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Early intervention with erythropoietin does not affect the outcome of acute kidney injury (the EARLYARF trial)

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We performed a double-blind placebo-controlled trial to study whether early treatment with erythropoietin could prevent the development of acute kidney injury in patients in two general intensive care units. As a guide for choosing the patients for treatment we measured urinary levels of two biomarkers, the proximal tubular brush border enzymes γ-glutamyl transpeptidase and alkaline phosphatase. Randomization to either placebo or two doses of erythropoietin was triggered by an increase in the biomarker concentration product to levels above 46.3, with a primary outcome of relative average plasma creatinine increase from baseline over 4 to 7 days. Of 529 patients, 162 were randomized within an average of 3.5 h of a positive sample. There was no difference in the incidence of erythropoietin-specific adverse events or in the primary outcome between the placebo and treatment groups. The triggering biomarker concentration product selected patients with more severe illness and at greater risk of acute kidney injury, dialysis, or death; however, the marker elevations were transient. Early intervention with high-dose erythropoietin was safe but did not alter the outcome. Although these two urine biomarkers facilitated our early intervention, their transient increase compromised effective triaging. Further, our study showed that a composite of these two biomarkers was insufficient for risk stratification in a patient population with a heterogeneous onset of injury.

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KEYWORDS: acute renal failure; clinical trial; erythropoietin; randomized controlled trials

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Acute kidney injury (AKI), is a major complication of patients admitted to the intensive care unit (ICU), with a mortality of 30–80%.[1] There have been no successful intervention trials after diagnosis of AKI.[2] Failure to replicate the successful experimental treatment of AKI has been attributed to the 48- to 72-h delay in diagnosis from using plasma creatinine (pCr) and the underlying heterogeneity of the etiology, duration, severity, and comorbidity among patients.[2]

Patient heterogeneity has been approached by consensus re-definition of AKI, grading of severity based on defined increases in pCr, reduction in glomerular filtration rate and urine output.[3,4] Population studies have shown that these new definitions predict further decline in renal function[5] and mortality.[6,7] Nevertheless, problems with reliability of creatinine in AKI, and absence of real-time measurement of glomerular filtration rate, have stimulated a search for alternative injury biomarkers.[8–11] Urinary biomarkers with an area under the receiver-operator-characteristic curve (AUC) value >0.9 that find use in prediction of the development of AKI include γ-glutamyltranspeptidase (GGT) × alkaline phosphatase (AP), kidney injury molecule-1, interleukin-18, liver fatty acid-binding-protein, and neutrophil–gelatinase-associated lipocalin.[12–16] None have been used to screen patients for treatment.

Whereas experimental treatments for AKI show promise,[17] erythropoietin is an experimentally validated renoprotective agent with an established safety profile in treating anemia.[18–21] Increased excretion of GGT and AP, two typical enzymes found in proximal tubules, implies injury to the brush border of the proximal tubules, a feature of early ischemic injury.[22–25] At the time this study was commenced these urinary enzymes were the only confirmed[12] and rapidly measurable renal injury biomarkers available.

EARLYARF was both a randomized, placebo-controlled, double-blind trial of the role of erythropoietin-β to ameliorate or prevent the development of AKI in ICU

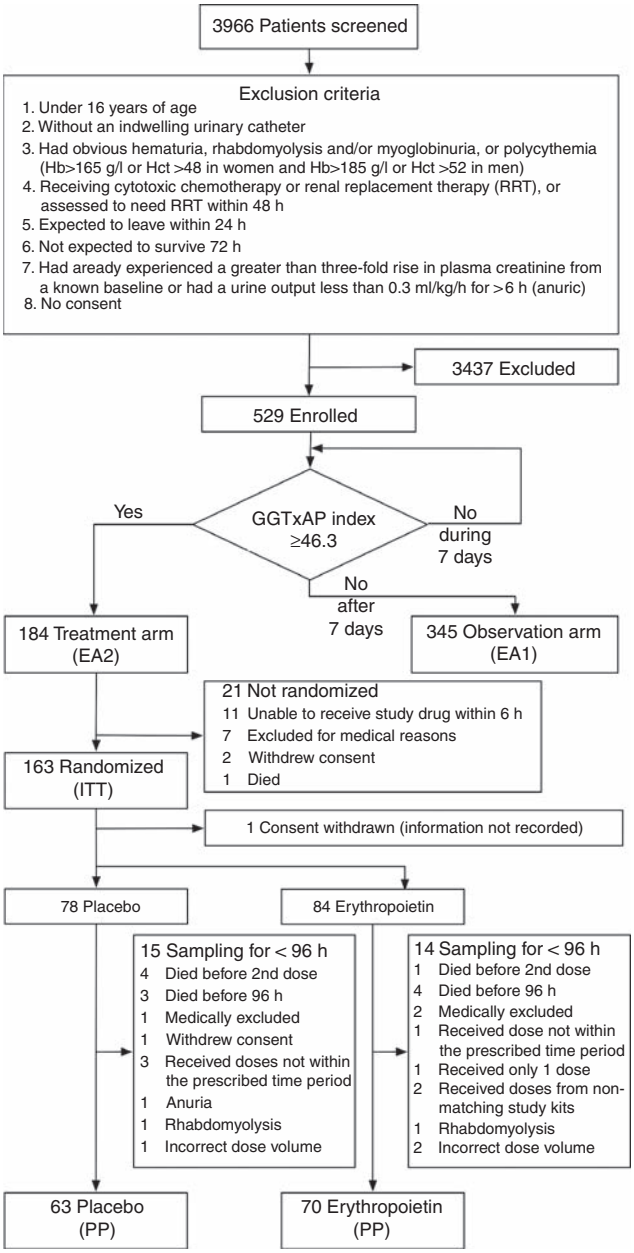

Figure 1 | Study protocol and patient flow.

patients, and a novel proof-of-concept study of the prospective use of GGT × AP to triage patients to early treatment of AKI.

RESULTS

Between 5 March 2006 and 8 July 2008, 3966 patients were screened and 499 general ICU and 30 cardiothoracic surgery patients were enrolled (Figure 1). Of 184 patients with an elevated GGT × AP index (EA2 cohort), 163 were randomized to placebo (78) or erythropoietin (84) and started treatment (note, 1 patient withdrew consent for recording data). Most patients were randomized after the first sample (103). The intervention trial cohort comprised the remaining 162 randomized patients. Patient exclusions are summarized in Figure 1. The triage trial comprised all patients.

