

Analyses of age, gender and other risk factors of erythropoietin resistance in pediatric and adult dialysis cohorts

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Abstract Previous studies often report lower responses to erythropoietin (EPO) therapy in pediatric patients on chronic dialysis than those of adults. Because of the greater capacity for hematopoiesis in the younger population, these studies may be confounded by poorly identified variables. Thus, we made parallel studies of pediatric and adult cohorts to explore the relationship between age, gender and other risk factors with EPO resistance. Thirty pediatric subjects (aged 8–20 years) and 66 adult subjects (aged 22–85 years) on chronic hemodialysis and EPO were enrolled. After stratification by 50th percentile of EPO response, the best predictive model was identified by backward elimination of the risk factors with the least contribution to the regression. Relationship between age, gender and EPO resistance was examined by analysis of covariance (ANCOVA). The most predictive model of EPO response for the pediatric cohort had, as the major variables, urea clearance \times dialysis duration/total body water (Kt/V), urea reduction ratio (URR), intact parathyroid hormone (iPTH), blood loss, normalized protein catabolic rates (nPCR) and

indices of malnutrition and inflammation, whereas adults had iron and folate deficiencies as the dominant variables. Although EPO resistance was more common in female subjects than in male subjects, relationship with neither age nor gender was significant. Furthermore, the prescription of a larger (initiating) EPO dose by pediatric physicians compared with adult nephrologists confounded the interaction between age and EPO resistance. In summary EPO resistance in the pediatric dialysis cohort was predicted by nutritional deficits, inflammation, poor dialysis, and hyperparathyroidism, while iron and folate deficits were the major determinants in adults. Although confounded by the pattern of EPO prescription, neither age nor gender was predictive of EPO resistance in the two study groups.

Keywords Age · Gender · EPO resistance · Anemia · Hemodialysis · Regression analysis

Introduction

The routine use of erythropoietin (EPO) in anemia control has reduced morbidity and mortality rates in patients with chronic kidney disease (CKD) [1–3]. Risk factors for EPO resistance include dosing modality, malnutrition, inflammation, hyperparathyroidism, and dialysis adequacy [4–9]. The non-modifiable predictors are age, gender, and race [4, 8, 10–12]. Recent study suggests an association of EPO resistance with higher mortality rates in dialysis subjects [13].

Compared with that in adults, EPO resistance is more commonly reported in retrospective data analysis of children on hemodialysis (HD) [8, 14, 15]. However, effects of confounding variables were seldom controlled in these studies [4]. In addition, these observations are not

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supported by the superior capacity for hematopoiesis in younger subjects [16].

Using multiple regression statistics, our study examined the influence of confounding variables in the risk-factor analysis of EPO resistance in dialysis cohorts. Furthermore, relationships between age, gender and EPO resistance were explored in an extended population of pediatric, adult and geriatric subjects.

Objectives (1) To determine the risk factors for EPO resistance in a parallel study of pediatric and adult dialysis cohorts, and (2) to analyze the contributory roles of age and gender in the two study groups.

Hypotheses (1) Young age per se is not an etiological factor in EPO resistance; the relationship is likely confounded by other variables. (2) Because of estrogenic influence and menstrual blood loss, larger EPO doses may be required for women than for men.

Methods

The study was approved by the Institutional Review Board (IRB) of the Montefiore Medical Center, Bronx, New York, USA. A total of 30 pediatric subjects who were on HD and EPO for a minimum of 2 months between July 2002 and January 2005 were enrolled. In addition, a cohort of 163 adults was stratified by gender and age group. As shown in Table 1, 12 subjects were then randomly selected from each of the age- and gender-specific strata. Six of the 72 adults were not suitable for analysis because of inadequate data.

