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Mediator effects of parameters of inflammation and neurogenesis from a *N*-acetyl cysteine clinical-trial for bipolar depression

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Abstract

Objective: This study aimed to explore effects of adjunctive treatment with *N*-acetyl cysteine (NAC) on markers of inflammation and neurogenesis in bipolar depression. *Methods:* This is a secondary analysis of a placebo-controlled randomised trial. Serum samples were collected at baseline, week 8, and week 32 of the open-label and maintenance phases of the clinical trial to determine changes in interleukin (IL)-6, IL-8, IL-10, tumour necrosis factor-α (TNF-α), C-reactive protein (CRP) and brain-derived neurotrophic factor (BDNF) following adjunctive NAC treatment, and to explore mediation and moderator effects of the listed markers. *Results:* Levels of brain-derived neurotrophic factor (BDNF), tumour necrosis factor-α (TNF-α), C-reactive protein (CRP), interleukins (IL) -6, 8, or 10 were not significantly changed during the course of the trial or specifically in the open-label and maintenance phases. There were no mediation or moderation effects of the biological factors on the clinical parameters. *Conclusion:* The results suggest that these particular biological parameters may not be directly involved in the therapeutic mechanism of action of adjunctive NAC in bipolar depression.

Significant outcomes

- *N*-acetyl cysteine (NAC) adjunctive treatment had no significant effect on the biological parameters evaluated.
- Although an improvement of depressive symptoms was seen in the clinical analysis we could not propose any mediation of response or remission by the biological factors.
- The study failed to support the hypothesis that the serum levels of BDNF, IL-6, IL-8, and IL-10, TNF-α, and CRP represent the pathways by which NAC mediates effects on depressive symptoms in this population.

Limitations

- The small sample size for the biological analyses may have affected the ability to detect subtle effects.
- The complexity of the pathways likely involved may also have precluded us from finding significant results.
- Timing of the assays may have missed earlier or later changes.

Introduction

Bipolar disorder (BD) is a lifelong, episodic and potentially progressive disorder, associated with medical and psychiatric comorbidities (1). BD was ranked by the World Health Organisation (WHO) as the illness with the second greatest effect on days out of role (2). Therapy is complex and differs for mania, depression, and euthymia, and between maintenance periods and acute episodes (3). Available pharmacological treatments are also associated with high rates of treatment resistance. Recurrent and persistent depressive episodes are particularly poorly addressed by available therapies (4).

New treatment options for BD exist, based on modulation of multiple targets thought to be involved in the pathophysiology of BD: glutamate receptors, oxidative stress, mitochondrial function, neuroprotective factors, and inflammatory pathways (5). NAC has been investigated for several psychiatric conditions and may be beneficial in the treatment of acute episodes in BD (6,7) and other neuropsychiatric disorders (8). NAC targets glutamatergic transmission, glutathione, and oxidative stress, neurotrophins, apoptosis, mitochondrial function, and inflammatory pathways (9–11), all described as altered in BD.

This report takes advantage of a placebo-controlled randomised trial to investigate both the effects of NAC on BDNF, IL-6, IL-8, IL-10, CRP, and TNF-α, and the relation between clinical response and changes in these circulating factors. As potential mechanisms underlying the improvement in depressive symptoms and functionality reported in the clinical part of the study (12,13), we hypothesise that NAC would increase serum levels of BDNF and the anti-inflammatory IL-10 and reduce the serum levels of the pro-inflammatory IL-6, IL-8, CRP, and TNF-α; moreover, that the changes in these circulating factors during the trial could be mediating and/or moderating the effects of NAC seen in the clinical outcomes of the trial.

Methods

Trial study design

This study examined blood samples provided from participants who took part in a trial of 2000 mg/day of adjunctive NAC for bipolar depression (ANZCTR: ACTRN12607000074493) detailed description of the clinical study design, procedures and sample characteristics has been published elsewhere (12,13). Briefly, participants were aged between 22 and 70 years of age and approximately two-thirds (67.8%) were female. Bipolar I disorder was the main diagnosis and the average length of illness since time of diagnosis was 10.0 years.

