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Adjunctive *N*-acetylcysteine in depression: exploration of interleukin-6, C-reactive protein and brain-derived neurotrophic factor

Hasebe K, Gray L, Bortolasci C, Panizzutti B, Mohebbi M, Kidnapillai S, Spolding B, Walder K, Berk M, Malhi G, Dodd S, Dean OM. Adjunctive *N*-acetylcysteine in depression: exploration of interleukin-6, C-reactive protein and brain-derived neurotrophic factor.

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Objective: This study aimed to explore effects of adjunctive *N*-acetylcysteine (NAC) treatment on inflammatory and neurogenesis markers in unipolar depression.
Methods: We embarked on a 12-week clinical trial of NAC (2000 mg/day compared with placebo) as an adjunctive treatment for unipolar depression. A follow-up visit was conducted 4 weeks following the completion of treatment. We collected serum samples at baseline and the end of the treatment phase (week 12) to determine changes in interleukin-6 (IL6), C-reactive protein (CRP) and brain-derived neurotrophic factor (BDNF) following NAC treatment.
Results: NAC treatment significantly improved depressive symptoms on the Montgomery–Asberg Depression Rating Scale (MADRS) over 16 weeks of the trial. Serum levels of IL6 were associated with reductions of MADRS scores independent of treatment response. However, we found no significant changes in IL6, CRP and BDNF levels following NAC treatment.
Conclusion: Overall, this suggests that our results failed to support the hypothesis that IL6, CRP and BDNF are directly involved in the therapeutic mechanism of NAC in depression. IL6 may be a useful marker for future exploration of treatment response.

Keywords: clinical trial; depression; inflammation; *N*-acetylcysteine; neurotrophic factor

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Significant outcomes

- Lower serum levels of interleukin-6 (IL6) at baseline were associated with a greater reduction of Montgomery–Asberg Depression Rating Scale (MADRS) score regardless of *N*-acetylcysteine (NAC) intervention.
- The study failed to support associations between serum levels of C-reactive protein (CRP), IL6 and brain-derived neurotrophic factor (BDNF), and changes in depressive symptoms.

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- Alternate mechanisms including mitochondrial energy generation and glutamate need to be examined as potential mechanisms of action of NAC.

Limitations

- Solely relying on levels of IL6 and CRP to index the mechanisms of action of NAC may fail to capture the mechanism of action of NAC.
- The statistical method to overcome the number of missing samples at endpoint, multiple imputation, may not be fully representative of the missing data.
- Relatively small sample size may have impacted ability to detect significant effects given the small clinical effects seen.

Introduction

Depression is a widely prevalent psychiatric disorder that imposes significant psychological and physical burdens on individuals and their families. Although there are several factors contributing to depression, the association between depression and inflammation has been well documented. Depression is a common comorbidity in patients with chronic inflammatory diseases (1–4). Moreover, people with major depressive disorder (MDD) also exhibit elevated levels of inflammatory mediators even in the absence of inflammatory comorbidities (5,6). Mechanistically, elevated inflammatory mediators affect oxidative biology, bioenergetics, neuronal structure and survival. Glial cells are activated by inflammatory mediators and trigger immune activation in the central nervous system (7). Prolonged immune activation can produce elevated levels of reactive oxygen species and neurotoxins which subsequently damage neuronal structure and survival (7,8). Furthermore, magnetic resonance imaging studies show structural changes in the hippocampus and white matter in brains of individuals diagnosed with depression, potentially demonstrating the consequences of continued immunoinflammatory response, and these changes appear to be associated with recurrence (9,10). These data imply that an agent that modulates inflammatory mediators could be a promising therapeutic agent for depression. One such agent is NAC.

NAC is an antioxidant which provides cysteine for synthesis of the most ubiquitous antioxidant in the brain, glutathione (11). NAC also has intrinsic anti-inflammatory effects, as illustrated by reduced plasma levels of pro-inflammatory cytokines in haemodialysis patients following NAC treatment (12). NAC in addition has direct effects on glutamate neurotransmission. Preclinical studies suggest that the antidepressant effects of NAC are blocked if an AMPA glutamate antagonist is co-administered, suggesting a key role of glutamate (13). NAC also reverses many models of mitochondrial dysfunction, which is noteworthy given the increased evidence of mitochondrial dysfunction in mood disorders.

NAC has been trialled in psychiatric disorders including obsessive–compulsive disorder, trichotillomania, skin picking, addictions, schizophrenia and bipolar disorders. Significantly, NAC has shown therapeutic efficacy by modulating negative mood states in these disorders and has been successfully trialled as an adjunctive therapy for bipolar depression (14–16). Furthermore, adjunctive NAC treatment showed efficacy for depressive symptoms in a recently conducted clinical trial (17). These findings suggest that negative mood states in these psychiatric disorders may share an underlying mechanism. Although NAC has potential efficacy in these disorders, what is unclear is which pathways are responsible for these therapeutic effects, for example, whether NAC might exert therapeutic effects through targeting glutamate, mitochondrial energy generation, neurogenesis, apoptosis, oxidative stress and/or modulating pro-inflammatory responses.

Aim

To further investigate the therapeutic effects of NAC on depressive mood, we conducted exploratory analyses of association between NAC, inflammatory mediators and depressive mood using serum samples collected during the NAC depression clinical trial (17). In the current study, we evaluated serum levels of IL6, CRP and BDNF. We selected IL6 and CRP as inflammatory markers due to their observed correlations with clinical depression (5,18). BDNF was chosen as lower levels of BNDF were found in depressive patients and increased BDNF was associated with improvement of depression scores (6,19–23). We thus evaluated; whether inflammatory and neurotrophic markers correlated with depression status; whether these markers had predictive value for a response to NAC treatment; and how NAC may modulate these markers and depressive symptoms. In this exploratory study, we hypothesised that decreases in the serum levels of IL6 and CRP and an increase in levels of BDNF would be associated with improvement of MADRS score, and adjunctive

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NAC treatment ameliorates serum levels of IL6 and CRP and enhances levels of BDNF that subsequently is linked to improvement of MADRS scores.