Anemia management Anemia was managed according to the guidelines of the National Kidney Foundation/Dialysis Outcome Quality Initiatives [17]. Dose of EPO was increased by 25% every 2–4 weeks to achieve a target hemoglobin (Hb) level of 12.0 g/dl. Ten intravenous doses of 100–125 mg iron were given with consecutive HD

sessions if iron saturation was < 20% and/or serum ferritin was < 100 ng/ml. If iron saturation was > 20% and/or serum ferritin > 100 ng/ml, 25–125 mg iron was administered weekly. If iron saturation was > 50% and/or serum ferritin > 800 ng/ml, iron therapy was withheld. In furtherance of an ongoing study, oral doses of 5 mg folate were given to all pediatric subjects with every HD session.

Statistics

Data were analyzed with the aid of Statistical Package for the Social Sciences (SPSS) software. All data sets were assessed for Gaussian distribution using the D'Agostino–Pearson's test for kurtosis, skewness and omnibus normality. All data were essentially normally distributed.

Demographic characteristics As shown in Table 1, demographic variables including age, gender, weight, height, body mass index (BMI), and ethnicity were compared in the two cohorts. Secondly, both groups (Table 2) were assessed for differences in dialysis adequacy, nutritional status, objective malnutrition inflammation score (OMIS), indices of bone disease, estimated blood loss (EBL), residual renal function (RRF), and type of vascular access [HD catheter or arterio-venous fistula (AVF)]. In addition, the pattern of EPO and iron prescription among the pediatric and adult physicians was examined (Table 3).

Univariate risk factor analysis To assess their etiological role, we analyzed previously identified risk factors in the pediatric and adult literature for each cohort. The risk factor distribution was stratified into two sub-sets (by 50th percentile of EPO requirements), while differences in their mean values were examined. Only the risk factors with a $P < 0.75$ were selected for regression studies. These included age, gender, race, iron and folate indices, EBL, urea reduction ratio (URR), urea clearance \times dialysis duration/total body water (Kt/V), RRF, normalized protein catabolic rates (nPCR), calcium (Ca), phosphorous (P) intact parathyroid hormone (iPTH), surgery grade, infection

Table 1 Demographic characteristics of the pediatric and adult study subjects on chronic dialysis (SD standard deviation, AA African American, H Hispanic, Cs Caucasian or Asian, M male, F female)

Study groups	Age (years)		Race/ethnicity			Gender		Diabetic	
	Range	Mean \pm SD	AA	H	Cs	M	F	Yes	No
Pediatrics ($n=30$)	8 – 20	16 \pm 3	12	16	2	16	14	0	30
Young adults ($n=23$)	21 – 45	40 \pm 6	12	9	2	10	13	3	20
Adults ($n=22$)	45 – 65	55 \pm 4	13	4	5	10	12	4	18
Elderly ($n=21$)	\geq 66	75 \pm 6	12	5	4	9	12	4	17
Total ($n=96$)	8 – 85	43 \pm 22	49	34	13	45	51	11	85

Table 2 Comparative distribution of the potential risk factors for EPO resistance in pediatric and adult dialysis cohorts (*CI* confidence interval, *Kt/V* urea clearance × dialysis duration/total body water, *iPTH* intact parathyroid hormone, *EBL* estimated blood loss, *RRF* residual renal function, *OMIS* objective malnutrition inflammation score)

Risk factors	Pediatrics (n=30)	Adults (n=66)	Mean difference	t-test (P)	95% CI	
Serum albumin (g/dl)	3.7±0.6	3.6±0.6	0.04	0.79	- 0.23	0.31
Urea reduction ratio ^a	1.6±0.5	1.85±0.3	- 0.25	0.01	- 0.43	- 0.07
Kt/V ^b	1.57±0.5	1.8±0.41	- 0.22	0.03	- 0.41	- 0.03
Serum transferrin (mg/dl)	162±32.4	155±53	7.21	0.49	- 13.6	28.0
iPTH (pg/ml)	614±526	656±424	- 42.6	0.67	- 243	157
Iron saturation (%)	33.3±8.3	36.2±16.2	- 2.99	0.34	- 9.2	3.2
EBL (ml)	124±91	55±24	69.0	0.001	46.0	93.0
Serum ferritin (mg/dl)	532±294	619±273	- 145.6	0.11	- 324.7	33.5
RRF ^c	0.47±0.5	0.55±0.5	- 0.08	0.47	- 0.3	0.14
Iron deficiency ^d	0.23±0.4	0.89±0.3	- 0.66	0.001	- 0.81	- 0.51
Folate deficiency ^e	0.20±0.4	0.87±0.3	- 0.67	0.001	- 0.85	- 0.50
OMIS	11.9±3.4	6.8±1.3	5.15	0.001	4.2	6.1

