesize that simvastatin administration reduces elevated levels of CRP and IL-6 and may have a beneficial effect on symptom severity in a high inflammation cluster.

# METHODS

*Participants*

This current study utilizes data from a prospective cohort study with participants from an earlier double-blind randomized placebo-controlled multi-centre trial. The primary results from this study were recently published[28]. Patients were 18-50 years of age, with a DSM-IV diagnosis of schizophrenia, schizoaffective, schizophreniform disorder or psychotic disorder not otherwise specified. First psychosis onset was no longer than 3 years ago. A total of 127 patients were included and 119 were randomized. Simvastatin or placebo administration was between baseline and 12 months and the follow-up period was 24 months with visits at baseline and after 1,3,6,9,12 and 24 months. Blood samples were collected at baseline as well as after 1,6,12 and 24 months. Complete study procedures and instruments are described per visit by Begemann et al[30].

*Immune biomarkers*

Blood was drawn in the morning of the baseline visit. We analysed the levels of a panel of inflammatory markers that have been associated with schizophrenia in recent meta-analyses[13,31–33] . High-sensitivity C-reactive protein (CRP) was measured in these samples through the central diagnostic laboratory of the UMC Utrecht and Groningen using the Siemens Atellica™ Solution turbidimetric immnoassay. The detection limit for CRP was defined as the lowest detected value within the standard curve. Samples below the detection limit were set to the half of this detection limit. The samples below the detection included 19 CRP with detection limit 0.5 (4,0%) from the UMC Utrecht and 8 CRP with detection limit 0.3 (1,7%) from the UMC Groningen.

In addition, serum was prepared and stored at -80$^0$C in aliquots within four hours after blood draw by the Central Biobank of the UMC Utrecht. At the end of the study, Interleukin(IL)-6, IL-8, IL-10, IL-1 Receptor Agonist (IL-1RA) were assessed using the Meso Scale Discovery U-PLEX Assay Platform (MSD Cat #K15067M, customized) and S-PLEX Human IL-6 Kit (MSD Cat# K151B3S), according to the manufacturer's protocol. All samples were detected within the standard range of the assay.

*Outcomes*

To examine possible differences in anti-inflammatory responses over time between simvastatin and placebo, we evaluated IL-6 and CRP levels. IL-6 levels were determined from serum at baseline, after 1 month and after 12 months. CRP was measured at baseline, after 1 month, 6 months and 12 months.

For the identification of a high-inflammation subgroup, we had two different approaches. First, we performed a cluster analysis to identify a high-inflammation cluster. Due to the fact that IL-10 is mainly anti-inflammatory, it was excluded for analysis. The clustering analysis included IL-6, IL-8, IL-1RA and CRP. Any missing values were replaced by the mean of the total. Only individuals with data of at least 3 out of 4 inflammatory markers were included. Biomarker values > 2 standard deviations from the group mean were considered outliers. If two or more values were identified as outliers, individuals were excluded from cluster analysis (N=2). A total of 94 Individuals were included in the cluster-analysis.

Second, a high CRP group was based on elevated levels of CRP at baseline. The cut-off was set at 3mg/mL because above this level, the risk of developing cardiovascular diseases is increased[34]. These high-inflammation groups were related to CRP levels over time. Furthermore, we evaluated total PANSS score as a predictor of symptom severity.

*Statistical analysis*

Statistical analyses were performed using R version 4.1.2 (www.R-project.org). We evaluated normality with Shapiro-Wilk normality test and evaluation of QQ-plots. Group differences in demographic variables were assessed using Chi-Square tests of independence for dichotomous variables and t-tests for continuous variables. All cytokine values were skewed and thus log transformed. Primary and secondary outcomes were tested using linear mixed effect models (LMMs) for repeated measurements with the *lme4* package. Time point, treatment, sex, BMI and study site were set as fixed factors, age and baseline CRP and PANSS scores as covariates and subject as a random factor. A binary variable indicating the allocation to the simvastatin or placebo-group was added to this model to evaluate the treatment effect. Subsequently, to test whether the treatment effects changes over time, a time*treatment interaction effect was added. When significant, group differences were compared at individual timepoints. Various assumptions (i.e. normality of residuals, normality of random effects, heteroscedasticity, homogeneity of variance and multicollinearity) of the linear mixed models were evaluated. To evaluate the relationship between LDL and CRP, a repeated measurement correlation (*rmcorr* package) was used.

For the cluster analysis we used the *kmeans* package to establish the optimal number of clusters. We used the *factoextra* package to evaluate overall model quality (silhouette width) and to perform cluster analysis. A Silhouette width <0.5 is considered weak[35] and therefore the required threshold was set at 0.5. The significance level for all statistical tests was P<.05, 2-tailed.