Methods

Participants

Participants were recruited between 2007 and 2011 in three locations in Australia; Geelong, Melbourne and Sydney. All participants provided informed written consent and the study was conducted according to Good Clinical Practice guidelines. The trial was registered on the Australian and New Zealand Clinical Trials Registry (ACTRN12607000134426) and was approved by the relevant Human Research Ethics Committees. The main trial paper has been published and includes all demographic data (17). The demographic information relevant to the current paper is outlined below.

Inclusion criteria required that participants fulfilled the Diagnostic and Statistical Manual of Mental Disorders, the fourth edition (DSM-IV-TR) diagnostic criteria for MDD with a single episode or recurrent episodes; had a score of ≥18 on the MADRS score at the time of entry into the study; were at least 18 years of age; had the capacity to consent to the study; and to follow its instructions and procedures. If undergoing treatment, participants were required to have 2 weeks of stable treatment (based on their primary treatment; medication or psychotherapy) before entry into the study. Exclusion criteria were as follows: a concurrent diagnosis of bipolar I or II disorder or bipolar disorder not otherwise specified; a primary clinical diagnosis of a personality disorder; failure in three or more adequate trials of antidepressant therapy or electroconvulsive therapy (ECT) for the current major depressive episode; presence of a known or suspected clinically unstable systemic medical disorder, including recent gastrointestinal ulcers; pregnant or breast feeding status; current users of >500 mg/day of NAC, 200 µg/day of selenium, or 500 IU/day of vitamin E; and/or history of anaphylactic reaction to NAC or any component of the preparation (17). Diagnosis was confirmed using a structured interview, the Mini-International Neuropsychiatric Interview – 5.

Participants were randomly allocated to treatment or placebo groups in a double blind manner. The treatment group received NAC, 2 × 500 mg capsules twice daily (total of 2 g/day) in addition to existing treatment for their major depressive episode. NAC was supplied by Zambon (Milan, Italy), and encapsulated by DFC-Pharmamed Pty Ltd (Sydney, Australia) in accordance with Good Manufacturing Practice guidelines. The clinical trial endpoints were at end of treatment (week 12) and post-treatment discontinuation (week 16 – washout). The biological samples were collected at baseline and the end of treatment (week 12) (17). In this study, the primary outcomes were effects of adjunctive NAC intervention on levels of inflammatory markers (CRP and IL6) and BDNF. Baseline biological status was used as a predictor variable for clinical outcomes at both end of treatment (week 12) and washout (week 16).

Biological measures and assay methodology (CRP, IL6 and BDNF)

Blood samples for the investigation of inflammatory markers and BDNF were drawn at baseline and treatment endpoint (week 12) in the study. Standard vacutainer blood collection tubes (BD; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used. The current study utilised serum which was drawn into tubes with no additive. Tubes were immediately centrifuged at 1006 g, and the supernatant collected as the serum sample. All samples were stored at −80°C until tested. Serum CRP concentrations were determined using an enzyme-linked immunosorbent assay (human CRP Quantikine ELISA, R&D Systems, Minneapolis, MN, USA). Intra- and inter-assay coefficients of variation for the CRP assay were 5.5% and 6.5%, respectively. Serum concentrations of IL6 were measured using a high-sensitivity ELISA (R&D Systems). Intra- and inter-assay coefficient were 7.3% and 7.7%, respectively. Serum BDNF concentrations were measured using a human BDNF Quantikine ELISA. Intra- and inter-assay coefficient were 4.1% and 9.3%, respectively. All assays were carried out in accordance with the manufacturer's instructions.

Statistical analyses

All analyses are based on a modified intention to treat (ITT) model. Parametric modelling was not appropriate due to skewness of CRP, IL6 and BDNF data and the presence of outliers. In order to perform ITT analysis multiple imputation was performed for all missing biological data at treatment endpoint (week 12). Quantile regression models aimed at estimating the conditional median with least absolute errors were performed with imputed endpoint serum data to compare the difference of biological correlates at baseline and endpoint between the control and NAC groups (24,25).

For comparing MADRS score (baseline, weeks 12 and 16) and levels of CRP, IL6 and BDNF (baseline and endpoint) between the NAC treatment and control groups, repeated measures split plot in time analysis of variance models were estimated using a generalised estimation equation (GEE) approach with exchangeable working correlation matrix. Data sets

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of CRP, IL6 and BDNF were transformed and ranked due to outliers and the skewed nature of the data. Intervention by follow-up interaction (IFI) impacts and their 95% confidence intervals (CIs) were estimated from GEEs and reported to evaluate model adjusted mean difference between NAC and placebo groups.

For responders analyses, responders were defined as a reduction of more than or equal to 50% of MADRS score from baseline to treatment endpoint (week 12). For remitters analyses, remitters were defined as less than or equal to MADRS score 7 at treatment endpoint and washout (weeks 12 and 16). Median (quantile) regression models were used to explore the relationship between levels of CRP, IL6 and BDNF at baseline in MADRS responders and remitters at the NAC treatment endpoint and washout. Statistical analyses were carried out using STATA (StataCorp, College Station, TX, USA). We set a significance level at 0.05.