Table 1 | Cohort characteristics on entry to ICU

	EA1 (GGT × AP ⩽46.3) (*n*=345)	EA2 (GGT × AP >46.3) (*n*=183)[b]
Age (yrs)	60 ± 18	61 ± 16
Sex (% Female)	36.8	45.4
Weight (kg)	81 ± 20	77 ± 19[d]
APACHE II score	17 ± 6	19 ± 7[e]
SOFA score	6.1 ± 2.7	6.6 ± 2.9
Time to first sample after entry to ICU (h)	3.5 ± 5.4	3.0 ± 4.3
Baseline plasma creatinine[a] (mg/dl)	0.91 (0.72–1.11)	0.79 (0.68–0.97)[f]
Baseline[a] eGFR (ml/min)	89 ± 36	96 ± 33[d]
Length of ICU stay (h)	145 ± 209	169 ± 210
%FENa <1% (*n*)	80.1 (270)	74.6 (135)
%FENa 1–2% (*n*)	13.4 (45)	10.5 (19)
%FENa >2% (*n*)	6.5 (22)	14.9 (27)[f]
Plasma creatinine (mg/dl)	1.0 (0.79–1.4)	1.0 (0.79–1.3)
Plasma cystatin-C (mg/l)	0.87 (0.65–1.24)	0.90 (0.73–1.24)
4 h Creatinine clearance (ml/min)	83 (50–128)	66 (43–104)[e]
AKI (%, AKIN)	26.7 (90)	30.1 (55)
BUN (mg/dl)	18 (12–28)	19 (13–26)
eGFR (ml/min)	73 (47–96)	73 (52–98)
Hematocrit	0.35 (0.30–0.39)	0.33 (0.29–0.38)
Hemoglobin	115 (100–131)	109 (95–126)
Lowest daily MAP	60 ± 12	60 ± 12
Highest daily MAP	96 ± 17	98 ± 19
24 h Urine output (ml)	2080 ± 1330	1860 ± 1240
GGT × AP	10 ± 11 6.1 (2.3–14)	466 ± 2390[g] 678 (21–169)[g]
Principal diagnostic group % (n)		
Abdominal aortic aneurysm rupture and repair	2.0 (7)	9.2 (17)
Abdominal surgery or inflammation	7.5 (26)	15.2 (28)
Burns	1.4 (5)	0
Cardiac arrest or failure	8.1 (28)	19.0 (35)
Cardiac surgery[c]	19.7 (68)	14.1 (26)
Collapse, cause unknown	0.9 (3)	0
Neurological surgery, injury or seizure or intracranial hemorrhage	17.7 (61)	7.1 (13)
Pulmonary or thoracic surgery or failure	13.0 (45)	10.3 (19)
Sepsis	19.1 (66)	19.0 (35)
Trauma	9.9 (34)	4.9 (9)
Other	0.6 (2)	0.5 (1)

Abbreviations: AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; AP, alkaline phosphatase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; FENa, fractional sodium excretion; GGT, γ-glutamyltranspeptidase; ICU, intensive care unit; MAP, mean arterial pressure.
Results are mean ± standard deviation or median (interquartile range).
[a]Baseline plasma creatinine and eGFR determined retrospectively as described in the text. There were no differences in the proportion of patients whose baselines was allocated by each of the various methods used to determine baselines between EA1 and EA2.
[b]EA2 patient data are pre-randomization; *n* was reduced to 162 after randomization.
[c]Includes the 30 CTS patients recruited before surgery (EA1, *n*=21; EA2, *n*=9).
Significance of EA1 versus EA2: d: $P<0.05$; e: $P<0.005$; f: $P<0.001$; g: $P<0.0001$.

The baseline characteristics are shown in Table 1 for all patients and in Table 2 for randomized patients. More patients in the placebo group ($n=31$, 40%) had AKI on randomization as compared with that in the EPO group ($n=23$, 27%), according to the Acute Kidney Injury Network (AKIN) creatinine changes criteria (not significant; Table 2). The EPO group patients were older ($P=0.011$); less likely to

Table 2 | ITT cohort characteristics

	Randomized (ITT)	
	Placebo (*n*=78)	EPO (*n*=84)
On entry to ICU		
Age (years)	58 ± 17	65 ± 14[d]
Sex (% female)	42.3	45.2
Weight (kg)	76 ± 18	78 ± 20
APACHE II Score	19 ± 7	20 ± 6
SOFA score	6.5 ± 2.9	6.7 ± 3.0
Time to first sample after entry to ICU (h)	2.2 ± 2.9	3.3 ± 5.0
Baseline plasma creatinine[a] (mg/dl)	0.79 (0.68–0.93)	0.79 (0.68–1.02)
Baseline[a] eGFR (ml/min)	96 (75–114)	91 (70–115)
Length of ICU stay (h)	84 (42–180)	86 (45–163)
%FENa <1% (*n*)	76.9 (*n*=60)	70.7 (*n*=58)
%FENa 1–2% (*n*)	7.7 (*n*=6)	12.2 (*n*=10)
%FENa >2% (*n*)	15.4 (*n*=12)	17.1 (*n*=14)
On randomization		
Plasma creatinine (mg/dl)	1.02 (0.78–1.36)	1.02 (0.70–1.33)
Plasma cystatin-C (mg/l)	0.92 (0.67–1.31)	1.02 (0.80–1.38)
4 h creatinine clearance (ml/min)	83 (50–104) (*n*=73)	65 (39–96) (*n*=74)
AKI % (*n*) (AKIN)	40 (31)	27 (23)
BUN (mg/dl)	17.2 (12.7–26)	19.9 (14.6–24.5)
eGFR (ml/min)	72 (52–99)	70 (51–104)
Hematocrit	0.33 (0.27–0.39)	0.33 (0.28–0.38)
Hemoglobin	111 (90–131)	104 (93–125)
Lowest daily MAP	66 ± 12	63 ± 13
Highest daily MAP	98 ± 20	96 ± 18
24 h urine output (ml)	2020 ± 1150	1820 ± 1120
GGT × AP	741 ± 3570 105 (66–242)	274 ± 473 82 (62–227)
Randomization time (h)[b]	3.4 ± 1.5	3.6 ± 1.5
Randomized on first sample % (*n*)	65.4 (51)	61.9 (52)
Principal diagnostic group % (n)		
Abdominal aortic aneurysm rupture and repair	10.3 (8)	9.5 (8)
Abdominal surgery or inflammation	11.5 (9)	17.9 (15)
Burns	0	0
Cardiac arrest or failure	20.5 (16)	20.2 (17)
Cardiac surgery[c]	15.4 (12)	14.3 (12)
Collapse, cause unknown	0	0
Neurological surgery, injury, or seizure or intracranial hemorrhage	12.8 (10)	3.6[d] (3)
Pulmonary or thoracic surgery or failure	10.3 (8)	7.1 (6)
Sepsis	11.5 (9)	23.8[d] (20)
Trauma	6.4 (5)	3.6 (3)
Other	1.3 (1)	0

Abbreviations: AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; AP, alkaline phosphatase; eGFR, estimated glomerular filtration rate; EPO, erythropoietin-β; GGT, γ-glutamyltranspeptidase; ICU, intensive care unit; ITT, Intention-To-Treat; MAP, mean arterial pressure.
Results are mean ± s.d., median (interquartile range), or % (*n*).
[a]Baseline plasma creatinine and eGFR determined retrospectively as described in the text. There were no differences between EA1 and EA2 in the proportion of patients whose baselines were allocated by each of the various methods used to determine baselines.
[b]After sampling for elevated GGT × AP (not necessarily the initial sample on ICU admission).
[c]Includes the 30 CTS patients recruited before surgery (placebo, *n*=4; EPO, *n*=4).
[d]Significance of EPO versus placebo: $P<0.05$.

