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ORIGINAL ARTICLE

Perioperative administration of high-dose recombinant human erythropoietin for delayed graft function prevention in kidney transplantation: a meta-analysis

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Keywords
delayed graft function, erythropoietin, kidney transplantation, meta-analysis.

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Summary

Delayed graft function (DGF) due to ischemia–reperfusion injury is a major early complication of kidney transplantation (KT). Recombinant human erythropoietin (rHuEPO) has been shown to exert nephroprotective action in animal models. We conducted a meta-analysis to explore the impact of rHuEPO on DGF in KT. Eligible studies comparing perioperative high-dose rHuEPO with placebo or no therapy for prevention of DGF were identified through MEDLINE, CENTRAL, and Transplant Library. Their design and data were assessed by two independent reviewers. Among 737 examined studies, four randomized controlled trials, involving 356 recipients of kidney allografts from deceased donors, fulfilled inclusion criteria. Statistical heterogeneity across studies was not significant ($P = 0.98$, $I^2 = 0\%$). In a random effects model, no significant difference was found in the occurrence of DGF (odds ratio: 0,74, 95% CI: 0.47–1.18, $P = 0.21$). At 4 weeks after KT, the rHuEPO group exhibited higher systolic blood pressure (mean difference: 6.47 mmHg, 95% CI: 1.25–11.68, $P = 0.02$). Perioperative, high-dose rHuEPO administration does not prevent DGF in deceased donor KT. Furthermore, it is associated with higher systolic blood pressure leading to safety concerns. Nonerythropoietic rHuEPO derivatives, designed for nephroprotective action without increasing cardiovascular risk, might prove an alternative but still are at early stages of development.

Introduction

Kidney transplantation (KT) is the treatment of choice for end-stage renal disease (ESRD) due to its beneficial effect on patient survival and quality of life [1,2]. The source of allografts is either living or deceased donors with the latter being further subdivided into those after brain or cardiac death. This classification is fundamental for prognostic reasons; deceased donor KT exhibits inferior outcomes compared with living donor KT, mainly because it is complicated by prolonged ischemia times. Subsequent reperfusion of an already ischemic graft might injure tubular epithelial cells leading to necrosis and apoptosis that manifest clinically as delayed graft function (DGF). This is generally defined as the need for dialysis within the first postoperative week and is probably the main early complication of deceased donor KT. DGF incidence in KT of grafts from brain or cardiac death deceased donors reaches 21,1% and 46,7%, respectively [3]. In addition, DGF has been correlated with 41% increased risk of graft loss and 38% higher acute rejection risk [4].

Methods for the effective prevention and treatment of ischemia–reperfusion injury (IRI) and resultant DGF include the (as much as possible) reduction in warm and/or cold ischemia time (CIT), the use of sophisticated mechanical devices or novel solutions for graft preservation during transportation and possibly the use of pharmacological compounds, such as recombinant human erythropoietin (rHuEPO). Endogenous erythropoietin is primarily

composed of fibroblast-like cells in the renal cortex as a response to reduced tissue oxygen supply. Through binding to $EPOR_2$ receptor on red blood cell precursors, erythropoietin inhibits apoptosis, thus increasing red blood cell survival and total mass [5].

The development and introduction of rHuEPO in clinical practice has revolutionized the management of chronic kidney disease-induced anemia [6]. Beyond its well-established effect on red blood cells, rHuEPO also possesses numerous, 'pleiotropic' properties, among which stand out cyto- and organ protection. Indeed, animal studies have shown that rHuEPO exerts nephroprotective action in IRI models when administered right before ischemia induction or shortly after reperfusion [7,8]. This occurs due to its anti-apoptotic, anti-inflammatory, and regenerative properties [9]. Moreover, clinical evidence suggests that rHuEPO might also exert beneficial effects on cardiac and nervous tissues [10,11]. All these cyto-protective actions are mediated by rHuEPO binding to a complex heteromeric receptor comprised of both $EPOR_2$ and β-common-receptor ($EPOR_2$-βCR_2) [12]. Intriguingly, higher rHuEPO concentration (2–20 nmol/l) seems to be needed to activate $EPOR_2$-βCR_2 compared to classic $EPOR_2$ (1–10 pmol/l). This implies that considerably increased rHuEPO dose is probably required to induce cyto- and organ protection rather than to stimulate erythropoiesis.

In the last two decades, DGF incidence has been steadily on the rise reflecting the increased utilization of grafts from advanced age donors with multiple comorbidities. According to USRDS (United States Renal Data System) registry data, overall DGF incidence peaked at 24% between 1998 and 2004, whereas it stood at 14,7% between 1985 and 1992 [13,14]. It is, therefore, obvious that interventions aiming to prevent and treat DGF are urgently needed. In an effort to shed light to the potential role of rHuEPO in this regard, we systematically reviewed the literature and conducted a meta-analysis of randomized controlled trials (RCTs) evaluating early, high-dose rHuEPO administration for DGF prevention.