A brief overview of the overarching clinical trial is outlined below. The study included an 8-week open-label phase followed by a 24-week randomised, double-blind, maintenance phase. All study treatments were provided in addition to treatment as usual. To be included in the study participants had to meet DSM-IV criteria for bipolar I, bipolar II, or bipolar disorder not otherwise specified and have current symptoms of depression with Montgomery-Asberg Depression Scale (MADRS) scores of ≥12 at baseline. Response to NAC in the open-label phase was not an inclusion criterion for the maintenance phase (week 8 to week 32) and all participants who completed the 8-week open-label trial were randomised in the maintenance phase. All participants provided informed written consent and the study was conducted according to Good Clinical Practice and approved by the relevant Human Research and Ethics Committees.

Clinical outcomes were assessed from baseline to week 8 (12) and baseline to week 32 (13). However, for this report the time points for the investigation of biological parameters were baseline (week 0), the start of the maintenance phase (week 8) and trial endpoint (week 32). All analyses were completed for the whole trial (W0–W32), the open-label phase (W0–W8), and the maintenance phase (W8–W32). The total number of participants in the clinical trial was 149; however, for this report there were missing blood samples due to withdrawal from the clinical trial, lack of sample collection (which was optional) or out of range in the biomarkers assay (Table 1). Interviews assessed a variety of outcomes including the MADRS.

Biomarkers

Standard vacutainer blood collection tubes (BD; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with no additive were used. Tubes were immediately centrifuged at $1006 \times g$ and serum was collected and stored at −80°C until tested. BDNF, CRP, and TNF-α were determined using enzyme-linked immunosorbent assays (human Quantikine ELISA, R&D Systems, Minneapolis, MN, USA). IL-6, IL-8, and IL-10 were determined using multiplex immunoassay system (Bio-Plex Pro™ Human Inflammation Assays; Bio-Rad, Gladesville, NSW, Australia). All assays were carried out following the manufacturer's instructions.

Statistical analysis

To investigate longitudinal changes in BDNF and interleukins and their impacts on MADRS score, linear mixed models were used. For potential mediators, change scores were calculated as percent change (absolute change divided by baseline value) for ease of comparison. In addition, paired *t*-tests were performed to examine biomarkers' change from baseline to week 8 and 32 in NAC and placebo groups. Between-group (i.e. NAC versus placebo) difference of biomarkers' change from baseline were also examined using independent sample *t*-tests. With each of these potential mediators, we reran the original Intention To Treat (ITT) analysis of the primary outcome variable using linear mixed model approach (i.e. fixed effect treatment group as a factor, logarithm of time as a covariate and two-way interaction of treatment group and time), with the suspected mediator entered into the model as a time-dependent covariate. Because of randomisation, all suspected moderators were expected to be independent from and temporally precede the randomly assigned treatment, so the remaining criterion to evaluate was a significant three-way interaction of mediator as a time varying factor, treatment, and time (14) in a linear mixed model that consists of group, logarithm of time, and the potential mediator as main effects, and all three possible two-way interactions, and the three-way interaction. Beta coefficients [odds ratios (ORs)] and 95% confidence intervals (CIs) for two-way interactions of potential moderator and treatment group were reported as average moderator impact across all time points. For effect modifications, the original ITT analysis of the primary outcome variable was implemented with the suspected moderator entered into the model as a time invariant covariate measured at baseline. Similarly, three-way interaction of baseline (time invariant) potential moderator, treatment, and time was examined. Beta coefficients (ORs) and 95% CIs of potential moderator and treatment group two-way interaction were reported as average moderator impact across all time points.

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Table 1. Biological factors explored as potential mediators of *N*-acetyl cysteine (NAC) affects