# RESULTS

*Demographics*

Demographic characteristics are shown in table 1. The simvastatin group did not significantly differ in terms of age, gender, smoking, BMI and Years of education. The groups were also similar in PANSS total, negative and general scores. However, the simvastatin group had higher baseline positive PANSS scores. The LMM corrected for this difference, by including the baseline scores as covariates.

| | **Simvastatin (N=61)** | | **Placebo (N=58)** | | **P-Value** |
|---|---|---|---|---|---|
| **Gender (female) [N(%)]** | 14 (23,0) | | 13 (22,4) | | 1 |
| **Smoking [N(%)]** | 40 (66,7)[1] | | 38 (65,5) | | 1 |
| | **Mean±SD** | **Range** | **Mean±SD** | **Range** | |
| **BMI** | 24,3±4,2 | 16,6-40,6 | 25±3,8 | 19,5-34,9 | 0.64 |
| **Age** | 26,4±5,8 | 18-46 | 28,0±7,9 | 18-50 | 0.12 |
| **Years of education** | 13,5±2,4 | 10-17 | 13,4±2,5 | 6-17 | 0.79 |
| **PANSS** | | | | | |
| *Total* | 58,9±13,0 | 36-83 | 56,8±15,0 | 33-97 | 0.36 |
| *Positive symptoms* | 14,1±5,2 | 7-30 | 12,4±4,1 | 7-24 | **0.041** |
| *Negative symptoms* | 14,8±4,9 | 7-28 | 14,9±5,4 | 7-31 | 0.93 |
| *General psychopathology* | 30,0±13,0 | 18-46 | 29,6±7,6 | 17-48 | 0.70 |

**Table 1** Demographic and clinical parameters at baseline in the simvastatin and the placebo group (N=119).
1: missing 1

*Inflammatory markers*

CRP showed a significant treatment effect ($X^2(1)=5,33$, estimated mean difference= -0,14, P=.02; figure 1). The time*treatment effect was not statistically significant ($X^2(1)=0,05$, P=.97). For IL-6, the treatment effect ($X^2(1)=0,31$, P=.58) and the treatment*time interaction effect ($X^2(1)=0,51$, P=0,47) were not significant.

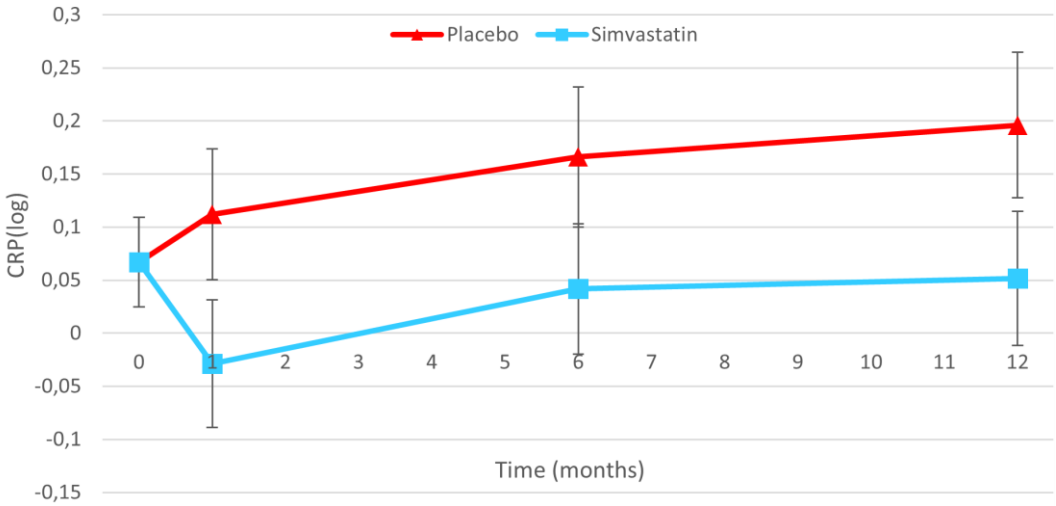


**Figure 1** *Mean CRP levels for the simvastatin and CRP group.*
This figure shows the change in CRP in the simvastatin and placebo group over 12 months. Means are estimated by a linear mixed effect model including sex, BMI, smoking, age, study site and time*treatment as covariates and displayed with standard errors.

*High inflammation sub-groups*

High inflammation k-means clustering led to an optimal clustering of 2 groups. The size of the groups and means of the inflammatory markers are summarized in table 4 (appendix). Silhouette width was 0.29 (figure 4, appendix). The silhouette width implies the quality of the clustering link and can vary between -1 and +1. The silhouette width did not reach the threshold of 0.5 and therefore, the clustering based on baseline cytokine levels of IL-6, IL-8, IL-1RA and CRP did not have enough discriminative power and was not further analysed.

