# Acetylcysteine has No Mechanistic Effect in Patients at Risk of Contrast-Induced Nephropathy - A Failure of Academic Clinical Science

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# Acetylcysteine has No Mechanistic Effect in Patients at Risk of Contrast-Induced Nephropathy - A Failure of Academic Clinical Science

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**ABSTRACT (424 WORDS)**

Contrast-induced nephropathy (CIN) is a major complication of imaging in patients with chronic kidney disease (CKD). The publication of an academic RCT (n=83) reporting oral (N)-acetylcysteine (NAC) to reduce CIN led to >70 clinical trials, 23 systematic reviews, and two large RCTs showing no benefit. However, no mechanistic studies were conducted to determine how NAC might work; proposed mechanisms included renal artery vasodilatation and antioxidant boosting. We evaluated the proposed mechanisms of NAC action in participants with healthy and diseased kidneys. Four sub-studies were performed. Two randomised, double-blind, placebo-controlled, three-period cross-over studies (n=8) assessed the effect of oral and intravenous (IV) NAC in healthy kidneys in the presence/absence of iso-osmolar contrast (iodixanol). A third cross-over study in CKD3 patients (n=8) assessed the effect of oral and IV NAC without contrast. A three-arm randomised, double-blind, placebo-controlled parallel-group study, recruiting CKD3 patients (n=66) undergoing coronary-angiography, assessed the effect of oral and IV NAC in the presence of contrast. We recorded systemic (blood pressure, heart-rate) and renal (renal blood flow [RBF], glomerular filtration rate [GFR]) haemodynamics, and antioxidant status, plus biomarkers of renal injury in CKD3 patients undergoing angiography. Primary outcome for all studies was RBF over 8h after start of IV NAC/placebo. NAC at doses used in previous trials of renal prophylaxis was essentially undetectable in plasma after oral administration. In healthy volunteers, IV NAC, but not oral NAC, increased blood pressure (mean area-under-the-curve [AUC] arterial pressure [MAP]: mean difference 29 h.mmHg, p=0.019 vs. placebo), heart-rate (28 h.bpm, p<0.001), and RBF (714h.mL/min, 8.0% increase, p=0.006). Renal vasodilatation also occurred in the presence of contrast (RBF 917h.mL/min, 12% increase, p=0.005). In CKD3 patients without contrast, only a rise in heart-rate (34h.bpm, p=0.010) and RBF (288 h.mL/min, 6.0% increase, p=0.001) occurred with IV NAC, with no significant effect on blood pressure (MAP rise 26 h.mmHg, p=0.156). Oral NAC showed no effect. In CKD3 patients receiving contrast, IV NAC increased blood pressure (MAP rise 52 h.mmHg, p=0.008) but had no effect on RBF (151 h.mL/min, 3.0% increase, p=0.470), GFR (29h.mL/min/1.73m², p=0.122), or markers of renal injury. Neither IV nor oral NAC affected plasma antioxidant status. We found oral NAC to be poorly absorbed and have no reno-protective effects. Intravenous, not oral, NAC caused renal artery vasodilatation in healthy volunteers but offered no protection to CKD3 patients at risk of CIN. These findings emphasise the importance of mechanistic clinical studies before progressing to RCTs for novel interventions. Thousands were recruited to academic clinical trials without the necessary mechanistic studies being performed to confirm the approach had any chance of working.

## INTRODUCTION

Radiographic contrast has been used since the 1950s to enhance medical imaging in diagnostic and interventional procedures. Although risk from radiocontrast is generally low, patients with chronic kidney disease (CKD), particularly in the setting of diabetes or intravascular volume depletion, are at risk of developing contrast-induced nephropathy (CIN).[1-4] There is no universally accepted definition for CIN[5] and, partly as a result, its reported incidence varies from 3 to 19%.[1,2,6,7] Importantly, CIN has been associated with increased length of hospital stay, adding $10,000 on average to a US hospital admission,[8] as well as increased morbidity and mortality.[2,6,7,9,10] The underlying mechanisms of CIN are poorly understood.[1,11] Reduced renal blood flow (RBF) due to afferent renal artery constriction, leading to ischaemic kidney injury, as well as direct injury by oxygen free radicals have been proposed.[12-14] Thus, preventative strategies, including intravenous (IV) hydration and bicarbonate, focus on maintaining RBF and reducing oxidative stress.[11] However, data supporting these strategies are limited.[15,16]

In 2000, a small RCT (n=81) reported less CIN in patients undergoing diagnostic CT contrast radiography after receiving oral acetylcysteine (NAC) 600 mg.[17] NAC is a vasodilator [18] and considered to be an antioxidant,[19] supporting biological plausibility of efficacy. The results of the trial were accepted and led to a mass of papers on the role of NAC in CIN and the conduct of more than 70 RCTs testing oral (less frequently IV) NAC that recruited over 18,000 patients by 2020 (**figure 1**).[20] The trials were followed by multiple systematic reviews calling for larger studies. Finally, two large RCTs of patients undergoing angiography compared oral NAC with placebo (ACT,[21] n=2,308; PRESERVE,[22] n=4,993) and showed no effect of oral NAC. After the ACT study, in 2013, an international consensus report stated that neither oral nor IV NAC should be administered for CIN prophylaxis.[23] However, systematic reviews continue to report that NAC shows promise for preventing CIN (for example, ref [20]). A 2019 National Institute for Health and Care Excellence (NICE) review of the evidence found no evidence for benefit from NAC but recommended that more clinical research be performed.[16]

Unfortunately, the many RCTs on NAC renal prophylaxis performed to date have been done without any prior mechanistic studies of how NAC affects both healthy and damaged kidneys and without pharmaco-dynamic dose-finding studies. Systematic review of the literature revealed no studies that included mechanistic secondary analyses to explain the reported effects. To definitively identify the optimal role of NAC, if any, there is a need to determine how NAC affects kidneys in patients with CKD, to identify the ideal

dose, route of administration, and outcome measure, based on its mechanism of action. Previous studies have used changes in serum creatinine to detect NAC's effect. However, if contrast causes renal vasoconstriction and NAC vasodilatation, NAC itself may cause a reduction in serum creatinine concentration,[24,25] suggesting that changes in creatinine are not the best marker of NAC effect. Until mechanistic studies are done, there is a risk that NAC will be discarded without adequate testing, or that yet more time and money will be spent setting up yet more RCTs. The aim of this study was therefore to determine how NAC affects renal haemodynamics and oxidant status in healthy volunteers and in patients with CKD stage III (CKD3), a population at risk of CIN.

## METHODS

Full details of the protocol have been published previously.[26] The studies were performed in the Wellcome Trust Clinical Research Facility and the Coronary Angiography Suite, Royal Infirmary of Edinburgh. Approval of the local research ethics committee and written informed consent of each subject were obtained. The investigations conformed to the principles outlined in the Declaration of Helsinki. This study was registered with European Clinical Trials Database (EudraCT 2006-017800-10) and ClinicalTrials.gov (NCT00558142).

### *Study design*

The overall study comprised of 4 sub-studies (**Figure S1**). **Studies 1** and **3** were randomised, double-blind, double-dummy placebo-controlled three-period cross-over studies, each recruiting eight healthy volunteers to compare the effect of oral NAC, IV NAC and placebo on renal and systemic haemodynamics with and without contrast, with at least a two-week wash-out interval between study arms. In **Study 3**, the protocol was as for **Study 1** but participants also received a single 100 mL dose of iodixanol (Visipaque 320, an iso-osmolar non-ionic radiocontrast agent used to show the coronary arteries) by IV injection. The protocol was replicated in **Study 2** (without contrast) in subjects with CKD3; estimated glomerular filtration rate (eGFR) 30 to <60mL/min/1.73m$^2$),[27] with a similar two-week wash-out interval.

**Study 4** was a three-arm randomised, double blind, double-dummy placebo-controlled study (n=22 in each arm) comparing placebo, oral, and IV NAC in patients with CKD3 undergoing elective coronary angiography. A parallel group design was selected because multiple contrast administrations to patients with CKD3 were considered unethical. Dose and timing of iodixanol in Study 4 was determined by the interventional cardiologist carrying out the procedure; these were therefore outwith control of the research protocol and varied between study arms. Iodixanol was selected because it has a low incidence of CIN complications.

### *Subjects*

Healthy, non-smoking, male subjects over the age of 45 years who were not taking regular medicines were eligible for recruitment to **Studies 1** and **3**. Subjects in **Study 1** were able to participate in **Study 3** provided that >3 months had elapsed (n=4). Male patients with stable CKD3, and such patients awaiting elective coronary angiography, were eligible for recruitment to **Studies 2** and **4**, respectively; participants could do both studies with a 3-month interval (n=6). CKD patients were allowed to continue their prescribed

medications but omitted metformin and diuretic therapy from the day prior to the study as per local clinical guidelines. Exclusion criteria included clinically significant co-morbidity, thyroid disease, asthma, atopy or myasthenia gravis, and history of allergy or sensitivity to NAC or contrast medium.[26] Participants were enrolled by researchers from Jul 2008 to Dec 2014, follow up was for 72 h. The trial stopped when all planned participants had been recruited and studied. The study recruited only male volunteers. Previous experience has shown that regular timed voiding by female participants receiving multiple infusions is difficult while maintaining volunteer privacy.[26]

***Interventions and randomisation***

Participants received all three interventions separately for **Studies 1-3**, with the sequence in which they received the treatments randomised. Participants in **Study 4** were randomised to receive one of IV NAC, oral NAC, or placebo. Placebo was intravenous 0.9% saline or oral lactose tablets; patients randomised to placebo received both. Randomisation was done by the company supplying NAC/placebo capsules (Tayside Pharmaceuticals) using a random number table with a 1:1:1 allocation ratio (no blocking); the patients were allocated to study day (**Studies 1-3**) or arm (**Study 4**) by pharmacists. The investigators recording data in the clinical research facility were blind to allocation.

No definitive data exist to guide the optimal dosing regimen or route of administration of NAC. We chose a revised IV dosing regimen (100 mg/kg over 2 h followed by 100 mg/kg over 5 h) based on a regimen effective in treating paracetamol poisoning, and associated with a low rate of adverse reactions[28] (selection discussed in the methods paper [26]). For the oral NAC regimen, we used 1200 mg twice daily (BD) the day before and the day of the study (total dose 4.8 g, 53.3 mg/kg in a 90 kg study participant; double the dose used in the original paper [17] and the dose often used in subsequent RCTs). All treatments and laboratory analyses were blind to subjects and investigators; placebo capsules were matched to oral NAC, while IV saline alone was administered as the placebo infusion. Visually it was not possible to distinguish the NAC and placebo infusions.

***Haemodynamic measurements***

Blood pressure and heart rate were measured with an appropriate size cuff using a calibrated oscillometric sphygmomanometer (PMS Instruments Ltd, UK). Consecutive measurements were taken at each time point until two consecutive measurements each of pulse, systolic and diastolic blood pressure within 10 bpm or 10 mmHg of each other were achieved. The means of these two measurements were then used for analysis.