Patients and methods

We searched through the electronic databases MEDLINE (US National Library of Medicine, National Institutes of Health), CENTRAL (Cochrane Central Register for Controlled Trials), and Transplant Library (Centre for Evidence in Transplantation) for eligible RCTs that were published till December 1, 2013. Two different search themes combined with the Boolean operator AND were used. The first theme involved terms relative to erythropoietin (i.e., erythropoietin, epoetin, erythropoiesis-stimulating agents), and the second theme involved terms relative to KT (i.e., renal transplantation, kidney transplantation). Furthermore, reference lists of the retrieved RCTs were scrutinized for additional citations.

Two of the authors (GV and TIK) independently assessed abstracts of initially detected studies to identify eligible RCTs. In the next stage, the full text of selected RCTs was thoroughly reviewed. The inclusion criteria were satisfied if RCTs had compared: high-dose rHuEPO administration with placebo or no drug for DGF prevention, in the perioperative period of KT, namely from hours before until 2 weeks after KT. The use of rHuEPO for anemia management was considered as an exclusion criterion (irrespective of the presence of graft dysfunction or not). Any disagreement between the two authors was resolved through discussion under supervision of a senior researcher (AA). Quality of included RCTs as well as potential for bias was assessed using Cochrane's Collaboration risk of bias tool, as described by Higgins *et al.* [15]. Corresponding level of evidence was analyzed and presented in a summary of findings table, according to the GRADE system, also by Cochrane Collaboration [16].

Occurrence of DGF, defined as the need for dialysis within the first week after KT (except for treatment of hyperkalemia), was set as primary outcome. Secondary outcomes were divided into clinical outcome (all-cause mortality, biopsy-proven acute rejection, all-cause graft loss, graft loss due to thrombosis, and total thrombotic episodes) and surrogate outcome [estimated glomerular filtration rate (eGFR) and systolic and diastolic blood pressure (SBP and DBP)]. For categorical outcomes, odds ratio (OR) with 95% confidence intervals (CI) was estimated using the random effects model. For continuous outcomes, mean difference (MD) with 95% CI was calculated, also with the use of the random effects model. Statistical heterogeneity was assessed by Cochran's Q-test and I^2 index. For sensitivity analyses, we removed each study separately calculating every time OR or MD for related outcomes and examined whether this resulted in any significant change. Statistical analyses were performed with Review Manager, version 5.1 (RevMan, computer program, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011).

Results

Of a total of 737 studies that were initially detected (Fig. 1), 724 were rejected after abstract review because they did not refer to RCTs. The full text of thirteen articles [17–29], which reported RCTs, was assessed for eligibility. Eight studies [17–24] were excluded because they were performed in different clinical contexts and did not use relevant endpoints. The study by Yasari *et al.* [25]. did examine the impact of rHuEPO administration on DGF but each single rHuEPO dose was substantially lower than that used in the

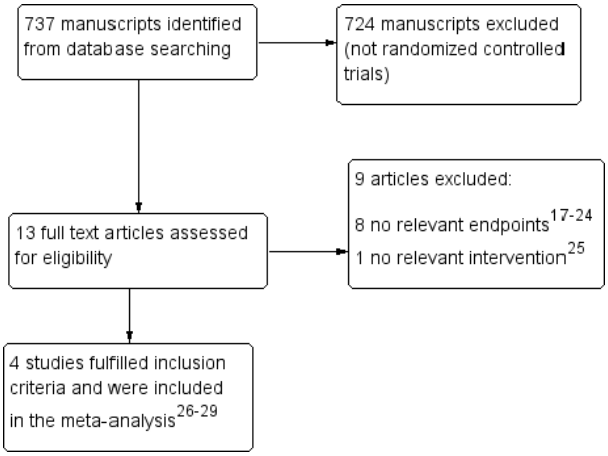

Figure 1 Flow diagram of systematic literature search.

studies which were finally eligible (2000 IU compared with 30 000–40 000 IU). It was considered that including this study in the meta-analysis would introduce significant clinical heterogeneity and, therefore, it was excluded. The remaining four studies [26–29] fulfilled inclusion criteria and were added in the meta-analysis.

From the risk of bias summary graph (Fig. 2), it is concluded that overall quality of the included studies ranges from low to moderate. Risk of bias exists for several different categories of bias. As an illustration, the randomization method is not reported in two studies [27,28]. Moreover, in all four studies [26–29], the method of allocation concealment is unclear. In particular, for the study by Martinez *et al.* [26], there is a high risk of bias because it was an open-label study and there was no use of placebo in the control group.

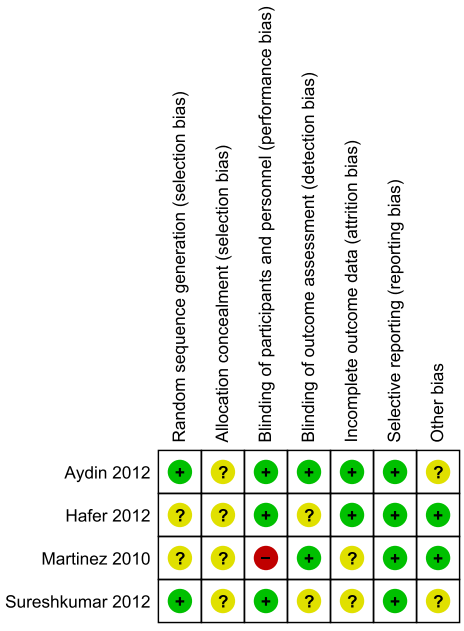

Figure 2 Risk of bias summary graph. Symbol explanation: (+): low risk of bias, (?): unclear risk of bias, (−): high risk of bias.