Results

Baseline characteristics of the sample are shown in Table 1. The treatment and placebo groups did not differ on demographic variables, clinical characteristics, body mass index (BMI) or collection sites. Of the total number of participants through the clinical trial ($n = 252$), we obtained baseline serum CRP and BDNF for 63 participants in the placebo group and 58 in the NAC group. For IL6, 62 participants in the placebo group had analyses and 56 participants in the NAC group. At treatment endpoint (week 12) serum CRP and BDNF were measured in 32 participants in the placebo group and 28 participants in the NAC group. For serum IL6, 30 samples were analysed in the placebo group and 26 in the NAC group. Missing samples were due to treatment withdrawal from the primary clinical trial or non-attendance for pathology collection [which was an optional component of the primary clinical trial (17)].

Table 1. Baseline demographic and clinical characteristics

	Total ($n = 121$)	Placebo ($n = 63$)	NAC ($n = 58$)
Demographic characteristics			
Collection site			
Geelong [*N* (%)]	46 (38.0)	16 (25.4)	30 (51.7)
Melbourne [*N* (%)]	4 (3.3)	3 (4.8)	1 (1.7)
Sydney [*N* (%)]	71 (58.7)	44 (69.8)	27 (46.6
Age (year)			
Mean (SD)	49.6 (12.5)	48.8 (12.5)	50.4 (12.5)
[range]	[20–77]	[21–77]	[20–75]
Gender			
Female [*N* (%)]	80 (66.1)	41 (65.1)	39 (67.2)
Male [*N* (%)]	42 (33.9)	22 (34.9)	19 (32.8)
Marital status			
Married [*N* (%)]	59 (48.8)	28 (44.4)	31 (53.5)
Single [*N* (%)]	28 (23.1)	17 (27.0)	11 (19.0)
De facto [*N* (%)]	10 (8.3)	6 (9.5)	4 (6.9)
Divorced [*N* (%)]	18 (14.9)	8 (12.7)	10 (17.2)
Separated [*N* (%)]	3 (2.5)	2 (3.2)	1 (1.7)
Widowed [*N* (%)]	3 (2.5)	2 (3.2)	1 (1.7)
Clinical characteristics			
MADRS			
Baseline [Mean (SD)]	27.49 (5.88)	28.11 (5.89)	26.83 (5.85)
[*N*]	[$n = 121$]	[$n = 63$]	[$n = 58$]
Endpoint [Mean (SD)]	16.01 (10.13)	18.78 (10.61)	13.13 (8.81)
[*N*]	[$n = 96$]	[$n = 49$]	[$n = 47$]
Washout [Mean (SD)]	17.19 (16.00)	20.46 (8.34)	13.78 (9.87)
[*N*]	[$n = 94$]	[$n = 48$]	[$n = 46$]
Duration of illness (year)			
[Mean (SD)]	14.7 (12.1)	15.4 (15.2)	13.9 (9.8)
[range]	[1–54]	[1–53]	[1–43]
Number of comorbidities			
None [*N* (%)]	35 (28.9)	21 (33.9)	14 (25.0)
1 Comorbidity [*N* (%)]	33 (27.3)	20 (32.3)	11 (19.6)
2 Comorbidities [*N* (%)]	30 (24.8)	10 (16.1)	19 (33.9)
3 Comorbidities [*N* (%)]	18 (14.9)	8 (12.9)	10 (17.9)
4 Comorbidities [*N* (%)]	5 (4.1)	3 (4.8)	2 (3.6)
BMI			
Underweight [*N* (%)]	3 (2.5)	2 (3.2)	1 (1.7)
Normal weight [*N* (%)]	46 (38.0)	24 (38.1)	22 (37.9)
Overweight [*N* (%)]	36 (29.8)	22 (34.9)	14 (24.1)
Obese [*N* (%)]	26 (21.5)	12 (19.1)	14 (24.1)
Missing [*N* (%)]	10 (8.2)	3 (4.8)	7 (12.1)
Medication at baseline [% (*n*)]			
No psychotrophic medication [*N* (%)]	33 (27.3)	17 (27.0)	16 (27.6)
Antidepressant [*N* (%)]	82 (67.8)	45 (71.4)	37 (63.8)
Benzodiazepine [*N* (%)]	10 (8.3)	5 (7.9)	5 (8.6)
Antipsychotic [*N* (%)]	9 (7.4)	4 (6.3)	5 (8.6)
Mood stabiliser [*N* (%)]	5 (4.1)	4 (6.3)	1 (1.7)

BMI, body mass index; MADRS, Montgomery–Asberg Depression Rating Scale; NAC, *N*-acetylcysteine. BMI was categorised < 18.50 = underweight, 18.50 to <25.00 = normal weight, 25.00 to <30.00 = overweight and >=30.00 = Obese.

NAC treatment and serum levels of CRP, IL6 and BDNF

To evaluate effects of adjunctive NAC treatment on serum levels of CRP, IL6 and BDNF, differences in these markers from baseline to endpoint in each treatment group were assessed. Median regression models indicated that the levels of serum CRP, IL6 and BDNF at baseline did not differ from levels of corresponding markers at endpoint in either the NAC or placebo groups (Table 2). The changes in levels of CRP, IL6 and BDNF between baseline and endpoint were compared between the NAC and placebo treatment groups, however, the changes in levels of each biological marker over time did not vary between groups (Table 2).