***Clearance studies***

RBF and GFR were formally measured by renal clearance of para-aminohippuric acid (PAH) and inulin, respectively, as previously described.[29] Well-hydrated participants arrived fasted, a standard light breakfast was given, and participants asked to empty their bladder. Following loading doses of PAH and inulin, a maintenance infusion was given at 120 mL/h throughout the study. After a 2 h equilibration period, the IV infusion of NAC or placebo was commenced, and volunteers administered the 3rd dose of oral NAC or placebo (having ingested two doses the previous day – compliance checked on arrival). Participants in **Study 3** received 100 mL of radiographic contrast IV after completion of the first 2 h NAC/placebo infusion before starting the 5 h infusion. Participants in **Study 4** received IV contrast (volume decided by the interventional cardiologist according to clinical need) during angiography at variable times after starting the NAC/placebo infusion. At the time the contrast was first given, the clock was reset, and samples collected for 6 h as the NAC was infused. After completion of the 5 h NAC infusion, the infusion of PAH and inulin was continued for one further hour before the study ended.

Blood pressure and pulse rate were recorded every 30 min throughout the study (every 60 min after contrast in Study 4, due to presence of an arterial catheter entry site, usually in a radial artery) and blood samples obtained every hour (plus 15 and 30 min after contrast administration). Volunteers were asked to void urine every 120 min. The final dose of oral NAC/placebo was administered during the evening of the study day when the patients were usually home. Participants returned at 24 and 72 h for repeat blood and urine samples. Renal and systemic indices were calculated as previously described[26].

***Outcome measures***

The primary outcome for all four sub-studies was a change in RBF over 8 h after administration of IV NAC/placebo. Changes in GFR, tubular function (through assessment of fractional excretion of sodium), blood and urine biomarkers of renal damage, plasma cysteine, cellular glutathione, oxidative balance & systemic haemodynamic measurements were also assessed at multiple timepoints (**Table S1**).

***Laboratory analyses***

Para-aminohippuric acid (PAH) was measured by HPLC with fluorescence detection (see supplementary information for details on all assays). Inulin was measured colourimetrically by reaction with resorcinol. Urine KIM-1 and NGAL were measured using ELISA (DY1750, DY1757) and plasma cystatin C using DuoSet

ELISA (DY1196; all R&D Systems). Serum and urine creatinine were measured using the hospital's clinical laboratory and by the Jaffe method, respectively. Plasma oxygen radical absorbance capacity (ORAC) was measured using Cell Biolabs kit. Total thiol (NAC, cysteine, glutathione) concentrations ([reduced + oxidised]) were measured using a modified version of a published method.[30]

***Statistical analysis***

The study was powered on the basis of a mean (SD) RBF in patients with CKD3 of 600 ± 100 mL/ min.[29] Eight subjects in the cross-over studies would allow a 16% change in RBF for an active arm compared to placebo to be detected at 80% power (alpha of 0.05). The parallel group study had a 90% power (alpha of 0.05) with n=22 participants per arm to show a 30% change in RBF in patients with CKD.

A statistical analysis plan (SAP) was written before the statistician had access to the trial data. All analyses used an intention to treat (ITT) population. The primary analysis fitted random coefficient models to assess whether there were differences in the treatment effects (IV NAC vs. placebo, oral NAC vs. placebo), and whether these differences were constant with time. Since these models had convergence issues, the results from the secondary analysis were taken as the primary results (as per the SAP). For the secondary analysis, the treatment effects were considered by calculating the area under the curve (AUC) for RBF from baseline to 8 hours after the IV NAC/placebo was administered for each time period. For **Studies 1-3**, linear mixed models were fitted to the RBF AUC data with baseline RBF, treatment and period as fixed effects and patients as a random effect. For **Study 4**, a linear regression model was fitted with baseline RBF and treatment as covariates. These models were also fitted to the secondary outcomes. The point estimates and 95% confidence intervals (CI) for the adjusted difference in mean AUC for IV NAC vs. placebo and oral NAC vs. placebo are presented as treatment effects. All p-values and 95% Cis are two-sided with no adjustment made for multiple comparisons. Analyses were performed in SAS version 9.4.

***Role of the funding source and registration***

The study funder had no role in the design of the study, data collection, analysis or interpretation, or writing of the report. All authors had full access to the study data and the corresponding author had responsibility for the decision to submit for publication. This trial was registered with European Clinical Trials Database (EudraCT number 2006-017800-10) and ClinicalTrials.gov (identifier NCT00558142) before any patient was recruited.

## RESULTS

Eight healthy volunteers completed **Studies 1** (no contrast) and **3** (contrast), while eight patients with stable CKD3 completed **Study 2** (no contrast) (**figure 2**). Sixty-six patients with stable CKD3 undergoing elective coronary angiography completed **Study 4**. Two and three participants were withdrawn from **Study 1** and **2** after randomisation (see figure 2 for reasons), while one participant in **Study 2** had an eGFR >60 at recruitment and was therefore excluded. Seven participants were withdrawn from **Study 4** after randomisation and were replaced (figure 2); five participants had eGFR >60 at recruitment and were therefore excluded. All participants who completed the study were analysed.

CKD patients were older than healthy volunteers and had higher serum creatinine concentrations and lower ORAC status (**table 1**). CKD patients undergoing elective angiography had higher systolic blood pressure at baseline (before administration of IV NAC/placebo). Baseline variables were similar at the start of each study period (for the crossover studies) and between study arms (for the parallel groups study) (**Table S2**).

In **Study 4**, patients receiving placebo, oral NAC, and IV NAC were administered median (IQR) contrast doses of 118 (84 to 221) mL, 115 (94 to 183) mL, and 138 (90 to 193) mL, a median of 118 (103 to 179) min, 142 (118 to 151) min, and 130 (105 to 173) min after the beginning of the IV NAC or saline placebo infusion.

### *Pharmacokinetics of oral and IV NAC*

In healthy volunteers without contrast (**Study 1**), plasma NAC concentration increased from baseline to a mean (SD) peak of 235 ± 27 μM at 2.5 hours following IV administration (AUC 1278 [+/-132] μM.h, p<0.0001 *vs.* baseline for AUC, **figure 3A**). Mean peak concentrations were 100-fold lower following oral administration (2.5 ± 0.7 μM at 1 hour, p=0.01 *vs.* baseline; p<0.0001 *vs.* IV NAC, **figure 3B**). NAC concentrations after IV administration were modestly higher in the other studies of patients with CKD3 and/or with contrast compared to healthy volunteers without contrast in **Study 1**: **Study 2** (AUC 1633 [+/-124] μM.h, p=0.070 vs Study 1), **Study 3** (AUC 1946 [+/-198] μM.h, p=0.014), and **Study 4** (AUC 1992 [+/-355] μM.h, p=0.245) (**figure 3A**).

### *Effects on NAC on systemic haemodynamics*

NAC had modest effects on systemic haemodynamics. In the absence of contrast, administration of IV NAC, but not oral NAC, increased heart rate (28 [16 to 40] h.bpm, p<0.001 vs. placebo), systolic BP (62 [26 to 98]

h.mmHg, p=0.003), and MAP (29 [6 to 53] h.mmHg, p=0.019), of healthy volunteers (**Study 1**), but only the heart rate (34 [10 to 57] h.bpm, p=0.010) of participants with CKD3 (**Study 2**) (**table 2, figure 4, Figures S2-S3**).

When contrast was administered, IV NAC caused a larger increase in heart rate (46 [26 to 66] h.bpm, p<0.001 vs placebo) and blood pressure (MAP 59 [44 to 74] h.mmHg, p<0.001) of healthy volunteers (**Study 3**) (**table 2**). In patients with CKD3 undergoing angiography (**Study 4)**, IV NAC increased blood pressure (MAP 52 [14 to 90] h.mmHg, p=0.008) but no clear increase in heart rate (26 [-2 to 53] h.bpm, p=0.064). Oral NAC was associated with increased blood pressure (MAP 40 [2 to 79] h.mmHg, p=0.041) in CKD3 patients receiving contrast (**table 2**).

***Effects on NAC on renal haemodynamics and function***

Intravenous NAC increased RBF in healthy volunteers, in the absence (**Study 1**) (714 [254 to 1,175] h.mL/min, 8.0% increase, p=0.006 vs placebo) and presence (**Study 3**) (917 [352 to 1,481] h.mL/min, 12.0% increase, p=0.005) of contrast (**table 2**, **figure 5 A,C**). Oral NAC had no such effect (without contrast: 212 [-239 to 663] h.mL/min, 2.4% increase, p=0.325; with contrast: 35 [-539 to 610] h.mL/min, 0.5% increase, p=0.894). Intravenous NAC increased renal blood flow in CKD3 participants without contrast to a lesser degree than in healthy volunteers (**Study 2**) (288 [153 to 424] h.mL/min, 6.0% increase, p=0.001) but not with contrast (**Study 4**) (151 [-264 to 565] h.mL/min, 3.0% increase, p=0.470) (**figure 5 B,D**). IV NAC increased the GFR of healthy volunteers receiving contrast (**Study 3**) (147 [77 to 217] h.mL/min/1.73m², 19.7% increase, p=0.001), but not of patients with CKD3 (**Study 4**) (29 [-8 to 65] h.mL/min/1.73m², 6.3% increase, p=0.122) (**table 2**, **figure 5E-H**). In all 4 study groups, effective filtration fraction was unaffected by oral or IV NAC, while fractional excretion of sodium was increased by IV NAC only in patients receiving contrast (**table 2**, **Figures S4-S5**).

In **Study 4**, we assessed the effects of NAC on biomarkers of renal injury after contrast in CKD3 patients. Only six patients (four receiving placebo, one oral NAC, one IV NAC) showed acute kidney injury at 72 h (serum creatinine concentration >170 μmol/L). None of the biomarkers consistently indicated injury after contrast (**figure 6**). There were no statistically significant differences in concentration of serum creatinine or plasma cystatin C, or in the urinary KIM-1/creatinine or NGAL/creatinine ratios, between study arms (**Table S3**).

***Effects of NAC on antioxidant status***

Intravenous, but not oral, administration of NAC caused a marked increase in plasma cysteine as a breakdown product of acetylcysteine in all 4 studies (**figure 7A-D**) but no increase in white cell glutathione (formed from cysteine) (**Figure S6**). Neither IV nor oral NAC increased ORAC in any of the studies (**table 2**, **figure 7E-H**).

***Harms***

In **Studies 1-3**, 6, 4 and 2 participants reported adverse events. Two were serious adverse events, neither of which were considered to be associated with acetylcysteine (one episode of angina three days after the study day, requiring admission for angiography; one transient ischaemic event at home between study days, admitted for 24 h with full resolution). In **Study 4**, there were 22 adverse events, with 6 considered to be serious (1x myocardial infarction after recruitment before starting the study, 1x angina during study, 1x exacerbation of heart failure during study, 1x bradycardia during angiography, 1x angina secondary to coronary artery dissection during angiography, 1x episode of angina following study day).

## DISCUSSION

We show here that the oral NAC dose used in many reported RCTs results in barely detectable plasma NAC and cysteine concentrations and has no effect on renal haemodynamics or oxidant status, while IV NAC causes renal vasodilatation only in those patients with healthy kidneys who would not be expected to benefit. We found no mechanistic evidence that NAC can benefit patients at risk of CIN. Conducting unnecessary RCTs of interventions with no possibility of benefit leaves participants exposed only to any harms. It also has an opportunity cost for (at best) delaying RCTs of an intervention that might be effective and safe.

