ORIGINAL ARTICLE

Effects of early high-dose erythropoietin on acute kidney injury following cardiac arrest: exploratory *post hoc* analyses from an open-label randomized trial

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ABSTRACT

Background. Acute kidney injury (AKI) is frequent in patients resuscitated from cardiac arrest (CA) and may worsen outcome. Experimental data suggest a renoprotective effect by treating these patients with a high dose of erythropoietin (Epo) analogues. We aimed to evaluate the efficacy of epoetin alpha treatment on renal outcome after CA.

Methods. We did a *post hoc* analysis of the Epo-ACR-02 trial, which randomized patients with a persistent coma after a witnessed out-of-hospital CA. Only patients admitted in one intensive care unit were analysed. In the intervention group, patients received five intravenous injections of Epo spaced 12 h apart during the first 48 h, started as soon as possible after resuscitation. In the control group, patients received standard care without Epo. The main endpoint was the proportion of patients with persistent AKI defined by Kidney Disease: Improving Global Outcomes criteria at Day 2. Secondary endpoints included the occurrence of AKI through Day 7, estimated glomerular filtration rate (eGFR) at Day 28, haematological indices and adverse events.

Results. A total of 162 patients were included in the primary analysis (74 in the Epo group, 88 in the control group). Baseline characteristics were similar in the two groups. At Day 2, 52.8% of the patients (38/72) in the intervention group had an AKI, as compared with 54.4% of the patients (46/83) in the control group (P = 0.74). There was no significant difference between the two groups regarding the proportion of patients with AKI through Day 7. Among patients with persistent AKI at Day 2, 33% (4/12) in the intervention group had an eGFR <75 mL/min/1.73 m^2 compared with 25% (3/12) in the control group at Day 28 (P = 0.99). We found no significant differences in haematological indices or adverse events.

Conclusion. After CA, early administration of Epo did not confer any renal protective effect as compared with standard therapy.

Keywords: cardiopulmonary resuscitation, renal function, reperfusion injury

INTRODUCTION

Cardiac arrest (CA) and resuscitation may cause multiple organ dysfunctions due to ischaemia–reperfusion (IR) injury, which can lead to the so-called 'post-CA syndrome [1]. This IR insult is known to have major effects on kidneys. IR acute kidney injury (AKI) results from endothelial and vascular injuries provoked by activation of various inflammatory cytokines, inflammation involving tubular epithelium and immune cell subgroups and abnormal repair processes, including incomplete repair of tubular cell and fibrosis formation [2]. The prevalence of AKI in CA patients ranges from 11 to 49% [3–5], depending on the employed definition of AKI. Severe AKI has been shown to be associated with a higher risk of both mortality and poor neurological outcomes [5–7]. Despite the high prevalence of AKI after CA and its poor prognosis, there is no renoprotective strategy recommended in recent guidelines for post-resuscitation care [8].

Erythropoietin (Epo) is a glycoprotein whose main role is to support erythropoiesis in bone marrow. In recent years, additional supplementary organ protection has been observed, in particular for the brain, heart and kidneys [9]. Previous animal studies have demonstrated its protective effect against AKI secondary to the IR mechanism by decreasing apoptosis, oxidative stress and inflammation [10–12]. These physiological effects are due to the binding of Epo to receptors found on renal tubular and collecting duct cells, activating multiple signalling pathways and leading to the transcription of promitogenic and anti-apoptotic genes [13]. No clinical trial has demonstrated Epo reduces the risk of AKI among patients with critical illness or perioperative care [14, 15]. It should be noted, however, that the Epo doses tested in these trials were substantially lower than those tested in experimental studies.

Neuroprotective effects of an Epo analogue (epoetin alpha) have recently been evaluated in a large trial (Epo-ACR-02) that enrolled patients successfully resuscitated from an out-of-hospital CA (OHCA). No benefit was observed regarding survival rate and neurological outcome [16], but analysis of renal outcomes is of interest as the dose of Epo in the Epo-ACR-02 trial was higher than that in previously reported trials. We therefore aimed to explore, *post hoc*, in a subgroup of the Epo-ACR-02 trial population, whether there are any renoprotective effects of Epo therapy on patients with resuscitated CA.

MATERIALS AND METHODS

Study design

This study is a *post hoc* analysis of the multicentre, single-blind, randomized controlled trial Epo-ACR-02 that evaluated the safety and efficacy of a high dose of Epo in patients resuscitated

from an OHCA [16]. The study received ethics committee approval by CPP Ile de France III, Paris-Tarnier Cochin, Paris, France and was performed between October 2009 and July 2013 in 20 French hospitals.