	Total											
	Baseline (W0)				Maintenance (W8)				End of trial (W32)			
	n	Mean (SD)	Range	Median	*n*	Mean (SD)	Range	Median	*n*	Mean (SD)	Range	Median
BDNF	93	20.40 (8.9)	4.8–50.1	20.26	62	21.48 (8.6)	4.4–42.8	20.48	35	20.28 (10.8)	0.00–46.64	20.27
CRP	40	4.45 (5.6)	0.09–19.3	1.44	11	5.40 (4.77)	0.06–14.99	6.54	28	3.93 (4.67)	0.10–17.91	1.63
TNF-α★	89	0.0012 (0.27)	−0.63 to 0.98	0.0022	62	0.0135 (0.23)	−0.74 to 0.51	0.0569	33	0.0470 (0.2863)	−0.078 to 0.46	0.0283
IL-6	14	20.64 (26.99)	1.26–79.10	7.86	13	13.46 (16.94)	3.17–52.42	5.69	8	12.91 (15.22)	2.72–47.18	6.21
IL-8	51	22.96 (37.76)	1.41–191.5	7.46	28	14.23 (15.29)	1.58–70.97	9.63	13	10.14 (8.61)	2.10–31.40	7.41
IL-10	16	97.95 (186.60)	4.48–750.6	19.01	12	42.25 (29.58)	4.39–140.0	19.31	6	80.74 (125.82)	7.12–324.66	17.91
						NAC						
BDNF	46	21.67 (9.17)	4.92–50.12	21.19	31	21.27 (8.37)	4.43–42.88	20.41	16	22.38 (12.59)	0.00–46.64	24.45
CRP	20	3.7 (4.7)	0.47–19.35	1.31	6	5.67 (5.88)	0.37–14.99	4.35	14	3.84 (3.44)	0.25–10.51	3.27
TNF-α★	45	−0.0094 (0.27)	−0.63 to 0.85	−0.0074	31	0.0124 (0.21)	−0.38 to 0.39	0.0599	16	0.0382 (0.28)	−0.53 to 0.46	0.0982
IL-6	6	20.87 (29.35)	4.97–79.10	6.46	7	12.51 (16.66)	5.04–50.22	5.69	3	6.10 (3.17)	3.8–9.72	4.79
IL-8	27	24.05 (40.34)	1.84–191.5	9.16	12	15.60 (19.12)	1.84–70.97	10.43	5	8.60 (7.07)	2.10–20.17	7.41
IL-10	9	46.45 (58.74)	4.73–170.3	19.20	8	40.43 (46.32)	4.39–127.2	18.46	3	13.12 (10.32)	7.12–25.05	7.21
						Placebo						
BDNF	47	19.17 (8.61)	4.89–39.34	19.01	31	21.69 (8.95)	7.71–41.84	20.54	19	18.52 (9.09)	1.47–36.85	18.72
CRP	20	5.15 (6.46)	0.09–18.95	2.09	5	5.07 (3.67)	0.06–9.17	6.54	14	4.03 (5.79)	0.10–17.91	1.10
TNF-α★	44	0.0120 (0.26)	−0.55 to 0.98	0.0416	31	0.0147 (0.26)	−0.74 to 0.51	0.0512	17	−0.12 (0.26)	−0.78 to 0.26	−0.0630
IL-6	8	20.46 (27.15)	1.26–71.27	8.69	6	14.57 (18.79)	3.17–52.42	7.69	5	17.0 (18.57)	2.72–47.18	6.74
IL-8	24	21.74 (35.47)	1.41–130.4	6.93	16	13.21 (12.25)	1.58–50.10	8.96	8	11.10 (9.78)	2.36–31.40	7.09
IL-10	7	164.17 (270.84)	4.48–750.6	18.83	4	45.91 (63.09)	5.02–140.01	19.31	3	148.36 (160.48)	10.77–324.66	109.65

BDNF, brain-derived neurotrophic factor (pg/ml); CRP, C-reactive protein (ng/ml); TNF- α, tumour necrosis factor alpha (pg/ml); IL, interleukin (pg/ml); SD, standard deviation; *n*, number of subjects per time point.
★Values transformed to logarithm due to skewness.

For responder and remitters analysis, responders were defined as the participants who had more than or equal to 50% reduction in MADRS scores from baseline to the end of the trial. Remitters were those who at the end of the trial presented MADRS scores of less than or equal to 7. Logistic regression models were used to examine the mediator effect of biological factors (BDNF, TNF-α, CRP, IL-6, IL-8, and IL-10) on the NAC treatment at baseline, maintenance, and end of trial time points that were evaluated by two-way interaction of mediator and treatment. ORs and 95% CIs were reported as effect size. For all analyses, two-tailed type I error of 0.05 was considered.

Post-hoc power analyses were performed to examine the linear mixed models' statistical power to detect important change in MADRS score through potential mediators. The power analysis takes into account the study design (i.e. a RCT with two arms and two post baseline measures) and assumed repeated measures analysis of covariance setting with group, time and potential mediator as main effects, and possible interactions. Simulation techniques using PASS software was used for this purpose (15). A range of 3–5 minimum detectable between-group mean difference was considered as important effect size for the MADRS score outcome. There was more than 80% power to detect minimum effect size of 3.5 and 4 for BDNF and TNF-α, respectively, but there was less than 80% to detect a minimum effect size of 5 score for CRP, IL-6, IL-8, and IL-10.

