Original Article

Improved estimation of glomerular filtration rate by serum cystatin C in preventing contrast induced nephropathy by N-acetylcysteine or zinc—preliminary results

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Abstract

Background. Prevention of contrast media (CM) induced nephropathy (CIN) by prophylaxis (e.g. N-acetylcysteine; NAC) is controversially discussed. Up to now, assessment of kidney function has been based on measurements of serum creatinine, although this biomarker has several limitations. We investigated NAC and zinc (Zn) for the prevention of CIN by monitoring creatinine and cystatin C.

Methods. In a prospective, placebo-controlled, double blind trial, patients with moderately impaired kidney function receiving low-osmolar, non-ionic CM were randomly assigned to an oral treatment for 2 days with 1.2 g/day of NAC (n = 19), for 1 day with 60 mg/day of Zn (n = 18) or placebo (n = 17). All patients received peri-procedurally 1 ml/kg/h of 0.45% saline for 24 h. At baseline, prior to exposure of CM, 2 and 6 days after CM, creatinine and cystatin C were measured.

Results. There was no difference in the incidence of CIN, but a significant drop in creatinine (P < 0.05) was observed in all patients during volume expansion. Cystatin showed no increase after CM and it was normalized to the baseline values in all groups at the study end. In contrast, 2 days after CM there was a significant rise in cystatin C in the Zn (P = 0.012) and the placebo (P = 0.041) group, whereas NAC prevented this deterioration of kidney function.

Conclusions. Cystatin C seems to reflect CM-induced changes in kidney function better than creatinine. NAC and Zn have no effect in preventing CIN by the standard definition, but based on cystatin C we can confirm a preventive effect of NAC. It appears mandatory to assess kidney function by cystatin C in CIN intervention trials, because relying on creatinine can be misleading.

Keywords: contrast-induced nephropathy; cystatin C; NAC; volume expansion; zinc

Introduction

Approximately 80 million doses of iodinated contrast media (CM) were prescribed worldwide in 2003, making CM amongst the most commonly used medications in the history of medicine [1]. The value of these agents is unquestioned, but they are an important cause of acute kidney injury (rise in serum creatinine ≥ 0.5 mg/dl or ≥ 25%) [2]. Indeed, contrast-induced nephropathy (CIN) is one of the most common forms of acute kidney injury, and the third most frequent cause of hospital acquired renal failure. The incidence of CIN, which is 0.6 to 2.3% in the general population and higher in patients with cardiovascular disease, has a high associated mortality rate, e.g. as high as 34% in some reports [2–4]. Moreover, even a small deterioration of kidney function can increase mortality [5–7]. It is important therefore to detect changes of renal function after administration of CM and to develop strategies for prevention.

A change in serum creatinine concentration is the accepted method for detecting changes of renal function in patients receiving CM. As a breakdown product of muscle, however, serum creatinine concentration is influenced by a variety of non-renal factors, including body weight, nutritional status, race, age and gender. Furthermore, creatinine is insensitive for detecting reductions in kidney function, which may deteriorate more than 50% before serum creatinine exceeds the normal range [8,9]. A recent study questioned, in fact, whether possible renoprotective effects of N-acetylcysteine (NAC), in patients receiving CM, could be assessed at all by measuring serum creatinine [10]. Cystatin C is another surrogate for GFR and cystatin C may be superior to creatinine as a marker for GFR, be a more accurate measure of drug-induced changes in renal function and have particular advantages in the elderly because concentrations in blood are not influenced by age, gender or muscle mass [11–14].

In order to clarify the relative importance of cystatin C in the monitoring of renal function in studies regarding the controversial issue of pharmacological prevention (e.g. by NAC) of CIN, we performed a small randomized,
placebo-controlled, double blind trial in patients with mild to moderately impaired kidney function, receiving CM for coronary angiography, and we assessed GFR by measuring concomitantly serum levels of creatinine and cystatin C.

**Methods**

**Study population**

Between March 2004 and March 2006, patients with mild to moderately impaired kidney function undergoing coronary angiography were screened in our Department of Internal Medicine with the following inclusion criteria: (1) older than 18 years of age, (2) serum creatinine ≥ 1.2 mg/dl or a creatinine clearance < 50 ml/min (measured by a 12- or 24-h urine collection). Exclusion criteria were: (1) acute inflammatory disease, (2) medication with NSAID or metformin up to 3 days before entering study, (3) abnormal findings in physical examinations, e.g. signs of dehydration or inflammation.