The Epo-ACR-02 trial included adult patients (18–80 years of age) resuscitated from witnessed OHCA of presumed cardiac cause, who remained unconscious (Glasgow Coma Scale <7) after sustained return of spontaneous circulation (ROSC). The time from CA to ROSC was <60 min for eligible patients.

The main exclusion criteria were evidence of extracardiac cause of arrest (trauma, sepsis, acute respiratory insufficiency and asphyxia), previous or chronic treatment with Epo or analogues, pregnancy, rapidly fatal underlying disease (expected life duration <6 months) and patients with no medical insurance (according to French legislation).

Eligible patients were included as soon as possible, either in the resuscitation theatre by the prehospital emergency medical service (EMS) or by the intensive care unit (ICU) team at the time of hospital admission. After being screened for eligibility, patients were randomly assigned in a 1:1 ratio to the intervention or to the control group. Intervention assignments were stratified by site. In this *post hoc* study, we only analysed patients who were admitted to the medical ICU at Cochin Hospital (Paris, France), which was the largest participating centre in the Epo-ACR-02 trial and in whom kidney outcome was assessed. Among patients included in the Epo-ACR-02 trial, those with chronic kidney disease (CKD) and those who died within the first 24 h after ICU admission were excluded from the present analysis. CKD was defined by a glomerular filtration rate <60 mL/min/1.73 m^2 [17] and was retrieved from the medical history.

Post-CA care

In the intervention group, patients received a first intravenous dose of epoetin alpha as soon as possible after ROSC, followed by four injections every 12 h during the first 48 h. Each injection was 40 000 U, resulting in a maximal dose of 200 000 U in total. In the control group, patients received standard care without any Epo medication. In the ICU, all patients were treated according to standard resuscitative guidelines, including early percutaneous coronary intervention (PCI) and targeted temperature management when indicated. Indication for renal replacement therapy (RRT) at ICU admission has been previously described [3].

Outcome

The primary endpoint of the study was the presence of AKI at Day 2, which is usually considered as being the most coherent

timing according to previous clinical studies on AKI after OHCA [18]. We used Stages 2 and 3 of the Kidney Disease: Improving Global Outcomes (KDIGO) criteria to define AKI [19]. According to this definition, patients were classified as KDIGO 2 or 3 if serum creatinine showed, respectively, a 2- or 3-fold increase compared with baseline or reached an absolute level of 4 mg/dL for KDIGO 3. For all analysed patients, pre-CA creatinine was back-calculated through the Modification of Diet in Renal Disease (MDRD) equation assuming a 75 mL/min/1.73 m^2 estimated glomerular filtration rate (eGFR), as recommended [20]. The urine output criteria were an output <0.5 mL/kg/h for >12 h for KDIGO 2 and an output <0.3 mL/kg/h for 24 h or anuria for at least 12 h for KDIGO 3. Patients who received RRT within the first 48 h were considered to have met the criteria for KDIGO 3 irrespective of other criteria.

The secondary outcome measures were the incidence of AKI at Day 2 among the two groups according to the KDIGO stage, the occurrence of AKI through Day 7 and the presence of AKI at Days 2 and 7 with AKI defined by at least a 2-fold increase in serum creatinine compared with baseline or the use of RRT, the presence of AKI at Day 2 using admission creatinine as pre-CA creatinine, comparison of the creatinine value between the two groups through Day 28, KDIGO stages based on urine output and creatinine results in each group, the haematopoietic effects of Epo through Day 28 or ICU discharge [haemoglobin, haematocrit, platelet levels and proportion of patients receiving red blood cell (RBC) transfusions] and the renal recovery of patients with AKI at Day 2 based on Day 28 eGFR estimated with the MDRD equation. Patients with eGFR >75 mL/min/1.73 m^2 were considered to have recovered normal renal function. All serious adverse events (SAEs) were screened until Day 60. To make sure that no patient with unknown CKD was included in the final analysis, we performed a sensitivity analysis in the group of patients with normal serum creatinine at admission (i.e. <1.5-fold from back-calculated pre-CA serum creatinine).

Statistical analysis

Baseline and follow-up characteristics were described as median (interquartile range) for continuous variables and numbers (percentages) for categorical variables. The Wilcoxon signed-rank test was used to compare distributions of continuous variables between the intervention group and the control group. For categorical variables, chi-squared tests or Fisher's exact tests were performed. A P-value <0.05 was considered statistically significant. Statistics were determined with SAS version 9.4 software (SAS Institute, Cary, NC, USA).