For the high CRP clustering, the cut-off at 3mg/mL CRP resulted in the following groups: placebo-normalCRP (pnCRP)(N=45), placebo-highCRP (phCRP)(N=11), simvastatin-normalCRP (snCRP)(N=46) and simvastatin-highCRP (shCRP)(N=14)(Table 2). In total, 25 of the 119 patients (21%) had elevated CRP baseline levels. LMM analysis showed a significant treatment*CRP status effect ($X^2(7)=28,284$, P=.0002)(figure 2). At baseline, phCRP and pnCRP differ significantly, as well as shCRP and snCRP. This significant difference remains between pnCRP and phCRP. In the simvastatin group, this effect between high vs normal diminishes after treatment. Table 3 shows a complete overview of all the estimated means and P-values. A repeated measurement correlation did not show a significant correlation between LDL and CRP in the high-inflammation simvastatin group ($R^2(23)=0,30$, P=.07, 95% CI:-0.04 to 0.57(table 5, appendix).

| | | |
|---|---|---|
| **Placebo** | Normal | 45 |
| | High | 11 |
| **Simvastatin** | Normal | 46 |
| | High | 11 |

**Table 2** *Sample sizes of the different CRP clusters*

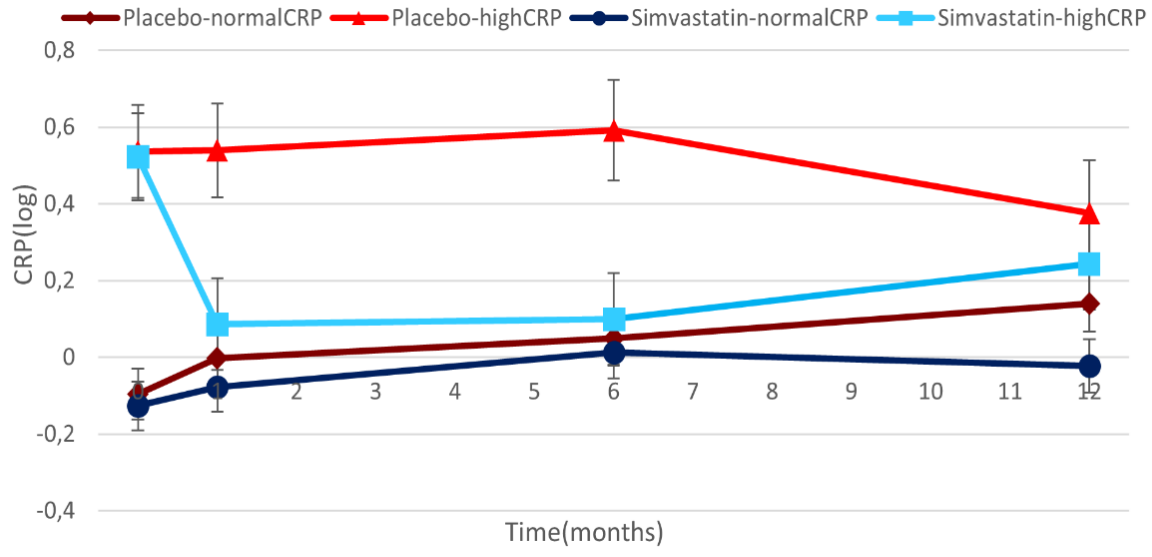


**Figure 2** *Mean CRP levels for the different groups.*

This figure shows the change in CRP in the high and low inflammation cluster of either the simvastatin or placebo group. Means are estimated by a linear mixed effect model including sex, BMI, smoking, age, study site and time*treatment as covariates and displayed with standard errors.

| | *Placebo high CRP vs normal CRP* | | | *Simvastatin high CRP vs low CRP* | | |
|---|---|---|---|---|---|---|
| | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* |
| ***Baseline*** | *0,63243* | *0,1334* | ***0,0001*** | *0,64919* | *0,1246* | ***<.0001*** |
| ***1 month*** | *0,54122* | *0,1345* | ***0,0021*** | *0,16407* | *0,1308* | *0,9816* |
| ***6 months*** | *0,54137* | *0,145* | ***0,0057*** | *0,08735* | *0,1321* | *1* |
| ***12 months*** | *0,23594* | *0,1515* | *0,8994* | *0,26626* | *0,1336* | *0,6132* |

**Table 3** *Estimated mean change in different CRP clusters*

Estimated mean difference in CRP in the CRP high vs CRP low groups for each timepoint within treatment conditions (simvastatin vs placebo). Means are estimated by a linear mixed model and displayed with standard errors.

*Symptom severity*

We used the total PANSS score to evaluate the symptom severity in the CRP cut-off groups. Figure 3 shows the estimated means of the total PANSS scores. The only significant effect was found in the snCRP group between 1-6 months of treatment (estimated mean difference = -6,50, P =0.01). A complete table of all the mean changes within groups are shown in the appendix (table 7). Furthermore, comparisons in both the simvastatin and the placebo group between high and low CRP status did not yield any significant results (data not shown).