The last two decades has seen more than 18,000 patients recruited to over 75 RCTs of mostly low dose oral NAC to prevent CIN, in turn meta-analysed by more than 20 different groups. Two large RCTs, including over 7,000 patients, ultimately showed that oral NAC does not prevent CIN. The whole 'enterprise' was started by a high impact journal publishing a single under-powered RCT performed by an academic research group. Unlike typical drug development in industry, there were no pre-clinical studies and no early phase clinical trials to clarify possible mechanisms and effective doses before the first phase 3 trial was performed.

In our study, we examined the potential mechanisms by which NAC may affect renal function that could be protective versus CIN. We found that IV NAC increased heart rate and blood pressure in patients with CKD3 undergoing angiography – at relatively high risk for CIN – but had no effect on their renal haemodynamics, in particular renal blood flow. By contrast, IV NAC did increase renal blood flow in healthy volunteers with normal renal function.

The total dose of oral NAC we used (1,200 mg BD for two days, 60 mg/kg for an 80 kg individual) is about 5-fold lower than the IV dose and resulted in a 100-fold lower blood NAC concentration around the time of contrast administration and no effect on renal haemodynamics. This blood concentration was barely detectable, in contrast to IV NAC. These findings indicate that one of NAC's proposed mechanisms of protection against CIN – vasodilatation of the afferent arteries – is invalid in the patients at risk of developing CIN.

We also found that IV and oral NAC did not increase cellular glutathione concentrations or produce a clinically significant rise in plasma antioxidant capacity, as measured by ORAC. This suggests that NAC does not protect kidneys using its other proposed mechanism of action – that of boosting protective plasma antioxidant capacity.[19] However, the status of NAC as a direct antioxidant has been challenged, particularly at clinically relevant concentrations [31] and its potential to induce intracellular antioxidant effects might only relate to cells or individuals depleted of glutathione.[30] However, NAC delivery by both routes did increase thiol availability in plasma (NAC, cyst(e)ine) and intracellular (NAC, cyst(e)ine, GSH) compartments, as well as the MAP. These results suggest that a physiological effect may be conferred by the relatively small changes in thiol availability from oral NAC and that substantially higher plasma concentrations do not alter the extent of the MAP effect or protect against contrast-induced renal dysfunction. Further work is required to better understand the pharmacokinetic/dynamic effects of NAC and its metabolites.

**Limitations**

This study was not designed to assess the efficacy of NAC in preventing CIN – its purpose was to undertake the mechanistic investigations that logically should have preceded the 70+ RCTs on >18,000 participants that failed to show NAC was effective in preventing CIN. CIN was uncommon in the study - only six patients in Study 4 showed a rise in serum creatinine concentration at 72 h of >170 mol/L, and there were no changes in plasma cystatin-C or urinary KIM-1 and NGAL. These are sensitive measures of acute kidney injury. In a recent meta-analysis,[32] serum cystatin C had a sensitivity and specificity of 0.87 and 0.86,

respectively, for diagnosing AKI. For urinary KIM-1, these figures have been estimated at 0.84 and 0.78, respectively,[33] and for urine NGAL sensitivity and specificity are 0.82 and 0.90, respectively.[34] These figures will vary depending on the nature of acute or chronic kidney injury. A lack of change in these measures in the current study is likely because patients were asked to hydrate well before the procedure, modest amounts (Study 3: 100 mL; Study 4: median 130 mL) of an iso-osmolar contrast agent were used, and each patient received around 220 mL IV fluid per hour during the 9 hours surrounding the contrast administration.[35] The study recruited only male volunteers; however, we do not know of any reason why the results will not be as relevant for women as men.[26]

This study was designed to assess mechanistic signals on (i) healthy volunteers and those with CKD, (ii) using oral or IV NAC, and (iii) with and without a contrast medium, on RBF and a range of secondary outcomes – NAC concentration, systemic haemodynamics (including HR and MAP), renal haemo-dynamics (including GFR), and antioxidant properties (including cysteine and ORAC). It was difficult to confidently assess the required sample sizes to be adequately powered for all these investigations and, with limited resources, we chose to recruit n=8 to each of the 3-by-3 crossover studies and n=66 to the 3-arm parallel groups study. That our first choice of statistical model – the random coefficients model – failed to converge on occasion indicated a lack of information, and hence too small a sample size. However, for all the important issues we were able to rule out clinically important differences, and hence demonstrate that the putative mechanisms were unfounded and could not underpin the extensive suite of RCTs that overall failed to detect benefit for NAC. They failed to detect benefit because that benefit is not there, which would have been clear and obvious if this study had been done first, and not last.

Baseline values for comparison were taken after participants had taken oral NAC for 24 h, meaning that the effect of these first two doses of NAC was excluded from the statistical analysis. However, NAC was barely detectable at baseline in patients taking oral NAC, there was no evidence that oral NAC affected glutathione or antioxidant status, and participants were similar at baseline whether they had received oral NAC or not (Table S2).

**Implications**

We have shown here that oral NAC could never have benefited patients at risk of CIN through the proposed mechanisms and that the initial finding was likely a false positive. The single study published in a high impact journal[17] has resulted in an immense waste of funds (many millions of pounds at a cost of 1,000-10,000

pounds to recruit one patient), time, and also opportunity, since the patients could have been recruited to trials more likely to show benefit. This story shows vividly the weaknesses of stand-alone small-scale academic RCTs that are not part of a development process. The initial RCT should not have started a series of clinical trials without careful evaluation of whether the medicine could ever have worked.

There have been improvements since the 1990s. Now all grant & ethics applications request information on RCTs that have gone before. Systematic reviews of evidence are more commonly done. However, in this case, there were no previous studies to review and this requirement would therefore have made no difference. The problem was the wholesale acceptance of the result and the mass set-up of small RCTs worldwide, encouraged by the systematic review 'industry'. It illustrates the weaknesses of systematic reviews, many of which suggested that oral NAC might prevent CIN. Two large RCTs showed that it offered no benefit. The study complements the discourse around research wastage[36] and the harms of unthinking systematic reviews.[37,38]

**In conclusion,** this study shows that oral NAC is poorly absorbed and can offer no mechanistic benefit to kidneys affected by contrast, despite more than 18,000 patients being recruited to trials testing this intervention. Intravenous NAC did cause renal artery vasodilatation but only for healthy kidneys, not for kidneys in patients with CKD3 who are at higher risk of CIN. This study illustrates the dangers of designing clinical trials without clear proof of how an intervention might work and how the outcome should be measured.

**STUDY HIGHLIGHTS**

**What is the current knowledge on the topic?** There is uncertainty whether oral or intravenous acetylcysteine prevents contrast induced nephropathy. A single small RCT in 2000 triggered a wave of >70 clinical trials, 23 systematic reviews, and two large RCTs ultimately showing no effectiveness, but no mechanistic studies to determine whether NAC might possibly work.

**What question did this study address?** Does oral or intravenous acetylcysteine increase renal blood flow or reduce oxidative stress in chronic kidney disease patients at risk of contrast nephropathy?

**What does this study add to our knowledge?** Oral acetylcysteine at doses previously used in RCTs has no reno-protective effects; it neither increased blood flow to the kidneys nor protected against oxidative stress. Intravenous acetylcysteine did cause renal artery vasodilatation, increasing blood flow to kidneys, in healthy volunteers but not in patients with kidney disease who are at risk of contrast nephropathy. Intravenous acetylcysteine offered no protection against oxidative stress.

**How might this change clinical pharmacology or translational science?** This study emphasises the research and financial waste that may result when RCTs are initiated without mechanistic clinical studies to confirm potential benefit.

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**CONTRIBUTOR AND GUARANTOR INFORMATION**

ME, EAS, JMBR, and ILM wrote the manuscript; ME, EAS, ND, DNB, SC, NU, JG, DJW, and ILM designed the research; EAS, KR, EEM, KH, JW, TG, LB, SC, APT, NRJ, NU, AT, ILM and ME performed the research; EAS, JMBR, ND, JN, ILM, and ME analysed the data. GR contributed new analytical tools.

ME and ES are guarantors and accept full responsibility for the work and study conduct, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

**DATA SHARING**

The study protocol has been published.[26] A fully anonymised trial dataset will be made available to other researchers after the publication of the full trial report. Requests should first be directed to Michael Eddleston (Chief Investigator). Written proposals will be assessed and a decision made about the appropriateness of the use of data. A data sharing agreement will be put in place before any data will be shared.

**REFERENCES**

1. Parfrey P. The clinical epidemiology of contrast-induced nephropathy. *Cardiovascular and interventional radiology* 2005; **28 Suppl 2**: S3-11.

2. Weisbord SD, Chen H, Stone RA, et al. Associations of increases in serum creatinine with mortality and length of hospital stay after coronary angiography. *Journal of the American Society of Nephrology : JASN* 2006; **17**(10): 2871-7.

3. Brown JR, Rezaee ME, Nichols EL, Marshall EJ, Siew ED, Matheny ME. Incidence and in-hospital mortality of acute kidney injury (AKI) and dialysis-requiring AKI (AKI-D) after cardiac catheterization in the National Inpatient Sample. *Journal of the American Heart Association* 2016; **5**(3): e002739.

4. Gorelik Y, Bloch-Isenberg N, Yaseen H, Heyman SN, Khamaisi M. Acute kidney injury after radiocontrast-enhanced computerized tomography in hospitalized patients with advanced renal failure: a propensity-score-matching analysis. *Investigative radiology* 2020; **55**(10): 677-87.

5. Solomon R. Acute kidney injury: antioxidants do not PRESERVE kidney function after contrast exposure. *Nature reviews Nephrology* 2018; **14**(3): 148-9.

6. Tsai TT, Patel UD, Chang TI, et al. Contemporary incidence, predictors, and outcomes of acute kidney injury in patients undergoing percutaneous coronary interventions: insights from the NCDR Cath-PCI registry. *JACC Cardiovascular interventions* 2014; **7**(1): 1-9.

7. Brown JR, Solomon RJ, Sarnak MJ, et al. Reducing contrast-induced acute kidney injury using a regional multicenter quality improvement intervention. *Circ Cardiovasc Qual Outcomes* 2014; **7**(5): 693-700.

8. Subramanian S, Tumlin J, Bapat B, Zyczynski T. Economic burden of contrast-induced nephropathy: implications for prevention strategies. *J Med Econ* 2007; **10**(2): 119-34.

9. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *Journal of the American Society of Nephrology : JASN* 2005; **16**(11): 3365-70.

10. James MT, Samuel SM, Manning MA, et al. Contrast-induced acute kidney injury and risk of adverse clinical outcomes after coronary angiography: a systematic review and meta-analysis. *Circulation Cardiovascular interventions* 2013; **6**(1): 37-43.

11. Barrett BJ, Parfrey PS. Clinical practice. Preventing nephropathy induced by contrast medium. *N Engl J Med* 2006; **354**: 379-86.

12. Murphy SW, Barrett BJ, Parfrey PS. Contrast nephropathy. *Journal of the American Society of Nephrology : JASN* 2000; **11**(1): 177-82.

13. Persson PB, Hansell P, Liss P. Pathophysiology of contrast medium-induced nephropathy. *Kidney international* 2005; **68**(1): 14-22.