Missing data were dealt with as follows: complete case analysis if <5%, multiple imputations by equation chained if between 5% and 30% and variable excluded if >30%. In case of multiple imputation by equation chained, data were imputed using an imputation model repeated 10 times. An analysis model was fit in each of the 50 imputed datasets separately and these 50 datasets were pooled and give overall sets of estimates and corresponding standard errors according Rubin's rules [21]. Patients dead before landmark times (Day 7 or Day 28) were not considered at risk and consequently were not analysed.

Ethics approval and consent to participate

The study received ethics committee approval from CPP Ile de France III, Paris-Tarnier Cochin, Paris, France.
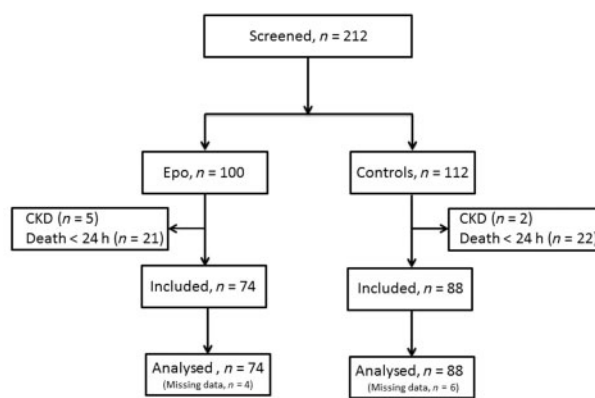
Epo: erythropoietin; CKD: chronic kidney disease

FIGURE 1: Flow chart.

RESULTS

A total of 212 patients were screened for their participation in this *post hoc* study: 100 were randomly assigned to the Epo group and 112 to the control group. Five patients with CKD in the Epo group and 2 in the control group were excluded, as 21 patients in the Epo group and 22 in the control group died within the first 24 h after ICU admission (Figure 1). The characteristics of the excluded patients are summarized in the Supplementary data, Table S1.

Patients

The Epo and control groups had similar pre-randomization characteristics (Table 1). The median age of patients was 60.7 years in the Epo group and 55.6 years in the control group. A total of 81.1% of patients in the Epo group and 72.7% of the control group were male. Bystanders provided cardiopulmonary resuscitation in 61.7% of cases. A shockable rhythm (ventricular fibrillation or non-perfusing ventricular tachycardia) was the most frequent monitored cardiac electrical activity at the first EMS presentation (49.4%).

No significant difference between treatment groups with respect to ICU management was observed (Table 1). At ICU admission, therapeutic hypothermia was performed or continued in 97.5% of patients. The cause of the OHCA was considered of cardiac origin in 140 patients (86.4%) and an early PCI with coronary stenting was performed in 69 patients (42.6%).

Intervention

In the interventional group, the median time (Q1–Q3) between ROSC and first Epo injection was 1.4 (0.7–2.8) h. The second injection (Hour 12) was administered to 72 patients (97.3%), the third one (Hour 24) to 70 patients (94.6%). Since some patients died after the first 24 h, the fourth injection (Hour 36) was given to 69 patients (93.2%) and the fifth (Hour 48) to 66 patients (89.16%).

Outcome

There was no significant difference between the two groups regarding the primary endpoint: at Day 2, 52.8% of the patients (38/72) in the intervention group had an AKI as compared with 54.4% of the patients (46/83) in the control group (P = 0.74). The proportion of patients in each level of the KDIGO score was not