Correlations between serum levels of CRP, IL6 and BDNF, and demographic data

Correlations between serum levels of CRP, IL6 and BDNF, and duration of illness, comorbidities and

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Table 2. Comparisons of serum levels of C-reactive protein (CRP), interleukin-6 (IL6) and brain-derived neurotrophic factor (BDNF) at baseline to endpoint

	Placebo ($N = 63$)								NAC ($N = 58$)								Between group comparison		
	Baseline			Endpoint			Baseline vs. Endpoint		Baseline			Endpoint			Baseline vs. Endpoint				
	Mean	Median	SD	Mean	Median	SD	*p* value Model adjusted median [95% CI]		Mean	Median	SD	Mean	Median	SD	*p* value Model adjusted median [95% CI]		Model adjusted median	*p* value	95% CI
CRP (mg/l)	3.58	1.41	5.37	3.20	1.8	4.27	$p = 0.790$ −0.09 mg/l [−0.831 to 0.643]		4.10	2.50	4.68	3.91	1.75	5.29	$p = 0.412$ −0.57 mg/l [−2.246 to 1.098]		−0.48	$p = 0.485$	[−1.9999 to 1.03998]
IL6 (pg/ml)	3.50	1.01	8.49	2.76	1.21	4.82	$p = 0.924$ 0.03 pg/ml [−0.637 to 0.697]		2.68	0.97	8.3	2.77	1.14	6.37	$p = 0.624$ 0.17 pg/ml [−0.549 to 0.891]		0.14	$p = 0.769$	[−0.8384 to 1.11945]
BDNF (ng/ml)	23.03	22.73	7.47	22.15	21.90	6.72	$p = 0.468$ 1.42 ng/ml [−2.928 to 1.385]		21.74	20.78	9.29	24.51	22.51	10.26	$p = 0.494$ 1.42 ng/ml [−3.144 to 5.981]		2.19	$p = 0.258$	[−1.6974 to 6.07852]

CI, confidence interval.

BMI were analysed. However, there were no significant correlations (data not shown).

Potential mediation of clinical efficacy (MADRS) by biological markers (CRP, IL6 and BDNF)

The primary results from this clinical trial (17) found that adjunctive NAC treatment had positive effects at washout (week 16) – reflected by changes in MADRS score. This study further evaluated whether levels of CRP, IL6 and BDNF may have had mediator effects on the association between 12 weeks of NAC treatment and MADRS score. In this analysis, two separate time points for MADRS score were included: endpoint (week 12) and washout (week 16), which was 4 weeks after discontinuation of NAC treatment. Follow-up by intervention interaction impacts for MADRS score and CRP, IL6 and BDNF were assessed separately, including; (a) the interaction between NAC intervention and week 12 follow-up and week 16 washout for MADRS score controlling for baseline MADRS score; (b) the effects of baseline levels of CRP, IL6 and BDNF on MADRS score outcome from baseline to endpoint (weeks 12 and 16) controlling for MADRS score at baseline; and (c) the marginal interaction between baseline levels of CRP, IL6 and BDNF, and NAC intervention from baseline to endpoint (weeks 12 and 16) controlling for MADRS score at baseline.

MADRS score was significantly improved in the NAC treatment group at follow-up (week 12) compared with MADRS score at baseline [IFI impact = −4.1997; $p = 0.04$; 95% CI (−8.214 to −0.185)] and at washout (week 16) [IFI impact = −5.4451; $p = 0.002$; 95% CI (−8.927 to −1.963)], consistent with the findings of the overall trial. The main effects of baseline levels of biological markers on MADRS score outcome from baseline to endpoint (weeks 12 and 16) were assessed. Lower levels of IL6 at baseline were significantly related to a reduction of MADRS score from baseline to endpoint (weeks 12 and 16) [model adjusted mean changes in MADRS score = 0.029; $p = 0.042$; 95% CI (0.0011–0.0588)]. However, this effect of levels of IL6 at baseline to predict a reduction of MADRS score was not related to NAC intervention as our analysis indicated that the interaction between NAC intervention and levels of IL6 were not significant. Changes in MADRS score from baseline to endpoint (weeks 12 and 16) were not associated with either baseline CRP levels [model adjusted mean changes in MADRS score mean = 0.005; $p = 0.27$; 95% CI (−0.004 to 0.015)] nor baseline BDNF levels [model adjusted mean changes in MADRS score = 0.006; $p = 0.67$; 95% CI([−0.022 to 0.034]) (Table 3a). We then evaluated the marginal interaction effects

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Table 3a. Effects of baseline serum levels of C-reactive protein (CRP), interleukin-6 (IL6) and brain-derived neurotrophic factor (BDNF) on Montgomery–Asberg Depression Rating Scale (MADRS) score from baseline to endpoint (weeks 12 and 16) controlling by MADRS score at baseline

	Model adjusted mean changes in MADRS*	*p* value	95% CI
CRP	0.005	0.265	−0.004 to −0.015
IL6	0.029	0.042	0.001 to 0.059
BDNF	0.006	0.669	−0.022 to 0.034

CI, confidence interval.

Generalised estimation equation model was used to explore intervention impact on MADRS score (week 12 follow-up and week 16 washout), levels of each target (CRP, IL6 and BDNF) at baseline and endpoint. Data sets of CRP, IL6 and BDNF were transformed ranked data set due to outliers.

* Unstandardised regression β coefficient on ranked serum levels.

Table 3b. Marginal interaction effects of baseline serum levels of C-reactive protein (CRP), interleukin-6 (IL6) and brain-derived neurotrophic factor (BDNF) and *N*-acetylcysteine (NAC)/placebo treatments from baseline to endpoint (weeks 12 and 16) controlling for Montgomery–Asberg Depression Rating Scale (MADRS) score at baseline

	Model adjusted mean changes in MADRS*	*p* value	95% CI
CRP	0.008	0.830	−0.011 to −0.027
IL6	0.010	0.315	−0.009 to 0.030
BDNF	0.0001	0.990	−0.018 to 0.018

CI, confidence interval.