Results

The two treatment groups were similar on demographic, clinical and functioning measures at baseline. The longitudinal models did not reveal significant changes in the biological parameters; BDNF, TNF-α, CRP, IL-8, IL-6, and IL-10, between baseline and week 32, or in the open-label or maintenance phases individually (there were no interaction effects of time by treatment group, Tables 2 and 3).

There were also no significant three-way interactions (correlations) between any of the biological measures and MADRS scores, either at baseline only (examining effect modification), at the open-label phase, during maintenance or when all data was analysed. Changes in MADRS scores were only associated with time, not with any of the biological markers (Table 3).

To investigate whether the levels of circulating factors were mediators of remission or response status, a logistic regression

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Table 2. Change in potential mediators through the trial period

	NAC																					
	Whole trial (W0–W32)							Open-label (W0–W8)							Maintenance (W8–W32)							
	n	Mean (SD)	Range	Median	Difference W32–W0*			*n*	Mean (SD)	Range		Median	Difference W8–W0*		*n*	Mean (SD)	Range	Median	Difference W32–W8*		
		% Change			Mean	95% CI			% Change			Mean	95% CI			% Change			Mean	95% CI		
BDNF	15	0.3788 (0.713)	−0.98 to 1.79	0.1804	3.58	−2.52 to 9.69		30	0.1813 (0.775)	−0.64 to 2.45	−0.0369	−0.75	−4.52 to 3.00		14	0.2219 (1.12)	−1.0 to 3.14	0.0670	0.49	−6.02 to 7.01		
CRP	14	0.8263 (2.08)	−0.94 to 7.08	0.3360	−0.44	−3.81 to 2.92		6	0.9719 (1.60)	−0.67 to 3.95	0.7269	3.19	−1.72 to 8.10		0	–	–	–	–	–		
TNF-α★	15	−6.28 (23.96)	−92.6 to 5.2	−0.3513	−0.05	−0.18 to 0.07		29	1.60 (9.46)	−13.1 to 45.6	0.0670	0.013	−0.04 to 0.069		13	0.0194 (1.48)	−2.9 to 2.24	−0.0457	−0.03	−0.15 to 0.09		