Table 3 | Intervention trial efficacy of erythropoietin

	Placebo	EPO	*P*
(a) Continuous (non-categorical) variables			
Primary outcome			
ITT population	*(n=78)*	*(n=84)*	
RAVC	17 ± 44	23 ± 49	0.40
	9.0 (−9.4–33.6)	10.7 (−1.8–32.2)	0.36
PP population	(*n*=63)	(*n*=70)	
RAVC	12 ± 38	22 ± 46	0.20
	8.2 (−11–30)	11 (−1.9–29)	0.20
Secondary outcomes	(*n*=78)	(*n*=84)	
Length of ICU Stay (h)	84 (43–80)	86 (45–163)	0.91
Length of hospital stay (days)	27 (14–48)	22 (14–45)	0.69
(b) *Categorical variables* % (*n*)			
AKI in 7days	48.7 (38)	48.8 (41)	1.0
AKI AKIN-creatinine[a]	47.4 (37)	45.2 (38)	0.88
AKI AKIN-UO[a]	51.3 (40)	70.2 (59)	0.016
AKI AKIN-Total[b]	69.2 (54)	78.6 (66)	0.21
AKI RIFLE-creatinine[c]	19.2 (15)	23.8 (20)	0.57
Dialysis in 30 days	3.8 (3)	6.0 (5)	0.72
Death in 7 days	16.7 (13)	10.7 (9)	0.36
Death in 30 days	21.8 (17)	19.0 (16)	0.70

Abbreviations: AKI, acute kidney injury; AKIN, acute kidney injury network; EPO, erythropoietin-β; ICU, intensive care unit; PP, Per-Protocol; RAVC, Relative Average Value of Creatinine; UO, urine output.
Results are mean ± s.d., median (interquartile range), or % (*n*).
[a]AKIN-creatinine and percentage increase definitions include the first plasma creatinine measurement beyond 48 h (mean 57 ± 13 h).
[b]AKI AKIN-Total: The sum of AKI AKIN-creatinine, AKI AKIN-UO, and dialysis in 7 days. AKI in 7 days: At least a 50% rise or a 0.3-mg/dl (26.4-μmol/l) rise from baseline or dialysis within 7 days of randomization.
[c]AKI RIFLE-creatinine: At least a 50% rise from baseline sustained for at least 24 h within 7 days of randomization.

have had neurological surgery, injury or seizure or intracranial hemorrhage ($P<0.05$); and more likely to have had sepsis ($P<0.05$; Table 2).

Intervention efficacy

There was no significant difference in the primary outcome between the groups for either the Intention-To-Treat (ITT) or Per-Protocol (PP) cohort (Table 3), and no significant difference after correction for age, neurological surgery, injury or seizure or intracranial hemorrhage, and sepsis ($P=0.58$). There was no significant difference in secondary outcomes with the exception of the incidence of AKI (at or within approximately 48 h of randomization) on the basis of the urine output criteria alone (AKI AKIN-UO, 50.8 versus 70%), with lower incidence in the placebo group (Table 3). These analyses were not different for the PP cohort (data not shown). None of the creatinine-based variables showed any significant difference between the groups, and pCr relative to baseline decreased with time from randomization for both the groups (Figure 2). In the subgroup of randomized patients who did not have AKI on randomization ($n=104$), the Relative Average Value of Creatinine (RAVC) was significantly higher in the EPO group (8.5 ± 27 ($n=61$) compared with -4.6 ± 18 ($n=47$) for the placebo group, $P=0.004$) as was AKI AKIN-UO (see Appendix). There were

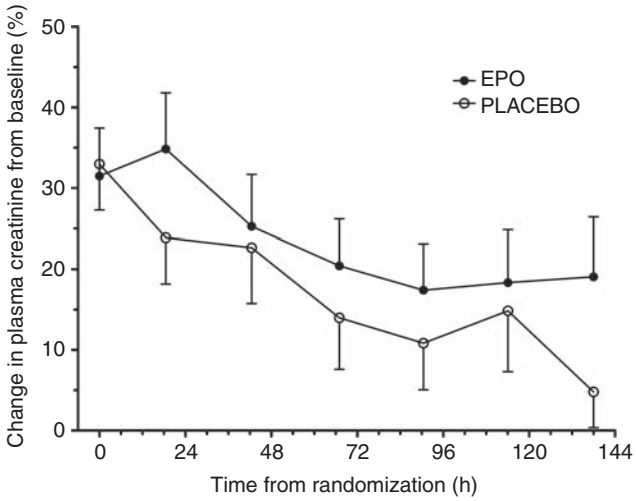

Figure 2 | Change in plasma creatinine after intervention with EPO (closed circles) or placebo (open circles) in patients triaged using an increased urinary GGT × AP index. The error bars indicate the standard errors of the mean.

no differences in the length of ICU or hospital stay, need for dialysis, or mortality.

Survival curve analysis for survival and need for dialysis showed that there was no significant difference regarding 180 days between EPO and placebo (hazard ratio for survival: 0.95 (95% confidence interval (CI) 0.52–1.7), log rank test $P = 0.85$); hazard ratio for dialysis: (0.98 (95% CI 0.53–1.8), log rank test $P = 0.94$).

There were no significant differences between the EPO and placebo groups regarding incidence of grade-3 or higher adverse events (AEs) except for a larger number of acidosis and alkalosis episodes in EPO-treated patients ($P < 0.003$) (see Appendix). As there was no time-based association and as both acidosis and alkalosis were more frequent in the EPO group, these events appear of doubtful clinical significance. There was no difference in the incidence of EPO-specific AEs in the placebo and EPO groups.

Triage efficacy

Randomization occurred 3.5 ± 1.6 h after procurement of urine sample with an increased GGT × AP index with 97.5% under the 6-h goal, Table 1. Most patients were randomized after the first sample (64%, $n = 105$). This was achieved in 6.3 ± 4.2 h after admission to ICU. An increased GGT × AP index selected patients (EA2) with higher APACHE II score, more likely to have an fractional sodium excretion > 2% and lower creatinine clearance on admission (Table 1). The EA2 cohort was more likely than EA1 to experience a negative outcome in the first week (AKI according to RIFLE, dialysis, or death; Table 4; $P = 0.0006$) with a relative risk of 1.5 (CI 1.2–1.9). Each of RIFLE, dialysis, and death were individually significant ($P < 0.05$). The EA2 cohort also had a higher RAVC from admission and longer hospital stay in the ICU.

The predictive value of GGT × AP on admission for AKI was poor and for renal replacement therapy and death it was low (Table 5). Without the potential confounding of AKI on entry, the AUCs improved with GGT × AP becoming moderately predictive for renal replacement therapy (AUC = 0.69).

Three typical situations where GGT × AP identified cases of AKI in advance of change in pCr are illustrated in Figure 3a and c, and contrasted with three cases where GGT × AP failed to diagnose AKI that subsequently developed (Figure 3b and d).