Generalised estimation equation model was used to explore intervention impact on MADRS score (week 12 follow-up and week 16 washout), levels of each target (CRP, IL6 and BDNF) at baseline and endpoint. Data sets of CRP, IL6 and BDNF were transformed ranked data set due to outliers.

* Unstandardised regression β coefficient on ranked serum levels for baseline serum levels and NAC/placebo treatments interaction with NAC group as reference category.

between baseline levels of biological markers and NAC intervention from baseline to endpoint (weeks 12 and 16) controlling for MADRS score at baseline. Levels of CRP, IL6 and BDNF did not differ between baseline and endpoint by NAC intervention; levels of CRP [model adjusted mean rank difference between NAC and placebo at endpoint = 0.0003; $p = 0.98$; 95% CI (−0.019 to 0.019)]; levels of IL6 [model adjusted mean rank difference between NAC and placebo at endpoint = 0.013; $p = 0.67$; 95% CI (−0.046 to 0.072)]; and levels of BDNF [model adjusted mean rank between NAC and placebo at endpoint = −0.017; $p = 0.56$; 95% CI (−0.075 to 0.040)] (Table 3b).

Responders and remitters analysis

Median regression models were used to analyse differences in levels of CRP, IL6 and BDNF between responders and non-responders, and between remitters and non-remitters. Responders were defined as participants who had more than or equal to a 50% reduction of MADRS scores from baseline to endpoint. Baseline CRP, IL6 and BDNF levels in responders did not differ from non-responders; CRP: model adjusted median difference at baseline between responders and non-responders 1.49 mg/l, $p = 0.27$, 95% CI (−1.1556 to 4.139); IL6: model adjusted median differences at baseline between responders and non-responders = −0.002 pg/ml, $p = 0.997$, 95% CI (−1.162 to 1.158); and BDNF: model adjusted median differences at baseline between responders and non-responders = −0.92 ng/ml, $p = 0.84$, 95% CI (−10.011 to 8.175). For exploratory analysis, levels of CRP, IL6 and BDNF at endpoint in responders and non-responders were also assessed. Endpoint CRP, IL6 and BDNF levels in responders did not differ from non-responders; CRP: model adjusted median differences at endpoint between responders and non-responders = 1.13 mg/l, $p = 0.28$, 95% CI (−0.931 to 3.199); IL6: model adjusted median differences at endpoint in responders and non-responders =0.314 pg/ml, $p = 0.73$, 95% CI (−1.511 to 2.138); and BDNF: model adjusted median differences at endpoint between responders and non-responders = 3.435 ng/ml, $p = 0.48$, 95% CI (−6.170 to 13.040). For remitters analysis, participants in each treatment group were categorised either remitters or non-remitters (final MADRS score <7). Baseline CRP, IL6 and BDNF levels in remitters did not differ from non-remitters; CRP: model adjusted median differences at baseline between remitters and non-remitters = −1.117 mg/l, $p = 0.56$, 95% CI (−4.875 to 2.640); IL6: model adjusted median differences at baseline between remitters and non-remitters = −0.218 pg/ml, $p = 0.749$, 95% CI (−1.567 to 1.131); and BDNF: model adjusted median differences at baseline between remitters and non-remitters = 0.898 ng/ml, $p = 0.87$, 95% CI (−10.330 to 12.126). Endpoint CRP, IL6 and BDNF levels in remitters did not differ from non-remitters; CRP: model adjusted median differences at endpoint between remitters and non-remitters = −0.39 mg/l, $p = 0.82$, 95% CI (−3.854 to 3.070); IL6: model adjusted median differences at endpoint between remitters and non-remitters = −0.70 pg/ml, $p = 0.83$, 95% CI (−7.008 to 5.609); and BDNF: model adjusted median differences at endpoint between remitters and non-remitters = 3.207 ng/ml, $p = 0.48$, 95% CI (−5.795 to 12.208). Characteristics of responders and remitters can be found in Supplementary Table S1.

Discussion

This study evaluated the potential mechanisms of action of adjunctive NAC treatment on depressive symptoms. We explored serum levels of CRP, IL6 and BDNF to index the inflammatory and

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neurotrophic elements of NAC's actions. Adjunctive NAC treatment improved depressive symptoms, which was consistent with the findings from the overall trial and a meta-analysis (17). However, our data did not show significant effects of adjunctive NAC on levels of CRP, IL6 or BDNF following 12 weeks of treatment. This study therefore does not provide support for the hypothesis that the operative pathway of NAC in depression is via CRP, IL6 or BDNF. What is unclear is whether this result suggests alternative mechanisms of action via alternate pathways impacting inflammation or neurotrophins, or whether this result is an artefact of assay sensitivity and study power, or whether the effects on depression are via entirely other pathways. In particular, the modest clinical effect sizes may compromise the power of detecting a moderating effect of subtle biomarker changes.

Interestingly, the GEE model indicated that lower baseline IL6 levels were significantly associated with reductions of MADRS scores from baseline to treatment endpoint and washout, but the effect was not related to NAC intervention. These findings imply that lower levels of IL6 at baseline appear to predict a reduction of MADRS scores. A possibility remains that the reduction of MADRS score at follow-up which was associated with baseline levels of IL6 might be attributable to ongoing concomitant medications. As NAC intervention was adjunctive therapy, our clinical population continued on their ongoing medications. A meta-analysis showed that antidepressants can reduce levels of IL6 (26). However, this finding should be interpreted with caution, given that our findings that failed to find differences in levels of IL6 between baseline and endpoint in the placebo and NAC intervention groups, and that levels of IL6 at baseline did not differ between responders and non-responders.