Table 1. Baseline characteristics

Variable	Epo (n = 74)	Controls (n = 88)	P-value
Age (years), median (IQR)	60.7 (49.4–68.4)	55.6 (48.5–63.2)	0.12
Male	64 (81.1)	64 (72.7)	0.21
First-monitored rhythm			0.63
Asystole	24 (32.9)	22 (25.9)	
Pulseless electrical activity	6 (8.2)	7 (8.2)	
Shockable (VF or non-perfusing VT)	36 (49.3)	44 (51.8)	
Perfusing rhythm	7 (9.6)	12 (14.1)	
Unknown	1 (1.4)	3 (3.4)	
Location of CA			0.61
Public place	39 (52.7)	40 (45.5)	
Place of residence	28 (37.8)	34 (38.6)	
Other	7 (9.5)	14 (15.9)	
Cause of arrest			
Cardiac	64 (86.5)	76 (87.4)	0.87
Neurological	2 (2.7)	2 (2.3)	0.87
Respiratory	3 (4.1)	5 (5.8)	0.62
Intoxication	0	0	.
Miscellaneous	5 (6.7)	4 (4.6)	0.55
Bystander performed cardiopulmonary resuscitation	45 (60.8)	55 (62.5)	0.87
Time from collapse to ROSC (min), mean (IQR)	23 (15–30)	21 (15–33)	0.80
Admission SAPS 2 score, mean (IQR)	65 (57–73)	60 (54–69)	0.06
Admission creatinine level (μmol/L), mean (IQR)	94 (49–125)	86 (47–114)	0.38
Need for catecholamine at admission			
Dobutamine	0	0	.
Epinephrine	33 (44.6)	40 (45.5)	0.91
Norepinephrine	2 (2.7)	6 (6.8)	0.23
Epinephrine or norepinephrine	35 (47.3)	46 (52.3)	0.53
Need for catecholamine during the first 24 h			
Dobutamine	6 (8.1)	10 (11.4)	0.60
Epinephrine	24 (32.4)	39 (44.3)	0.15
Norepinephrine	17 (23)	28 (31.8)	0.22
Epinephrine or norepinephrine	39 (52.7)	59 (67.1)	0.08
Cooling	74 (100)	84 (95.5)	0.06
Early PCI	30 (40.5)	39 (44.8)	0.58
Day 28 mortality	38 (51.3)	47 (53.4)	0.79

Values are presented as or *n* (%) unless stated otherwise.
SAPS, simplified acute physiological score; VF, ventricular fibrillation; VT, ventricular tachycardia.

different between the two groups, even when AKI was defined using creatinine criterion or the need for RRT (Table 2), as well as using urine output (Supplementary data, Table S2). The proportion of patients with AKI at Day 2 was still not different between the control and intervention group when using admission creatinine as the pre-CA creatinine (Supplementary data, Table S3).

During hospitalization in the ICU, there was no significant difference between the two groups regarding the proportion of patients with AKI through Day 7 with AKI defined by creatinine criterion or the use of RRT: 45.9% of patients (34/74) in the intervention group had AKI during the first 7 days of hospitalization versus 33% of patients (29/88) in the control group (P = 0.09). At Day 2, 18.6% of patients required RRT: 19.4% in the intervention group as compared with 17.9% in the control group (P = 0.84). At Day 7, 6.8% of the patients (5/74) in the intervention group still had AKI compared with 9.1% of the patients (8/88) in the control group (P = 0.77). Creatinine values were not different between the two groups at admission, Days 2, 7 and 28 or discharge (Supplementary data, Table S4).

In the group of patients with a normal serum creatinine at admission, there was no significant difference regarding the

Table 2. Presence of AKI at Day 2 according to treatment allocation

Variable	Epo (n = 72)	Controls (n = 83)	P-value
KDIGO 0	33 (45.8)	36 (43.4)	
KDIGO 1	1 (1.4)	1 (1.2)	
KDIGO 2	14 (19.4)	19 (22.9)	0.96
KDIGO 3	24 (33.3)	27 (32.5)	
AKI	38 (52.8)	46 (54.4)	0.74
AKI_c	14 (19.4)	16 (19.5)	0.99

Values are presented as *n* (%).
AKI_c, acute kidney injury based on creatinine criterion.

main endpoint: 44.1% of patients (26/59) in the intervention group had AKI at Day 2 as compared with 46.6% of patients (34/73) in the control group (P = 0.86).

Overall, the Day 28 mortality rate was 52.5% (85/162) and 62% (52/84) for patients with persistent AKI at Day 2. Among the 32 patients with AKI at Day 2 still alive at Day 28, Day 28 creatinine was available in 24. The eGFR estimated by the MDRD equation was <75 mL/min/1.73 m² in 33% of patients (4/12) in the