The characteristics of the four included studies are presented in Table 1. Of a total of 356 patients, 176 received treatment with rHuEPO and 180 served as controls. In all four studies, the source of renal allografts was deceased donors. In the study by Aydin *et al.* [28], allografts came exclusively from deceased donors after cardiac death, so the risk of DGF was higher by definition, because of the longer warm ischemia time and the accompanying more severe IRI. Data about donor renal function were provided by (i) Martinez *et al.* [26], in the study of whom, donor MDRD eGFR at organ recovery was 91.6 ± 39.5 ml/min and 92.3 ± 36.0 ml/min ($P = 0.93$) in rHuEPO and control groups, respectively (according to the DGF-USRDS [30] prognostic score, the initial risk of DGF was over 60% in both study groups), (ii) Aydin *et al.* [28], who reported that donor serum creatinine at organ recovery was 0.86 ± 0.58 mg/dl and 0.93 ± 0.57 mg/dl ($P = 0.55$), respectively, and c) Sureshkumar *et al.* [29], in the study of whom, donor serum creatinine at organ recovery was 1.14 ± 0.85 mg/dl and 1.18 ± 0.90 mg/dl ($P = 0.84$), respectively. In the same study, the proportion of extended criteria donors rose to 30.6% and 61.2%, in rHuEPO and control groups, respectively ($P = 0.21$).

Donor age and CIT presented wide variability across studies. In the study by Sureshkumar *et al.* [29], donor age was 39.0 ± 17.0 and 41.0 ± 17.0 years in rHuEPO and control groups, respectively ($P = 0.63$), whereas in the study by Martinez *et al.* [26], donor age was 65.3 ± 9.4 and 65.1 ± 8.4 years, respectively ($P = 0.75$). CIT varied across a range of 12.5 ± 0.6 and 13.4 ± 0.8 h in rHuEPO and control groups, respectively, ($P = 0.1876$) in the study by Hafer *et al.* [27], to 24.1 ± 6.1 and 26.3 ± 8.0 h, respectively, ($P = 0.21$) in the study by Sureshkumar *et al.* [29].

Details of rHuEPO treatment and immunosuppressive regimens are demonstrated in Table 2. Epoetin α was used in two [27,29] of the studies and epoetin β in the remaining two [26,28]. Total rHuEPO dosage ranged from 40 000 to 120 000 IU, and each single rHuEPO dose ranged from 30 000 to 40 000 IU among studies. Sureshkumar *et al.* [29] administered a sole rHuEPO dose, whereas in the other three studies, a total of three or four rHuEPO doses were given. Timeline of rHuEPO administration varied considerably: First dose was given as early as 3 h prior to surgery and last dose as late as 14 days postoperatively. In three studies [26–28], induction immunosuppression included an interleukin-2 receptor inhibitor (daclizumab or basiliximab) and maintenance immunosuppression was

Table 1. Characteristics of the studies included in the meta-analysis. Data are presented as percentages or mean ± standard deviation (in the study by Hafer *et al.* [27], mean ± standard error of the mean).

Study	Martinez *et al.* [26]		Hafer *et al.* [27]		Aydin *et al.* [28]		Sureshkumar *et al.* [29]	
Design	Randomized Open-label Multicenter		Randomized Double blind Single-center		Randomized Double blind Single-center		Randomized Double blind Single-center	
Follow-up time (months)	3		12		12		1	
Study groups	rHuEPO	Controls	rHuEPO	Controls	rHuEPO	Controls	rHuEPO	Controls
Patients	51	53	44	44	45	47	36	36
Age (years)	60.0 ± 7.7	58.9 ± 9.5	53.6 ± 1.8	49.8 ± 1.6	51.0 ± 14.0	56.0 ± 12.0	58.0 ± 11.0	56.0 ± 13.0
Gender (% males)	66.7%	56.6%	56.8%	59.1%	71.0%	70.0%	56.0%	53.0%
BMI (kg/m^2)	25.1 ± 4.6	23.8 ± 4.1	25.3 ± 0.6	25.9 ± 0.6	n/a	n/a	27.8 ± 5.4	28.3 ± 6.4
Cold ischemia time (hours)	18.8 ± 4.9	19.9 ± 6.9	12.5 ± 0.6	13.4 ± 0.8	17.0 ± 4.0	17.0 ± 4.0	24.1 ± 6.1	26.3 ± 8.0
Donor type	Deceased donors, DGF risk>60%		Deceased donors		Deceased donors after cardiac death		Deceased donors, ECD allografts:	
							30.6%	61.2%
Donor age (years)	65.3 ± 9.4	65.1 ± 8.4	n/a	n/a	45.0 ± 13.0	49.0 ± 17.0	39.0 ± 17.0	41.0 ± 17.0
Donor renal function	91.6 ± 39.5 (eGFR, ml/min)	92.3 ± 36.0 (eGFR, ml/min)	n/a	n/a	0.86 ± 0.58 (sCr, mg/dl)	0.93 ± 0.57 (sCr, mg/dl)	1.14 ± 0.85 (sCr, mg/dl)	1.18 ± 0.90 (sCr, mg/dl)

BMI, body mass index; DGF, delayed graft function; ECD, extended criteria donors; eGFR, estimated glomerular filtration rate; rHuEPO, recombinant human erythropoietin; n/a, not available; sCr, serum creatinine.