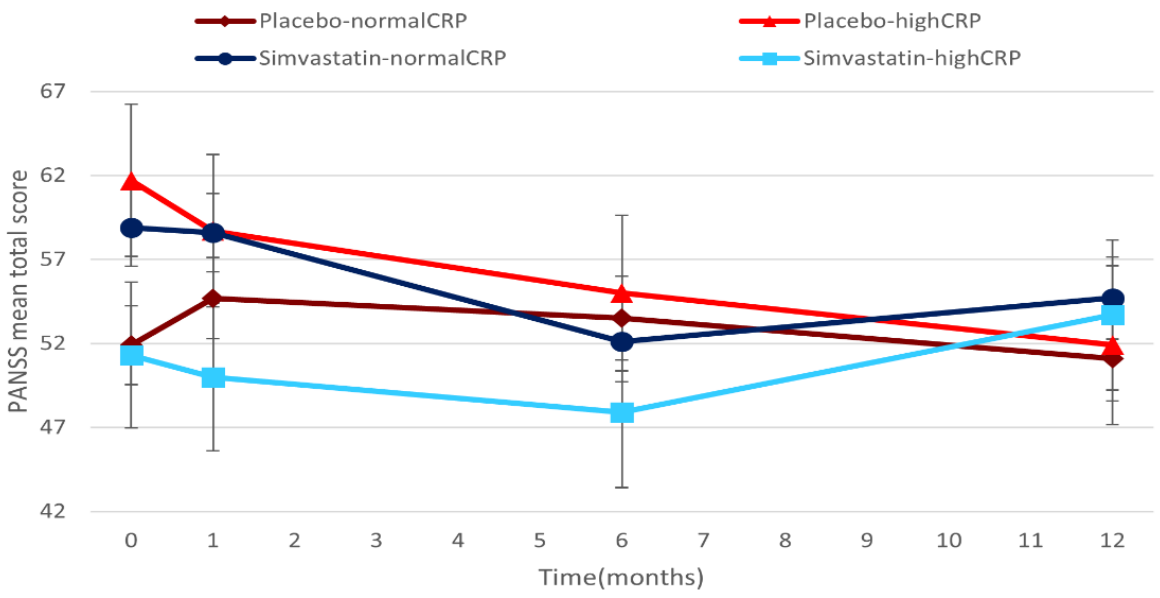


***Figure 3*** *Mean PANSS total scores for the different groups.*

Mean symptom severity (PANSS total score) for the high and normal CRP clusters of both the placebo and simvastatin groups. Means are estimated by a linear mixed model and displayed with standard errors.

# DISCUSSION

This study aimed to explore the effect of simvastatin administration on inflammatory markers in SSD patients, as additional analyses to a previous RCT. In addition, we attempted to identify a high inflammation cluster group with cluster analyses and a CRP cut-off to examine the possible effects of simvastatin on symptom severity in a high inflammation cluster. Simvastatin showed a significant CRP-lowering effect compared with placebo, which remained constant over time (i.e. no time*treatment effect). IL-6 failed to show any significant results. High inflammation clustering on IL-6, IL-8, IL-1RA and CRP did not result in suitable clusters. A subgroup consisting of patients with elevated CRP baseline levels (>3mg/L) showed a significant treatment*time effect. Simvastatin normalized high CRP levels after 1 month of treatment and this effect persisted throughout the treatment period. In the placebo group, elevated levels of CRP remained significant after 1 and 6 months of treatment. After 12 months, this effect was normalized. A repeated measurement correlation found that LDL and CRP were not correlated. Regarding secondary outcomes, snCRP PANSS total scores lowered significant between 1 and 6 months of treatment. This is in line with previous analyses on this data by Sommer et al., which found a decline in the PANSS total score after 6 months of simvastatin treatment[28]. Next to this effect, no other changes were found in the total PANSS scores between or within different groups.

Previous trials found a reduction in CRP levels after statin treatment in patients with a prior history of myocardial infarction[36], hyperlipidemia[37] or healthy peers[38]. However, to our knowledge, no trials exist which tested the effect of statins on CRP in SSD patients over time. We are the first study to investigate the levels of the pro-inflammatory biomarker CRP after simvastatin administration in a longitudinal study. Our results show that simvastatin administration induces a CRP lowering effect. No correlation between CRP and LDL was found. Although the beneficial effect of statins are mainly mediated by their lipid-lowering effects, studies showed that mechanism of statins may extend beyond their ability to lower cholesterol. Several trials argued that statins have anti-inflammatory effects independent of their lipid lowering capacities[37,39,40]. This is in line with our results and therefore, it can be suggested that simvastatin leads to a reduction of CRP independent of lipid lowering in SSD patients.

