N-Acetylcysteine does not artifactually lower plasma creatinine concentration

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Abstract

Background. All randomized controlled trials of N-acetylcysteine (NAC) in contrast media-induced nephropathy used creatinine as a marker of renal function. However, it has been suggested that NAC may lower plasma creatinine levels independent of any effects on glomerular filtration rate (GFR).

Methods. At a tertiary hospital 110 cardiac surgical patients were randomly allocated to peri-operative infusion of NAC (300 mg/kg over 24 h, N = 30) or placebo (N = 80). We compared the plasma concentrations of creatinine, cystatin C and urea, the plasma creatinine/plasma cystatin C ratio and the estimated GFR at baseline and at 24 and 72 h after commencement of the infusion. We measured urinary creatinine excretion at 24 h.

Results. At baseline, the plasma creatinine/plasma cystatin C ratio did not differ between the NAC and placebo group (0.90 versus 0.92; P = 0.94). There was no significant difference in the plasma creatinine/plasma cystatin C ratio for the NAC and placebo group either during or after NAC infusion at 24 h (1.03 versus 1.00; P = 0.78) and 72 h (0.94 versus 0.89; P = 0.09). Those allocated to NAC showed no difference in urinary creatinine excretion when compared to placebo (P = 0.24).

Conclusions. The results of our study do not demonstrate that NAC artifactually lowers creatinine measured using the Jaffé method. (ClinicalTrials.gov, NCT00332631, NCT00334191)

Keywords: acute kidney injury; contrast-induced nephropathy; creatinine; cystatin C; N-acetylcysteine

Introduction

Contrast media-induced nephropathy (CIN) is common in patients with impaired renal function. Although results remain controversial, several randomized controlled trials (RCTs) [1–3] support hydration in combination with N-acetylcysteine (NAC) as a preventive strategy for CIN especially in high-risk patients [4,5]. The reported beneficial effects are based on the absolute or relative change in plasma creatinine concentration as the primary study-specific surrogate for the diagnosis of CIN. However, the possibility was raised that the above findings may be due to a direct effect of NAC on plasma creatinine concentration rather than an improved glomerular filtration rate (GFR) [6,7].

Recently, in addition to plasma creatinine, investigators have used plasma cystatin C to simultaneously assess GFR during NAC administration [6]. Cystatin C concentrations appear—in contrast with plasma creatinine concentrations—to be independent of age, gender and muscle mass [8,9]. In a study of healthy volunteers, changes in the plasma creatinine concentration and estimated GFR occurred following NAC administration despite lack of changes in plasma cystatin C concentration.

This observation has powerful clinical implications because it calls into question both the standard approach used to evaluate the effect of NAC on CIN and the belief that NAC attenuates CIN.

In response to these concerns, we used the results from two randomized double-blind, placebo-controlled trials in cardiac surgical patients comparing 30 NAC-treated patients with 80 patients receiving placebo [10]. We compared the effect of NAC to that of placebo on simultaneously measured creatinine and cystatin C concentrations.

Subjects and methods

Patients

Patients were considered for our study when non-emergency cardiac surgery with the use of cardiopulmonary
bypass was planned and if they fulfilled at least one of the following criteria for an increased risk of post-operative acute kidney injury (AKI) [11]: age above 70 years, pre-operative plasma creatinine above 120 µmol/L, insulin-dependent diabetes mellitus, concomitant valve surgery, moderate to severe left ventricular dysfunction or redo cardiac surgery.

Placebo patients were pooled from two RCTs directed at the prevention of post-operative AKI. Patients of the two RCTs were not different in demographic variables and co-morbidities.

In placebo patients of both trials and in the NAC patients, the same inclusion and exclusion criteria applied and the same treatment protocol was used. Exclusion criteria comprised allergies to NAC, plasma creatinine concentration above 300 µmol/L, intake of steroids and emergency cardiac surgery. For detailed characterization of patients’ inclusion and exclusion criteria, study design, intervention and data collection, we refer to our recent publication [10].

The institutional ethics committee at the Austin Hospital, Melbourne, approved both RCTs. Informed written consent was obtained from all patients.

### Intervention

Patients were randomly allocated to intravenous high-dose NAC infusion (300 mg/kg bodyweight in 1.7 L of 5% glucose solution over 24 h; N = 30) or to placebo (1.7 L of 5% glucose solution over 24 h, N = 80) commencing immediately following the induction of anaesthesia. Investigators, surgeons and intensivists were blinded to treatment allocation, which was concealed until data analysis was completed.