14. Makhija R, Divyaveer S. Angiography with sodium bicarbonate and acetylcysteine. *The New England journal of medicine* 2018; **378**(18): 1748.

15. Stacul F, Adam A, Becker CR, et al. Strategies to reduce the risk of contrast-induced nephropathy. *The American journal of cardiology* 2006; **98**(6A): 59K-77K.

16. Guideline Updates Team. National Institute for Health and Care Excellence: Clinical Guidelines. Acute kidney injury: prevention, detection and management. London: National Institute for Health and Care Excellence (UK); 2019.

17. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic contrast agent-induced reductions in renal function by acetylcysteine. *N Engl J Med* 2000; **343**: 180-4.

18. Sunman W, Hughes AD, Sever PS. Anaphylactoid response to intravenous acetylcysteine. *Lancet* 1992; **339**(8803): 1231-2.

19. Stacul F. Reducing the risks for contrast-induced nephropathy. *Cardiovasc Intervent Radiol* 2005; **28 Suppl 2**: S12-S8.

20. Xie W, Liang X, Lin Z, Liu M, Ling Z. Latest clinical evidence about effect of acetylcysteine on preventing contrast-induced nephropathy in patients undergoing angiography: a meta-analysis. *Angiology* 2021; **72**: 105-21.

21. ACT Investigators. Acetylcysteine for prevention of renal outcomes in patients undergoing coronary and peripheral vascular angiography: main results from the randomized Acetylcysteine for Contrast-induced nephropathy Trial (ACT). *Circulation* 2011; **124**(11): 1250-9.

22. Weisbord SD, Gallagher M, Jneid H, et al. Outcomes after Angiography with Sodium Bicarbonate and Acetylcysteine. *The New England journal of medicine* 2018; **378**(7): 603-14.

23. Lameire N, Kellum JA. Contrast-induced acute kidney injury and renal support for acute kidney injury: a KDIGO summary (Part 2). *Critical care (London, England)* 2013; **17**: 205.

24. Hoffmann U, Fischereder M, Kruger B, Drobnik W, Kramer BK. The value of N-acetylcysteine in the prevention of radiocontrast agent-induced nephropathy seems questionable. *J Am Soc Nephrol* 2004; **15**: 407-10.

25. Huang JW, Lahey B, Clarkin OJ, et al. A systematic review of the effect of N-acetylcysteine on serum creatinine and cystatin C measurements. *Kidney international reports* 2021; **6**: 396-403.

26. Sandilands EA, Cameron S, Paterson F, et al. Mechanisms for an effect of acetylcysteine on renal function after exposure to radio-graphic contrast material: study protocol. *BMC clinical pharmacology* 2012; **12**: 3.

27. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2002; **39**(2 Suppl 1): S1-266.

28. Bateman DN, Dear JW, Thanacoody HK, et al. Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial. *Lancet* 2014; **383**(9918): 697-704.

29. Goddard J, Johnston NR, Hand MF, et al. Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: a comparison of selective and combined endothelin receptor blockade. *Circulation* 2004; **109**(9): 1186-93.

30. Treweeke AT, Winterburn TJ, Mackenzie I, et al. N-Acetylcysteine inhibits platelet-monocyte conjugation in patients with type 2 diabetes with depleted intraplatelet glutathione: a randomised controlled trial. *Diabetologia* 2012; **55**: 2920-8.

31. Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. *Pharmacology & therapeutics* 2014; **141**: 150-9.

32. He Y, Deng Y, Zhuang K, Li S, Xi J, Chen J. Predictive value of cystatin C and neutrophil gelatinase-associated lipocalin in contrast-induced nephropathy: A meta-analysis. *PloS one* 2020; **15**(4): e0230934.

33. Li Q, Huang Y, Shang W, Zhang Y, Liu Y, Xu G. The predictive value of urinary Kidney Injury Molecular 1 for the diagnosis of contrast-induced acute kidney injury after cardiac catheterization: a meta-analysis. *Journal of interventional cardiology* 2020; **2020**: 4982987.

34. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clinical journal of the American Society of Nephrology : CJASN* 2008; **3**(3): 665-73.

35. Rudnick MR, Kesselheim A, Goldfarb S. Contrast-induced nephropathy: how it develops, how to prevent it. *Cleveland Clinic journal of medicine* 2006; **73**: 75-87.

36. Macleod MR, Michie S, Roberts I, et al. Biomedical research: increasing value, reducing waste. *Lancet* 2014; **383**: 101-4.

37. Horton R. Offline: The gravy train of systematic reviews. *Lancet* 2019; **394**: 1790.

38. Roberts I, Ker K. How systematic reviews cause research waste. *Lancet* 2015; **386**: 1536.

39. Giacoppo D, Gargiulo G, Buccheri S, et al. Preventive strategies for contrast-induced acute kidney injury in patients undergoing percutaneous coronary procedures: evidence from a hierarchical Bayesian network meta-analysis of 124 trials and 28 240 patients. *Circulation Cardiovascular interventions* 2017; **10**: e004383.

**Figure legends**

**Figure 1: A. Number of publications reporting clinical use of acetylcysteine for CIN and B. number of RCTs and patients recruited in published systematic reviews and meta-analyses of studies assessing the effectiveness of NAC in CIN 1990-2020.**

There were no clinical or mechanistic studies of NAC published in the decade before Tepel's publication in 2000. This paper was followed by a dramatic increase in the number of studies and systematic reviews, only beginning to fall after the publication of the negative ACT RCT in 2011.The largest meta-analysis (search performed 21 Sep 2018) reported 74 RCTs and recruitment of 14,635 patients in 2017.[39] In part B, each dot/diamond represents a single published meta-analysis including all known participants (excluding systematic reviews addressing sub-populations of trials). The most recent large RCT (Preserve; n=4,993)[22] was not included in any of the meta-analyses; the top right point (dark blue, n=19,628) represents the sum of patients in the largest meta-analysis plus this RCT. Key: Green diamond: number of studies in each systematic review (left y-axis); Blue circles: number of participants in each systematic review (right y-axis).

**Figure 2: CONSORT flow diagram.**

Number analysed refers to the RBF primary outcome. * One participant had a myocardial infarction on his way to hospital, one underwent emergency coronary angiography just before the study day, five participants had eGFR >60 at recruitment and were withdrawn, while five withdrew consent before the study day. ** In Study 1, one participant developed high blood pressure during the first study arm and was withdrawn, while one withdrew consent after the first study day. In Study 2, two participants withdrew consent before the first study started, one withdrew consent after the first study day, and one had eGFR >60 at recruitment.

**Figure 3: Pharmacokinetics of NAC. A:**IV NAC vs placebo (only one set of placebo results is visible as all overlap), **B:** oral NAC vs placebo. Data are mean (+/- SD) plasma concentrations (μM)**. Study 1:** healthy volunteers, without contrast (blue circles); **Study 2:** CKD3 patients, without contrast (red diamonds); **Study 3:** healthy volunteers, with contrast (purple squares); **Study 4:** CKD3 patients, with contrast (green triangle). NAC concentrations in patients receiving placebo are shown with

grey circles. Does of NAC administered were IV: 200 mg/kg over 7 h; oral: 1200 mg BD for 2 days, starting the morning before the procedure.

**Figure 4: Systemic haemodynamics in (Left Column) healthy volunteers (HV) and (Right Column) CKD3 patients, without (A,B,E,F) and with (C,D,G,H) contrast. A-D:** mean (SD) heart rate (beats per min); **E-H:** mean (SD) mean arterial pressure (MAP, mmHg). Participants received intravenous NAC (red triangles), oral NAC (blue squares), or double placebo (green circle). Black arrows indicate the timing of the contrast administration in the appropriate groups.

**Figure 5: Renal blood flow and glomerular filtration rate in (Left Column) healthy volunteers (HV) and (Right Column) CKD3 patients, without (A,B,E,F) and with (C,D,G,H) contrast. A-D:** mean (SD, dotted lines) renal blood flow (RBF, mL/min); **E-H:** mean (SD, dotted lines) glomerular filtration rate (GFR, mL/min/1.73m$^2$). Participants received intravenous NAC (red triangles), oral NAC (blue squares), or double placebo (green circle). Black arrows indicate the timing of the contrast administration in the appropriate groups.

**Figure 6: Blood and urinary renal biomarkers in CKD3 patients receiving contrast**. **A:** mean (SD, dotted lines) serum creatinine concentration (μmol/L); **B:** mean (SD, dotted lines) plasma cystatin-C concentration (mg/L); **C:** mean (SD, dotted lines) urinary KIM-1/creatinine ratio; **D:** mean (SD, dotted lines) urinary NGAL/creatinine ratio. Participants received intravenous NAC (red triangles), oral NAC (blue squares), or double placebo (green circle).

**Figure 7: Plasma antioxidant status in (Left Column) healthy volunteers (HV) and (Right Column) CKD3 patients, without (A,B,E,F) and with (C,D,G,H) contrast. A-D:** mean (SD, dotted lines) plasma (de-acetylated) cystine concentration (mg/L); **E-H:** mean (SD, dotted lines) plasma oxygen radical absorbance capacity (ORAC, TEAC/μg inulin). Participants received intravenous NAC (red triangles), oral NAC (blue squares), or double placebo (green circle).

**Table 1: Study participant demographics at recruitment.**

| | Study 1<br>Healthy volunteers<br>No contrast | Study 2<br>CKD3 patients<br>No contrast | Study 3<br>Healthy volunteers<br>With contrast | Study 4<br>CKD3 patients<br>With contrast |
|---|---|---|---|---|
| **Age, y** | 58.4 ± 5.9 | 71.1 ± 4.8 | 56.9 ± 9.3 | 72.7 ± 6.3 |
| **Weight, kg** | 91.7 ± 19.1 | 85.5 ± 5.9 | 82.2 ± 11.0 | 88.8 ± 14.3 |
| **BMI, kg/m²** | 27.7 ± 4.7 | 28.5 ± 2.6 | 25.8 ± 2.9 | 29.5 ± 4.3 |
| **Diabetes mellitus, n** | 0 | 5 (62.5%) | 0 | 25 (37.9%) |
| **Heart rate, bpm** | 55.5 ± 3.2 | 67.1 ± 15.3 | 67.8 ± 15.1 | 62.2 ± 10.2 |
| **Systolic BP, mmHg** | 135.1 ± 7.2 | 136.6 ± 13.7 | 134.3 ± 14.4 | 149.2 ± 22.0 |
| **Diastolic BP, mmHg** | 77.9 ± 10.4 | 74.4 ± 8.5 | 74.9 ± 10.3 | 71.7± 10.6 |
| **MAP, mmHg** | 97.0 ± 8.5 | 95.1 ± 8.0 | 94.7 ± 9.9 | 97.6 ± 11.1 |
| **Serum creatinine, μmol/L** | 77.4 ± 22.4 | 136.8 ± 22.2 | 80.3 ± 7.0 | 129.4 ± 19.7 |
| **eGFR** | >60 | 46.3 ± 7.5 | >60 | 48.4 ± 7.5 |

Data are displayed as mean ± SD. BMI: body mass index; BP: blood pressure; eGFR, estimated glomerular filtration rate; MAP, mean arterial pressure.