^a < 65=1; ≥ 65=2
^b < 1.2=1; ≥ 1.2=2
^c No=0; Yes=1
^d No=0; Yes=1
^e No=0; Yes=1

scores, pubertal status (pediatrics), diabetic mellitus (adults) and malnutrition inflammation indices. Excluded from the analyses (*P*>0.75) were primary renal disease, human immunodeficiency virus (HIV) status, HD duration, use of angiotensin modifiers, thyroid dysfunction, nephrotic state, and vascular access modality.

Multiple regression studies The EPO resistance index (defined as the number of units of EPO divided by the Hb level in grams per deciliter) was the dependent variable. All the risk factors with *P*<0.75 (identified above) were initially included in the regression. The best set of predictors was determined by a serial elimination of variables with the least contribution to the significance (F value) of the model. The basic prerequisites for an accurate multivariate analysis (including goodness of fit) were met. Multi-collinearity (measure of variable similarity) was minimized by acceptance of only the predictors with a variance inflation factor (VIF)<10. We avoided overfitting by seeking a minimum of ten possible outcomes for each variable. Separate analyses were performed for

variables with similar attributes, e.g. Kt/V vs URR. In such cases, equivalent statistical results are reported as an entity.

- (1) Age vs EPO resistance index (ERI). The linear correlation between ERI and age was determined by Pearson’s statistic. The confounding effect of the different EPO dosing for children and adults was avoided by the use of analysis of covariance (ANCOVA). Unlike Student’s *t*-test, ANCOVA was predicated on variance rather than the mean, thereby eliminating error that might have resulted from EPO doses.
- (2) Gender vs ERI. The mean difference between the ERIs for each age category by gender, pre- and post-puberty, and menstrual status was examined for both study groups.

Composite scoring of variables To facilitate (quantitative) regression analysis, we scored surgical procedures, infection severity, folate and iron deficits, malnutrition and inflammation indices (see Appendices). Indices for the

Table 3 Comparative analysis of initial EPO prescription between pediatric and adult renal physicians (*EPO* erythropoietin, *CI* confidence interval, *Hb* hemoglobin)

EPO dosing parameters	Pediatric EPO dosage (units)			Adult EPO dosage (units)			t-Test (P)
	Mean	Number	95% CI	Mean	Number	95% CI	
Hb 9–10 g/dl	17,778	6	12,253–23,302	6,272	11	4063–8482	< 0.0001
Hb 11–12 g/dl	19,524	21	15,280–23,768	3,508	42	1325–3006	< 0.0001

scoring of the malnutrition–inflammation complex in adults were adapted for the pediatric population by exclusion of subjective items [18].

Sample size The number of pediatric subjects ($n=30$) required to determine the predictors of EPO resistance was estimated on the basis of a 30% prevalence (pilot study), statistical power of 0.80, and a $P<0.05$. Because of the wide prevalence rates (5–67%) of EPO resistance found in the adult literature, twice the number of the pediatric sample ($n>60$) was selected for study.

Results

Baseline demographic characteristics of the study groups

- (a) Pediatric cohort. As illustrated by Table 1, the male-to-female ratio was 1:1, mean age was 16 ± 3.2 years, average weight was 51.4 ± 16.5 kg, and the mean BMI was 21.4 ± 5 kg/cm². Eight subjects had focal glomerulosclerosis (26.7%), six had chronic glomerulonephritis (20%), five had HIV nephropathy (16.7%), and four had renal dysplasia (13.3%), while two patients each had membrano-proliferative (6.7%), rapidly progressive (6.7%) or lupus glomerulopathy (6.7%). Four (13.3%) patients had suffered at least one episode of renal loss from graft rejection. Duration of dialysis was < 6 months in nine subjects (30%), 6–72 months in 17 (56.7%), and > 72 months in two (6.7%) patients.
- (b) Adult subjects. There were 29 men and 37 women with mean ages of 57 ± 15 years and 56 ± 15 years, respectively. Their mean weight was 73.5 ± 6.1 kg, mean height 165.5 ± 6.5 cm, and BMI was 27 kg/cm². Polysulfone membrane dialyzers were used for all subjects.