### Peri-operative management

Patients were haemodynamically monitored throughout cardiac surgery and post-operatively in the intensive care unit. After heparinization the ascending aorta and right atrium were cannulated for cardiopulmonary bypass. Myocardial protection was by antegrade and retrograde, intermittent, warm blood cardioplegia. Heparinization was reversed using 1 mg protamine sulfate per 100IU heparin. Milrinone and norepinephrine were used to optimize cardiac output and systemic perfusion pressures as necessary. Peri-operative haemodynamic management targeted a cardiac output of >2.5 L/min/m² and a mean arterial blood pressure of above 75 mmHg.

### Data collection

Blood samples were collected for measurement of plasma creatinine, plasma cystatin C and plasma urea at baseline, at 24 h and at 72 h after commencement of study medication. Urine was sampled for creatinine measurement at 24 h and at 72 h after commencement of study medication. Fluid input and output data were collected and fluid balance was calculated. Intra-operative mean arterial pressure values were recorded electronically. In addition, we calculated the EuroScore, which takes into account age, sex, pre-operative co-morbidities, complexity and acuity of cardiac surgery [12].

### Measurement of surrogate parameters of renal function

Plasma creatinine and urinary creatinine concentrations were measured utilizing the Jaffe method (Beckman Coulter SYNCHRON LX System, Beckman Coulter, Inc., Brea, CA, USA). The coefficient of variation of plasma creatinine measurement was 3.9% for plasma creatinine concentrations of 44 µmol/L and 1.9% for plasma creatinine concentrations of 530 µmol/L. Urea was measured on a Beckman Synchron LX 20 (Beckman Coulter Inc., Fullerton, CA 92835, USA). Urease in the reagent converts urea (non-ionic) to ammonium and bicarbonate ions. The rate of increase of solution conductivity is proportional to the urea concentration. The coefficient of variation of plasma urea measurement was 5.1% for plasma urea concentrations of 5.4 mmol/L and 2.5% for plasma urea concentrations of 42.8 mmol/L. The coefficient of variation of urinary creatinine measurement was 2.3% to 2.6%. The analytical sensitivity of plasma creatinine for a confidence interval of 95% was 8.8 µmol/L and 1.1 mmol/L for plasma urea.

Plasma cystatin C was measured using nephelometric technology on a Beckman Image Analyser (Beckman Coulter, Inc., Brea, CA, USA). The reagents and calibrators used were produced by DakoCytomation (DAKO, Botany, NSW, Australia). The coefficient of variation of plasma cystatin C measurement was 4.5% at a concentration of 0.7 mg/L and 4.6% at a concentration of 2.5 mg/L. The analytical sensitivity of the plasma cystatin C assay was 0.4 mg/L.

The estimated GFR was calculated using the modified MDRD (modification of diet in renal disease) formula [13].

### Statistical analysis

Data were tested for normal distribution by use of histograms. As data lacked normal distribution, we used the Mann–Whitney U-test for between-group comparisons and for comparison within groups we used the Friedman test. Dichotomous data were compared by the use of Fisher’s exact test. Values are presented as medians with 25th to 75th percentiles and as Box-and-Whisker plots in the figures. To increase the power of the present study, we pooled the placebo groups from two randomized double-blind, placebo-controlled trials in cardiac surgical patients comparing 30 NAC-treated patients with a total of 80 patients receiving placebo. Power was increased to compensate for greater signal-to-noise ratio in patients compared to healthy subjects. We considered P values ≤ 0.05 to be statistically significant.

### Results

We compared 30 patients receiving NAC infusion with 80 patients receiving placebo. Baseline characteristics of the patients with regard to demographic, clinical, surgical and intra-operative characteristics were well balanced in both groups (Table 1). There were no statistically significant between-group differences in fluid intake, output and balance from baseline to 48 h post-operatively (Table 2).