IL-6	0	–	–	–	–	–		3	0.0715 (0.47)	−0.37 to0.58	−0.0018	−8.66	−52.30 to 34.96		1	−0.1211 (–)	−0.12 to0.12	−0.1211	–	–		
IL-8	5	0.6263 (0.35)	0.14–1.06	0.7417	3.70	−1.22 to 8.63		9	0.9194 (1.79)	−0.53 to 5.41	0.75	6.95	−9.00 to 22.92		1	−0.3183 (–)	−0.32 to0.32	−0.3183	–	–		
IL-10	1	−0.1773 (–)	−0.18 to0.18	−0.1773	–	–		5	0.3696 (0.82)	−0.25 to 1.58	−0.1238	−8.01	−43.71 to 27.68		3	0.1673 (0.39)	−0.06 to0.62	−0.0587	0.21	−5.38 to 5.81		
							Between-group difference†							Between-group difference†							Between-group difference†	
							Mean	CI						Mean	CI						Mean	CI
	Placebo																					
BDNF	17	0.1166 (0.94)	−0.92 to 3.19	−0.0434	−1.44	−7.43–4.53	0.26	−0.33–0.77	30	0.1578 (0.46)	−0.75–1.19	0.1117	1.10	−1.90–4.10	0.02	−0.28–0.36	17	0.0928 (0.70)	−0.92–1.54	−0.0204	−1.94	−7.35–3.46	0.12	−0.52–0.86
CRP	14	0.3672 (0.88)	−0.86–2.68	0.0917	0.28	−1.28 to 1.85	0.45	−0.54 to 1.67	5	−0.3132 (0.46)	−0.81 to0.28	−0.5163	−3.82	−11.45 to 3.8	1.28	0.19–2.63	0	–	–	–	–	–	–	–
TNF-α★	15	−0.3170 (1.12)	−2.66 to 2.40	0.00	−0.04	−0.10 to 0.02	−5.97	−20.37 to 0.98	27	0.7691 (5.78)	−7.33 to 28.50	−0.0959	0.04	−0.00 to 0.09	0.83	−2.94 to 5.09	15	−0.4111 (1.53)	−3.46 to 3.0	−0.3806	−0.05	−0.16 to 0.04	0.43	−0.78 to 1.54
IL-6	3	−0.4056 (0.26)	−0.68 to0.16	−0.3804	−19.63	−82.47 to 43.21	–	–	2	.7273 (1.11)	−0.06 to 1.52	0.7273	−0.75	−34.61 to 33.10	−0.65	−1.71 to 0.44	3	−0.0860 (0.22)	−0.31 to0.15	−0.1000	−2.49	−9.97 to 4.99	−0.03	−0.26 to 0.18
IL-8	3	−0.0113 (0.72)	−0.76 to0.69	0.0396	−32.51	−175.69 to 110.67	0.63	−0.11 to 1.45	8	2.12 (4.63)	−0.72 to 13.33	0.4056	2.92	−9.71 to 15.56	−1.20	−4.79 to 1.47	2	−0.5784 (0.49)	−0.93 to0.23	−0.5784	−16.31	−192.35 to 159.72	0.26	−0.08 to 0.60
IL-10	2	−0.5167 (0.07)	−0.57 to0.47	−0.5167	−260.84	−2359.6 to 1837.9	0.33	0.28–0.39	2	1.04 (1.92)	−0.32 to 2.40	1.04	−26.28	−522.0 to 469.5	−0.67	−2.43 to 1.07	2	0.4643 (0.96)	−0.22 to 1.15	−0.4643	−12.30	−241.7 to 217.1	−0.29	−1.20 to 0.66