The local ethics committee and regulating government authorities approved the study and we registered the study (ClinicalTrials.gov: NCT00399256). Written informed consent was obtained from all patients.

**Study protocol**

All patients were interviewed and examined at the beginning and the end of the double blind study. Furthermore they were instructed at study entry to avoid intake of alcohol, nicotine and caffeine during the study period.

Fifty-eight eligible patients were randomized and 54 were analysed (3 patients in the Zn group discontinued the study because of diarrhoea and 1 patient in the placebo group refused to participate without giving any reason). Patients were randomly assigned to receive either orally NAC (600 mg twice daily; n = 19) on the day before (d-1) and the day of injection of CM (d0); Zn (60 mg once daily; n = 18) on d-1 or placebo (n = 17) for 2 days. Every patient took the same amount of specially prepared capsules two times daily, so the patients received matching placebos to fill up the dosing points. All patients received a periprocedural intravenous infusion (‘volume expansion’) of 1 ml/kg/h with 0.45% saline for 24 h (12 h before and 12 h after exposure to CM). A non-ionic low-osmolar contrast medium (Iomeperole 350, Altana Pharma AG, Konstanz, Germany) was used in all patients.

Serum levels of creatinine were measured enzymatically and GFR was estimated by the MDRD formula (male: GFR = 186 × (serum creatinine)^−1.154 × (age)^−0.203, female: GFR = 186 × (serum creatinine)^−1.154 × (age)^−0.203 × 0.742). Serum cystatin C was measured by nephelometry and GFR was estimated by GFR = 74.835/(serum cystatin C)^−1.333 [15].

**Study end-points**

The primary outcome measure was development of CIN and the prevention by Zn or NAC. According to the literature CIN was defined as a ≥25% or ≥0.5 mg/dl increase in serum creatinine.

The secondary outcome was the difference of creatinine and cystatin C in the three groups.

**Statistical analysis**

The sample size estimation is based on the study of Durham et al. with a mean serum creatinine of 2.4 mg/dl and a standard deviation of 0.5 mg/dl [16]. To detect a ≥20% difference in serum creatinine with a power of >95% and an Alpha of 0.01 this resulted in 40 patients for each group.

The randomization scheme was generated by using the website www.randomization.com.

To test for the difference in the clinical characteristics and their distributions between the three groups, the Kruskal-Wallis test and Fisher’s exact test were applied. To investigate the time course of the two kidney function tests (creatinine and cystatin C) in each treatment group, a non-linear mixed effects regression model was used, which allows us to account for the longitudinal nature of the data [17]. P-values <0.05 were considered as significant. Statistical computations were carried out with the statistical package R together with the R-library nlme for mixed effects models (www.r-project.org).

**Study termination**

Midway through accumulation of the planned number of study patients there was an interim analysis which showed increased episodes of diarrhoea in the Zn group. Furthermore there were no changes in creatinine after CM exposure. Although there were no prospectively established stopping rules the study was halted after the clinical concern of exposure in the Zn group to the increased risk of diarrhoea.

**Results**

Characteristics of the patients included in the trial have been summarized in Table 1. The three groups were comparable concerning distribution of sex, body weight, age, diabetes, coronary artery disease, baseline renal function tests (creatinine, estimated GFR-MDRD, cystatin C, estimated GFR by cystatin C, urea) and volume of CM. All patients exhibited a mild to moderate impairment of kidney function (mostly chronic kidney disease in stage 3). There were no differences in routine laboratory values (haemoglobin, glucose, HbA1c, CRP, ESR).