Table 3. Haematopoietic effects according to treatment allocation

	Admission			Day2			Day7			Day28/ICU discharge		
Variable	Epo (n = 74)	Control (n = 88)	P-value	Epo (n = 72)	Control (n = 83)	P-value	Epo (n = 32)	Control (n = 42)	P-value	Epo (n = 55)	Control (n = 56)	P-value
Haemoglobin (g/dL)	12.9 (12.1–13.7)	12.7 (11.6–13.7)	0.27	11.7 (10.6–13.3)	11.8 (10.6–12.9)	0.45	10.7 (9.5–12)	10.2 (9.4–11.2)	0.16	10.4 (9–11.6)	10.6 (9.8–11.9)	0.22
Haematocrit (%)	38 (35–41)	37.1 (35–40)	0.39	35 (32–39)	35 (32–38)	0.76	31 (28–35)	30 (27–32)	0.05	28 (25–34)	32 (29–35)	0.10
Platelets (10^9/L)	173.5 (137–218)	183 (133–225)	0.41	153 (124–186)	161 (124–195)	0.52	165 (137–205)	188 (139–224)	0.42	158 (120–195)	160 (126–238)	0.53
Transfused patients cumulative, n (%)	2 (2.7)	3 (3.4)	0.80	2 (2.8)	4 (4.8)	0.52	8 (24.2)	7 (16.3)	0.40	4 (7.3)	2 (3.6)	0.48

Values are presented as median (interquartile range) unless states otherwise.

intervention group compared with 25% (3/12) in the control group (P = 0.99).

We found no significant difference between the two groups on biological data related to haematological indices of Epo and the proportion of patients receiving RBC transfusions through Day 28 or ICU discharge (Table 3). Among patients with persistent AKI at Day 2, 26% in each group (10/38 in the intervention group versus 12/46 in the control group) received at least one RBC transfusion during ICU hospitalization (P = 0.98).

At least one SAE was observed in 19 patients (25.7%) in the Epo group versus 12 patients (13.6%) in the control group (P = 0.07) (Table 4). Thrombotic complications occurred in nine patients (12.2%) in the Epo group versus four (4.6%) in the control group (P = 0.09). The most frequent thrombotic event was deep venous thrombosis, which was detected in seven patients in the intervention group versus two patients in the control group (P = 0.08).

DISCUSSION

In this *post hoc* and exploratory analysis of the Epo-ACR-02 trial, we did not find any renoprotective effect of a high dose of Epo analogue added to standard therapy in survivors of OHCA. The Epo treatment was not associated with a higher rate of complications compared with standard care, even if a trend for a higher rate of thrombotic events was observed in Epo-treated patients.

Lack of efficacy

This clinical study could not reproduce previous positive experimental studies that have shown promising data on the renoprotective effects of recombinant human Epo against IR injury. Indeed, *in vivo* and *in vitro* data, using histological and biochemical assessments, showed that Epo could reduce glomerular dysfunction and tubular injury by decreasing apoptosis, oxidative stress and inflammation [10–13, 22–34]. These protective effects were suspected in case of sepsis-related AKI [35], contrast-induced nephropathy [36], kidney transplantation [37] and haemorrhagic shock [38]. However, clinical trials have produced conflicting results regarding the renoprotective effect of Epo in AKI. Results from these studies were inconsistent, partly because they involved single-site trials with a small number of subjects and used small doses of Epo. The EARLYARF trial, a double-blinded randomized trial, assessed Epo in 162 AKI high-risk patients admitted to the ICU (mostly after major surgery). There was no difference in creatinine serum level evolution

Table 4. SAEs according to treatment allocation

Variable	Epo (n = 74)	Controls (n = 88)	P-value
Patients with at least one SAE	19 (25.7)	12 (13.6)	0.07
Categories			
Cardiovascular	6 (8.1)	2 (2.3)	0.14
Neurological	2 (2.7)	4 (4.6)	0.70
Pulmonary	0	0	–
Metabolic	1 (1.4)	1 (1.1)	1.00
Infectious	1 (1.4)	0 (0)	0.46
Thrombotic	9 (12.2)	4 (4.6)	0.09
Other	4 (5.4)	4 (4.6)	1.00
Details of thrombotic complications			
Venous thrombosis	7 (9.5)	2 (2.3)	0.08
Acute coronary stent thrombosis[a]	2 (6.7)	0	0.21
Other arterial thrombosis	2 (2.7)	2 (2.3)	1.00

Values are presented as n (%).
[a]Among patients with PCI, percentage calculated for 30 patients in the Epo group.

within 7 days after ICU admission between the placebo and the Epo group [14]. The efficacy and safety of Epo administration have also been evaluated for the prevention of cardiac surgery–associated AKI. Two randomized trials have shown that prophylactic administration of Epo reduced the incidence of AKI after elected coronary artery bypass grafting in patients without high-risk factors for AKI [39, 40]. In the study by Song *et al.* [39], Epo administration to 36 patients at the time of anaesthetic induction reduced the incidence of AKI and improved postoperative renal function compared with the 35 patients in the control group. Tasanarong *et al.* [40] found that intravenous administration of Epo to 50 patients 3 days before surgery and at the time of surgery reduced the incidence of AKI compared with the 50 patients in the control group. The additional administration of Epo 3 days before cardiac surgery may explain these positive results, as it may have improved the antioxidant property of Epo, which increases the number of circulating young RBCs and raises the level of erythrocyte antioxidant enzymes [13]. These preliminary data need additional and sufficiently powered studies to confirm the renoprotective effect of prophylactic Epo administration, especially since other studies on cardiac surgery patients have not highlighted such positive results [15, 41–43].