GGT × AP peaked at approximately 6 h after insult (Figure 4a) and had a median (interquartile range) duration of 12.3 (11.3–26.3) hours, which was above the threshold for all EA2 patients. Heterogeneity in time from insult to first sample reduced the triage efficacy by reducing the discriminatory ability of the first sample (see Figures 4b and c). Subdividing patients into three groups according to time from insult (< 6 h, 6 to 12 h, and > 12 h) showed that the maximum AUC for AKI was 6 to 12 h after putative insult (AUC = 0.69 (CI 0.55–0.84); Figure 4d). Similarly the predictive AUCs for dialysis and death were higher in the 6- to 12-h subgroup (Table 5).

DISCUSSION

This is the first randomized, placebo-controlled, double-blind trial of intervention in AKI with EPO. This trial allowed an assessment of the safety of high-dose EPO and provided insight into its potential benefit. As experimental studies had shown renoprotection by EPO up to 6 h after reperfusion following ischemia,[21] we aimed to randomize patients within 6 h of detection of kidney injury after entry into ICU.

There was no biologically significant renoprotection or amelioration of AKI after EPO. There was an imbalance in age and sepsis and neurological events between the EPO and placebo groups, which occurred by chance. However, correction for these made no difference to the outcome. A subgroup analysis of patients without AKI on randomization suggested that EPO may negatively affect renal function, resulting in a higher RAVC. As the study was not powered for such a subgroup analysis, this result must be viewed with caution. EPO was not associated with increase in clinically significant serious AEs. In particular, there was no evidence for increased intravascular thrombosis or sequelae thereof. This is relevant to 24 other currently registered clinical trials using EPO (International trial registry data http://www.who.int/trialsearch; accessed 16 March 2009). It supports the absence of harm reported in the pilot stroke neuroprotection study.[26] This contrasts with a significantly higher mortality in the EPO arm in a more adequately powered, completed stroke study.[27] As in the EARLYARF trial, both stroke studies used similar high doses of EPO (100 000 U over 3 days), but the lower than expected mortality in the placebo arm of the Ehrenreich study suggests this outcome must also be viewed with caution.

This is the first early intervention (also known as secondary prevention[28]) study to test the concept that a urinary biomarker can triage patients at high risk of AKI to early treatment.[28] The product of GGT and AP was selected

Table 4 | Triage trial efficacy of GGT × AP: comparison of outcomes

	EA1 (*n*=345)	EA2 (*n*=183)	*P*
ICU length of stay (h)	69 (42–162)	91 (46–190)	0.070
RAVC from admission	14 ± 38	25 ± 51	0.0094
	7.0 (−7.0 to 21.6)	10.7 (−3.4 to 34.8)	0.056
Outcomes within 7 days of entry (EA1) or raised GGT × AP (EA2), % (n)			
AKI RIFLE-creatinine	15.1 (52)	23.0 (42)	0.031
Dialysis	2.3 (8)	6.0 (11)	0.047
Death	8.1 (28)	14.2 (26)	0.034
AKI RIFLE-creatinine or dialysis or death	21.4 (74)	35.5 (65)	0.0006

Abbreviations: AKI, acute kidney injury; ICU, intensive care unit; RAVC, relative average value of creatinine.
Results are mean ± s.d., median (inter-quartile range), or % (*n*).
AKI RIFLE-creatinine: At least a 50% increase from baseline sustained for at least 24 h within 7 days of ICU entry.

Table 5 | Triage trial efficacy of GGT × AP on admission to ICU: comparison of all patients with those without AKI on admission and those 6–12 h from insult

AUC (95% CI)	All patients	Not AKI (AKIN) on admission (*n*=383)	Between 6 to 12 hours of insult and not AKI on admission (*n*=103)
AKI AKIN-creatinine	0.55 (0.50 to 0.60)	0.57 (0.50 to 0.64)	0.69 (0.55 to 0.82)
AKI RIFLE-creatinine	0.55 (0.49 to 0.62)	0.63 (0.52 to 0.75)	0.68 (0.45 to 0.90)
Dialysis	0.62 (0.48 to 0.76)	0.69 (0.53 to 0.86)	0.75 (0.52 to 0.98)
Death in 7 days	0.63 (0.55 to 0.72)	0.66 (0.55 to 0.76)	0.73 (0.48 to 0.99)
Death in 30 days	0.59 (0.52 to 0.66)	0.62 (0.54 to 0.71)	0.71 (0.50 to 0.92)

Abbreviations: AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; AUC, area under the receiver-operator-characteristic curve; CI, confidence interval; ICU, intensive care unit.
AKI AKIN-creatinine: At least a 50% or 0.3 mg/dl increase from baseline sustained by the 1st sample following 48 hours ICU entry.
AKI RIFLE-creatinine: At least a 50% increase from baseline sustained for at least 24 h within 7 days of ICU entry.

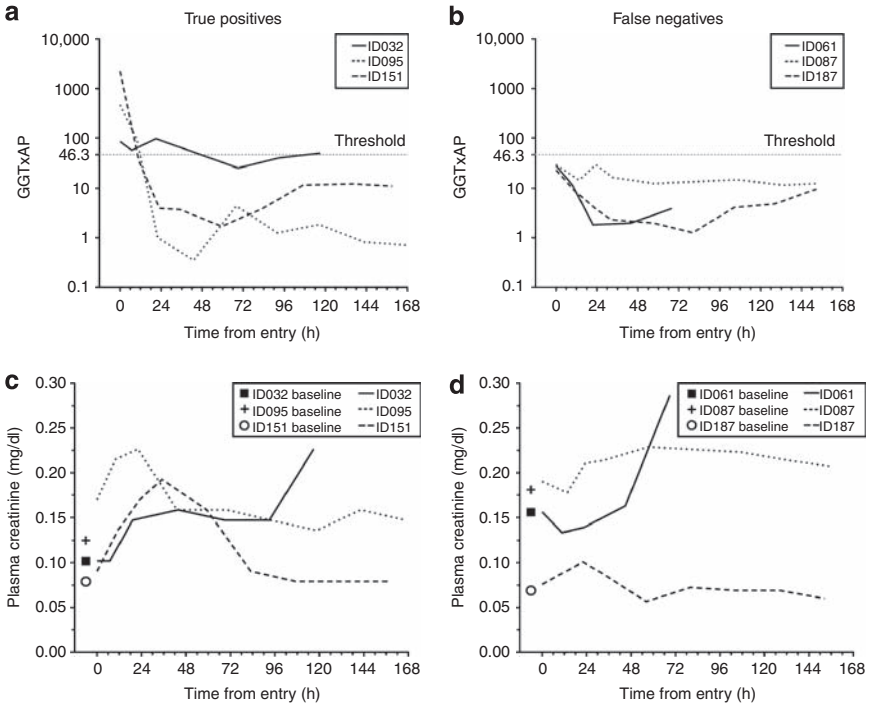

Figure 3 | Case studies. Examples of true positives (**a**, **c**) and false negatives (**b**), and (**d**) for AKI (AKIN definition) defined by triaging with GGT × AP. Key: True Positives: (i) ID032: 85-year-old female with respiratory failure, received placebo, AKI by 24 h, died on day 6; (ii) ID095: 67-year-old female with cardiac arrest, received EPO, AKI by 12 h, survived; (iii) ID151: 71-year-old male, ruptured abdominal aortic aneurysm, received EPO, AKI by 12 h, survived. False negatives: (i) ID061: 72-year-old female with respiratory failure, AKI by day 3, died on day 3; (ii) ID087; 72-year-old female with sepsis, AKI by day 2, survived; (iii) ID187: 24-year-old female following neurosurgery, AKI by 24 h, survived.