BDNF, brain-derived neurotrophic factor (pg/ml); CRP, C-reactive protein (ng/ml); TNF-α, tumour necrosis factor alpha (pg/ml); IL, interleukin (pg/ml); SD, standard deviation; *n*, number of subjects per time point; NAC, *N*-acetyl cysteine.
Per cent change reflects the difference between endpoint and baseline values divided by baseline values.
★Values transformed to logarithm due to skewness.
*Paired *t*-test.
†Independent *t*-test.

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Table 3. Examining mediator effect and effect modification of biological factors on Montgomery-Asberg Depression Scale (MADRS)

	Placebo	NAC	Placebo	NAC	Effect modification			Mediator effect		
Mediator	High		Low			Interaction**	95% CI		Interaction††	95% CI
BDNF					$p = 0.479^{\star}$	*20.42*	*18.53–22.30*	$p = 0.073^{\star}$	*10.41*	*7.43–13.40*
Mean (SD)	25.16 (5.94)	27.03 (6.65)	13.86 (4.81)	16.53 (5.36)	$p = 0.313^{\dagger}$	*21.48*	*19.27–23.69*	$p = 0.066^{\dagger}$	*19.94*	*17.96–21.91*
n	49	47	48	46	$p = 0.289^{\ddagger}$	*20.45*	*17.25–23.65*	$p = 0.265^{\ddagger}$	*10.91*	*8.47–13.36*
CRP					$p = 0.815^{\star}$	*4.45*	*2.78–6.11*	$p = 0.419^{\star}$	*10.35*	*7.28–13.42*
Mean (SD)	6.50 (6.02)	6.63 (4.22)	0.66 (0.42)	0.78 (0.42)	$p = 0.599^{\dagger}$	*5.37*	*2.00–8.74*	$p = 0.504^{\dagger}$	*16.95*	*13.96–19.94*
n	20	20	19	20	$p = 0.821^{\ddagger}$	*3.93*	*2.08–5.79*	$p = 0.511^{\ddagger}$	*12.28*	*6.47–18.08*
TNF-α★					$p = 0.207^{\star}$	*0.001*	*−0.054* to *0.056*	$p = 0.568^{\star}$	*10.93*	*7.87–14.0*
Mean (SD)	0.18 (0.17)	0.20 (0.17)	−0.21 (0.18)	−0.19 (0.15)	$p = 0.824^{\dagger}$	*0.014*	*−0.052* to *0.079*	$p = 0.797^{\dagger}$	*20.23*	*18.20–22.26*
n	46	46	46	26	$p = 0.129^{\ddagger}$	*−0.044*	*−0.132* to *0.043*	$p = 0.676^{\ddagger}$	*10.72*	*8.35–13.10*
IL-6					$p = 0.851^{\star}$	*20.67*	*8.40–32.93*	$p = 0.320^{\star}$	*11.558*	*4.66–18.50*
Mean (SD)	29.99 (24.06)	21.82 (25.87)	4.03 (1.91)	4.95 (0.56)	$p = 0.895^{\dagger}$	*13.54*	*−0.088* to *27.17*	$p = 0.088^{\dagger}$	*23.39*	*17.66–29.13*
n	10	9	9	7	$p = 0.575^{\ddagger}$	*11.55*	*−1.43* to *24.54*	$p = 0.593^{\ddagger}$	*12.15*	*5.98–18.32*
IL-8					$p = 0.996^{\star}$	*22.9*	*14.48–31.31*	$p = 0.996^{\star}$	*9.69*	*4.85–14.52*
Mean (SD)	29.04 (33.34)	32.76 (41.27)	4.84 (2.02)	4.68 (1.96)	$p = 0.996^{\dagger}$	*14.41*	*2.18–26.64*	$p = 0.950^{\dagger}$	*19.41*	*16.71–22.11*
n	24	24	24	20	$p = 0.611^{\ddagger}$	*9.85*	*1.82–17.88*	$p = 0.241^{\ddagger}$	*9.64*	*5.64–13.63*
IL-10					$p = 0.536^{\star}$	*105.31*	*33.20–177.4*	$p = 0.174^{\star}$	*5.75*	*−1.78* to *13.30*
Mean (SD)	242.9 (242.3)	70.33 (53.41)	11.0 (6.24)	7.75 (3.52)	$p = 0.334^{\dagger}$	*43.17*	*−47.55* to *133.9*	$p = 0.055^{\dagger}$	*21.81*	*16.43–27.19*
n	7	10	7	10	$p = 0.112^{\ddagger}$	*80.74*	*15.09–146.3*	$p = 0.873^{\ddagger}$	*11.12*	*5.90–14.10*

BDNF, brain-derived neurotrophic factor (pg/ml); CRP, C-reactive protein (ng/ml); TNF-α, tumour necrosis factor alpha (pg/ml); IL, interleukin (pg/ml); SD, standard deviation; *n*, number of subjects per time point; NAC, *N*-acetyl cysteine.
Effect modification – MMRM for longitudinal change; mediator effect – MMRM for mediation of clinical efficacy; High – levels of biomarker above the median; Low – levels of biomarker below the median.
★Values transformed to logarithm due to skewness;
*Whole trial.
†Open-label phase.
‡Maintenance phase.
**Two-way interaction of treatment group and potential effect modifier as a time invariant variable measured at baseline.
††Two-way interaction of treatment group and potential mediator as a time-dependent variable measured at baseline, week 8, and week 32.

Table 4. Mediator analysis on 32-week Montgomery-Asberg Depression Scale (MADRS): remission and response status