There also remains the possibility that ongoing medications may work in conjunction with the effects of NAC on these markers. Previous studies have shown that established antidepressant treatments have the capacity to influence inflammatory markers. Yoshimura et al. (6) reported that selective serotonin reuptake inhibitors (SSRI) and serotonin noradrenaline reuptake inhibitors (SNRIs) were associated with levels of IL6 and BDNF. Eight weeks of SSRIs (paroxetine, sertraline and fluvoxamine) or SNRI (milnacipran) treatment significantly reduced levels of IL6 and increased plasma levels of BDNF in participants who achieved a 50% reduction or more on the Hamilton Rating Scale for depression. Furthermore, higher plasma levels of IL6 were related to responsivity to SSRIs or SNRI treatments. Findings from Yoshimura et al. (6) indicated that plasma levels of IL6 and BDNF were associated with reduction in depressive symptoms following SSRI or SNRI treatments, and thus the mechanism of action of these drugs may involve both IL6 and BDNF. Although limited information regarding the cohort characteristics of this study was available, the age (39.6 year compared with average age 49.6 years in our clinical sample) and the sample size were different from our clinical population, raising the possibility that levels of IL6 and BDNF could fluctuate with age. The plasma IL6 concentration of depressed participants at baseline in Yoshimura et al. (6) was in a range between 2.5 and 3 pg/ml. On the other hand, the mean level of IL6 was 3.11 pg/ml and the median level of IL6 was 0.99 pg/ml in our clinical population. Considering the highly skewed distribution of IL6 concentration in our study, a comparison of the median of levels IL6 in our cohort to the range of levels of IL6 in Yoshimura et al. (6) indicates that there is a considerable difference in levels of IL6, which may be age related. In this regard, Saddadi et al. (12) reported that changes in serum levels of IL6 in response to NAC treatment in subjects whose age was under 40 years differed from subjects whose age was 40 years or over.

NAC treatment reduced levels of IL6 and CRP in chronic kidney disease patients. Nascimento et al. showed that 8 weeks of NAC treatment (600 mg × two times daily) significantly reduced plasma levels of IL6 but not plasma levels of CRP in peritoneal dialysis patients (27). Three months of NAC treatment, 600 mg twice a daily, also reduced serum levels of IL6 and CRP in patients with end-stage renal disease (12). Although both studies included study populations of relatively small size, demographic characteristics in these study populations and our study were very similar in terms of age and gender. However, the dose of NAC in these studies was slightly higher than our study. Furthermore, levels of IL6 at baseline (pre-treatment) in Nascimento et al. and Saddadi et al. were higher compared with our clinical population. The difference in the capacity of NAC to alter inflammatory markers may therefore reflect baseline IL6 levels, with low levels potentially not amenable to further reduction. There may also be dose effects or differences in the underlying inflammatory burden in these disorders.

For levels of BDNF, our cohort had the mean of 22.42 ng/ml at baseline which is considerably higher compared with a range between 1 and 1.5 ng/ml in Yoshimura et al. study (6). Bus et al. (28) found that serum levels of BDNF declined with age in females in a community population but levels in males remained stable. The authors also found serum BDNF levels were lower with increased levels of depressive symptoms. This conflicts with the comparisons of our study and Yoshimura et al. as

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our cohort was older than Yoshimura et al. and the plasma levels of BDNF at baseline in our study appeared to be higher than Yoshimura et al. However, antidepressant use may have contributed to an increase in plasma levels of BDNF (22,23). Our cohort had a long duration of illness, with a mean of 14.7 years, and had ongoing standard pharmacotherapy including SSRIs, mood stabilisers, atypical antipsychotics and sedatives, and therefore these medications may contribute to higher levels of BDNF at baseline in this study. There were 51 depressed participants (31 SSRI- or SNRI-responsive; 20 SSRI- or SNRI-refractory patients; compared with 30 healthy controls) in Yoshimura et al. (6) compared with 121 in our cohort. Small sample size also could lead to Type I or Type II errors. Uher et al. (29) also reported that serum levels of CRP predicted different outcomes following Escitalopram (SSRI) and Nortriptyline (SNRI) treatment. Our clinical population appeared to be older and BMI scores appeared to be higher compared with the Uher et al. (29) study population. These differences could be confounding factors that contribute to the finding that serum levels of CRP in Uher et al. (29) were lower than in our population. In our clinical population, mean CRP level was 3.83 mg/l and median CRP level was 2.03 mg/l, whereas Uher et al. (29) reported mean CRP level was 1.30–1.66 mg/l. Apart from differences in demographic characteristics between studies, a notable difference was that our study was using NAC as an adjunctive therapy. Therefore, there is a possibility that the mechanism of action of NAC may interact with current medications, which may affect levels of CRP, IL6 and BDNF. Although levels of CRP, IL6 and BDNF might be modulated by current antidepressants, these markers may not indicate the efficacy of NAC treatment.

SSRIs reduce the production of Th2 type cytokines such as IL6 and regulate cellular immune response (30), whereas SNRIs suppress Th1 type cytokines such as TNFα and modulate humoral balance (30). On the other hand, the mechanism of action of NAC differs distinctly from simply suppressing production of Th1 and Th2 cytokines. NAC is also a potent antioxidant and the therapeutic capacity of NAC to restore mitochondrial dysfunction has been well documented in preclinical studies (31–34). Mitochondrial dysfunction could mediate increases in production of reactive oxidative and nitrosative species, and subsequently contribute to increases in production of pro-inflammatory cytokines, DNA damage and lipid peroxidation (11,33,35).

NAC also has the capacity to modulate glutamate flux in the CNS, a feature with particular relevance to depression considering the accumulating evidence for the antidepressant effects of glutamatergic agents such as ketamine (36). NAC has effects on glutamate cysteine exchange as well as on the glutamate transporter GLT1 (37). Lastly, there is preclinical evidence that the glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is involved in the antidepressant effects of NAC (13). The glutamate pathway merits closer scrutiny as a major operative pathway.