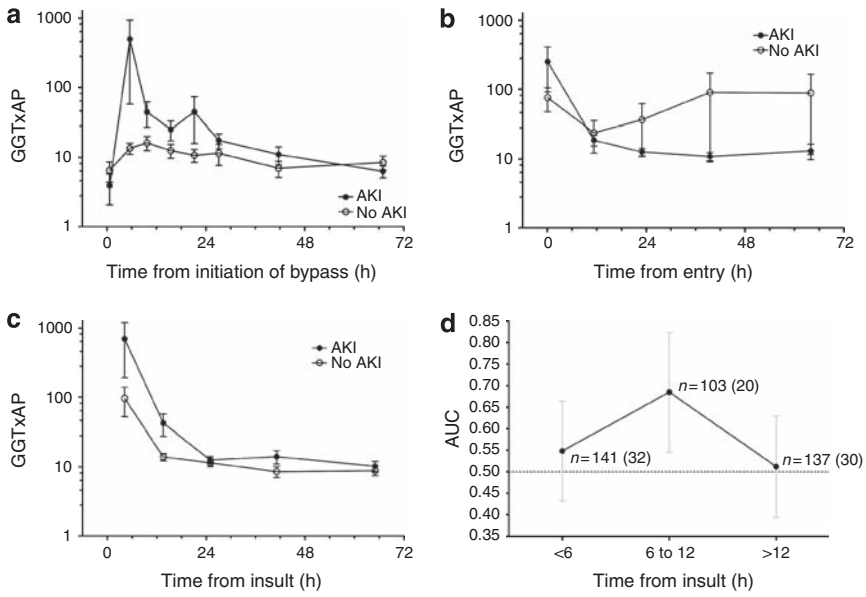

Figure 4 | Urinary GGT × AP index (mean ± s.e.m.) in patients who did not receive EPO and who either developed (closed circles) or did not develop AKI (AKIN definition, open circles). Time courses are shown for (**a**) cardiopulmonary bypass (CPB) patients ($n = 93$) timed from the start of CPB (on-pump; time 0 measurements taken pre-operatively); (**b**) all patients, including cardiothoracic surgery patients, timed from enrolment at ICU entry; and (**c**) as for panel **b**, but timed from a *post hoc* estimate of time of renal insult. Panel **d** shows the predictive area under the curve (AUC ± 95% CI) of the GGT × AP ROC curve for developing AKI for three groups of patients grouped according to time from insult who were not AKI on entry. n = number in each group (number of AKI).

as it had been shown to be highly predictive of AKI (AUC = 0.94 (CI: 0.73–0.99)) in a small pilot study and confirmed as sensitive (100%) and specific (91%) in an additional cohort at a cut-off of 46.3.[12] GGT and AP were the only rapidly measurable renal injury biomarkers available at the time this project was commenced. The cut-off of 46.3 was selected to include all possible cases of AKI and therefore expected to include some false positives. The widespread availability of GGT and AP assays also suggested easy clinical translational benefit should utility be confirmed.

A GGT × AP value greater 46.3 selected a high-risk group with more severe illness and at greater risk of AKI, dialysis, or death, and with an elevated level of renal injury biomarker out of a general ICU population. Almost all patients were randomized within 6 h. However, although moderately predictive of dialysis, the overall utility of GGT × AP as a triaging tool was limited. On admission to ICU, the incidence of AKI (AKIN definition) in the observation arm and the intervention arms was similar (26.7 versus 30.1%, $P = 0.42$; Table 1). Whereas subsequent incidence in the observation arm was lower (18.6 versus 27.3%, $P = 0.06$), the high overall incidence of AKI in the observation arm represents a high false negative rate for prediction of AKI by GGT × AP. This suggests that the time course of GGT × AP elevation was brief enough to lower the GGT × AP below the threshold before ICU entry, or that the threshold was inappropriately high. Both individual cases (Figure 3c and d) and re-plotting the GGT × AP profiles against the time from insult instead of the time after ICU admission (Figure 4b and c) support the suggestion that the brief time course was responsible for the high false negative rate. Given the short detection window, the delay produced by screening only on entry to ICU (rather than earlier) appears to have been responsible for the high false negative rate.

Biomarker performance in more heterogeneous (ie, less selected) groups, with variable severity and variable sampling relative to injury time, may be a reason why follow-up biomarkers studies often report lower predictive values than highly discriminatory initial reports.[8] Nevertheless, the predictive value of GGT × AP for AKI in patients without AKI on admission was low. This was not an artifact of definitions of baseline or AKI different from those in the pilot study as the AUC for AKI using definitions identical to those in the pilot study was still poor (0.65 (CI 0.55–0.75)). If patients were grouped according to the time following renal insult, then Figure 4d (and Table 5) shows that the predictive value of GGT × AP in patients without AKI on admission increases up to 12 h after which it becomes non-predictive. These changes mimic the profile of GGT × AP changes after cardiopulmonary bypass surgery (CPB) (Figure 4) and highlight the likely 'window of opportunity' during which this particular biomarker could be used for triaging. Other potential biomarkers will also have a window, which may explain some of the variations in the prognostic performance of other biomarkers at a single time point.[28]

The timing of screening at ICU admission, and imperfect triaging into the intervention arm, limits the conclusions that can be drawn regarding the efficacy of early intervention with EPO. The intervention trial became, in effect, an EPO trial of critically ill patients, with a typical overall incidence of AKI of

33%. Although the time to randomization of those randomized after the first sample, 6.3 ± 4.2 h, was brief as compared with that in other intervention trials (e.g. references Allgren *et al.*[29] and Hirschberg *et al.*[30]), but at a median of 12.9 (9.6–26.8) hours following renal insult in the majority of patients, it was still more than 6 h, the upper limit suggested in experimental AKI.[21]

Thus the potential therapeutic renoprotective benefit of EPO is not excluded by this study. As a snapshot of a realistic general ICU population, this study highlights the need to understand the individual time course of each injury biomarker and supports simultaneous use of multiple biomarkers with different profiles to identify the stage of injury.[8,31,32]

An increased GGT × AP index on admission to ICU was modestly predictive of renal replacement therapy and slightly less predictive of death within 7 or 30 days. Apart from association with AKI, a reason for this association with increased early mortality is unknown. An association of plasma GGT with cardiovascular and all-cause mortality in chronic kidney disease was recently attributed to oxidative stress.[33]

Since the inception of this trial other biomarkers have shown promise as potential triaging tools and even point-of-care testing is being developed (e.g., for plasma neutrophil—gelatinase-associated lipocalin and urinary kidney injury molecule-1[34]). Retrospective testing has shown that urinary neutrophil–gelatinase-associated lipocalin, kidney injury molecule-1, interleukin-18, and liver fatty acid-binding-protein are highly predictive of AKI after cardiac surgery.[14,15,35,36] Urinary neutrophil–gelatinase-associated lipocalin and interleukin-18 have also been shown to be predictive of AKI in the emergency department and the ICU, respectively.[13,37]

The study uncovered other limitations relevant to future biomarker-based trials. As diagnosis of reversible pre-renal AKI is retrospective,[37] such patients could not be excluded by early triaging on the basis of a biomarker of injury alone. A low fractional sodium excretion in the majority of randomized patients suggests that renal sodium reabsorption was preserved despite biomarker evidence of injury. While this highlights our lack of understanding of what pre-renal AKI actually means (see reference Bellomo *et al.*[38]), if such patients were volume-responsive, this would reduce the power of the study with respect to EPO efficacy.