Surrogate parameters of renal function before and after cardiac surgery are shown in Figures 1–5. We found no
Table 1. Pre- and intra-operative characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>N-Acetylcysteine (n = 30)</th>
<th>Placebo (n = 80)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>68 (60–77)</td>
<td>72 (64–76)</td>
<td>0.7</td>
</tr>
<tr>
<td>Sex, female/male, n</td>
<td>7/23</td>
<td>26/54</td>
<td>0.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.6 (23.5–30.6)</td>
<td>27.1 (24.2–30.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>8 (27)</td>
<td>24 (30)</td>
<td>0.7</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>19 (63)</td>
<td>50 (63)</td>
<td>0.9</td>
</tr>
<tr>
<td>Arterial hypertension, n (%)</td>
<td>25 (83)</td>
<td>66 (83)</td>
<td>0.9</td>
</tr>
<tr>
<td>Recent myocardial infarction, n (%)</td>
<td>5 (17)</td>
<td>16 (20)</td>
<td>0.7</td>
</tr>
<tr>
<td>Peripheral vascular disease, n (%)</td>
<td>5 (17)</td>
<td>7 (9)</td>
<td>0.2</td>
</tr>
<tr>
<td>Carotid disease, n (%)</td>
<td>4 (13)</td>
<td>7 (9)</td>
<td>0.5</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>9 (30)</td>
<td>23 (29)</td>
<td>0.9</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease, n</td>
<td>5 (17)</td>
<td>17 (21)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pre-operative plasma creatinine (µmol/L)</td>
<td>93.5 (73.5–115.3)</td>
<td>88.5 (73.5–102.5)</td>
<td>0.2</td>
</tr>
<tr>
<td>EuroScore</td>
<td>5 (3–7)</td>
<td>6 (5–7)</td>
<td>0.2</td>
</tr>
<tr>
<td>Characteristics of cardiac surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valve surgery</td>
<td>13 (43)</td>
<td>36 (45)</td>
<td>0.9</td>
</tr>
<tr>
<td>Coronary artery bypass grafting surgery</td>
<td>10 (33)</td>
<td>20 (25)</td>
<td>0.4</td>
</tr>
<tr>
<td>Valve and coronary surgery</td>
<td>6 (20)</td>
<td>17 (21)</td>
<td>0.9</td>
</tr>
<tr>
<td>Complex cardiac surgery, n (%)</td>
<td>1 (3)</td>
<td>8 (10)</td>
<td>0.3</td>
</tr>
<tr>
<td>Duration of cardiopulmonary bypass (min)</td>
<td>108 (93–144)</td>
<td>129 (99–188)</td>
<td>0.2</td>
</tr>
<tr>
<td>Intra-operative mean arterial pressure (mmHg)</td>
<td>69 (63–75)</td>
<td>66 (62–70)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 2. Fluid intake and urinary output from baseline to 48-h post-operatively

<table>
<thead>
<tr>
<th>Variables</th>
<th>NAC (n = 30)</th>
<th>Placebo (n = 80)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid intakea (mL)</td>
<td>7340 (5790–7970)</td>
<td>7270 (6030–8440)</td>
<td>0.4</td>
</tr>
<tr>
<td>Urinary output (mL)</td>
<td>4920 (4000–5680)</td>
<td>5100 (4420–6120)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

between-group differences for the pre- and post-operative concentrations of plasma creatinine, plasma cystatin C or plasma urea from baseline to 24 h and 72 h post-operatively (Figures 1–3). Further, there were no between-group differences in either the estimated GFR (Figure 4) or the plasma creatinine/plasma cystatin C ratio (Figure 5).

Post-operative plasma creatinine changed in parallel in both groups (Figure 1). Post-operative plasma creatinine

Fig. 1. Plasma creatinine concentration in the N-acetylcysteine (shaded) and placebo (white) group before and at 24 h and 72 h after cardiac surgery. P values for group comparison (Mann–Whitney U-test) are shown in the figure. Comparison of values over time within each group (Friedman test): NAC-treated patients P < 0.001, Placebo patients P < 0.001.
values were higher in the NAC group at all times; however, this difference was not significant. Similarly, there were no between-group differences for plasma cystatin C values at baseline, at 24 h and at 72 h post-operatively (Figure 2).

We found no differences in the plasma urea concentration and plasma creatinine-based estimated GFR at baseline, 24 h and 72 h after commencement of infusion (Figures 3 and 4).

There were no between-group differences in the plasma creatinine/plasma cystatin C ratio (Figure 5). At baseline, the median plasma creatinine/plasma cystatin C ratio was 0.90 in the NAC group and 0.92 in the placebo group.
Fig. 4. Plasma creatinine-based estimated glomerular filtration (eGFR) in the \( N \)-acetylcysteine (shaded) and placebo (white) group before and at 24 h and 72 h after cardiac surgery. \( P \) values for group comparison (Mann–Whitney U-test) are shown in the figure. Comparison of values over time within each group (Friedman test): NAC-treated patients \( P < 0.001 \), Placebo patients \( P < 0.001 \).