We also assessed whether levels of CRP, IL6 and BDNF at baseline predicted the response to adjunctive NAC treatment, through responders and remitters analyses. Neither baseline levels of IL6, CRP and BDNF nor endpoint levels of IL6, CRP and BDNF were associated with more than or equal to 50% of reduction of MADRS score or remission at follow-up. Our findings consistently indicated that levels of CRP, IL6 and BDNF were not related to adjunctive NAC treatment outcome nor the severity of depressive symptoms.

The results from multilevel mixed-effects linear regression models were consistent with the findings that adjunctive NAC treatment had a positive effect on the improvement of MADRS score at treatment endpoint and washout (17). However, this effect was independent from levels of CRP, IL6 and BDNF. The greater the clinical effect size, the greater the statistical power to detect an operative mechanism – that the trials primary effect was modest compromises this capacity. Our findings from a comprehensive series of analyses indicated that CRP, IL6 and BDNF may not intersect with the pathways by which NAC exerted therapeutic effects on depressive symptoms in this population. Therefore, solely relying on levels of inflammatory markers to measure effectiveness of NAC may not be reflective of the mechanism of action of NAC. A combination of a wide range of markers including indicators of oxidative stress and glutamatergic function could provide a more comprehensive view of the efficacy of NAC and also the underlying pathophysiology of depression. Another limitation in this study was the number of missing samples at endpoint. We used multiple imputations to overcome this limitation, however, this could affect our results. Imputed data were based on values from other participants, which may not necessarily be fully representative of the missing data.

In summary, 3 months of adjunctive NAC treatment improved depressive symptoms in patients with major depression. Lower levels of IL6 at baseline were associated with a greater reduction of MADRS score regardless of NAC intervention. However, the finding needs to be interpreted cautiously as it contradicts other findings in this study that failed to find differences in serum levels of IL6 at baseline between responders and non-responders, and levels of IL6 did not differ between

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baseline and endpoint in both placebo and treatment groups. Overall, our results failed to provide support for an association between serum levels of CRP, IL6 and BDNF, and changes in depressive symptoms in our clinical sample, and suggest that alternate mechanisms including mitochondrial energy generation and glutamate need to be examined as potential mechanisms of action.

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Conflicts of Interest

O.M.D. has received grant support from the Brain and Behavior Foundation, Marion and EH Flack Trust, Simons Autism Foundation, Australian Rotary Health, Stanley Medical Research Institute, Deakin University, Brazilian Society Mobility Program, Lilly, NHMRC, Australasian Society for Bipolar and Depressive Disorders and Sevier. She has also received in kind support from BioMedica Nutraceuticals, NutritionCare and Bioceuticals. M.B. has received Grant/Research Support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, MBF, NHMRC, Beyond Blue, Rotary Health, Geelong Medical Research Foundation, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Meat and Livestock Board, Organon, Novartis, Mayne Pharma, Servier and Woolworths, has been a speaker for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck, Merck, Pfizer, Sanofi Synthelabo, Servier, Solvay and Wyeth, and served as a consultant to Astra Zeneca, Bioadvantex, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck Merck and Servier. M.B. is a co-inventor on two provisional patents regarding the use of NAC and related compounds for psychiatric indications, assigned to the Mental Health Research Institute. S.D. has received Grant/Research Support from the Cooperative Research Centre, Simons Autism Foundation, Stanley Medical Research Foundation, NHMRC, Beyond Blue, Rotary Health, Eli Lilly, Glaxo SmithKline, Organon, Novartis, Mayne Pharma, Servier and Fondation FondaMental, has been a speaker for Eli Lilly, has served as an advisory board member for Eli Lilly and Servier and has received conference travel support from Servier.

Ethical Standards

All participants provided informed written consent and the study was conducted according to Good Clinical Practice guidelines. The trial was registered on the Australian and New Zealand Clinical Trials Registry (ACTRN12607000134426) and was approved by the relevant Human Research Ethics Committees.

Supplementary Material

To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2017.2

References

1. Celano CM, Huffman JC. Depression and cardiac disease: a review. Cardiol Rev 2011;**19**:130–142.
2. Iaquinta M, McCrone S. An integrative review of correlates and predictors of depression in patients with rheumatoid arthritis. Arch Psychiatr Nurs 2015;**29**:265–278.
3. Kozdag G, Yalug I, Inan N et al. Major depressive disorder in chronic heart failure patients: does silent cerebral infarction cause major depressive disorder in this patient population? Turk Kardiyol Dern Ars 2015;**43**:505–512.
4. Rusu F, Dumitrascu DL. Four years follow-up of patients with irritable bowel syndrome. Rom J Intern Med 2015;**53**:63–72.
5. Hiles SA, Baker AL, de Malmanche T, Attia J. A meta-analysis of differences in IL-6 and IL-10 between people

https://doi.org/10.1017/neu.2017.2 Published online by Cambridge University Press