Table 2. Characteristics of rHuEPO administration and immunosuppression regimens.

Study	Martinez *et al.* [26]	Hafer *et al.* [27]	Aydin *et al.* [28]	Sureshkumar *et al.* [29]
rHuEPO type	Epoetin β	Epoetin α	Epoetin β	Epoetin α
Placebo use in controls	No	Yes (normal saline)	Yes (normal saline)	Yes (normal saline)
Total rHuEPO dosage	120 000 IU	120 000 IU	≈100 000 IU	40 000 IU
Single rHuEPO dose	30 000 IU	40 000 IU	33 000 IU	40 000 IU
No. of doses	4	3	3	1
rHuEPO administration timeline	0.5–3 h before KT 12–24 h after KT 7 days after KT 14 days after KT	At reperfusion 7 days after KT 14 days after KT	3 h before KT 24 h after KT 48 h after KT	At reperfusion
Induction immunosuppression	Basiliximab	Basiliximab	Daclizumab	Basiliximab or alemtuzumab or ATG
Maintenance immunosuppression	Tacrolimus, MMF, prednisone	Tacrolimus or cyclosporine, MMF, prednisone	Cyclosporine from day 4 after KT, MMF, steroids	Tacrolimus or cyclosporine, MMF, early steroids withdrawal

ATG, antithymocyte globulin; rHuEPO, recombinant human erythropoietin; KT, kidney transplantation; MMF, mycophenolate mofetil.

a triple regimen consisting of a calcineurin inhibitor (cyclosporine or tacrolimus), mycophenolate mofetil, and corticosteroids. In the study by Sureshkumar *et al.* [29], induction agent was either basiliximab or alemtuzumab or antithymocyte globulin and maintenance regimen consisted of a calcineurin inhibitor (cyclosporine or tacrolimus) and mycophenolate mofetil together with early corticosteroid withdrawal. Data on calcineurin inhibitor's level monitoring were provided by Hafer *et al.* [27] and Aydin *et al.* [28]. No statistically significant difference in cyclosporine or tacrolimus levels was found between rHuEPO and control groups at assessed time-points.

Among various outcomes examined in each of the four included studies, DGF was consistently assessed in all of them. The classical definition of DGF as the need for dialysis within the first week after KT was generally used, either totally unaltered [26,29] or with minor additions. These slightly expanded definitions of DGF were noted in the two

studies by (i) Hafer *et al.* [27] who defined DGF as urine output of less than 500 ml in the first 24 h after KT and/or need for dialysis because of graft dysfunction within the first week after KT and (ii) Aydin *et al.* [28] who defined DGF functionally if the serum creatinine level increased, remained unchanged, or decreased by less than 10% per day during three consecutive days for more than 1 week and classically by the need for dialysis in the first week after KT.

All four studies reported data regarding predefined clinical outcome measures, and related forest plots are depicted in Fig. 3. There was no statistically significant difference in the occurrence of DGF between patients treated with rHuEPO and controls (OR: 0.74, 95% CI: 0.47–1.18, $P = 0.21$). Statistical heterogeneity across studies was not significant ($P = 0.98$, $I^2 = 0\%$). In the study by Aydin *et al.* [28], DGF rates were higher than those in the other three studies (36/45 = 80% and 39/47 = 83% in rHuEPO and control groups, respectively). This noteworthy finding can be attributed to the aforementioned fact that in the study by Aydin *et al.* [28], the origin of allografts was exclusively deceased donors after cardiac death. Excluding this study and performing the meta-analysis with the data from the rest three studies does not alter the result (OR: 0.73, 95% CI: 0.44–1.22, $P = 0.23$). There was also no statistically significant difference between patients treated with rHuEPO and controls in all-cause mortality (OR: 1.00, 95% CI: 0.25–4.05, $P = 1.00$), biopsy-proven acute rejection (OR: 0.81, 95% CI: 0.44–1.49, $P = 0.49$), graft loss (OR: 1.03, 95% CI: 0.43–2.47, $P = 0.94$), graft loss due to thrombosis (OR: 1.05, 95% CI: 0.30–3.64, $P = 0.94$), and thrombotic episodes (OR: 1.56, 95% CI: 0.58–4.19, $P = 0.38$). Statistical heterogeneity was likewise minimal across studies for all of these outcomes.

At 4 weeks after KT, MDRD eGFR was recorded in two studies [26,29] (forest plots, Fig. 4). We did not find a statistically significant difference between rHuEPO group and controls (MD: −0.62 ml/min, 95% CI: −5.41 to 4.17, $P = 0.80$). Hafer *et al.* [27] and Aydin *et al.* [28] extended follow-up time until 12 months; the former used CKD-EPI eGFR to estimate renal function and the latter used the endogenous creatinine clearance. As a result, their findings are not comparable. It is worth mentioning, however, that in the study by Aydin *et al.* [28], renal function at 12 months was significantly better in rHuEPO group (endogenous creatinine clearance: 68 ± 23 ml/min in rHuEPO-treated patients versus 57 ± 25 ml/min in controls, $P = 0.04$).