Fig. 5. Plasma creatinine/plasma cystatin C ratio in the \( N \)-acetylcysteine (shaded) and placebo (white) group before and at 24 h and 72 h after cardiac surgery. \( P \) values for group comparison (Mann–Whitney U-test) are shown in the figure. Comparison of values over time within each group (Friedman test): NAC-treated patients \( P = 0.016 \), Placebo patients \( P < 0.001 \).

\( N = \text{30} \)   \( N = \text{80} \)

\( P = 0.94 \). The plasma creatinine/plasma cystatin C ratio increased to 1.03 after 24 h of NAC infusion and to 1.00 after 24 h of placebo; \( P = 0.78 \) (Figure 5). It nearly returned to baseline values in both groups at 72 h (Figure 5).

There was no significant difference in the change in the plasma creatinine/cystatin C ratio between the NAC and placebo groups from baseline to 24 h \( [7.7\% \ (0.4–21.7\%) \ \text{versus 9.6\%} \ (−2.2−22.5\%), \ P = 0.89] \). There was a trend for a greater increase in the plasma creatinine/cystatin C ratio from baseline to 72 h in the NAC group \( [7.8\% \ (−13.5−37.1\% \ \text{versus −0.9\%} \ (−17.6−12.9\%), \ P = 0.08] \).

The urinary creatinine concentration was 0.025 mg/L (0.013–0.046) after 24 h of NAC infusion and 0.035 mg/L (0.02–0.055) after 24 h of placebo infusion (\( P = 0.24 \)).
Discussion

We tested whether NAC exerts a direct plasma creatinine-lowering effect. We did this by simultaneously measuring plasma creatinine, plasma cystatin C and urinary creatinine concentrations. We found that, compared with placebo, NAC infusion did not affect plasma creatinine/plasma cystatin C ratios, changes in creatinine/cystatin C ratios, urinary creatinine/plasma cystatin C ratio, post-operative estimated GFR and plasma urea/plasma creatinine ratios. These observations challenge the view that NAC administration might lower plasma creatinine by mechanisms unrelated to its effects on GFR.

In several RCTs, NAC has been shown to attenuate CIN, especially when given at high-dose and in high-risk patients [1–5]. Plasma creatinine or plasma creatinine-based estimates of GFR have been used as the primary outcome measure in the majority of such trials. However, the value of plasma creatinine in the assessment of renal function in NAC-treated patients has more recently become a matter of controversy [6,7]. In a study of 50 healthy volunteers treated with oral NAC a decrease in plasma creatinine was found to occur without similar changes in cystatin C values. This observation has major clinical implications and, if true, it would call into question the findings of at least 13 positive RCTs (including >1800 patients) of NAC for the prevention of CIN.

The mechanisms responsible for the above observations, however, remain unclear. The authors hypothesized that NAC might directly lower plasma creatinine by an increase in creatinine excretion, a decrease in creatinine production or by interference with laboratory measurement [6]. Unfortunately, such doubts about the use of creatinine as surrogate of GFR during NAC therapy were not addressed by the most recent study of NAC in the prevention of CIN [5,7]. Thus, the issue remains unresolved.

We have used a RCT design, comparing placebo to NAC infusion during and after cardiac surgery, in order to eliminate bias and to ensure clinical relevance. With this approach, clear alterations in GFR occurred in both groups, as would be the case with CIN. This approach allowed us to test the relationship between plasma creatinine and plasma cystatin C within a wide spectrum of values for both.

We found that the concerns raised about a direct effect of NAC on plasma creatinine were not sustained: the relationship between plasma creatinine and plasma cystatin C did not change when comparing NAC to placebo. It also did not change when we used the estimated GFR or plasma urea to further assess these changes.