**Table 2. Changes in systemic and renal haemodynamics and oxidant status for participants receiving oral or IV NAC, with or without contrast.**

| | NO CONTRAST | | | | | | CONTRAST | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study 1: HV | | | Study 2: CKD3 | | | Study 3: HV | | | Study 4: CKD3 | | |
| | Estimate (95% CI) | P value | No. (%) of part., no. of (%) obs. | Estimate (95% CI) | P value | No. (%) of part., no. (%) of obs. | Estimate (95% CI) | P value | No. (%) of part., no. (%) of obs. | Estimate (95% CI) | P value | No. (%) of part., no. (%) of obs. |
| **PRIMARY OUTCOME** | | | | | | | | | | | | |
| **AUC RBF, h.mL/min** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 65 (98.5), 65 (98.5) |
| IV NAC | 9590.8 (9168.4, 10013.2) | | | 5105.4 (4714.0, 5496.9) | | | 8529.4 (8096.7, 8962.1) | | | 5228.4 (4935.4, 5521.3) | | |
| Oral NAC | 9088.6 (8668.6, 9508.6) | | | 4909.3 (4517.8, 5300.7) | | | 7648.2 (7213.3, 8083.2) | | | 5038.9 (4740.0, 5337.9) | | |
| Placebo | 8876.5 (8452.5, 9300.5) | | | 4817.3 (4426.0, 5208.5) | | | 7612.8 (7185.1, 8040.6) | | | 5077.8 (4785.8, 5369.8) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 714.3 (254.1, 1174.5) | 0.006 | | 288.2 (152.6, 423.8) | 0.001 | | 916.6 (351.9, 1481.2) | 0.005 | | 150.6 (-263.9, 565.0) | 0.470 | |
| Oral - placebo | 212.1 (-239.0, 663.2) | 0.325 | | 92.0 (-42.2, 226.2) | 0.159 | | 35.4 (-539.1, 609.9) | 0.894 | | -38.9 (-456.3, 378.6) | 0.853 | |
| **SECONDARY OUTCOMES** | | | | | | | | | | | | |
| **AUC SBP, h.mmHg** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 66 (100), 66 (100) |
| IV NAC | 1172.3 (1145.1, 1199.4) | | | 1203.4 (1114.8, 1292.0) | | | 1140.6 (1117.3, 1163.9) | | | 1174.6 (1130.2, 1219.0) | | |
| Oral NAC | 1117.4 (1090.6, 1144.2) | | | 1155.6 (1067.4, 1243.7) | | | 1027.3 (1004.2, 1050.5) | | | 1161.1 (1116.7, 1205.6) | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Placebo | 1110.7 (1083.9, 1137.6) | | | 1159.5 (1071.6, 1247.5) | | | 1032.3 (1009.4, 1055.3) | | | 1099.6 (1055.2, 1144.0) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 61.5 (25.5, 97.5) | 0.003 | | 43.8 (-15.8, 103.4) | 0.133 | | 108.3 (79.9, 136.7) | <0.001 | | 75.0 (12.3, 137.8) | 0.020 | |
| Oral - placebo | 6.7 (-28.4, 41.7) | 0.680 | | -4.0 (-60.2, 52.2) | 0.877 | | -5.0 (-33.7, 23.7) | 0.704 | | 61.5 (-1.3, 124.3) | 0.055 | |
| **AUC MAP, h.mmHg** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 65 (98.5), 65 (98.5) |
| IV NAC | 834.7 (817.8, 851.6) | | | 820.2 (762.3, 878.0) | | | 807.7 (787.4, 828.0) | | | 785.3 (758.2, 812.4) | | |
| Oral NAC | 813.9 (797.3, 830.5) | | | 790.5 (732.8, 848.1) | | | 748.9 (728.5, 769.3) | | | 773.5 (745.7, 801.2) | | |
| Placebo | 805.3 (788.7, 821.9) | | | 794.5 (736.9, 852.0) | | | 748.4 (728.2, 768.7) | | | 733.1 (706.1, 760.1) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 29.4 (5.5, 53.4) | 0.019 | | 25.7 (-11.6, 63.0) | 0.156 | | 59.3 (44.2, 74.3) | <0.001 | | 52.2 (13.9, 90.4) | 0.008 | |
| Oral - placebo | 8.7 (-14.7, 32.0) | 0.447 | | -4.0 (-39.5, 31.5) | 0.808 | | 0.5 (-15.0, 15.9) | 0.948 | | 40.4 (1.6, 79.1) | 0.041 | |
| **AUC DBP, h.mmHg** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 66 (100), 66 (100) |
| IV NAC | 666.3 (650.3, 682.4) | | | 628.1 (582.5, 673.7) | | | 642.2 (621.1, 663.2) | | | 591.6 (569.7, 613.6) | | |
| Oral NAC | 661.8 (646.0, 677.5) | | | 608.2 (562.7, 653.7) | | | 612.8 (591.5, 634.0) | | | 570.8 (548.9, 592.6) | | |
| Placebo | 652.5 (636.8, 668.2) | | | 612.1 (566.6, 657.5) | | | 604.9 (583.7, 626.1) | | | 548.1 (526.3, 569.9) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - | 13.9 (-8.8, 36.5) | 0.215 | | 16.1 (-13.8, 45.9) | 0.258 | | 37.3 (18.7, 55.8) | 0.001 | | 43.5 (12.5, 74.6) | 0.007 | |

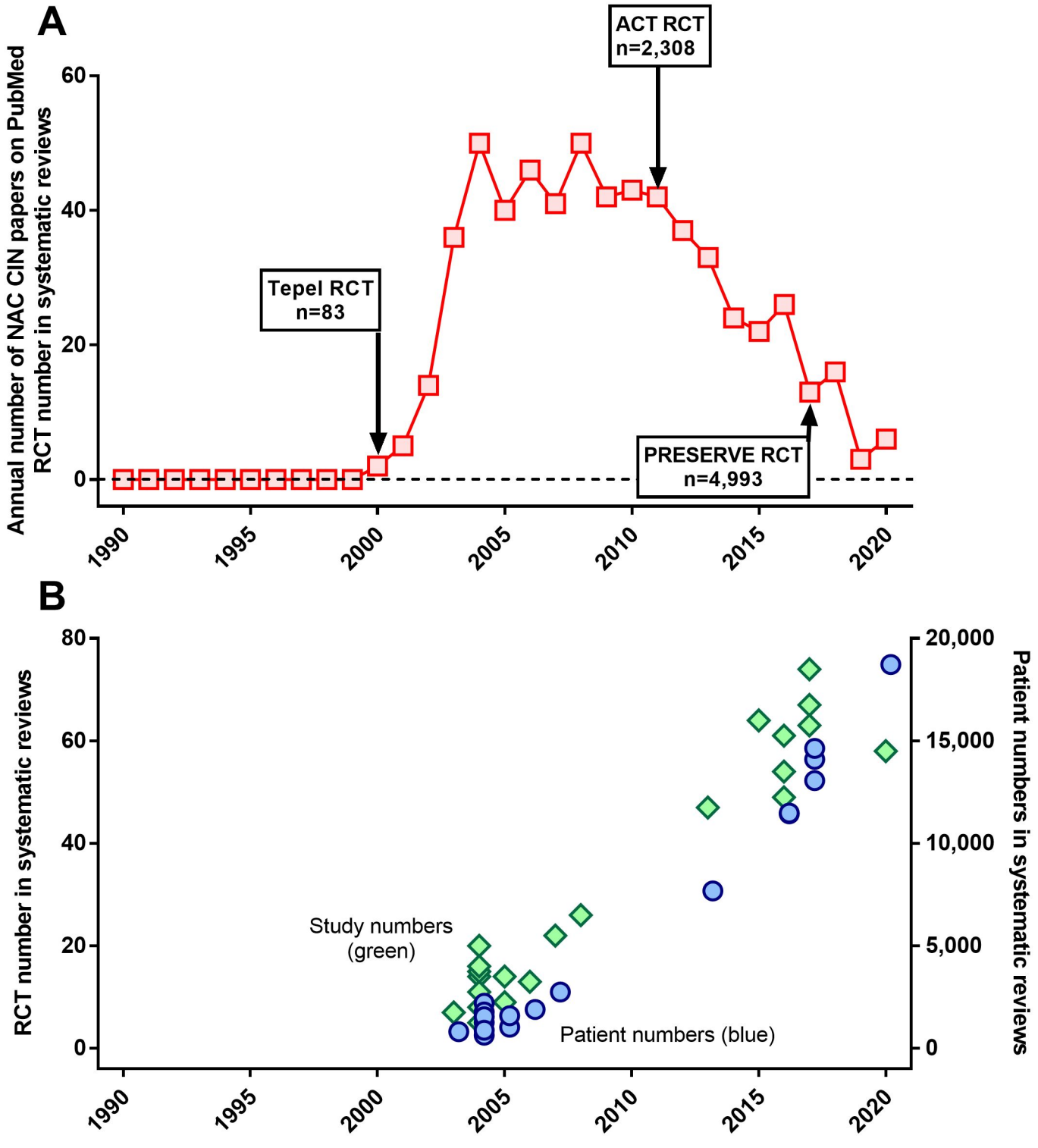
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| placebo | | | | | | | | | | | | |
| Oral - placebo | 9.3 (-12.9, 31.4) | 0.390 | | -3.9 (-32.6, 24.9) | 0.769 | | 7.9 (-11.3, 27.1) | 0.388 | | 22.7 (-8.2, 53.5) | 0.147 | |
| **AUC Heart rate, h.bpm** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 66 (100), 66 (100) |
| IV NAC | 450.2 (442.0, 458.3) | | | 520.5 (494.4, 546.6) | | | 503.6 (484.8, 522.3) | | | 475.6 (456.3, 494.9) | | |
| Oral NAC | 423.5 (415.4, 431.6) | | | 476.6 (450.5, 502.7) | | | 458.2 (439.3, 477.1) | | | 444.9 (425.6, 464.2) | | |
| Placebo | 422.2 (414.1, 430.2) | | | 486.9 (460.9, 512.9) | | | 457.7 (439.1, 476.4) | | | 450.0 (430.8, 469.2) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 28.0 (16.2, 39.8) | <0.001 | | 33.6 (9.8, 57.3) | 0.010 | | 45.9 (25.8, 65.9) | <0.001 | | 25.6 (-1.6, 52.7) | 0.064 | |
| Oral - placebo | 1.4 (-10.3, 13.0) | 0.801 | | -10.3 (-33.7, 13.1) | 0.354 | | 0.5 (-20.0, 21.0) | 0.960 | | -5.1 (-32.3, 22.1) | 0.709 | |
| **AUC GFR, h.mL/min/1.73m²** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 65 (98.5), 65 (98.5) |
| IV NAC | 982.2 (925.4, 1039.0) | | | 444.8 (415.7, 474.0) | | | 891.2 (842.0, 940.4) | | | 486.4 (460.4, 512.3) | | |
| Oral NAC | 900.0 (845.0, 955.0) | | | 443.8 (415.0, 472.5) | | | 806.7 (757.0, 856.3) | | | 479.8 (453.1, 506.4) | | |
| Placebo | 952.9 (895.8, 1010.0) | | | 438.9 (410.5, 467.3) | | | 744.4 (695.0, 793.8) | | | 457.6 (431.7, 483.5) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 29.3 (-27.8, 86.4) | 0.285 | | 5.9 (-30.8, 42.6) | 0.731 | | 146.8 (77.1, 216.5) | 0.001 | | 28.7 (-7.9, 65.3) | 0.122 | |
| Oral - placebo | -52.9 (-102.6, -3.3) | 0.038 | | 4.8 (-30.8, 40.4) | 0.770 | | 62.3 (-8.7, 133.2) | 0.080 | | 22.1 (-15.2, 59.4) | 0.240 | |
| **AUC EFF, h.%** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 65 (98.5), 65 |