Between the three groups there was no significant difference in the incidence of CIN on Day 2, after exposure to CM based on standard definitions. Rise in creatinine of ≥0.5 mg/dl: n = 1, 1 and 2 in placebo-, NAC- and Zn-group. A rise in creatinine ≥ 25%: n = 2, 1 and 3 in placebo-, NAC- and Zn-group. In Figure 1 the time- and treatment-dependent values of serum creatinine and cystatin C are illustrated. Three different results in the time pattern of both assessments are obvious:

1. There is a significant drop in serum creatinine in all three groups by volume expansion when comparing d-1
Table 1. Patients characteristics: (mean ± SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group (n = 17)</th>
<th>NAC (n = 19)</th>
<th>Zinc (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>66.8 ± 10.4</td>
<td>71.5 ± 9.5</td>
<td>67.2 ± 11.4</td>
<td>ns*</td>
</tr>
<tr>
<td>Male sex no. (%)</td>
<td>70</td>
<td>79</td>
<td>72</td>
<td>ns**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.6 ± 4.5</td>
<td>26.5 ± 3.9</td>
<td>28.8 ± 3.5</td>
<td>0.038*</td>
</tr>
<tr>
<td>CAD no. (%)</td>
<td>88</td>
<td>68</td>
<td>50</td>
<td>ns**</td>
</tr>
<tr>
<td>Diabetes no. (%)</td>
<td>35</td>
<td>26</td>
<td>28</td>
<td>ns**</td>
</tr>
<tr>
<td>Hypertension no. (%)</td>
<td>76</td>
<td>68</td>
<td>100</td>
<td>0.026**</td>
</tr>
<tr>
<td>Volume of contrast medium (ml)</td>
<td>219 ± 105</td>
<td>187 ± 88</td>
<td>173 ± 85</td>
<td>ns*</td>
</tr>
<tr>
<td>Laboratory examinations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>135.1 ± 18.0</td>
<td>116.5 ± 33.1</td>
<td>125.2 ± 25.2</td>
<td>ns*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>145.5 ± 47.8</td>
<td>135.8 ± 37.8</td>
<td>117.0 ± 35.0</td>
<td>ns*</td>
</tr>
<tr>
<td>HbA1c (%Hb)</td>
<td>6.4 ± 1.1</td>
<td>6.6 ± 1.3</td>
<td>5.9 ± 0.7</td>
<td>ns*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.65 ± 0.65</td>
<td>1.51 ± 0.23</td>
<td>1.60 ± 0.49</td>
<td>ns*</td>
</tr>
<tr>
<td>GFR (MDRD) (ml/min)</td>
<td>44 ± 12</td>
<td>48 ± 9</td>
<td>47 ± 12</td>
<td>ns*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>57 ± 35</td>
<td>58 ± 25</td>
<td>58 ± 22</td>
<td>ns*</td>
</tr>
<tr>
<td>Cystatin C (mg/l)</td>
<td>1.51 ± 0.52</td>
<td>1.48 ± 0.26</td>
<td>1.58 ± 0.64</td>
<td>ns*</td>
</tr>
<tr>
<td>GFR (cystatin C) (ml/min)</td>
<td>53 ± 19</td>
<td>49 ± 12</td>
<td>50 ± 21</td>
<td>ns*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>63.3 ± 29.7</td>
<td>64.7 ± 20.9</td>
<td>63.8 ± 42.2</td>
<td>ns*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.68 ± 0.41</td>
<td>0.77 ± 0.55</td>
<td>0.71 ± 0.74</td>
<td>ns*</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>22.3 ± 17.9</td>
<td>32.7 ± 22.6</td>
<td>40.9 ± 32.5</td>
<td>ns*</td>
</tr>
</tbody>
</table>

* Kruskal–Wallis test.
** Fisher’s exact test.

2. If all patients are analysed together there is a significant (P = 0.008) increase of serum cystatin C on d2 after exposure to CM compared to baseline (d-1). In the sub-group analysis this rise is significant for Zn (P = 0.012) and placebo (P = 0.041) but not in the NAC group.

3. There is no significant deterioration in either kidney function tests (serum creatinine and serum cystatin C) in all groups between baseline (d-1) and at the study end (d-6) as shown in Figure 1.

No dose effect (volume of CM) on the deterioration of either kidney function tests could be observed.

**Discussion**

Our randomized, placebo-controlled trial is the first attempt to prove the therapeutic benefit of NAC and Zn by monitoring concomitantly two independent serum markers of GFR (cystatin C and creatinine). In the primary outcome measure of this study, NAC and Zn have no effect in preventing CIN by the standard definition—but in the secondary outcome measure NAC prevents small increases of cystatin C. The major finding is that determination of serum creatinine is of limited value in assessing the renoprotective potential of a prophylactic intervention and serum cystatin C seems to be the more reliable parameter for estimating GFR.