NAC might influence serum creatinine in healthy subjects [6] while not demonstrated to do so in patients. However, several observations appear relevant to the interpretation of our findings. First, cystatin C has recently been established as a marker of renal function in cardiac surgical patients [14]. Second, plasma cystatin C may, like creatinine, be secreted into urine by tubules [15,16]. Theoretically, NAC might, therefore, interfere with both cystatin C and creatinine measurements simultaneously, thus leading to unchanged ratios. However, the similarity in ratios between patients randomized to placebo and NAC contradicts this notion. Third, the differences between our findings and those reported in normal volunteers [6] may relate to minor, yet statistically significant changes in plasma creatinine with NAC [6], which were missed by the earlier assay used in volunteers to measure cystatin C (type II error). The fact that in the study of normal volunteers, the decrease in plasma urea concentration was discordant with the decrease in plasma creatinine, but discordant with the unchanged cystatin C supports this possibility. Fourth, with regard to a possible effect of NAC towards decreased production of creatinine via modulation of the activity of the enzyme creatinkinase [6], it must be noted that creatinphosphatase is non-enzymatically converted into creatinine [17]. Accordingly, to our knowledge, it is speculative that creatinkinase activity might influence plasma creatinine concentration [18].

Hoffmann et al. [6] found NAC to reduce serum creatinine with both enzymatic and non-enzymatic (Jaffé) methods of creatinine measurement. In an ex-vivo experiment using the modified Jaffé method for the measurement of plasma creatinine, Izzedine et al. [19] did not find any influence of NAC on creatinine concentration unless NAC concentration exceeded 50 g/L plasma [19], a concentration several orders of magnitude higher than that conceivably achieved during NAC infusion. We used the modified Jaffé method and also did not find any effect on plasma creatinine. It seems biologically unlikely that NAC given orally at low dose would induce tubular secretion of creatinine or decrease production in normal individuals, but not do so when given intravenously at 10 times the dose in patients. Such high-dose NAC infusion used in our study should have maximized our ability to see its previously proposed direct plasma creatinine-lowering effect.

Both groups were well balanced reducing the possible effect of confounding variables (including hydration status and haemodynamic stability) on renal function. Also, urinary creatinine was measured to evaluate whether NAC increased tubular creatinine secretion. In fact, on average, patients receiving placebo excreted 231 mg creatinine versus 149 mg after NAC (which is a 35% reduction after NAC). This phenomenon is in contrast to the assumption that NAC may increase tubular creatinine excretion and still needs to be explored. Finally, we chose sampling times following current practice for reporting plasma creatinine concentrations in studies testing NAC for the prevention of CIN [1,5]. These times were similar to those reported in volunteers [6]. On the other hand, our primary hypothesis was directed to test the effectiveness of NAC as drug to protect
renal function in cardiac surgical patients. Thus, the assessment of its direct effects on plasma creatinine represented a secondary hypothesis. Similar to many trials of CIN, we administered NAC intravenously, not orally as in the study of volunteers. Oral NAC may have different biological effects [20]. However, these effects would likely be compensated for by our high-dose NAC infusion (more than 10 times the dose used in the previous study [6]), which provides more predictable bioavailability compared to oral NAC [20]. Results from the patients in the present study may not apply to patients with pre-existing renal impairment undergoing contrast media-based diagnostic or interventional procedures. However, our patients developed AKI, as is the case for CIN. Our study was not specifically powered to detect a specific differential effect of NAC on the creatinine/cystatin C ratio and the signal-to-noise ratio might be greater in patients compared to healthy subjects. This makes a type II error possible. However, given the mean values found and their standard deviation, we calculated that—with our sample size (30 patients treated with NAC versus 80 patients receiving placebo)—we had a >90% power of detecting a >15% difference in plasma creatinine/plasma cystatin at an alpha of 0.05. Moreover, if NAC had a creatinine-lowering effect and no direct effect on cystatin C, the logical consequence would be that the post-operative NAC/cystatin C ratios at 24 and 72 h should have been lower in the NAC group compared to placebo. The opposite was the case with the relevant trend almost reaching statistical significance (Figure 5).

Finally, cardiac surgery induces the transient release of creatinine through muscle injury (incision and retraction). This effect could artificially increase plasma creatinine concentrations. The finding that the creatinine/cystatin C ratio increased at 24 h after surgery and returned to baseline after 72 h supports this possibility. This effect might also mask the proposed direct creatinine-lowering effect of NAC. However, the development of similar changes in the placebo group makes this unlikely.

In summary, we tested whether NAC treatment has a direct, GFR-unrelated, plasma creatinine-lowering effect. We did this by simultaneously measuring plasma cystatin C concentration. We found that NAC infusion did not affect plasma creatinine/plasma cystatin C ratios or increased urinary creatinine concentration compared to placebo. The results of our study do not demonstrate that NAC artifactually lowers creatinine measured using the Jaffé method.

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Conflict of interest statement. None declared.

References


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