Distribution of potential risk factors

The distribution of potential risk factors for EPO resistance was compared in the two groups. As shown by Table 2, a greater number of pediatric subjects had URR values < 65, but more of the adults had a minimum daily urinary output of 100 ml (defined as residual renal function), while nutritional indices (e.g. serum albumin) were not significantly different. Furthermore, in spite of a lower volume of blood loss, more adults had higher scores on the indices of iron deficiency. In addition, while 80% of the pediatric cohort used HD catheters as vascular access, five had AVFs. The ratio was reversed for the adults, with AVF in

56% and catheter use in 44%. There was a six-times more likelihood of greater blood loss in subjects with HD catheters (mean= 132.5 ± 96 ml) than those with AVFs (85 ± 38 ml) ($P=0.02$).

Prescribed doses of EPO The mean dose of EPO prescribed (for hemoglobin 11–12 g/dl) at the onset of HD for the pediatric group (19,524 U) was six-times larger than that for adults (3,508 U) (Table 3). In contrast, except for the larger doses of iron given to the adults with a saturation range of 36–45%, equivalent amounts were prescribed for both groups. Hence, the mean doses of iron for saturation < 26% and 26–35% were 119 mg [95% confidence interval (CI) = 79–159 mg] and 95 mg (95% CI= 67–122 mg), while corresponding adult doses were 120 mg (95% CI= 102–139 mg) and 90 mg (95% CI=76–105 mg). Similarly, there was no significant difference in the amounts of iron prescribed for serum ferritin ranges 101–500 mg/dl ($P=0.41$), 501–1,000 mg/dl ($P=0.72$), and > 1,000 mg/dl ($P=0.3$).

(1) Predictors of EPO resistance in pediatric patients

- (a) Univariate analysis. Student's *t*-test on the mean differences of each risk factor distributed across two subsets (of 50th percentile ERI) yielded the following: serum ferritin ($P=0.002$), blood loss ($P=0.03$), Kt/V ($P=0.006$), serum albumin ($P=0.02$), serum transferrin ($P=0.03$), inflammation score ($P=0.002$), and Tanner pubertal status ($P=0.02$). The predictors with $P > 0.05$ but < 0.50 were age ($P=0.3$), gender ($P=0.2$), folate deficiency ($P=0.1$), iPTH ($P=0.5$), iron saturation ($P=0.5$), URR ($P=0.1$), C-reactive protein ($P=0.5$), and infection score ($P=0.5$) (Table 4).
- (b) Multiple regression. Multiple regression analysis of all the risk factors (with $P<0.75$) had a coefficient of determination (R^2) of 0.95 and an F value of 6.6 ($P=0.77$). With the use of backward elimination, URR, Kt/V, blood loss, nPCR, inflammation scores, iPTH, serum ferritin and infection scores emerged as the most predictive (model) of EPO resistance ($R^2=0.8$, $F=10.5$, and $P<0.0001$).