Numerous controlled studies have been performed with NAC and based on more recent meta-analysis, it appears that this drug has some minor, probably dose-dependent renoprotective effect [18–25]. In all these interventional studies, the claimed renoprotective potential was solely based on measurements of serum creatinine. This is somewhat surprising as there is an ongoing discussion on the limitations of this surrogate parameter of kidney function: the ongoing discussion of interference in creatinine determination by NAC and inaccuracy of serum creatinine in reflecting GFR [8–10]. A more reliable marker of GFR is represented by cystatin C and it has been proposed to include serum level monitoring of cystatin C when assessing kidney function in patients undergoing cardiac catheterization [11–14,26–28]. The time course of cystatin C in CIN has recently been described, and it appears to be advisable to monitor cystatin C in studies when assessing the renoprotective effect.
potential of a prophylactic intervention [10,29]. With this novel approach, some more definite answers might be generated in the controversial issue of the therapeutic value of NAC in preventing CIN.

The exact underlying pathophysiology of CIN is still unknown and most likely several factors, including vasoconstriction resulting in medullar ischaemia, direct contrast media effects on renal tubular cells and toxic damage caused by reactive oxygen species (ROS) act in concert to cause CIN [30]. As ROS seems to play a crucial role in the pathogenesis it is not surprising that agents with antioxidant properties (e.g. NAC) have been employed for preventing CIN. For this reason we have included Zn in our placebo-controlled, comparative trial, as this agent has the potential to act as an ‘endogenous’ antioxidant via increasing metallothionein [31].

In our study low-osmolar and not iso-osmolar (the most ‘modern’) CM, as would be recommended based on the small NEPHRIC (Nephrotoxicity in High-risk Patients) study in such a high-risk patient population, was used [32]. However, several recently published trials comparing iso-osmolar and low-osmolar CM have not been able to confirm these results [33–35].

The results of this study indicate that determination of serum creatinine is of limited value in assessing unequivocally the renoprotective potential of a drug. Based on these measurements (Figure 1) we were able to observe a significant drop of serum creatinine in all three groups after volume expansion before CM exposure. This represents most probably a temporary increase in GFR, and does not necessarily translate into alterations of cystatin C because the kinetics of this marker are slower. The reduction of serum creatinine is of special interest because CIN is defined by an increase in creatinine in relation to baseline serum creatinine, which is measured either before volume expansion or before CM exposure. It is important to verify these two different time points for the correct interpretation of the results in such trials.

In contrast to creatinine, the results of cystatin C are in good agreement with the expected time pattern of kidney injury: CM induced a transient impairment in GFR in a more sensitive way than measurable by creatinine. Prophylactic treatment with placebo or Zn resulted in a deterioration of kidney function (<25%) on Day 2, which is completely reversible after 6 days, but prophylactic treatment with NAC could prevent this small but significant (P < 0.05) increase in cystatin C, as levels of cystatin C remained relatively stable during the entire monitored time period. This indicates that basal kidney function was maintained despite exposure to CM by NAC.

The study was terminated midway because of an increased incidence of diarrhoea in the Zn group, a well known, but not as serious expected side effect of Zn. Because of small patient numbers with a limited power our data are preliminary and cannot prove the benefit of one of the interventions. Focussing on cystatin C our results, even if generated from small groups of patients, confirm that NAC has some value for the prevention of acute kidney injury. It has been shown by several authors that even small changes in deterioration of kidney function can have an impact on mortality [5,6]. This has been confirmed recently in a large database of 27 608 patients with exposure to CM during coronary angiography [7]. Therefore the prevention of small changes in kidney function as seen in our study with cystatin C and NAC could be important. Furthermore, we think that the present definition of CIN (based on changes of serum creatinine) needs to be re-evaluated and discussed.

In conclusion, we recommend the prophylactic use of NAC and prefer cystatin C as the more reliable parameter for estimating GFR. Guidelines based solely on measurements of serum creatinine might have some limited impact for the tested therapeutic regimens in preventing CIN.

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Conflict of interest statement: None declared.

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