Experimental and clinical trials mostly evaluated short-term effects of Epo through 48 h. One study of a rat model indicated that Epo protects against AKI until Day 7, but at a supraphysiological

dose (5000 IU/kg) stimulates progressive fibrosis beyond what is normally seen in repair with IR-induced AKI at Day 28 [44]. Thus Epo could increase CKD in the long term.

Here we observed that 48 h after admission, AKI was present in 54% of patients, which is consistent with previous results from studies on AKI in CA patients [3, 7]. Several possibilities may explain the failure to show the expected reduction of AKI in this study. First, the optimal timing of Epo administration for renal protection has not yet been established in the clinical setting. Experimental and clinical studies have shown that administration of Epo before, rather than after, ischaemic injury is effective in attenuating renal injury. Delayed administration of Epo after reperfusion showed renal protection effects in some animal studies [22], but not in a clinical trial. Moreover, Sharples *et al.* [23] found that Epo administration 30 min after reperfusion in rats undergoing IR injury partially protected the kidneys, whereas Epo administration 30 min before ischaemia and 5 min before reperfusion was more effective.

Second, the dose that was employed can also be debated. The chosen dosage (200 000 U >48 h) is the highest among clinical studies evaluating the renal protection effects of Epo, in which doses range from 300 to 700 UI/kg (with a maximal dose of 100 000 U in the EARLYARF study [14]). This dosage was also the dose employed in the pilot study of Epo-ACR-02, in which pharmacokinetic parameters appear similar to those reported in healthy subjects [45]. However, this dosage is much lower than the dose of 5000 UI/kg frequently used in preclinical studies. In clinical trials, we are limited by the effect of Epo on erythropoiesis, leading to thrombotic complications. However, by using a lower dose, we may limit the effect of Epo on Epo receptors responsible for organ protection.

Side effects

In this *post hoc* analysis, we found no significant increase of SAEs in the intervention group as compared with controls. However, there were more thrombotic events in Epo-treated patients and the lack of significance is probably due to inadequate power. Also, we did not find any differences concerning the haematopoietic effects of Epo between the two groups. Regarding the small size of our analysed population, we cannot assert that injections of a high dose of Epo are considered to be safe. One alternative could be the use of non-haematological derivatives such as carbamylated Epo that produce cellular and organ protection in animal studies [31].

Limitations

This study has several limitations. First is the definition of early AKI by the KDIGO classification, because diuresis in the ICU is not a specific criterion reflecting renal function and because RRT is sometimes used to optimize metabolic control in severe acidotic patients. Moreover, use of the eGFR determined by the MDRD equation to assess the Day 28 creatinine clearance can also lead to a misevaluation of renal function, as significant changes between estimated and calculated eGFR have been reported at ICU discharge [46]. Second, since this study is a *post hoc* analysis, data concerning survival and renal function at Day 28 have been collected retrospectively, and thus some data were missing. Third, to assess the pre-CA creatinine, we used a back-calculated creatinine through the MDRD equation assuming a 75 mL/min/1.73 m^2 eGFR for every patient, whereas their anterior renal function was unknown. As KDIGO stages are partly determined by creatinine increase compared with the

baseline, it could have created a bias in the proportion of AKI at Day 2. However, sensitivity analysis using admission creatinine values showed similar results. Finally, the Epo-ACR-02 study was an open-label trial while this study is a single-centre trial on a small sample of patients with an exploratory design, which limits generalization of our results.

CONCLUSION

In patients resuscitated from CA, early administration of Epo compared with standard therapy during the first 48 h did not confer any renoprotective effect.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

Availability of data and material

A.C. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

AUTHORS' CONTRIBUTIONS

M.D., A.B., S.K., E.V., O.J. and A.G. were responsible for data monitoring. A.V.-B., G.C., and P.B. were responsible for the data and safety monitoring board. A.C., N.D. and O.H. contributed to the study concept and design. L.G. and A.C. contributed to the drafting of the article. J.C. performed statistical analysis.

All the authors contributed to the review and revisions of the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

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