(2) Predictors of EPO resistance in adult patients

- (a) Univariate analysis. The variables with P less than 0.51 for the adult subjects are as follows: iron saturation < 25% ($\chi^2=-0.3$, $P=0.01$), folate deficiency ($\chi^2=-0.3$, $P=0.05$), iron deficiency ($\chi^2=-0.3$, $P=0.1$), Ca \times P ion products ($P=0.1$), EBL ($P=0.3$), iPTH ($P=0.3$), RRF [odds ratio=1.7 (95% CI 0.5 - 5.9); $P=0.3$], URR ($P=0.5$), Kt/V ($P=0.4$), OMIS ($P=0.3$), serum transferrin ($P=0.5$), and ferritin ($P=0.4$).
- (b) Multiple regression analysis (with ERI as dependent variable) on the adult data, with age, gender,

Table 4 Best predictive (regression) model of EPO resistance in a pediatric cohort on chronic maintenance dialysis (ANOVA analysis of variance)

Risk factors	Upper (U) vs lower (L) 50th percentiles of EPO requirements				
	Mean difference (U–L)	95% CI	Beta coefficient	t-Test	P*
Constant	–	–	–	4.20	0.001
Intact parathyroid hormone (pg/ml)	190.1	108–250	0.20	2.52	0.02
Malnutrition–inflammation score	2.8	–0.52 to 4.5	0.06	0.28	0.78*
Urea reduction ratio	– 1.9	– 3.9 to 1.0	– 0.72	– 2.72	0.01
Kt/V	– 0.22	– 0.4 to 0.04	– 1.08	– 4.11	0.001
Protein catabolic rate	0.10	– 0.05 to 0.26	– 0.40	– 3.73	0.001
Serum ferritin (mg/dl)	338	95–580	0.29	2.52	0.02
Infection score	0.40	0.25–0.8	0.25	2.24	0.035
Volume of blood loss (ml)	12.3	– 80 to 56.3	0.28	1.50	0.15*
Regression summary (ANOVA)	R ² =0.80			F=10.5	0.0001

Beta coefficient = relative effect of risk factor on EPO resistance

Kt/V = urea clearance x dialysis duration/ total body water

R² (coefficient of determination) = proportion of variance accounted for by the model

F (ratio of the sum of squares of the residuals) = statistical significance of the model

*P value estimates contribution of the predictor to the overall regression model. Thus P>0.05 for a co-variate is important with regards to achieving the stated F-statistic

race, iron profile, folate indices, serum albumin, blood loss, dialysis adequacy, RRF, Ca×P ion product and iPTH as predictors, yielded a model with R²=0.95 (F=1.4; P=0.5). The best set of predictors (Table 5) after backward elimination were low serum transferrin, low iron saturation and folate deficiency status (F=5.1, P=0.001).

Age, gender and EPO responses

Age and ERI Analysis of covariance on the mean ages of pediatric (16±3.2 years) and adult (57±15.6 years) subjects and the corresponding ERI yielded no statistical signifi-

cance (Table 6). Similarly, there was no difference in the frequency distribution of the risk factors by 50th-percentile ERI across the four age groups of children and adults. Furthermore, neither the correlation between ERI and the age of pediatric patients (r=–0.13) nor that of adult patients (r=0.03) was significant.

Gender and EPO resistance There were no significant differences in the ERI values of male and female subjects in each age stratum (Table 7). Similarly, the difference in the mean ERI of 2,056 (95% CI=1,439–2,621) for the pre-pubertal children and 2,138 (95% CI=1,079–3,135) for the post-pubertal children was not significant (P=0.9). With similar hemoglobin mass, there was no difference in the ERI (P>0.5) for either eight of 14 adolescents (2,431±

Table 5 Best predictive (regression) model of EPO resistance in adult subjects on chronic maintenance dialysis (ANOVA analysis of variance)

Risk factors	Upper (U) vs lower (L) 50 percentiles of EPO requirements				
	Mean difference (U–L)	95% CI	Beta coefficient	t-Test	P*
Constant	–	–	–	4.30	0.0001
Iron saturation (% in serum)	– 0.15	– 0.05 to 0.36	0.13	1.06	0.296*
Folate deficiency	– 0.18	0.01–0.38	0.42	2.74	0.009
Serum transferrin (mg/dl)	– 0.21	0.07–0.35	0.33	2.68	0.010
Model summary (ANOVA)	R ² =0.40			F=5.1	0.001