	Placebo	NAC	Placebo	NAC			
Mediator	Remitter		Non-remitters		Interaction**	OR††	95% CI
BDNF					$p = 0.477$*	*1.044*	*0.928–1.174*
Mean (SD)	25.16 (5.94)	20.68 (9.77)	20.22 (8.94)	23.10 (9.29)	$p = 0.392^{\dagger}$	*1.055*	*0.933–1.193*
n	39	44	55	44	$p = 0.336^{\ddagger}$	*0.957*	*0.876–1.046*
CRP					$p = 0.198$*	*1.097*	*0.953–1.264*
Mean (SD)	2.88 (4.20)	2.99 (3.00)	5.77 (6.42)	5.14 (5.37)	$p = 0.468^{\dagger}$	*1.127*	*0.816–1.556*
n	14	20	25	20	$p = 0.193^{\ddagger}$	*1.146*	*0.933–1.407*
TNF-α★					$p = 0.450$*	*17.281*	*0.011–27.886*
Mean (SD)	−0.03 (0.25)	−0.0017 (0.21)	−0.0092 (0.27)	0.0215 (0.26)	$p = 0.860^{\dagger}$	*0.474*	*0.000–1.900*
n	34	42	55	44	$p = 0.655^{\ddagger}$	*0.304*	*0.002–56.727*
IL-6					$p = 0.256$*	*0.974*	*0.932–1.019*
Mean (SD)	24.78 (25.44)	35.53 (35.65)	13.56 (19.04)	7.61 (5.37)	$p = 0.243^{\dagger}$	*0.904*	*0.763–1.071*
n	7	4	12	11	$p = 0.348^{\ddagger}$	*0.939*	*0.824–1.071*
IL-8					$p = 0.910$*	*0.999*	*0.984–1.015*
Mean (SD)	16.04 (25.71)	17.60 (38.75)	16.55 (27.42)	19.50 (22.22)	$p = 0.322^{\dagger}$	*1.029*	*0.973–1.088*
n	21	23	25	19	$p = 0.854^{\ddagger}$	*0.987*	*0.864–1.129*
IL-10					$p = 0.606$*	*1.002*	*0.995–1.008*
Mean (SD)	70.58 (81.05)	52.55 (55.99)	188.9 (301.8)	14.73 (13.71)	$p = 0.304^{\dagger}$	*0.963*	*0.896–1.035*
n	7	13	6	6	$p = 0.359^{\ddagger}$	*1.032*	*0.965–1.103*
	Responders		Non-responders				
BDNF					$p = 0.886$*	0.991	*0.877–1.120*
Mean (SD)	19.43 (9.02)	22.98 (10.51)	20.25 (8.65)	21.14 (8.86)	$p = 0.132^{\dagger}$	1.107	*0.970–1.263*
n	42	36	52	52	$p = 0.071^{\ddagger}$	0.902	*0.806–1.009*
CRP					$p = 0.214$*	*1.105*	*0.944–1.292*
Mean (SD)	3.25 (4.44)	2.65 (2.92)	5.39 (6.32)	5.01 (5.04)	$p = 0.468^{\dagger}$	*1.127*	*0.816–1.556*
n	12	16	27	24	$p = 0.309^{\ddagger}$	*1.112*	*0.907–1.363*
TNF-α★					$p = 0.834$*	*2.189*	*0.001–3262.3*
Mean (SD)	−0.0043 (0.27)	0.0072 (0.25)	−.0.0271 (0.26)	0.0121 (0.22)	$p = 0.850^{\dagger}$	*0.441*	*0.000–2123.0*
n	37	34	52	52	$p = 0.867^{\ddagger}$	*1.529*	*0.011–218.92*
IL-6					$p = 0.239$*	*0.973*	*0.929–1.018*
Mean (SD)	24.78 (25.44)	35.49 (35.68)	13.56 (19.04)	7.63 (5.39)	$p = 0.206^{\dagger}$	*0.917*	*0.802–1.049*
n	7	4	12	11	$p = 0.348^{\ddagger}$	*0.939*	*0.824–1.071*
IL-8					$p = 0.677$*	*0.997*	*0.981–1.012*
Mean (SD)	15.72 (25.10)	19.62 (44.67)	16.86 (28.0)	17.67 (20.41)	$p = 0.232^{\dagger}$	*1.054*	*0.967–1.148*
n	22	17	24	25	$p = 0.854^{\ddagger}$	*0.987*	*0.864–1.129*
IL-10					$p = 0.473$*	*1.003*	*0.995–1.010*
Mean (SD)	64.33 (77.09)	53.71 (55.01)	222.65 (324.66)	12.22 (14.47)	$p = 0.226^{\dagger}$	*0.889*	*0.734–1.076*
n	8	13	5	6	$p = 0.317^{\ddagger}$	*1.026*	*0.975–1.080*

OR, odds ratio (Exp (B)); 95% CI = 95% confidence interval for odds ratio; BDNF, brain-derived neurotrophic factor (pg/ml); CRP, C-reactive protein (ng/ml); TNF-α, tumour necrosis factor alpha (pg/ml); IL, interleukin (pg/ml); SD, standard deviation; *n*, number of subjects per time point.
Responders ≥50% reduction in MADRS scores; Remitters ≤7 on MADRS scores.
★Values transformed to logarithm due to skewness.
*Baseline levels.
†Maintenance levels.
‡End of trial levels.
**Interaction between treatment group and mediators on response and remission status in the logistic regression.
††Odds ratio for two-way interaction between treatment group and potential mediator.