Second, the appropriate endpoint in AKI intervention trials is controversial.[39–42] Surrogate endpoints are appropriate for early intervention studies. Categorical variables, such as AKIN or RIFLE stages, depend on change in pCr and are therefore secondary to changes in filtration function, and not directly related to structural injury. Nevertheless, they have been associated with increased mortality.[5,7,43,44] We relied on change in pCr as a surrogate endpoint, but used a continuous variable, RAVC, integrated over a long period of time (4–7 days), rather than a categorical variable measured at a single time point. In a simulated trial RAVC has been shown to more accurately reflect the difference between treated and placebo groups than categorical metrics, including RIFLE and AKIN.[45]

Third, both categorical and continuous variable definitions of AKI require a reliable estimate of baseline pCr. We prefer to determine baseline creatinine from measured rather than estimated variables using prior or *post hoc* creatinine measurements with defined rules. This is because using a formula based on age, gender, and race to estimate a baseline creatinine overestimates the prevalence of AKI and does not take into account an individual's renal function.[45,46] The alternative of using the first creatinine measure in the ICU does not distinguish between a patient with a high basal creatinine and one with already established AKI. In taking the lowest of the on-entry ($n = 98$), final ICU, or follow-up creatinine ($n = 142$) we attempted to identify the individual's normal creatinine ($n = 20$). The distribution of creatinine levels across each of these methods was identical. This approach is, of course, only appropriate for a *post hoc* analysis. Ideally for an early intervention trial, patients with AKI on entry should be triaged out. Where a pre-ICU baseline creatinine is readily available this is possible; however, when not available, it is not possible to use a retrospective strategy. One possible solution is to triage out patients with creatinine above a pre-determined level (say 1.5 mg/dl) and use the first creatinine measure as the baseline. An analysis of our primary outcome on the basis of a baseline of the on-entry creatinine level showed no significant difference between EPO and placebo ($P = 0.07$) treatment. The optimal strategy for defining the baseline and endpoint requires assessment in large multicenter cohorts.

The progressive decline in pCr in randomized patients may reflect the current emphasis on volume hydration in ICU patients,[47] and raises the possibility that the majority of patients had a degree of AKI, making evaluation of baseline creatinine values more difficult.

Finally, biomarker studies have not shown conclusively that indexing to urinary creatinine to account for dilution always improves diagnostic accuracy. Other solutions, such as calculating the biomarker excretion rate by multiplying urinary concentration by urine flow rate, need to be explored.

This is the first AKI trial to show the feasibility of rapid triaging to intervention with a urinary biomarker. Using a single composite biomarker with a brief post-injury profile was insufficient for risk stratification in a population with a heterogeneous onset of injury. A panel of biomarkers with different time profiles is desirable. The data suggest that brief exposure to high-dose EPO is safe in this population, but a larger trial is required to assess efficacy in AKI.

MATERIALS AND METHODS

Study population

Consecutive patients admitted to the general ICU of either Christchurch or Dunedin Hospital, or high-risk patients scheduled for cardiothoracic surgery with CPB, were screened for inclusion (see Figure 1 for exclusion criteria). Cardiothoracic surgery patients were consented electively if they met the criteria for increased risk of AKI:[48] pCr > 1.7 mg/dl or estimated glomerular filtration rate

25–50 ml/min, and scheduled to undergo CPB for valvular heart disease or coronary artery bypass grafting plus at least one of the following: extra-cardiac vascular disease, diabetes mellitus, ejection fraction <25%, use of a preoperative intra-arterial balloon pump, emergency surgery, or other surgery.

The study was approved by the multiregional ethics committee of New Zealand (MEC/050020029) and registered under the Australian Clinical Trials Registry (ACTRN012606000058572; http://www.actr.org.au). Screening on entry to ICU was by presumptive consent, followed by written consent from the patient or family.

Study design

The study comprised an intervention trial and a triage trial running concurrently. The intervention trial comprised only patients triaged to intervention (EA2) who were subsequently randomized to treatment (Figure 1). Patients not triaged to intervention comprised the observation arm (EA1). The triage trial was to compare all EA2 patients (EPO and placebo) with those in EA1 if there were no significant differences between treatments; if there were differences only placebo patients in EA2 would be used.

Intervention trial. The intervention trial was a prospective, randomized, double-blind, placebo-controlled, parallel-group, early intervention trial of intravenous EPO to ameliorate the development of AKI. Eligible patients were allocated to EPO or placebo (normal saline) groups in a 1:1 ratio, using a predefined computer-generated randomization sequence with permuted blocks stratified on center. Concealment was by pharmacist; pairs of identical 5-ml syringes containing EPO (50 000 U in 3 ml) or normal saline were prepared and stored. Patients received an intravenous dose of 500 U/kg to a maximum of 50 000 U, within 6 h of the time of collection the sample with increased GGT × AP and a second dose 24 h later. Weight was known or estimated from height. Patients, all medical staff, and investigators were masked to treatment.

The prospectively defined primary outcome was the RAVC, defined as the average pCr (pC) increase from baseline ($pC-pC_b$) as a percentage of baseline creatinine (pC_b).[45] That is

$$RAVC(\%) = 100\times\left(1/t\int_0^t (pC - pC_b)\mathrm{d}t\right)/pC_b$$

RAVC was calculated using the trapezoidal rule to estimate the incremental area above baseline from the first value in the ICU (time 0) to the last measure before death or on day 7 (time t).

Triage trial. Patients were triaged to EA2 if GGT × AP was >46.3 units (indexed to urinary creatinine; dimensionless); the threshold was determined from pilot study data to minimize false negatives (sensitivity was 100% in the pilot study).[12] GGT × AP was determined within 1 h of entry to ICU, at 12 h, 24 h, and daily to 7 days. It was hypothesized that early triaging by increased GGT × AP would allow randomization of patients at risk of a decrease in renal function to occur within 6 h. The outcome measures were the mean time to randomization following collection of urine sample with increased GGT × AP and identification of patients most at risk of AKI, dialysis, or death.