Beta coefficient = relative effect of risk factor on EPO resistance

R² =proportion of variance accounted for by the model

F test measures statistical significance of the model

Iron saturation < 25%=1, ≥ 25%=2

Folate deficiency: Yes=1, No=2

*P value estimates contribution of the predictor to the overall regression model. Thus, P>0.05 for a co-variate is important with regards to achieving the stated F-statistic

Table 6 The relationship between age and EPO resistance index among pediatric and adult cohorts

Variable	Children (<i>n</i> =30)	Adults (<i>n</i> =66)	ANCOVA
Age (years)	16±3.2	57±15.6	F=1.8
EPO U/Hb	2056±1285	353±250	P=0.2

1,683 vs 3,099±1,542) or seven of the 13 adults (301±176 vs 287±140) with and without regular menstrual cycles.

Discussion

Although there were similarities in the demographic attributes (e.g. gender and ethnicity) in the two study populations (Table 1), there were differences in their clinical characteristics. Whereas preliminary analysis had shown a lower dialysis dose and a greater (volume of) blood loss in pediatric subjects, statistical evidence showed that iron and folate deficiencies were more common in adults (Table 2). In addition, apparently due to a frequent catheter infection in pediatric subjects, a higher score was obtained for the malnutrition inflammation indices [5, 18–27]. Regression analysis of pediatric data had URR, Kt/V, blood loss, hyperparathyroidism, infection and inflammation scores as strong predictors of EPO resistance (Table 4). On the other hand, iron and folate deficits were the predominant variables in adults (Table 5). The occurrence of a potent set of predictors such as inflammation, malnutrition and poor dialysis (and the possible synergistic interaction between them) in pediatric subjects may explain its larger share of EPO requirement than the adults [20–23].

Earlier studies on dialysis subjects often failed to consider the impact of blood loss on EPO requirements. The larger amount of blood loss in our pediatric cohort was due to (a more frequent) laboratory sampling and use of HD catheters. Nevertheless, in spite of the disproportionate blood loss, iron deficiency was more prevalent in the adults [28]. Unlike EPO, intravenous dosing of iron was based on laboratory parameters [e.g. iron (Fe) saturation] rather than body weight in both groups. Consequently, because of the lower body mass of children, an equivalent dose of iron

invariably results in a larger share. Furthermore, as part of an ongoing study, pediatric subjects routinely received folate supplements, and, therefore, had a lower prevalence of folic acid deficiency [9].

However, the aforementioned differences in clinical characteristics (alone) do not justify the wide disparity in EPO requirement in both groups. In general, pharmacological dosing in pediatric patients is often based on body weight. Hence the initial EPO dose was calculated as 150 IU per kilogram of body mass. Once a large dose has been initiated, high maintenance dosing is perpetuated by the addition of a fraction of the original amount so that the hemoglobin targets recommended by the Kidney Disease Outcomes Quality Initiative (KDOQI) can be attained. On the other hand, for adults, the initial EPO dose is estimated on the basis of hemoglobin deficits (rather than body weight). Consequently, approximately 10,000–15,000 larger units of EPO (per subject) were prescribed by children's physicians than by adults' counterparts. Interestingly, even though there are limited data, there is no scientific basis for the use of body weight (rather than hemoglobin deficit) to derive EPO dose in pediatric subjects. In fact, as in adults, there is a median increase of 0.042 g/dl hemoglobin mass for every thousand units of EPO administered [29].

Unlike the findings in observational studies, there is no evidence to support the erroneous belief that younger subjects are intrinsically 'more resistant to EPO' than adults [6, 30]. In our study, we performed separate analysis on pediatric and adult data, and no relationship was found between EPO resistance and chronological age. If, indeed, there is a relationship, younger pediatric subjects (8–15 years) should have required larger doses than their older counterparts (16–21 years), but this was not the case. In like manner, there were no significant differences in the amount of EPO required by the three age categories of adults. However, in spite of a similar use of KDOQI protocol, there was a wide disparity in the prescribed doses of EPO by the children's and adults' physicians. In addition, while the combined pediatric and adult population showed a significant correlation between age and ERI, after data stratification by 50th-percentile (of EPO response), the influence of EPO prescription was effectively removed. Additional evidence in support of the confounding effect of EPO