Blood pressure was assessed at different prespecified time-points in each study. Only in the study by Martinez *et al.* [26] and Sureshkumar *et al.* [29], SBP was reported at 4 weeks after KT and it was significantly higher in rHuEPO group (MD: 6.47 mmHg, 95% CI: 1.25 to 11.68, $P = 0.02$, Fig. 4). No significant difference was found for DBP (MD: −0.35 mmHg, 95% CI: −3.48 to 2.78, $P = 0.83$, Fig. 4). Hafer *et al.* [27] did not examine the impact of rHuEPO on blood pressure, and Aydin *et al.* [28] did not detect a statistically significant difference in SBP or DBP at 6 weeks, 6 months, and 12 months after KT.

Sureshkumar *et al.* [29] examined the presence of novel biomarkers in urine to correlate them with the occurrence of DGF. Interleukin-18 and NGAL (neutrophil gelatinase-associated lipocalin) levels did not differ significantly between rHuEPO-treated patients and controls.

The study by Hafer *et al.* [27] was the only one in which protocol biopsies were performed (at 6 weeks and 6 months after KT). No difference was found in acute tubular necrosis, biopsy-proven acute rejection, and interstitial fibrosis between the two groups.

Data about the impact of rHuEPO on hemoglobin levels are contradictory: In two [28,29] of the studies, the groups did not differ longitudinally, whereas in the other two [26,27], hemoglobin levels were higher in rHuEPO-treated patients at 4 weeks after KT.

The summary of findings table according to the GRADE system [16] is displayed in Fig. 5. From the corresponding absolute risk differences, the numbers needed to treat (NNT) can easily be calculated. All sensitivity analyses, which were performed by removing each study separately, did not change significantly OR or MD for any of the clinical or surrogate outcomes, respectively.

Discussion

This meta-analysis comprises the first methodologically rigorous evaluation of the pooled results of four RCTs in 356 recipients of KT from deceased donors that compared high-dose rHuEPO administration versus placebo or no drug in the immediate perioperative period of KT. In a random effects model, this intervention had no significant impact on DGF. Although there was a trend in all studies in favor of rHuEPO, it failed to gain statistical significance in any of them and the same occurred when pooling the results to perform the meta-analysis. Furthermore, high-dose rHuEPO did not affect mortality, acute rejection, graft loss from all causes or from thrombosis, total thrombotic episodes, and renal function at 4 weeks after KT. Nevertheless, SBP was significantly higher in rHuEPO-treated patients at the same time-point.

Even though favorable results from animal experiments created expectations about rHuEPO nephroprotective potential, RCTs failed to confirm it. This mismatch can be partially explained by the fact that animal kidneys are healthy before IRI is instituted, do not suffer from concomitant immunosuppression, and do not undergo