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analysis was conducted using the levels of biological parameters in each visit as possible mediators. There were no significant correlations between circulating factor levels at baseline, at the start of maintenance phase, or end of trial levels and response or remission status (Table 4).

Discussion

We evaluated the effects of adjunctive NAC on biological parameters potentially involved in mechanisms underlying depressive episodes in BD. In this study, we measured the serum levels of BDNF, IL-6, IL-8, and IL-10, TNF-α, and CRP, as proxies of the putative neurotrophic and anti-inflammatory actions of NAC. Adjunctive NAC treatment did not show significant effects on any of these serum biological parameters levels over 32 weeks, suggesting that the effects of NAC in BD depression might not act directly through these neurotrophic and anti-inflammatory pathways.

NAC has been reported to decrease IL-6 levels in patients with chronic kidney disease (16), reduce the levels of inflammatory markers (IL-1β and TNF-α) and quench reactive oxygen species produced after lipopolysaccharide maternal prenatal injection in rodents (17) and reduce the activation of NF-kB in sepsis, associated with decreased levels of IL-8 but not IL-6 (18). All of these NAC actions appear to be dependent on the synthesis and replenishment of glutathione (GSH) levels and associated with the suppression of NF-κB activation (19), but not directly linked with circulating cytokine levels.

In the open-label phase, clinical assessment indicated reduced depressive symptoms and improved quality of life measurements with adjunctive NAC treatment (12,13), hence we investigated the relationship between clinical response and change in biological parameters. Our data did not show any mediation of response or remission by the biological factors evaluated, suggesting that the markers selected in this study cannot explain the relationship between the NAC adjunctive treatment and the clinical improvement. There remains the possibility that other medications may work in conjunction with the effects of NAC on these markers as all patients continued on treatment as usual.

For levels of BDNF for example, our cohort had the mean of 20 407 pg/ml at baseline which can be considered low when compared with the 23 320 pg/ml in the Rabie et al. study (20) that used the same methodology and had similar sample characteristics. However, the NAC group at the end of the trial had an increase in BDNF levels reaching a comparable mean of 22 385 pg/ml. Although this increase was not significant compared to the placebo group, it suggests that the treatment as usual may have contributed to the slight increase in serum levels of BDNF.

Therefore, these findings failed to support the hypothesis that the serum levels of BDNF, IL-6, IL-8, and IL-10, TNF-α, and CRP represent the pathways by which NAC mediates effects on depressive symptoms in this population. Apart from its anti-inflammatory effects, NAC also modulates brain redox processes via increasing GSH levels (21,22). Glutathione is thought to modulate redox-regulated signal transduction, regulate the immune response, prostaglandin and leukotriene metabolism, antioxidant defense, neurotransmitter signaling, and modulation of cell proliferation, which could contribute to the therapeutic effects of NAC through pathways not involving peripheral inflammatory signaling.

Although this was a relatively large study, sample sizes for biological analyses were smaller, and this is a significant limitation. The complexity of the pathways likely involved may also have precluded us from finding significant results. It is possible that mean symptom levels were too low in this study, as the mean MADRS score at inclusion was 19.7. It is also possible that the change over the assessment period was not driven by pharmacological effects, but by treatment as usual or placebo effects, obscuring the biological effects of NAC therapy. Timing of the assays may have missed earlier or later changes. The selected markers were selected based on the body of literature suggesting that these are implicated in the pathophysiology of psychiatric disorders and are key nodes in inflammatory pathways. However, it remains possible that more subtle changes in these or other markers have biological outcomes despite being below the operational range of currently available assays; although alterations in IL-6 and other cytokines have been consistently reported in BD the dynamic range of these changes is well below the levels observed in classical inflammatory states associated with infection, neoplasia or autoimmunity.

NAC has potential antidepressant effects (4,23,24) and its mechanisms of action may be widely distributed over several different pathways (10,11). Nevertheless, oxidative stress was the underpinning hypothesis leading to exploration of the role of NAC in psychiatric disorders, and this study did not evaluate this hypothesis. A wider range of markers including indicators of oxidative stress and glutamatergic function could provide a more comprehensive view of the efficacy of NAC on depressive symptoms in BD.

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Conflicts of Interest. None

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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 (ACTRN12607000074493) and was approved by the relevant Human Research Ethics Committees.

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