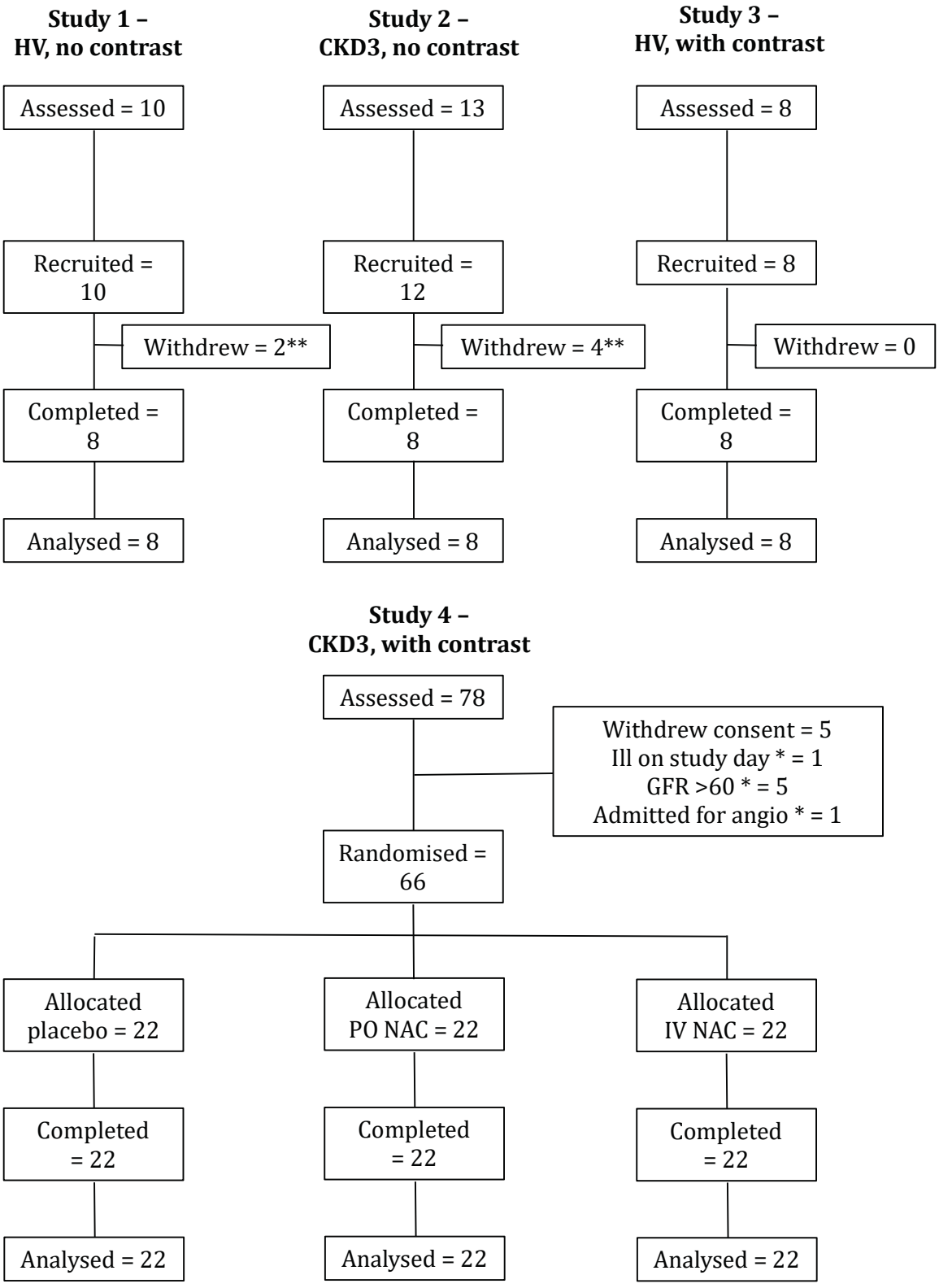
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | (98.5) |
| IV NAC | 83.4 (76.3, 90.5) | | | 71.7 (61.5, 81.9) | | | 82.7 (77.8, 87.6) | | | 79.7 (73.9, 85.5) | | |
| Oral NAC | 80.7 (74.2, 87.2) | | | 74.0 (63.7, 84.2) | | | 83.0 (77.8, 88.2) | | | 76.4 (70.5, 82.4) | | |
| Placebo | 87.2 (79.6, 94.7) | | | 75.0 (64.7, 85.2) | | | 80.4 (75.3, 85.6) | | | 75.0 (69.1, 80.8) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | -3.8 (-13.3, 5.8) | 0.414 | | -3.3 (-9.7, 3.2) | 0.264 | | 2.3 (-3.2, 7.7) | 0.385 | | 4.7 (-3.5, 13.0) | 0.254 | |
| Oral - placebo | -6.5 (-14.5, 1.6) | 0.107 | | -1.0 (-7.2, 5.1) | 0.698 | | 2.6 (-3.7, 8.8) | 0.393 | | 1.5 (-6.9, 9.8) | 0.727 | |
| **AUC UNaE, h.micromol/min** | | | 7 (87.5), 17 (70.8) | | | 8 (100), 23 (95.8) | | | 8, 22 | | | 56 (84.8), 56 (84.8) |
| IV NAC | 1797.8 (1346.1, 2249.5) | | | 1729.8 (1165.3, 2294.2) | | | 2170.5 (1966.5, 2374.5) | | | 2207.5 (1836.1, 2578.8) | | |
| Oral NAC | 1191.2 (754.6, 1627.9) | | | 1292.5 (773.1, 1812.0) | | | 1443.4 (1243.5, 1643.4) | | | 1363.8 (994.3, 1733.3) | | |
| Placebo | 1469.4 (1066.2, 1872.6) | | | 1063.8 (560.9, 1566.7) | | | 1321.5 (1107.4, 1535.6) | | | 1247.8 (880.4, 1615.2) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 328.4 (-276.8, 933.6) | 0.258 | | 666.0 (-98.2, 1430.1) | 0.080 | | 849.0 (613.8, 1084.3) | <0.001 | | 959.7 (438.7, 1480.7) | 0.001 | |
| Oral - placebo | -278.2 (-903.7, 347.3) | 0.349 | | 228.7 (-495.4, 952.9) | 0.493 | | 121.9 (-88.6, 332.5) | 0.224 | | 116.0 (-414.9, 646.9) | 0.663 | |
| **AUC ORAC, h.TEAC/mcg inulin** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 63 (95.5), 63 (95.5) |
| IV NAC | 70.3 (48.5, 92.1) | | | 56.3 (42.8, 69.7) | | | 55.1 (50.0, 60.2) | | | 48.3 (43.7, 52.9) | | |
| Oral NAC | 73.3 (51.5, 95.1) | | | 52.5 (39.3, 65.8) | | | 54.4 (49.3, 59.5) | | | 41.4 (36.8, 46.0) | | |
| Placebo | 78.5 (56.7, 100.3) | | | 62.0 (48.9, 75.0) | | | 56.0 (51.0, 61.1) | | | 41.0 (36.4, 45.6) | | |
| ***Treatment*** | | | | | | | | | | | | |

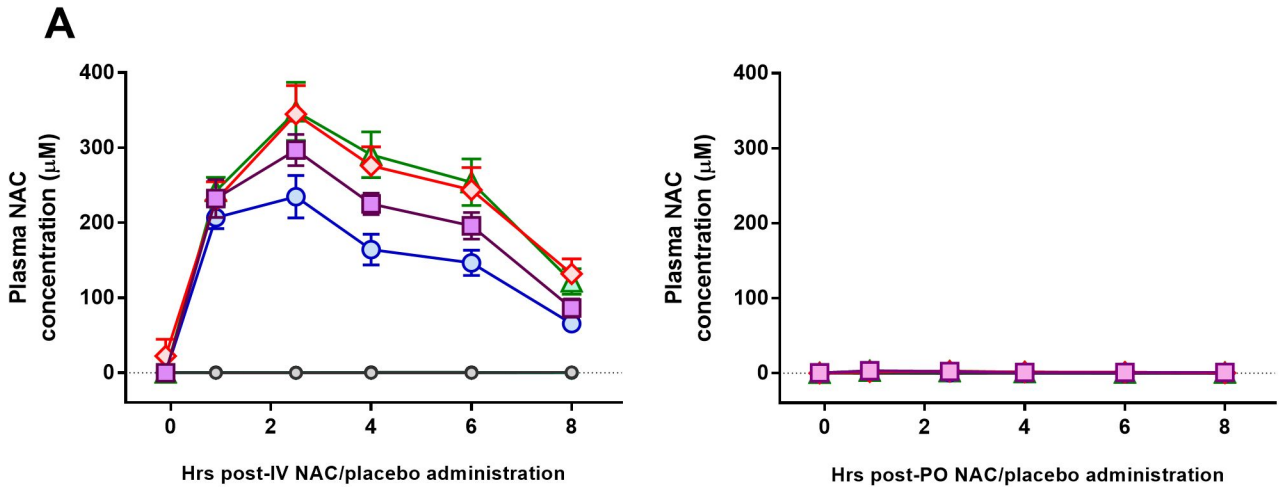
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***difference*** | | | | | | | | | | | | |
| IV NAC - placebo | -8.2 (-30.3, 13.9) | 0.426 | | -5.7 (-24.6, 13.2) | 0.536 | | -0.9 (-7.6, 5.7) | 0.765 | | 7.3 (0.8, 13.8) | 0.029 | |
| Oral - placebo | -5.2 (-27.3, 16.9) | 0.610 | | -9.4 (-27.9, 9.1) | 0.298 | | -1.7 (-8.4, 5.1) | 0.599 | | 0.3 (-6.2, 6.8) | 0.921 | |
| **AUC cysteine, h.mg/L** | | | 8 (100), 24 (100) | | | 8 (100), 23 (95.8) | | | 8 (100), 24 (100) | | | 66 (100), 66 (100) |
| IV NAC | 312.5 (275.6, 349.3) | | | 376.9 (332.2, 421.7) | | | 413.4 (356.3, 470.4) | | | 431.0 (400.3, 461.7) | | |
| Oral NAC | 85.8 (50.3, 121.3) | | | 127.6 (84.3, 170.9) | | | 85.4 (27.3, 143.5) | | | 124.8 (94.2, 155.3) | | |
| Placebo | 77.1 (41.5, 112.7) | | | 105.8 (60.7, 150.8) | | | 89.0 (31.0, 146.9) | | | 117.9 (86.9, 148.9) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | 235.4 (182.8, 288.0) | <0.001 | | 271.2 (205.9, 336.5) | <0.001 | | 324.4 (240.2, 408.5) | <0.001 | | 313.1 (269.0, 357.2) | <0.001 | |
| Oral - placebo | 8.7 (-40.9, 58.4) | 0.716 | | 21.9 (-39.3, 83.0) | 0.461 | | -3.6 (-90.0, 82.9) | 0.930 | | 6.9 (-36.9, 50.6) | 0.755 | |
| **AUC glutathione, h.nmol/mg** | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 8 (100), 24 (100) | | | 66 (100), 66 (100) |
| IV NAC | 266.0 (202.9, 329.0) | | | 431.0 (379.4, 482.7) | | | 263.8 (225.5, 302.1) | | | 345.7 (317.0, 374.3) | | |
| Oral NAC | 288.5 (225.3, 351.7) | | | 375.9 (325.0, 426.8) | | | 205.7 (166.9, 244.6) | | | 335.8 (307.5, 364.2) | | |
| Placebo | 295.2 (229.5, 361.0) | | | 365.8 (315.9, 415.7) | | | 259.2 (220.8, 297.7) | | | 329.5 (301.1, 357.8) | | |
| ***Treatment difference*** | | | | | | | | | | | | |
| IV NAC - placebo | -29.2 (-114.0, 55.6) | 0.470 | | 65.2 (-7.3, 137.8) | 0.075 | | 4.6 (-41.6, 50.7) | 0.832 | | 16.2 (-24.4, 56.7) | 0.428 | |
| Oral - placebo | -6.7 (-91.3, 77.9) | 0.867 | | 10.1 (-60.8, 81.0) | 0.768 | | -53.5 (-101.2, -5.8) | 0.031 | | 6.4 (-33.6, 46.3) | 0.751 | |