Remarkably, simvastatin augmentation had no effect on IL-6 levels. IL-6 induces the hepatic synthesis of CRP and they are therefore linked physiologically[41]. However, it is thought that in low-grade inflammation (as is often seen in schizophrenia patients) IL-6 and CRP are less influenced by each other compared to acute inflammation, due to different signalling routes of IL-6 and the different isoforms of CRP[42]. This may explain the discrepancy in simvastatin treatment effects on CRP and IL-6 reported in this study. CRP reduction was independent of lipid lowering and changes in IL-6 levels. A review article argued that statins induce reduction in CRP directly by inducing fractional catabolism of CRP[41], which may explain this lowering effect of simvastatin.

Several studies suggest that low-grade inflammation is present in only a subgroup of patients[8–10]. Evaluation of patients with elevated CRP levels at baseline led to some interesting results. Simvastatin was able to normalize CRP concentrations after 1 month of augmentation. However, the reduction of CRP in the high CRP cluster did not translate into total PANSS score. Several explanations can be found for these findings. The first and most obvious explanation is that a reduction of CRP is not an effective method to improve symptom severity in SSD. This is in line with other trials, which evaluated the association between symptom severity in patients with elevated CRP levels and anti-inflammatory agents such as prednisolone[46] and minocycline[47]. Secondly, evaluating solely CRP may not be a suitable way to define inflammation, since it is modulated via complex mechanisms. Therefore, it is possible that a CRP cut-off does not result in representative high-inflammation groups. Identification of a cluster by using multiple biomarkers may be a more appropriate method for characterizing inflammation compared to a single biomarker cut-off.

However, creating a high inflammation cluster appeared challenging in this study. Failure of reaching the overall model quality threshold can have several causes. First, the number of biomarkers we used were possibly not optimal. For this study, we had 5 inflammatory markers available and 4 were used for analysis. Cluster analyses performed by prior trials included 15[43], 20[44] or 798[45] markers. It is possible that a higher number of markers would result in a more suitable inflammation cluster. Second, the overall model quality can be manipulated by excluding patients with the least predictive power. We decided not to exclude more patients from analysis, because that would reduce our sample size and hereby the power of the model. For future research, when examining symptom severity in SSD patients, it may be interesting to create a high inflammation cluster including more markers and a greater sample size.

The sample size can definitely be considered a limitation in this study. Not only in the cluster analysis, but also in the high CRP cut-off groups, the sample sizes are considered small (N=11 and 14 for shCRP and phCRP, respectively). This results in little power of the findings we discussed. Next to these limitations, a few strengths of this study can be reported. First, to our knowledge, we are the first study to evaluate CRP levels in SSD patients after anti-inflammatory treatment in a longitudinal study. As mentioned before, although different trials exist which evaluated CRP after statin treatment over time in patients with a prior history of myocardial infarction, hyperlipidemia or healthy peers, no previous study examined this effect in SSD patients. Second, we used different methods for the creation of a high-inflammation subgroup. Due to the high complexity of the mechanisms behind inflammation, we considered the usage of several approaches highly important.

In conclusion, we demonstrated a reduction of the pro-inflammatory CRP after simvastatin treatment in SSD patients. This effect was more distinct in patients with elevated CRP at baseline, but was not associated with improvement of symptom severity. Future research may focus on the formation of a suitable high inflammation cluster and relate it to symptom severity.

# REFERENCES


1. Lewine RRJ, Fogg L, Meltzer HY. Assessment of Negative and Positive Symptoms in Schizophrenia. *Schizophrenia Bulletin*. 1983;9(3):368-376. doi:10.1093/schbul/9.3.368

2. Levi L, Bar Haim M, Burshtein S, et al. Duration of untreated psychosis and response to treatment: an analysis of response in the OPTiMiSE cohort. *European Neuropsychopharmacology*. 2020;32:131-135. doi:10.1016/j.euroneuro.2020.01.007

3. Haddad PM, Correll CU. The acute efficacy of antipsychotics in schizophrenia: a review of recent meta-analyses. *Therapeutic Advances in Psychopharmacology*. 2018;8(11):303-318. doi:10.1177/2045125318781475

4. Bucci P, Mucci A, van Rossum IW, et al. Persistent negative symptoms in recent-onset psychosis: Relationship to treatment response and psychosocial functioning. *European Neuropsychopharmacology*. 2020;34:76-86. doi:10.1016/j.euroneuro.2020.03.010

5. Bobes J, Arango C, Garcia-Garcia M, Rejas J. Prevalence of Negative Symptoms in Outpatients With Schizophrenia Spectrum Disorders Treated With Antipsychotics in Routine Clinical Practice. *The Journal of Clinical Psychiatry*. 2010;71(03):280-286. doi:10.4088/JCP.08m04250yel