Patient monitoring

Spot urine samples for GGT, AP, and creatinine were taken within 1 h of entry to ICU, at 12 h, 24 h, and daily to 7 days for all patients. Blood for creatinine and cystatin-C, and commencement of 4-h creatinine clearances were at the same time points. Blood and urine sodium was measured at 0 and 12 h. EA2 patient sampling continued for 7 days after increase in GGT × AP. Sampling continued in the wards for patients transferred from the ICU. EA2 patients were followed for serious AEs for 30 days. Urine output was recorded every 6 h while patients remained catheterized. Renal function (creatinine) and mortality were monitored in EA2 patients at 30 and 90 days.

The Data Safety Monitoring Board (DSMB) required AEs with severity scores >3 to be coded for severity, relatedness, onset, resolution time, and body system using the Common Terminology Criteria for Adverse Events (CTCAE) coding system (http://ctep.cancer.gov/forms/CTCAEv3.pdf). Physiological parameters including mean arterial pressure, hemoglobin, and potential EPO-related AEs (including hypertension, cardiac failure, thromboembolism, myocardial infarction, stroke, seizures, allergic response), were monitored for 30 days after randomization. Unmasking followed recording of the final AEs of the patient last enrolled.

Assays

Urine GGT, AP and creatinine, and pCr were assayed immediately. Creatinine concentrations were determined by the Jaffe Reaction, and GGT and AP concentrations, respectively, by γ-glutamyl-*p*-nitroanilide rate and *p*-nitrophenol rate reactions (IFCC method), with Abbott agents using an Architect ci8200 analyzer (Christchurch) or, with Roche reagents, on a Modular P Analyzer (Dunedin). There was no significant difference between the means or standard deviations of the reference samples between the laboratories.

Statistical methods

Intervention trial. The primary outcome and safety endpoints were evaluated in the ITT population. ITT patients were those with an increased GGT × AP index who had been randomized. As all randomized patients had received at least one dose of the study medication; the ITT and safety populations were identical. PP patients received both doses of study medication, needed at least 96 h for RAVC calculations, and thus, follow-up of at least four post-randomization pCr samples. Secondary outcomes compared efficacy using the ITT population.

A baseline creatinine was determined before unmasking using (in descending order of preference): (1) the most recent pre-ICU value between 30 and 365 days ($n=106$), or for elective cardiac surgery patients, the pre-surgery value ($n=33$); (2) a stable pre-ICU value >365 days for patients aged <40 years, (stable defined as within 15% of the lowest ICU measurement) ($n=7$); (3) pre-ICU >365 days and less than the initial creatinine on entry to ICU ($n=65$); (4) a pre-ICU value (between 3 and 39 days) less than or equal to the initial on-entry creatinine to ICU and not obviously in AKI ($n=57$); (5) the lowest of initial on-entry creatinine to ICU ($n=98$), the last ICU value ($n=148$), or the minimum value at follow-up to 365 days ($n=20$) total $n=528$).

With 65 patients per treatment group, the study was prospectively powered to detect a difference between RAVC means of 5.0% using unpaired t-test, with 80% power, assuming an s.d. of 0.11 mg/dl (10 μmol/l), an average baseline pCr of 1.13 mg/dl (100 μmol/l), and a two-tailed $\alpha=0.05$. Recruitment continued until there were 130 PP patients. Secondary outcomes, defined before unmasking, included RIFLE (≥50% increase in pCr within 7 days and sustained for 24 h) and AKIN (≥0.3 mg/dl or 50% increase in pCr within 48 h) categorical definitions of AKI, plasma cystatin-C, urine output, need for dialysis, death within 7, 30 or 90 days.

Triage trial. Triage efficacy was assessed by the mean time to randomization after increased GGT × AP as compared with the 6-h goal and by the ability of GGT × AP to predict outcomes related to renal function by AUC. These outcomes included categorical definitions of AKI (as above), need for dialysis, and death within 7 or 30 days. AUCs were also calculated for the subgroup that did not have AKI (AKIN definition) on admission. Additional analysis included comparison of the length of hospital stay and RAVC between EA1 and EA2. Time from insult was defined as the time period from the estimated time of a possible insult until the time of the first urine samples. The time of the possible insult was determined prior to unmasking by chart review (including ambulance records) where it was assumed all patients had a kidney insult and that the likely causes were cardiac arrest, time of onset of CPB, time of onset of anesthetic in other surgeries, time of documented hypotensive event, time of accident, and time of onset of illness symptoms.

Categorical outcomes were analyzed using χ^2 or Fisher's exact test and continuous variables were analyzed using independent *t*-tests (normally distributed) or Mann–Whitney *U*-tests (not normally distributed). The intervention trial was not powered for survival analysis of dialysis or survival. Nevertheless, we performed Kaplan–Meier analysis to 180 days and calculated the hazard ratios for survival and need for dialysis. Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, IL, USA), Matlab 2009a (MathWorks, Natick, MA, USA), and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). A two-tailed *P*-value <0.05 was taken to indicate statistical significance.

DISCLOSURE

ZHE received non-directed research funding from Roche Pharmaceuticals. All the other authors declared no competing interests.

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Appendix

Additional secondary outcomes of the intervention trial are presented in Table A1. For the subgroup of patients without AKI on randomization ($n = 108$), Table A2 shows the primary and main secondary outcomes. Table A3 shows the incidence of grade-3 or higher AEs in the placebo and EPO groups.

Table A1 | Intervention trial efficacy of erythropoietin: additional secondary outcomes

	Placebo ($n = 78$)	EPO ($n = 84$)	*P*
(a) Continuous (non-categorical) variables			
Peak plasma creatinine (mg/dl)	1.13 (0.79–1.56)	1.14 (0.89–1.61)	0.64
Time to peak plasma creatinine (hours)	44 (22–100)	39 (23–97)	0.90
Absolute increase from baseline to peak plasma creatinine (mg/dl)	0.30 (0.11–0.56)	0.27 (0.11–0.74)	0.50
Relative increase from baseline to peak plasma creatinine (%)	36 (14–71)	40 (17–82)	0.42
Plasma creatinine at 6 or 7 days (mg/dl) [n]	1.0 (0.89–1.22) [69]	1.0 (0.82–13) [76]	0.65
Plasma creatinine at 30 days (mg/dl) [n]	0.85 (0.70–1.11) [57]	0.90 (0.69–1.10) [60]	0.69
Plasma creatinine at 90 days (mg/dl) [n]	0.95 (0.79–1.10) [47]	0.85 (0.74–1.07) [56]	0.41
Length of ICU Stay (h)	84 (43–80)	86 (45–163)	0.91
Length of hospital stay (days)	27 (14–48)	22 (14–45)	0.69
Time to dialysis (h) (*n*)	53 ± 23 [3]	25 ± 17 [5]	0.088
RAVC maximum value in 7 days	18 (2.7–49)	18 (7–59)	0.44
Cystatin-C			
RAVC cystatin-C	5.0 (-8.7–18)	3.6 (-6.5–24)	0.61
RAVC maximum value in 7days	5.8 (−4.3 to 21)	9.5 (−0.6 to 24)	0.35
Peak cystatin-C (mg/l)	1.1 (0.89–1.6)	1.3 (0.97–1.7)	0.04
Relative increase to peak cystatin-C (%)	33 (13–47)	28 (13–50)	0.93
Time to peak cystatin-C (h)	108 (37–143)	93 (28–141)	0.61
(b) Categorical variables % (n)			
Increase of plasma creatinine $\geqslant 0.3$ mg/dl[a]	47.4 (37)	41.7 (35)	0.53
Increase of plasma creatinine 25%[a]	56.4 (44)	56.0 (47)	1.0
Increase of plasma creatinine 50%[a]	35.9 (28)	38.1 (32)	0.87
Increase of plasma creatinine 100%[a]	14.1 (11)	16.7 (14)	0.67
AKI AKIN-creatinine[a]	47.4 (37)	45.2 (38)	0.88
AKIN stage I[a]	33.3 (26)	28.6 (24)	
AKIN stage II[a]	9.0 (7)	9.5 (8)	
AKIN stage III[a]	5.1 (4)	7.1 (6)	
AKI AKIN-UO[a]	51.3 (40)	70.2 (59)	0.016
AKIN I UO[a]	9.0 (7)	9.5 (8)	
AKIN II UO[a]	32.1 (25)	50.0 (42)	
AKIN III UO[a]	10.3 (8)	10.7 (9)	
AKI AKIN-Total[b]	69.2 (54)	78.6 (66)	0.21
AKI RIFLE-creatinine[c]	19.2 (15)	23.8 (20)	0.57
RIFLE R	6.4 (5)	13.1 (11)	
RIFLE I	7.7 (6)	6.0 (5)	
RIFLE F	5.1 (4)	4.8 (4)	
Death in 90 days	24.4 (19)	23.8 (20)	1.0
Death or Dialysis in 30 days	23.1 (18)	22.6 (19)	1.0