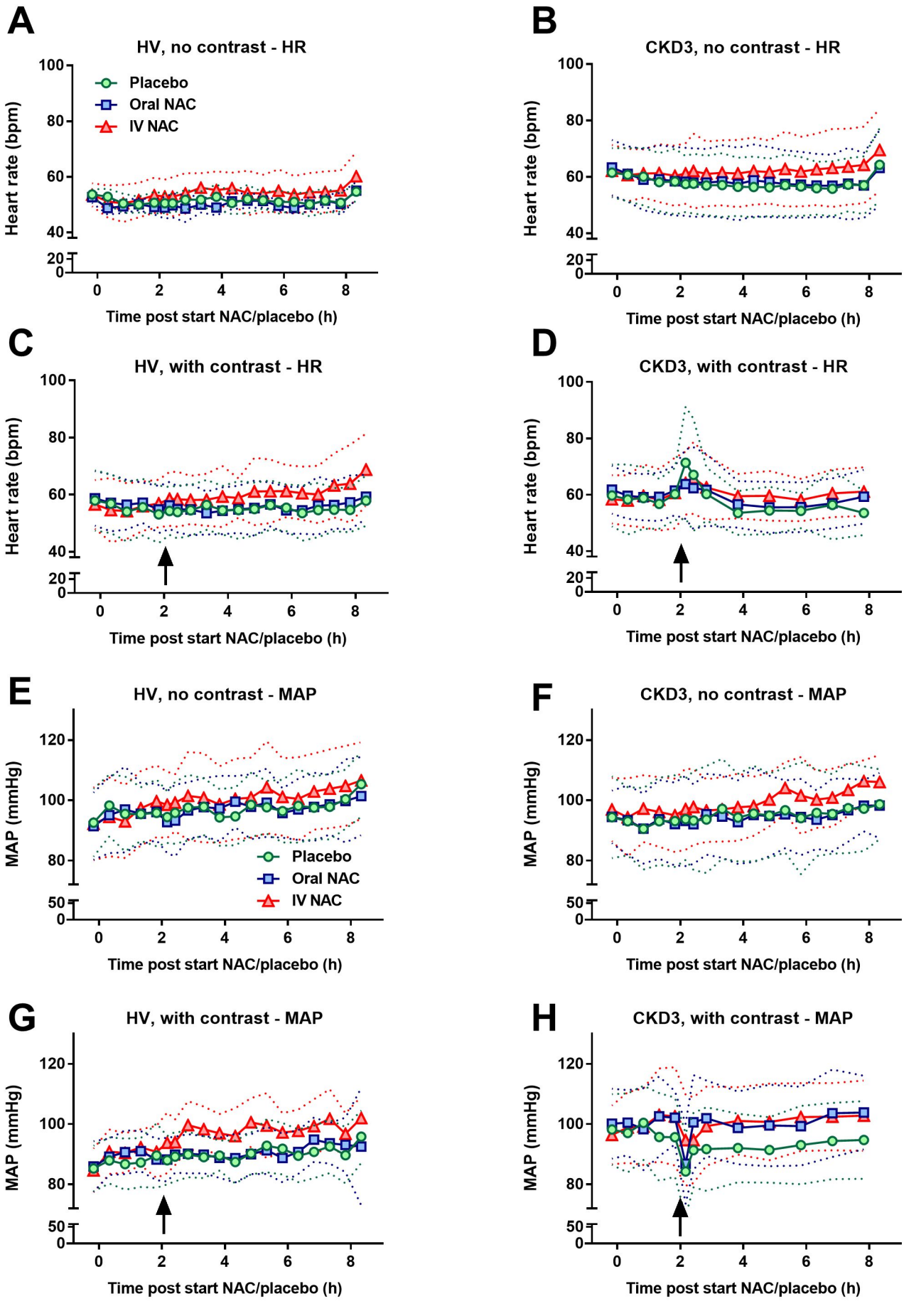
Comparisons are of mean area under the curve (AUC) values for IV NAC and oral NAC vs placebo, from start of the NAC/placebo infusion until 8 h later.

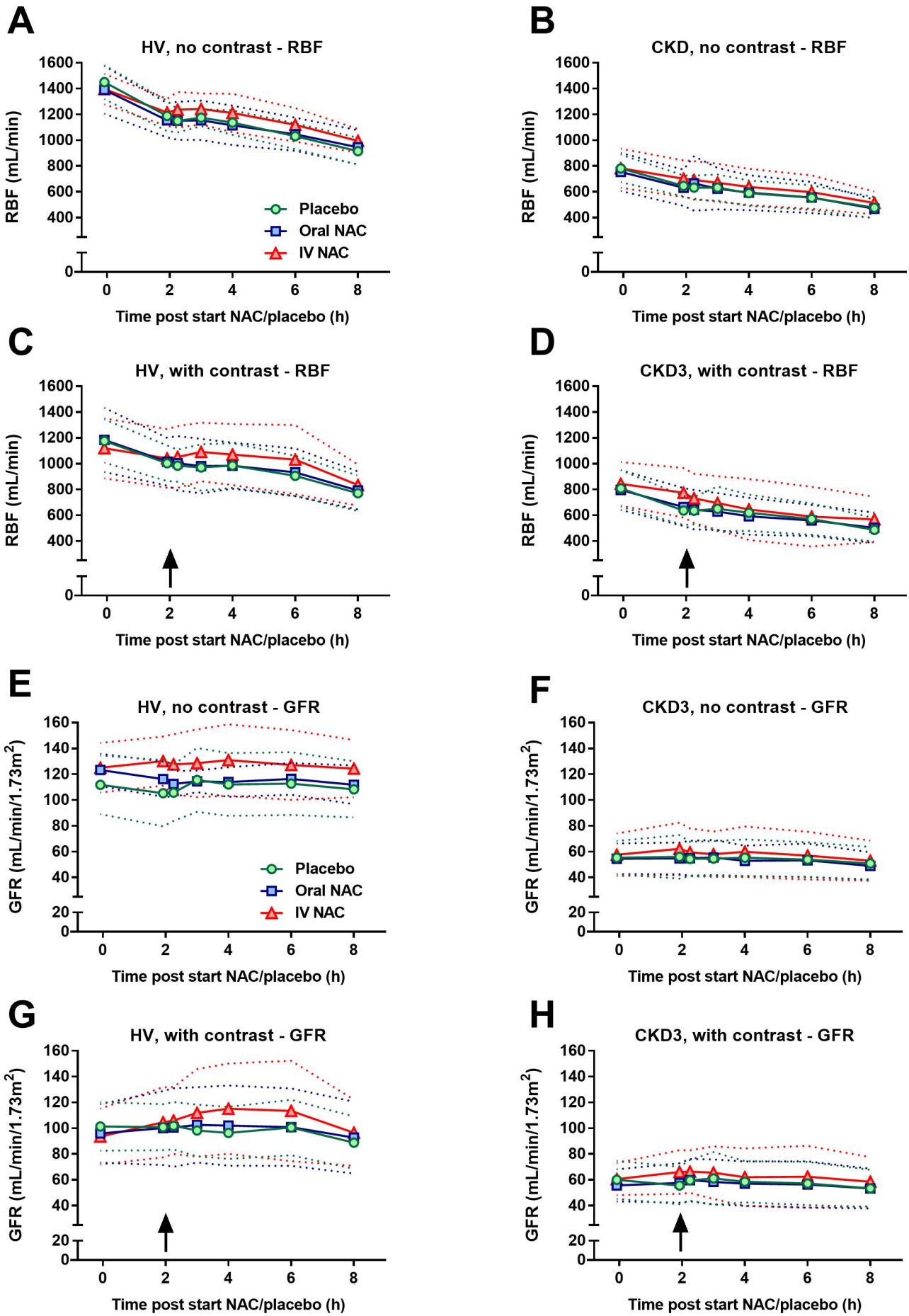
Abbreviations: CKD, chronic kidney disease; CI, confidence interval; no., number; part., participants; obs., observations; AUC, area under the curve; SBP, systolic blood pressure; h, hour; MAP, mean arterial pressure; DBP, diastolic blood pressure; RBF, effective renal blood flow; GFR, glomerular filtration rate; EFF, effective filtration fraction; UNaE, urine sodium excretion; ORAC, oxygen radical absorbance capacity.