6. de Berardis D, Rapini G, Olivieri L, et al. Safety of antipsychotics for the treatment of schizophrenia: a focus on the adverse effects of clozapine. *Therapeutic Advances in Drug Safety*. 2018;9(5):237-256. doi:10.1177/2042098618756261

7. Sommer IE, Tiihonen J, van Mourik A, Tanskanen A, Taipale H. The clinical course of schizophrenia in women and men—a nation-wide cohort study. *npj Schizophrenia*. 2020;6(1):12. doi:10.1038/s41537-020-0102-z

8. Trépanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. *Molecular Psychiatry*. 2016;21(8):1009-1026. doi:10.1038/mp.2016.90

9. Fond G, Resseguier N, Schürhoff F, et al. Relationships between low-grade peripheral inflammation and psychotropic drugs in schizophrenia: results from the national FACE-SZ cohort. *European Archives of Psychiatry and Clinical Neuroscience*. 2018;268(6):541-553. doi:10.1007/s00406-017-0847-1

10. Kahn RS, Sommer IE. The neurobiology and treatment of first-episode schizophrenia. *Molecular Psychiatry*. 2015;20(1):84-97. doi:10.1038/mp.2014.66

11. Fan X, Pristach C, Liu EY, Freudenreich O, Henderson DC, Goff DC. Elevated serum levels of C-reactive protein are associated with more severe psychopathology in a subgroup of patients with schizophrenia. *Psychiatry Research*. 2007;149(1-3):267-271. doi:10.1016/j.psychres.2006.07.011

12. Upthegrove R, Manzanares-Teson N, Barnes NM. Cytokine function in medication-naive first episode psychosis: A systematic review and meta-analysis. *Schizophrenia Research*. 2014;155(1-3):101-108. doi:10.1016/j.schres.2014.03.005

13. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Molecular Psychiatry*. 2016;21(12):1696-1709. doi:10.1038/mp.2016.3

14. Kunz M, Ceresér KM, Goi PD, et al. Serum levels of IL-6, IL-10 and TNF-α in patients with bipolar disorder and schizophrenia: differences in pro- and anti-inflammatory balance. *Revista Brasileira de Psiquiatria*. 2011;33(3):268-274. doi:10.1590/S1516-44462011000300010

15. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory Cytokine Alterations in Schizophrenia: A Systematic Quantitative Review. *Biological Psychiatry*. 2008;63(8):801-808. doi:10.1016/j.biopsych.2007.09.024

16. Meyer U, Schwarz MJ, Müller N. Inflammatory processes in schizophrenia: A promising neuroimmunological target for the treatment of negative/cognitive symptoms and beyond. *Pharmacology & Therapeutics*. 2011;132(1):96-110. doi:10.1016/j.pharmthera.2011.06.003

17. Inoshita M, Numata S, Tajima A, et al. A significant causal association between C-reactive protein levels and schizophrenia. *Scientific Reports*. 2016;6(1):26105. doi:10.1038/srep26105

18. Metcalf SA, Jones PB, Nordstrom T, et al. Serum C-reactive protein in adolescence and risk of schizophrenia in adulthood: A prospective birth cohort study. *Brain, Behavior, and Immunity*. 2017;59:253-259. doi:10.1016/j.bbi.2016.09.008

19. Sommer IE, van Westrhenen R, Begemann MJH, de Witte LD, Leucht S, Kahn RS. Efficacy of Anti-inflammatory Agents to Improve Symptoms in Patients With Schizophrenia: An Update. *Schizophrenia Bulletin*. 2014;40(1):181-191. doi:10.1093/schbul/sbt139

20. Dodd S, Maes M, Anderson G, Dean OM, Moylan S, Berk M. Putative neuroprotective agents in neuropsychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2013;42:135-145. doi:10.1016/j.pnpbp.2012.11.007

21. van der Most PJ, Dolga AM, Nijholt IM, Luiten PGM, Eisel ULM. Statins: Mechanisms of neuroprotection. *Progress in Neurobiology*. 2009;88(1):64-75. doi:10.1016/j.pneurobio.2009.02.002

22. Jain MK, Ridker PM. Anti-Inflammatory Effects of Statins: Clinical Evidence and Basic Mechanisms. *Nature Reviews Drug Discovery*. 2005;4(12):977-987. doi:10.1038/nrd1901

23. Kandelouei T, Abbasifard M, Imani D, et al. Effect of Statins on Serum level of hs-CRP and CRP in Patients with Cardiovascular Diseases: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Mediators of Inflammation*. 2022;2022:1-20. doi:10.1155/2022/8732360

24. Sierra S, Ramos MC, Molina P, Esteo C, Vázquez JA, Burgos JS. Statins as Neuroprotectants: A Comparative In Vitro Study of Lipophilicity, Blood-Brain-Barrier Penetration, Lowering of Brain Cholesterol, and Decrease of Neuron Cell Death. *Journal of Alzheimer's Disease*. 2011;23(2):307-318. doi:10.3233/JAD-2010-101179