Table 7 Differences in EPO resistance index between male and female pediatric and adult cohorts

Age (years)	Mean EPO resistance index [EPO (U/Hb g/dl)]						Student's <i>t</i> -test <i>P</i>
	Male subjects			Female subjects			
	Mean	Number	95% CI	Mean	Number	95% CI	
8–20	1,701.4	16	1,079–2313	3,557.4	14	2,499–3,186	0.1
22–45	249.9	10	191–301.3	301.3	13	220–367	0.3
46–65	279.3	10	139.6–419	330.7	12	198–463	0.6
> 66	271.9	9	198–345	370	12	191–602	0.4

dosing (on age and ERI) was the loss statistical significance upon re-analysis of the stratified data [4, 5, 27, 31–35].

The relationship between gender and EPO resistance in chronic uremia is often confounded by multiple factors. Thus, even though androgens potentiate bone marrow response to EPO, dialysis subjects are often functionally hypogonadic [36, 37]. On the other hand, estrogenic inhibition of erythropoiesis may be obviated by uremic ovarian toxicity. Furthermore, delayed puberty in chronic dialysis may modulate gender effects on EPO response [38]. Nevertheless, in spite of control for puberty and menstrual status, an observed trend of a lower EPO response among female subjects was not statistically significant, apparently because of a small sample size [36–41].

Finally, there is a gradual evolution of our understanding of the roles (and appropriate use) of EPO in the dialysis population. Contrary to popular belief, our study suggests that chronological age is not a limiting factor in (hematologic) response to EPO. Although we do not know the downside of excessive EPO dosing in the face of poor clinical response, an attempt to normalize hemoglobin mass (> 12 g/dl) in adults with cardiovascular and kidney diseases results in a greater mortality rate [13, 42]. Hence, except for the small-sized pediatric patients (weight < 20 kg), EPO dosing should be based on hemoglobin deficit rather than body mass. In addition, EPO requirements in pediatric subjects may be curtailed by limiting use of HD catheters, enhancing dialysis adequacy, improving nutrition, and minimizing laboratory blood sampling.

Conclusion

Poor response to EPO in the pediatric population was influenced by clinical characteristics (such as chronic inflammation, inadequate dialysis, hyperparathyroidism, and blood loss) rather than chronological age. In contrast, due to inadequate supplementation, iron and folate deficiencies were the major determinants in adults. Nevertheless, the relationship between age and EPO resistance was confounded by a disproportionate prescription of larger EPO doses for the pediatric cohort than for the adults. Because of the clinical significance of this finding, a multi-institutional prospective study that includes a sizable proportion of infants and young children is warranted.

Study limitations In spite of minimizing confounding interaction, risk-factor analysis by regression study does not establish a cause and effect relationship. On its face value, comparative analysis across the populations of pediatric and adult patients may have been prone to erroneous conclusions. However, the study was designed

to analyze the influence of common characteristics on EPO resistance (age and gender) in the two populations. In addition, a larger sample size of pre-pubertal children (and women of reproductive age) might have increased the credibility of the findings.

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Appendix

Appendix A. Objective malnutrition inflammation scores

- (a) Serum albumin: (1) ≥ 4 g/dl=0, (2) 3.5–3.9 g/dl=1, (3) 3.0–3.4 g/dl=2, (4) < 3.0 g/dl=3
- (b) Serum transferrin: (1) ≥ 250 mg/dl=3, (2) 200–249 mg/dl=2, (3) 150–199 mg/dl=1, (4) < 150 mg/dl=0
- (c) Body mass index: (1) ≥ 20 kg/m²=0, (2) 15–19.9 kg/m²=1, (3) 16–17.9 kg/m²=2, (4) < 16 kg/m²=3
- (d) Dialysis duration (years): (1) 0–1=0, (2) 1–3=1, (3) 3–5=2, (4) > 5 =3
- (e) Infection: (1) none=0, (2) low grade (e.g. line-induced bacteremia, upper respiratory tract infection)= $0.5 \times$ no. of events, (3) moderate (e.g. symptomatic line sepsis, pneumonia)= $1.0 \times$ no. of events, (4) severe (infection warranting hospitalization) = $1.5 \times$ no. of events
- (f) Dialysis adequacy: (1) URR $\geq 80\%$ =0, (2) 61–79%=1, (3) 41–60%=2, (4) $< 40\%$ =3
- (g) Serum ferritin: (1) 0–500 mg/dl=0, (2) 501–750 mg/dl=1, (3) 751–1,000 mg/dl=2, (4) $> 1,001$ mg/dl=3