Abbreviations: AKI, acute kidney injury; AKIN, acute kidney injury network; EPO, erythropoietin-β; ICU, intensive care unit; RAVC, Relative Average Value of Creatinine; UO, urine output.
Results are mean ± s.d., median (interquartile range), or % (*n*). Numbers are shown in square brackets where these differ from the initial cohort.
[a]AKIN-creatinine and percentage increase definitions include the first plasma creatinine measurement beyond 48 h (mean 57 ± 13 h).
[b]AKI AKIN-Total: The sum of AKI AKIN-creatinine, AKI AKIN-UO, and dialysis in 7 days.
[c]AKI RIFLE-creatinine: At least a 50% rise from baseline sustained for at least 24 h within 7 days of randomization.

Table A2 | Intervention trial efficacy of erythropoietin for the subgroup not AKI on randomization

	Placebo	EPO	P
(a) Continuous (non-categorical) variables			
Primary outcome			
ITT population	*(n=47)*	*(n=61)*	
RAVC	−4.6 ± 18	8.5 ± 27	0.004
	−3.4 (−17 to 9.3)	4.9 (−4.6 to 17)	0.008
Secondary outcomes			
Length of ICU Stay (h)	88 (42–181)	84 (46–132)	0.81
Length of hospital stay (days)	17 (8–39)	17 (12–39)	0.94
(b) Categorical variables % (n)			
AKI in 7days	14.9 (7)	29.5 (18)	0.11
AKI AKIN-creatinine[a]	12.8 (6)	24.6 (15)	0.15
AKI AKIN-UO[a]	44.7 (21)	68.9 (42)	0.018
AKI AKIN-Total[b]	48.9 (23)	70.5 (43)	0.029
AKI RIFLE-creatinine[c]	0.0 (0)	13.1 (8)	0.0093
Dialysis in 30 days	4.3 (2)	1.6 (1)	1.0
Death in 7 days	17.0 (8)	11.5 (7)	0.42
Death in 30 days	21.3 (10)	23.0 (14)	1.0

Abbreviations: AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; EPO, erythropoietin-β; ICU, intensive care unit; ITT, Intention-To-Treat; RAVC, relative average value of creatinine; UO, urine output.
Results are mean ± s.d., median (interquartile range), or % (*n*).
[a]AKIN-creatinine and percentage increase definitions include the first plasma creatinine measurement beyond 48 h (mean 57 ± 13 h).
[b]AKI AKIN-Total: The sum of AKI AKIN-creatinine, AKI AKIN-UO, and dialysis in 7 days. AKI in 7 days: At least a 50% rise or 0.3-mg/dl (26.4-μmol/l) rise from baseline or dialysis within 7 days of randomization.
[c]AKI RIFLE-creatinine: At least a 50% rise from baseline sustained for at least 24 h within 7 days of randomization.

Table A3 | Intervention trial 30 day safety analysis: serious adverse events (SAEs) according to Body System (ITT, *n*=162)

	Placebo					EPO				
			CTCAE grade					CTCAE grade		
SAEs body system	SAE (*n*)	Patients (*n*)	3	4	5	SAE (*n*)	Patients (*n*)	3	4	5
3. Blood/bone marrow	13	12	13			23	18	21	2	
4. Cardiac arrhythmias	12	12	7	5		5	5	4		1
5. Cardiac general	10	8	6	2	2	9	9	4	3	2
6. Coagulation						1	1	1		
8. Death (other)	7	7			7	9	9			9
9. Dermatology/skin						3	3	2	1	
11. Gastrointestinal	4	4	1	3		9	8	6	3	
13. Hemorrhage/bleeding	1	1	1							
15. Infection	13	12	12		1	26	21	23	3	
17. Metabolic/laboratory	11	8	11			32[a]	25	27	5	
18. Musculoskeletal	1	1	1							
19. Neurology	19	17	12	3	4	13	12	11	2	
21. Pain	3	3	3			1	1	1		
22. Pulmonary/upper respiratory	12	11	5	4	3	16	14	7	6	3
23. Renal/genitourinary	3	3	1	2		2	2	1	1	
27. Syndromes	1	1	1							
28. Vascular	5	5	4	1		5	5	3	1	1
Total	115	105	78	20	17	154	133	111	27	16
No SAEs		21					23			
EPO specific SAEs										
DVT	5	5	4	1		2	2	2		
SVT	3	3	2	1						
Pulmonary embolism	1	1			1	1	1		1	
Thromboembolism						1	1	1		
Ischemic						2	2	1		1
Hypertension	2	2	2			2	2	2		
Stroke	3	3	2	1		1	1		1	
Seizure	4	4	1	2	1	1	1	1		
Cardiac failure						1	1			1
Myocardial infarction	3	3	1	2						
Allergic response										
VF arrest	3	3		3						
Atrial fibrillation	6	6	5	1		3	3	3		
Total	30	30	17	11	2	14	14	10	2	2

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; EPO, erythropoietin-β.
[a]*P*=0.003. Although more frequent in the EPO group, metabolic disturbances were similar in both the groups except for acidosis and alkalosis: all shown as respective placebo and EPO pairings: acidosis: 0, 5; alkalosis: 1, 6; abnormal liver function tests: 0, 1; elevated alkaline phosphatase: 1, 0; elevated ALT: 0, 1; elevated AST: 1, 2; hyperglycemia: 0, 2; hyperkalemia: 2, 1; hypernatremia: 0, 2; hypoalbuminemia-intermittent: 0, 1; hypokalemia: 3, 5; hyponatremia: 2, 2; hypophosphatemia: 1, 4.