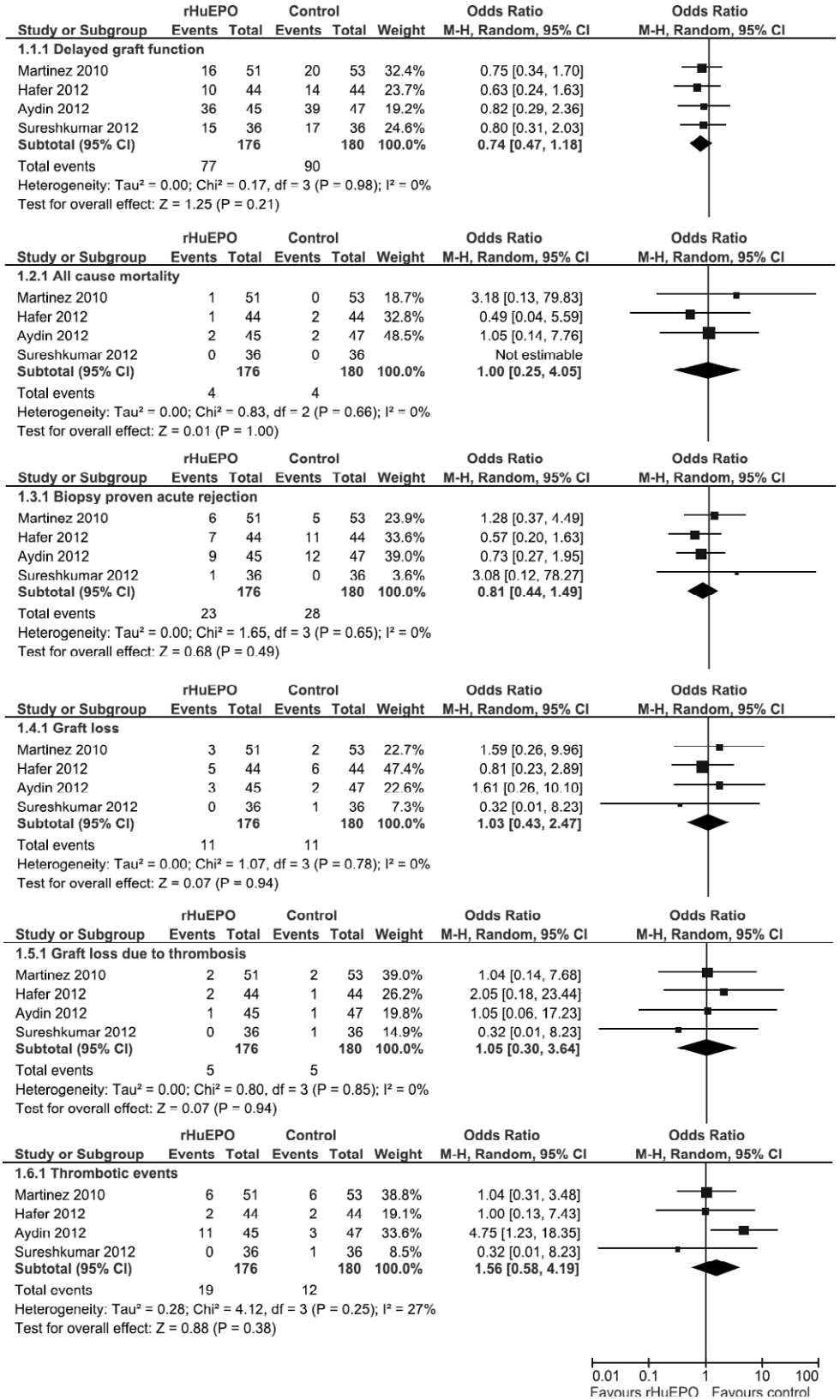

Figure 3 Forest plots of the effects of recombinant human erythropoietin (rHuEPO) on clinical outcomes in kidney transplant recipients.

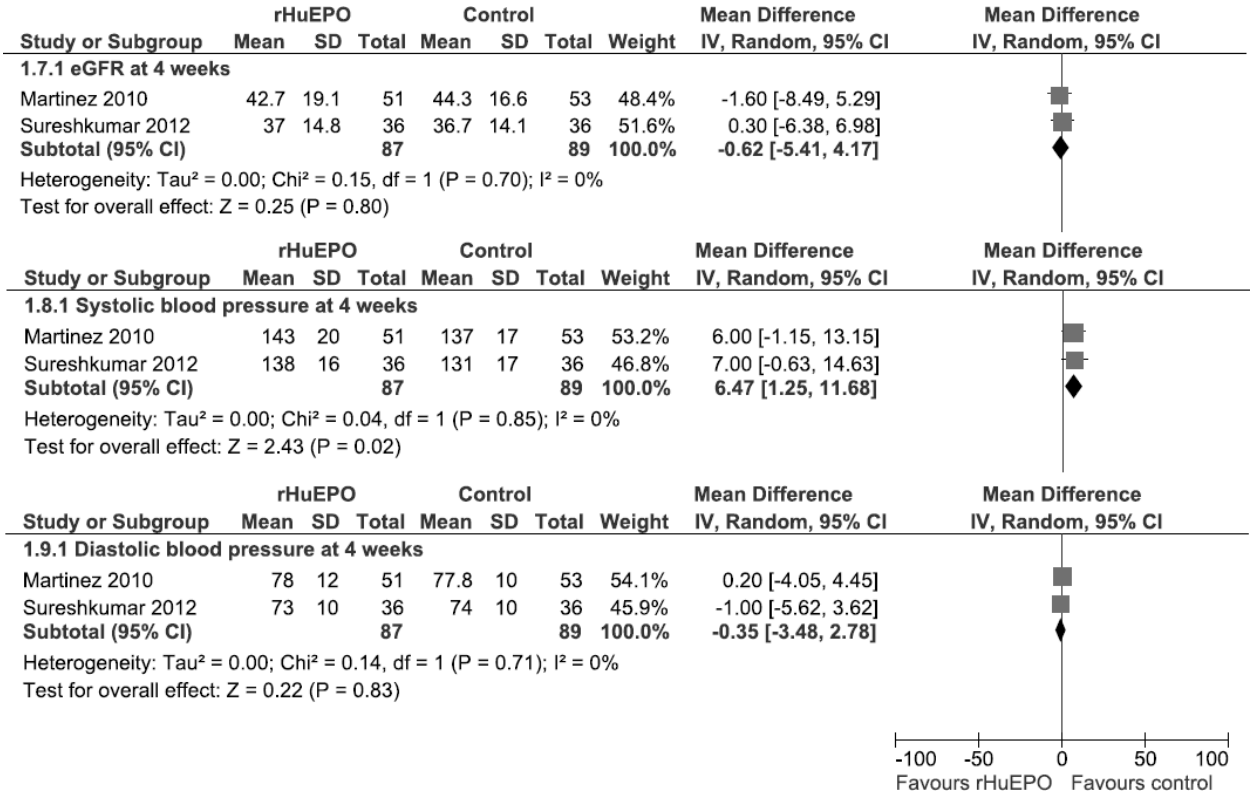

Figure 4 Forest plots of the effects of recombinant human erythropoietin (rHuEPO) on surrogate outcomes in kidney transplant recipients.

cold ischemia. The opposite happens in human studies, where patients with ESRD present with multiple comorbidities, must receive heavy immunosuppression (especially the first trimester after KT) and have prolonged CIT. Two more factors are equally important to interpret the negative results of the meta-analysis.