25. Tajik-Esmaeeli S, Moazen-Zadeh E, Abbasi N, et al. Simvastatin adjunct therapy for negative symptoms of schizophrenia. *International Clinical Psychopharmacology*. 2017;32(2):87-94. doi:10.1097/YIC.0000000000000159

26. Chaudhry IB, Husain N, Drake R, et al. Add-on clinical effects of simvastatin and ondansetron in patients with schizophrenia stabilized on antipsychotic treatment: pilot study. *Therapeutic Advances in Psychopharmacology*. 2014;4(3):110-116. doi:10.1177/2045125313511487

27. Ashrafi S, Ghaffari SM, Boostani H, et al. Adjunctive simvastatin treatment in schizophrenia patients; a double blind randomized and placebo controlled trial. *Journal of Preventive Epidemiology*. 2021;7(1):e02-e02. doi:10.34172/jpe.2022.02

28. Sommer IE, Gangadin SS, de Witte LD, et al. Simvastatin Augmentation for Patients with Early-Phase Schizophrenia-Spectrum Disorders: A Double-Blind, Randomized Placebo-Controlled Trial. *Schizophrenia Bulletin*. 2021;47(4):1108-1115. doi:10.1093/schbul/sbab010

29. Tjardo S P, Lyliana G N, Maya J L S, et al. Statins as an Adjuvant Therapy for Psychotic Disorders: Current Evidence with a Systematic Overview of Double-Blind Placebo Controlled Trials. *Journal of Brain Disorders*. 2017;1(1). doi:10.36959/524/325

30. Begemann MJH, Schutte MJL, Slot MIE, et al. Simvastatin augmentation for recent-onset psychotic disorder: A study protocol. *BBA Clinical*. 2015;4:52-58. doi:10.1016/j.bbacli.2015.06.007

31. de Witte L, Tomasik J, Schwarz E, et al. Cytokine alterations in first-episode schizophrenia patients before and after antipsychotic treatment. *Schizophrenia Research*. 2014;154(1-3):23-29. doi:10.1016/j.schres.2014.02.005

32. Frydecka D, Krzystek-Korpacka M, Lubeiro A, et al. Profiling inflammatory signatures of schizophrenia: A cross-sectional and meta-analysis study. *Brain, Behavior, and Immunity*. 2018;71:28-36. doi:10.1016/j.bbi.2018.05.002

33. Wang Z, Li P, Chi D, Wu T, Mei Z, Cui G. Association between C-reactive protein and risk of schizophrenia: An updated meta-analysis. *Oncotarget*. 2017;8(43):75445-75454. doi:10.18632/oncotarget.17995

34. Ridker PM. High-sensitivity C-reactive protein and cardiovascular risk: rationale for screening and primary prevention. *The American Journal of Cardiology*. 2003;92(4):17-22. doi:10.1016/S0002-9149(03)00774-4

35. Kaufman L, Rousseeuw P. *Finding Groups in Data: An Introduction to Cluster Analysis*. John Wiley & Sons, INC.; 1990.

36. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-Term Effects of Pravastatin on Plasma Concentration of C-reactive Protein. *Circulation*. 1999;100(3):230-235. doi:10.1161/01.CIR.100.3.230

37. Jialal I, Stein D, Balis D, Grundy SM, Adams-Huet B, Devaraj S. Effect of Hydroxymethyl Glutaryl Coenzyme A Reductase Inhibitor Therapy on High Sensitive C-Reactive Protein Levels. *Circulation*. 2001;103(15):1933-1935. doi:10.1161/01.CIR.103.15.1933

38. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-Reactive Protein for the Targeting of Statin Therapy in the Primary Prevention of Acute Coronary Events. *New England Journal of Medicine*. 2001;344(26):1959-1965. doi:10.1056/NEJM200106283442601

39. Plenge JK, Hernandez TL, Weil KM, et al. Simvastatin Lowers C-Reactive Protein Within 14 Days. *Circulation*. 2002;106(12):1447-1452. doi:10.1161/01.CIR.0000029743.68247.31

40. Albert MA, Danielson E, Rifai N, Ridker PM, for the PRINCE Investigators. Effect of Statin Therapy on C-Reactive Protein Levels. *JAMA*. 2001;286(1):64. doi:10.1001/jama.286.1.64

41. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships Among Circulating Interleukin-6, C-Reactive Protein, and Traditional Cardiovascular Risk Factors in Women. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2002;22(10):1668-1673. doi:10.1161/01.ATV.0000029781.31325.66

42. del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. *Brain, Behavior, and Immunity*. 2018;70:61-75. doi:10.1016/j.bbi.2018.02.013