Appendix B. Clinical and laboratory definitions

EPO resistance index ERI calculated as EPO dose (international units per kilogram per week) divided by a given value of hemoglobin concentration (grams per deciliter).

Folate deficiency Because of an overlap in the diagnostic accuracy of the red blood cell (RBC) parameters [e.g. mean corpuscular volume (MCV) and red cell distribution width (RDW) were poorly specific], folate deficiency (FD) was defined as (a) Mean serum folate and/or RBC folate ≤ 2 standard deviation score (b) Mean MCV ≥ 95 th percentile for age and gender and/or RDW ≥ 16 and (c) Normal serum vitamin B₁₂.

Iron deficiency Fe deficiency consists of (a) Mean Fe saturation $< 20\%$ (b) Mean serum ferritin < 100 mg/dl and (c) Mean MCV [and/or mean corpuscular hemoglobin (MCH)] < 5 th percentile for age and gender.

Malnutrition inflammatory score The objective malnutrition inflammatory score (OMIS), adapted from the comprehensive MIS [18], is a questionnaire designed to quantify nutritional deficiency and inflammatory burdens. Its components are primary renal disease, dialysis duration, dialysis adequacy, C-reactive protein, total iron binding capacity, serum ferritin, body mass index, serum albumin and transferrin.

Dialysis adequacy Urea reduction ratio (URR) = pre-dialysis blood urea nitrogen (BUN) – post-dialysis BUN/pre-dialysis BUN

Kt/V was determined by computer analysis (formal urea kinetic modeling) of the variables K, extrapolated from the mass transfer area coefficient (KoA) of the dialyzer, V, estimated total body water, and G, urea generation rate [or normalized protein catabolic rate (nPCR)].

Pubertal status Post-puberty was defined as (a) Tanner stage (breast and/or pubic hair) ≥ 2 , (b) plasma levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) consistent with the stage of puberty (c) and normal plasma free-thyroxine (FT₄) and thyroid-stimulating hormone (TSH). Plasma gonadotropins were measured by ultra-sensitive chemiluminometric assay. Tanner I, FSH > 0.65 ng/ml, LH > 0.75 ng/ml; Tanner II, FSH > 2.75 ng/ml, LH > 1.0 ng/ml; Tanner III, FSH > 2.25 ng/ml, LH > 1.0 ng/ml; Tanner IV, FSH > 2.5 ng/ml, LH > 2.5 ng/ml; Tanner V, FSH > 2.5 ng/ml, LH > 2.5 ng/ml.

Infection score Severity of infection: mild (e.g. line-induced bacteremia, upper respiratory tract infection) = 1, moderate (e.g. symptomatic line sepsis, pneumonia) = 2, and severe (infection warranting hospitalization) = 3.

Grade of surgery minor (e.g. vascular access placement or revision) = 1, major (e.g. nephrectomy, parathyroidectomy, appendectomy) = 3

Estimated blood loss Amount of blood collected for all laboratory procedures was measured; blood loss during dialysis (e.g. clotted circuit) was subjectively quantified by experienced dialysis nurses, and surgical blood loss was estimated by the attending surgeons. Blood loss was corrected for blood volume (BV) as EBL/BV. Calculated blood volume = 87 ml/kg for 7–18 years, and 72 ml/kg for > 18 years.

Residual renal function An estimated minimum daily urine output of 50 ml and 100 ml for study subjects below 10 years of age and above 10 years of age, respectively.

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