with and without depression: exploring the causes of heterogeneity. Brain Behav Immun 2012;**26**:1180–1188.
6. Yoshimura R, Hori H, Ikenouchi-Sugita A, Umene-Nakano W, Ueda N, Nakamura J. Higher plasma interleukin-6 (IL-6) level is associated with SSRI- or SNRI-refractory depression. Prog Neuropsychopharmacol Biol Psychiatry 2009;**33**: 722–726.
7. Czeh M, Gressens P, Kaindl AM. The yin and yang of microglia. Dev Neurosci 2011;**33**:199–209.
8. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007;**8**:57–69.
9. Samann PG, Hohn D, Chechko N et al. Prediction of antidepressant treatment response from gray matter volume across diagnostic categories. Eur Neuropsychopharmacol 2013;**23**:1503–1515.
10. Wisse LE, Biessels GJ, Stegenga BT et al. Major depressive episodes over the course of 7 years and hippocampal subfield volumes at 7 tesla MRI: the PREDICT-MR study. J Affect Disord 2015;**175**:1–7.
11. Dean O, Giorlando F, Berk M. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. J Psychiatry Neurosci 2011;**36**:78–86.
12. Saddadi F, Alatab S, Pasha F, Ganji MR, Soleimanian T. The effect of treatment with N-acetylcysteine on the serum levels of C-reactive protein and interleukin-6 in patients on hemodialysis. Saudi J Kidney Dis Transpl 2014;**25**: 66–72.
13. Linck VM, Costa-Campos L, Pilz LK, Garcia CR, Elisabetsky E. AMPA glutamate receptors mediate the antidepressant-like effects of N-acetylcysteine in the mouse tail suspension test. Behav Pharmacol 2012;**23**:171–177.
14. Berk M, Dean O, Cotton SM et al. The efficacy of N-acetylcysteine as an adjunctive treatment in bipolar depression: an open label trial. J Affect Disord 2011;**135**:389–394.
15. Lavoie S, Murray MM, Deppen P et al. Glutathione precursor, N-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. Neuropsychopharmacology 2008;**33**:2187–2199.
16. Magalhaes PV, Dean OM, Bush AI et al. N-acetylcysteine for major depressive episodes in bipolar disorder. Rev Bras Psiquiatr 2011;**33**:374–378.
17. Berk M, Dean OM, Cotton SM et al. The efficacy of adjunctive N-acetylcysteine in major depressive disorder: a double-blind, randomized, placebo-controlled trial. J Clin Psychiatry 2014;**75**:628–636.
18. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimaki M. Cumulative meta-analysis of interleukins 6 and 1beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. Brain Behav Immun 2015;**49**:206–215.
19. Matrisciano F, Bonaccorso S, Ricciardi A et al. Changes in BDNF serum levels in patients with major depression disorder (MDD) after 6 months treatment with sertraline, escitalopram, or venlafaxine. J Psychiatr Res 2009;**43**: 247–254.
20. Stelzhammer V, Haenisch F, Chan MK et al. Proteomic changes in serum of first onset, antidepressant drug-naive major depression patients. Int J Neuropsychopharmacol 2014;**17**:1599–1608.
21. Wolkowitz OM, Wolf J, Shelly W et al. Serum BDNF levels before treatment predict SSRI response in depression. Prog Neuropsychopharmacol Biol Psychiatry 2011;**35**: 1623–1630.
22. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. Int J Neuropsychopharmacol 2008;**11**: 1169–1180.
23. Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 2008;**64**:527–532.
24. Chamberlain G, Chamberlain G. Quantile regression, censoring, and the structure of wages: advances in econometrics. Cambridge: Cambridge University Press, 1994.
25. Koenker R. Quantile regression. New York: Cambridge University Press, 2005.
26. Hannestad J, DellaGioia N, Bloch M. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. Neuropsychopharmacology 2011;**36**:2452–2459.
27. Nascimento MM, Suliman ME, Silva M et al. Effect of oral N-acetylcysteine treatment on plasma inflammatory and oxidative stress markers in peritoneal dialysis patients: a placebo-controlled study. Perit Dial Int 2010;**30**:336–342.
28. Bus BA, Tendolkar I, Franke B et al. Serum brain-derived neurotrophic factor: determinants and relationship with depressive symptoms in a community population of middle-aged and elderly people. World J Biol Psychiatry 2012;**13**:39–47.
29. Uher R, Tansey KE, Dew T et al. An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. Am J Psychiatry 2014;**171**:1278–1286.
30. Martino M, Rocchi G, Escelsior A, Fornaro M. Immunomodulation mechanism of antidepressants: interactions between serotonin/norepinephrine balance and Th1/Th2 balance. Curr Neuropharmacol 2012;**10**:97–123.
31. Wright DJ, Renoir T, Smith ZM et al. N-acetylcysteine improves mitochondrial function and ameliorates behavioral deficits in the R6/1 mouse model of Huntington's disease. Transl Psychiatry 2015;**5**:e492.
32. Sandhir R, Sood A, Mehrotra A, Kamboj SS. N-acetylcysteine reverses mitochondrial dysfunctions and behavioral abnormalities in 3-nitropropionic acid-induced Huntington's disease. Neurodegener Dis 2012;**9**:145–157.
33. Guo J, Li Y, Chen Z et al. N-acetylcysteine treatment following spinal cord trauma reduces neural tissue damage and improves locomotor function in mice. Mol Med Rep 2015;**12**:37–44.
34. Sharma M, Kaur T, Singla SK. Protective effects of N-acetylcysteine against hyperoxaluria induced mitochondrial dysfunction in male wistar rats. Mol Cell Biochem 2015; **405**:105–114.
35. Morris G, Berk M. The many roads to mitochondrial dysfunction in neuroimmune and neuropsychiatric disorders. BMC Med 2015;**13**:68.
36. Krzyzanowska W, Pomierny B, Budziszewska B, Filip M, Pera J. N-acetylcysteine and ceftriaxone as preconditioning strategies in focal brain ischemia: influence on glutamate transporters expression. Neurotox Res 2016;**29**:539–550.
37. Roberts-Wolfe DJ, Kalivas PW. Glutamate transporter GLT-1 as a therapeutic target for substance use disorders. CNS Neurol Disord Drug Targets 2015;**14**:745–756.

https://doi.org/10.1017/neu.2017.2 Published online by Cambridge University Press