Timing of rHuEPO dosing is the first one, which varied considerably among included studies. None of them administered rHuEPO to the recipient earlier than 3 h before KT. Preclinical data suggest that rHuEPO nephroprotective action is exerted when the drug is given at least 30 minutes prior to ischemia until 6 h afterward. Considering that graft ischemia commences at the time of graft recovery, the obvious question arises whether pretreatment with rHuEPO would be more beneficial if administered as soon as possible. This means that rHuEPO could be even given to the donor at the interval between diagnosis of death and graft recovery or during graft transport under machine perfusion. Naturally resulting ethical and logistical dilemmas are evident in each case, and it is unlikely that this hypothesis will be tested in a RCT. As far as it concerns the prolongation of rHuEPO administration, there are no data that rHuEPO confers additional nephroprotection if IRI is extended beyond 48 h. However, two RCTs [26,27] included in the meta-analysis continued rHuEPO administration until day 14 after KT, which was futile as witnessed by the results.

The second critical factor to review in order to delineate our findings is actual rHuEPO dosing. Most animal studies used single doses over 1000 IU/kg body weight. In contrast, doses of 30 000 to 40 000 IU were used in RCTs; if we assume that the average patient body weight is approximately 70 kg, it results that each single rHuEPO dose in human studies was about 500 IU/kg body weight. This amount of rHuEPO is clearly lower compared with preclinical studies and likely inadequate to effectively activate the $EPOR_2$-βCR_2 [12] receptor. Despite the fact that the increase in rHuEPO dosing seems to be the logical next step, the risk of inducing adverse effects cannot be easily overlooked. A rHuEPO dose of 40 000 IU would be considered high enough in routine nephrology practice and would certainly alert the physician toward the emergence of hypertension and thromboembolic events.

Albeit we found that SBP is significantly higher at 4 weeks after KT, the true meaning of this finding is unknown. First of all, we need to take into account that in one [29] of only two studies analyzed for this outcome, SBP was marginally higher ($P = 0.05$) in rHuEPO-treated

High dose rHuEPO for prevention of delayed graft function in kidney transplant recipients.

Outcomes	No of Participants (studies) Follow up	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk with Control	Risk difference with High dose rHuEPO (95% CI)
Delayed graft function	356 (4 studies) 1 to 12 months	⊕⊕⊖⊖ **LOW**[2,3] due to risk of bias, imprecision	**OR 0.74** (0.47 to 1.18)	**Study population**[1]	
				500 per 1000	**75 fewer per 1000** (from 180 fewer to 41 more)
				Moderate[1]	
				425 per 1000	**71 fewer per 1000** (from 167 fewer to 41 more)
All cause mortality	356 (4 studies) 1 to 12 months	⊕⊕⊖⊖ **LOW**[2,3] due to risk of bias, imprecision	**OR 1** (0.25 to 4.05)	**Study population**[1]	
				22 per 1000	**0 fewer per 1000** (from 17 fewer to 62 more)
				Moderate[1]	
				21 per 1000	**0 fewer per 1000** (from 16 fewer to 59 more)
Biopsy proven acute rejection	356 (4 studies) 1 to 12 months	⊕⊕⊖⊖ **LOW**[2,3] due to risk of bias, imprecision	**OR 0.81** (0.44 to 1.49)	**Study population**[1]	
				156 per 1000	**26 fewer per 1000** (from 81 fewer to 60 more)
				Moderate[1]	
				172 per 1000	**28 fewer per 1000** (from 88 fewer to 64 more)
Graft loss	356 (4 studies) 1 to 12 months	⊕⊕⊖⊖ **LOW**[2,3] due to risk of bias, imprecision	**OR 1.03** (0.43 to 2.47)	**Study population**[1]	
				61 per 1000	**2 more per 1000** (from 34 fewer to 77 more)
				Moderate[1]	
				40 per 1000	**1 more per 1000** (from 22 fewer to 53 more)
Graft loss due to thrombosis	356 (4 studies) 1 to 12 months	⊕⊕⊖⊖ **LOW**[2,3] due to risk of bias, imprecision	**OR 1.05** (0.3 to 3.64)	**Study population**[1]	
				28 per 1000	**1 more per 1000** (from 19 fewer to 66 more)
				Moderate[1]	
				25 per 1000	**1 more per 1000** (from 17 fewer to 60 more)
Thrombotic events	356 (4 studies) 1 to 12 months	⊕⊕⊖⊖ **LOW**[2,3] due to risk of bias, imprecision	**OR 1.56** (0.58 to 4.19)	**Study population**[1]	
				67 per 1000	**34 more per 1000** (from 27 fewer to 164 more)
				Moderate[1]	
				55 per 1000	**28 more per 1000** (from 22 fewer to 141 more)
Estimated glomerular filtration rate at 4 weeks (ml/min, MDRD equation)	176 (2 studies) 1 to 3 months	⊕⊕⊖⊖ **LOW**[2,5] due to risk of bias, imprecision		The mean estimated glomerular filtration rate at 4 weeks ranged across control groups from **36.7 to 44.3 ml/min**[4]	The mean estimated glomerular filtration rate at 4 weeks in the intervention groups was **0.62 lower** (5.41 lower to 4.17 higher)
Systolic blood pressure at 4 weeks (mm Hg)	176 (2 studies) 1 to 3 months	⊕⊕⊖⊖ **LOW**[2,5] due to risk of bias, imprecision		The mean systolic blood pressure at 4 weeks ranged across control groups from **131.0 to 137.0 mm Hg**[4]	The mean systolic blood pressure at 4 weeks in the intervention groups was **6.47 higher** (1.25 to 11.68 higher)
Diastolic blood pressure at 4 weeks (mm Hg)	176 (2 studies) 1 to 3 months	⊕⊕⊖⊖ **LOW**[2,5] due to risk of bias, imprecision		The mean diastolic blood pressure at 4 weeks ranged across control groups from **74.0 to 77.8 mm Hg**[4]	The mean diastolic blood pressure at 4 weeks in the intervention groups was **0.35 lower** (3.48 lower to 2.78 higher)