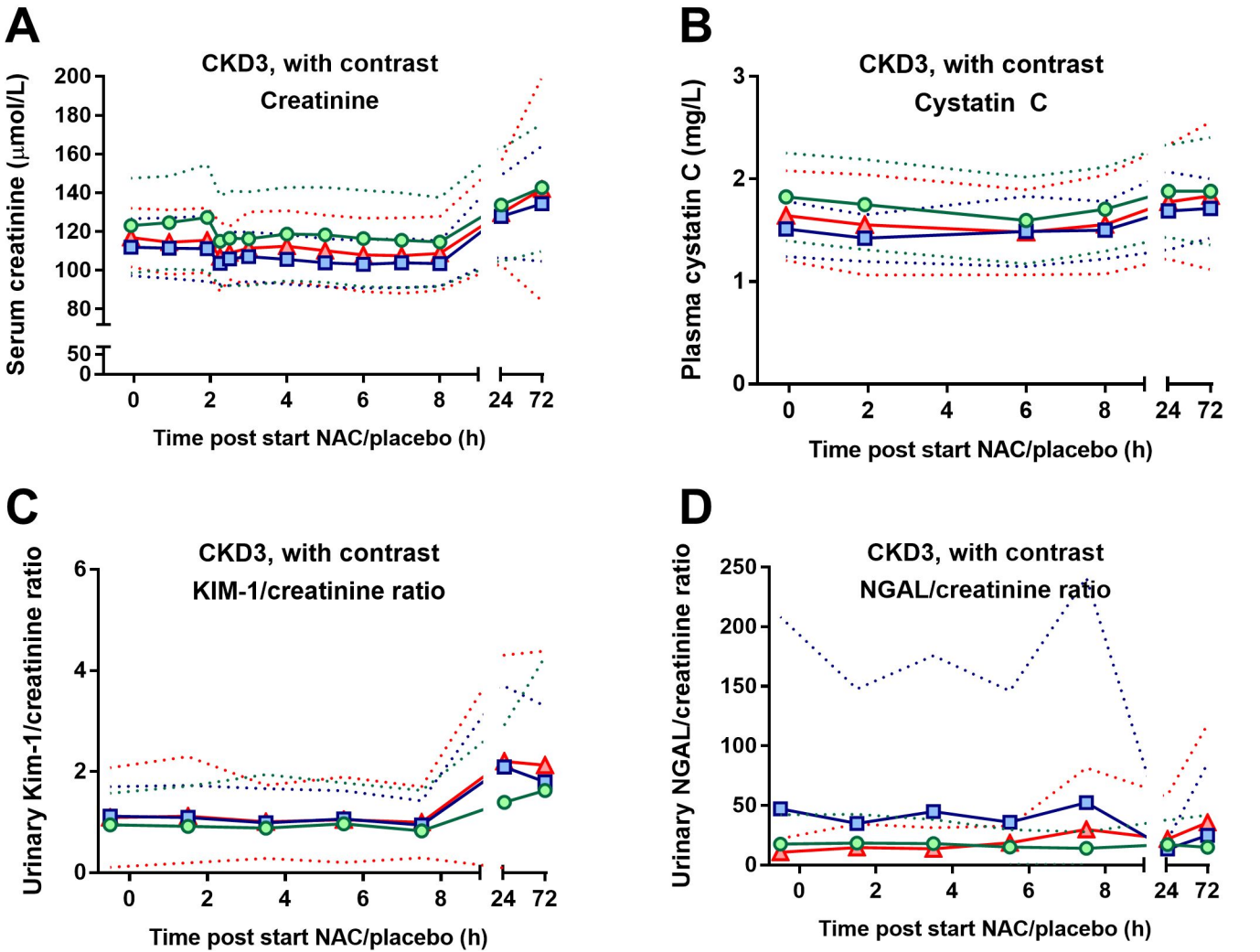

A
Annual number of NAC CIN papers on PubMed
RCT number in systematic reviews
60
40
20
0
Tepel RCT
n=83
ACT RCT
n=2,308
PRESERVE RCT
n=4,993
1990
1995
2000
2005
2010
2015
2020
B
RCT number in systematic reviews
80
60
40
20
0
20,000
15,000
10,000
5,000
0
Patient numbers in systematic reviews
Study numbers
(green)
Patient numbers (blue)
1990
1995
2000
2005
2010
2015
2020

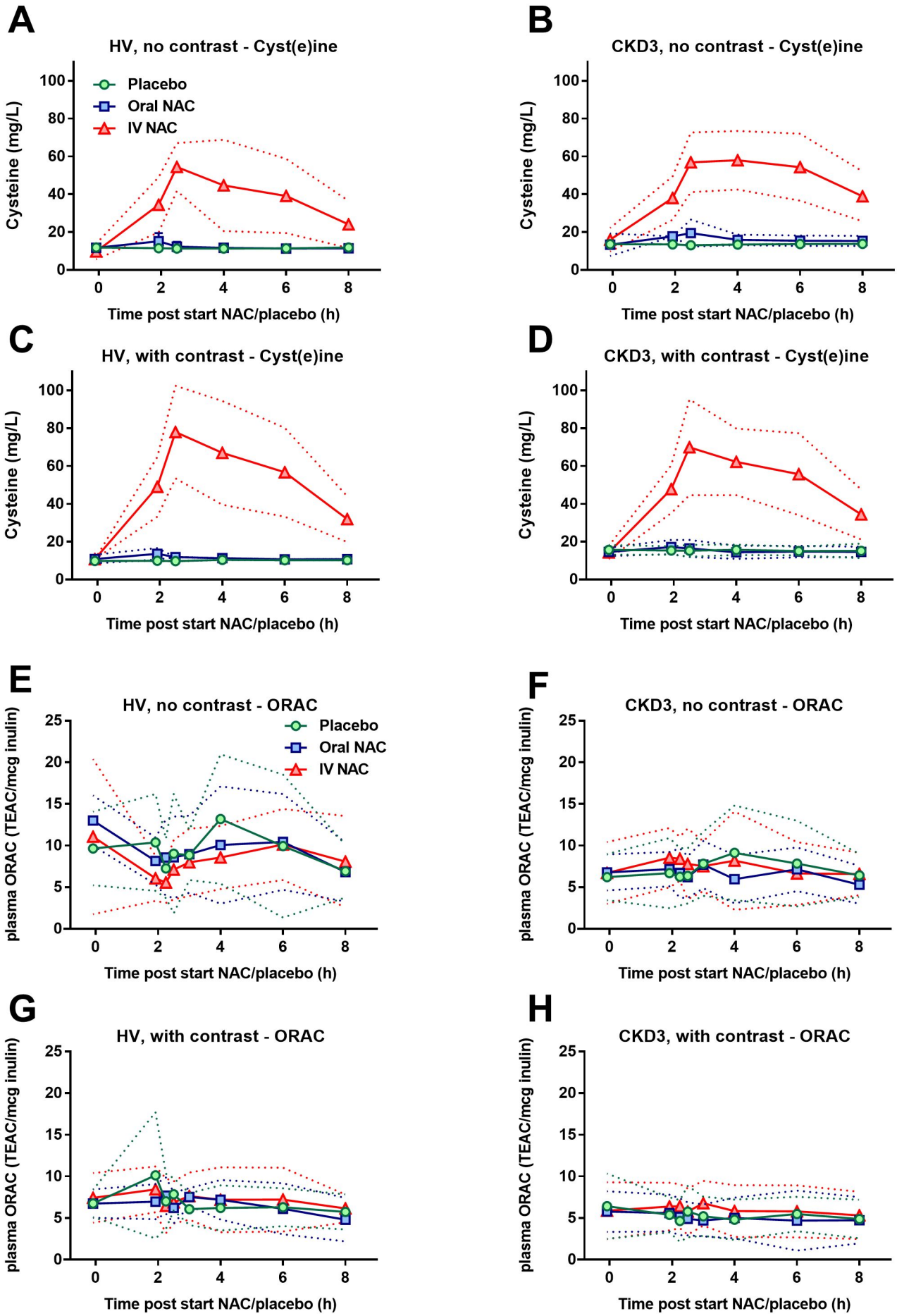

Study 1 –
HV, no contrast
Assessed = 10
Recruited = 10
Withdrew = 2**
Completed = 8
Analysed = 8
Study 2 –
CKD3, no contrast
Assessed = 13
Recruited = 12
Withdrew = 4**
Completed = 8
Analysed = 8
Study 3 –
HV, with contrast
Assessed = 8
Recruited = 8
Withdrew = 0
Completed = 8
Analysed = 8
Study 4 –
CKD3, with contrast
Assessed = 78
Withdrew consent = 5
Ill on study day * = 1
GFR >60 * = 5
Admitted for angio * = 1
Randomised = 66
Allocated placebo = 22
Completed = 22
Analysed = 22
Allocated PO NAC = 22
Completed = 22
Analysed = 22
Allocated IV NAC = 22
Completed = 22
Analysed = 22


A
Plasma NAC concentration (μM)
400
300
200
100
0
0
2
4
6
8
Hrs post-IV NAC/placebo administration
Plasma NAC concentration (μM)
400
300
200
100
0
0
2
4
6
8
Hrs post-PO NAC/placebo administration


A
HV, no contrast - HR
Placebo
Oral NAC
IV NAC
Heart rate (bpm)
Time post start NAC/placebo (h)
B
CKD3, no contrast - HR
Heart rate (bpm)
Time post start NAC/placebo (h)
C
HV, with contrast - HR
Heart rate (bpm)
Time post start NAC/placebo (h)
D
CKD3, with contrast - HR
Heart rate (bpm)
Time post start NAC/placebo (h)
E
HV, no contrast - MAP
MAP (mmHg)
Placebo
Oral NAC
IV NAC
Time post start NAC/placebo (h)
F
CKD3, no contrast - MAP
MAP (mmHg)
Time post start NAC/placebo (h)
G
HV, with contrast - MAP
MAP (mmHg)
Time post start NAC/placebo (h)
H
CKD3, with contrast - MAP
MAP (mmHg)
Time post start NAC/placebo (h)


A
HV, no contrast - RBF
RBF (mL/min)
Placebo
Oral NAC
IV NAC
Time post start NAC/placebo (h)
B
CKD, no contrast - RBF
RBF (mL/min)
Time post start NAC/placebo (h)
C
HV, with contrast - RBF
RBF (mL/min)
Time post start NAC/placebo (h)
D
CKD3, with contrast - RBF
RBF (mL/min)
Time post start NAC/placebo (h)
E
HV, no contrast - GFR
GFR (mL/min/1.73m2)
Placebo
Oral NAC
IV NAC
Time post start NAC/placebo (h)
F
CKD3, no contrast - GFR
GFR (mL/min/1.73m2)
Time post start NAC/placebo (h)
G
HV, with contrast - GFR
GFR (mL/min/1.73m2)
Time post start NAC/placebo (h)
H
CKD3, with contrast - GFR
GFR (mL/min/1.73m2)
Time post start NAC/placebo (h)


A
CKD3, with contrast
Creatinine
Serum creatinine (μmol/L)
200
180
160
140
120
100
80
50
0
0
2
4
6
8
24
72
Time post start NAC/placebo (h)
B
CKD3, with contrast
Cystatin C
Plasma cystatin C (mg/L)
3
2
1
0
0
2
4
6
8
24
72
Time post start NAC/placebo (h)
C
CKD3, with contrast
KIM-1/creatinine ratio
Urinary Kim-1/creatinine ratio
6
4
2
0
0
2
4
6
8
24
72
Time post start NAC/placebo (h)
D
CKD3, with contrast
NGAL/creatinine ratio
Urinary NGAL/creatinine ratio
250
200
150
100
50
0
0
2
4
6
8
24
72
Time post start NAC/placebo (h)


A
HV, no contrast - Cyst(e)ine
Placebo
Oral NAC
IV NAC
Cysteine (mg/L)
Time post start NAC/placebo (h)
B
CKD3, no contrast - Cyst(e)ine
Cysteine (mg/L)
Time post start NAC/placebo (h)
C
HV, with contrast - Cyst(e)ine
Cysteine (mg/L)
Time post start NAC/placebo (h)
D
CKD3, with contrast - Cyst(e)ine
Cysteine (mg/L)
Time post start NAC/placebo (h)
E
HV, no contrast - ORAC
Placebo
Oral NAC
IV NAC
plasma ORAC (TEAC/mcg inulin)
Time post start NAC/placebo (h)
F
CKD3, no contrast - ORAC
plasma ORAC (TEAC/mcg inulin)
Time post start NAC/placebo (h)
G
HV, with contrast - ORAC
plasma ORAC (TEAC/mcg inulin)
Time post start NAC/placebo (h)
H
CKD3, with contrast - ORAC
plasma ORAC (TEAC/mcg inulin)
Time post start NAC/placebo (h)