43. Lizano P, Lutz O, Xu Y, et al. Multivariate relationships between peripheral inflammatory marker subtypes and cognitive and brain structural measures in psychosis. *Molecular Psychiatry*. 2021;26(7):3430-3443. doi:10.1038/s41380-020-00914-0

44. Fillman SG, Sinclair D, Fung SJ, Webster MJ, Shannon Weickert C. Markers of inflammation and stress distinguish subsets of individuals with schizophrenia and bipolar disorder. *Translational Psychiatry*. 2014;4(2):e365-e365. doi:10.1038/tp.2014.8

45. Fillman SG, Cloonan N, Catts VS, et al. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Molecular Psychiatry*. 2013;18(2):206-214. doi:10.1038/mp.2012.110

46. Nasib LG, Gangadin SS, Rossum IW van, et al. The effect of prednisolone on symptom severity in schizophrenia: A placebo-controlled, randomized controlled trial. *Schizophrenia Research*. 2021;230:79-86. doi:10.1016/j.schres.2021.01.024

47. Deakin B, Suckling J, Barnes TRE, et al. The benefit of minocycline on negative symptoms of schizophrenia in patients with recent-onset psychosis (BeneMin): a randomised, double-blind, placebo-controlled trial. *The Lancet Psychiatry*. 2018;5(11):885-894. doi:10.1016/S2215-0366(18)30345-6

48. Levine SZ, Rabinowitz J. A population-based examination of the role of years of education, age of onset, and sex on the course of schizophrenia. *Psychiatry Research*. 2009;168(1):11-17. doi:10.1016/j.psychres.2008.05.005

# APPENDIX

| Cluster size | IL6 | CRP | IL1RA | IL8 |
|---|---|---|---|---|
| 38 | 0.8674962 | 0.8075048 | 0.6094499 | 0.2306975 |
| 56 | -0.5886582 | -0.5479497 | -0.4135553 | -0.1565447 |

**Table 4** *Inflammation clusters*
Clustering of the biomarkers IL-6, IL-1RA, IL-8 and CRP resulted in 2 clusters. Silhouette width = 0.29.


**Figure 4** *Cluster analysis*
A) Representation for clusters 1 (N=38) and 2 (N=56). B) Silhouette width for the different clusters.

| | PnCRP | | | PhCRP | | | SnCRP | | | ShCRP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* |
| ***Baseline*** | -0,09578 | 0,0653 | 0.000 | 0,53665 | 0,1215 | 0.000 | -0,1266 | 0,0635 | 0.000 | 0,52259 | 0,1134 | 0.000 |
| ***Change 0-1 months*** | 0,09374 | 0,0667 | 0,9523 | 0,00253 | 0,1304 | 1 | 0,0496 | 0,0654 | 0,9999 | -0,43552 | 0,1218 | **0,0108** |
| ***Change 1-6 months*** | 0,05229 | 0,0712 | 0,9999 | 0,05244 | 0,1395 | 1 | 0,08974 | 0,0692 | 0,9755 | 0,01302 | 0,1263 | 1 |
| ***Change 6-12 months*** | 0,09055 | 0,0752 | 0,9873 | -0,21488 | 0,1496 | 0,9438 | -0,0353 | 0,0727 | 1 | 0,14361 | 0,1249 | 0,9918 |

**Table 5** *Changes in CRP concentrations within the different groups*
Changes are with respect to the prior measurement. Means are estimated by a linear mixed effect model.

| | Normal CRP | Elevated CRP |
|---|---|---|
| **R** | 0.036 | 0.30 |
| **95% CI** | -0.1251506 - 0.1960346 | -0.04504091 - 0.5702414 |
| **P-value** | 0.66 | 0.07 |

**Table 6** *Repeated measurement correlation between LDL and CRP*
Repeated measurement correlation between baseline and 6 months of treatment

| | PnCRP | | | PhCRP | | | SnCRP | | | ShCRP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* | *Estimates* | *SE* | *P* |
| ***Baseline*** | 51,9 | 2,36 | 0.000 | 61,7 | 4,53 | 0.000 | 58,9 | 2,32 | 0.000 | 51,3 | 4,34 | 0.00 |
| ***Change 0-1 months*** | 2,856 | 1,77 | 0,8628 | -3 | 3,45 | 0,9995 | -0,289 | 1,71 | 1 | -1,351 | 3,16 | 1 |
| ***Change 1-6 months*** | -1,213 | 1,89 | 1 | -3,756 | 3,57 | 0,9959 | -6,503 | 1,81 | **0,01** | -2,09 | 3,27 | 1 |
| ***Change 6-12 months*** | -2,431 | 1,97 | 0,9817 | -3,054 | 3,75 | 0,9997 | 2,592 | 1,93 | 0,9616 | 5,833 | 3,31 | 0,7674 |

**Table 7** *Changes in PANSS total scores within the different groups*
Changes are with respect to the prior measurement. Means are estimated by a linear mixed effect model.