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] The assumed risk is the median control group risk across studies
[2] Inclusion of open-label trial
[3] Total number of events is less than 300
[4] The assumed score is provided by the range of the final values in the control groups
[5] Total population size is less than 400

Figure 5 Summary of findings table according to the GRADE system [16]. Abbreviations: rHuEPO: recombinant human erythropoietin.

patients already from baseline. Moreover, this particular study included 46% African Americans (in whom increased prevalence of essential hypertension is observed) and, therefore, its characteristics and results may not be generalizable to different populations. From our meta-analysis, it results that higher SBP was not directly accompanied by significantly more cardiovascular events in rHuEPO-treated patients, but this discrepancy can be attributed to insufficient follow-up times and small sample sizes. The study by Aydin *et al.* [28]. exacerbates uncertainty because it is the only one in which thrombotic events were more in rHuEPO group (11 vs. 3, $P = 0.02$); however, it failed to drive the pooled OR for thrombotic events toward a statistically significant result (Fig. 3). The question remains unanswered over the exact magnitude of rHuEPO toxicity in this context.

The previously mentioned finding of Aydin *et al.* [28] that renal function at 12 months was significantly better in rHuEPO group (when there was no difference between controls at 6 weeks, 3 months, and 6 months) deserves further evaluation. Is it likely that early administration of rHuEPO may inhibit development of graft fibrosis although it fails to restore renal function right after KT? Chronic fibrosis risk remains a substantial threat, even if initial graft function is fully recovered after ischemic insults to the graft [31]. In animal models of progressive interstitial fibrosis, it has been shown that erythropoiesis-stimulating agents can delay renal function decline [32,33]. A novel report, demonstrating that rHuEPO also possesses immunosuppressive properties, offers an alternative mechanism which could in part explain observed protective effects in KT [34]. Aydin *et al.* [28] speculated that, although high-dose rHuEPO is used to treat recent ischemic injury, its benefit extends far beyond the moment of administration. This is especially important for subsets of grafts at higher risk of dysfunction (i.e., those from deceased donors after cardiac death, such as in the study by Aydin *et al.* [28]). The hypothesis requires confirmation in future trials, because this particular study was not designed to address that matter.

The small number of included patients and studies may limit the robustness of the meta-analysis. On the other hand, summary results are pretty consistent with individual study results. Although nonsignificant, point estimates for the primary outcome of DGF are all on the same direction in favor of rHuEPO; this calls for a properly powered study with an adequate number of subjects included that could offer a definitive answer to the research question. Methodological rigor will be another prerequisite of this decisive, future study because we identified lack of reporting of randomization methods, allocation concealment, and lack of blinding in the studies included in the meta-analysis. Oversights like these reduce the study quality and undermine the provided level of evidence. Minor differences in patient and treatment characteristics inevitably lead to clinical heterogeneity of some extent. However, statistical heterogeneity proves to be quite low with I^2 index ranging from 0% (for most of the outcome analyses) to 27% and certainly enhances the strength of our conclusions.

Future research will likely focus on safety issues of erythropoiesis-stimulating agents. Thrombosis due to high-dose rHuEPO is caused by platelets activation and, mainly, by sudden increases in hematocrit. As it is already stated, the latter mechanism is mediated through $EPOR_2$ receptor, whereas cytoprotection is mediated through $EPOR_2$-βCR_2 receptor. Isolated activation of $EPOR_2$-βCR_2 would, theoretically, convey cytoprotection without inducing adverse effects. Exactly for this reason, new modified forms of rHuEPO have been developed, such as CEPO (carbamoylated EPO) and ARA290, which selectively bind to $EPOR_2$-βCR_2 receptor and have no erythropoietic actions. In preliminary IRI animal studies, CEPO and ARA290 attenuated acute tubular necrosis without hematologic and cardiovascular adverse effects [35–37].

According to our meta-analysis, nephroprotection—in the sense of preventing DGF—does not appear to be afforded by high-dose rHuEPO administration in the perioperative period of KT, and this strategy definitely cannot be recommended for routine clinical practice. It is correlated with higher SBP, and the resulting exact cardiovascular risk is unclear. Novel nonerythropoietic rHuEPO molecules, which were developed to lack cardiovascular adverse effects, have been promisingly tested in preclinical animal models of renal IRI. However, additional clinical studies in humans are certainly needed to fully explore their potential and to establish efficacy and safety definitively.

Authorship

GV and TIK: designed the study, collected, and analyzed data, and each wrote part of the article. AA: contributed to data analysis and manuscript edit.

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