

ORIGINAL REPORT

Factors precipitating erythropoiesis-stimulating agent responsiveness in a European haemodialysis cohort: case-crossover study[†]

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ABSTRACT

Purpose Hyporesponsiveness to erythropoiesis-stimulating agents (ESAs) is clinically and economically important in the treatment of anaemia in chronic kidney disease (CKD) patients. Previous studies focused on baseline predictors of ESA hyporesponsiveness, rather than factors associated with the transition to this state. Reversibility of ESA hyporesponsiveness has also not been studied previously.

Methods Case-crossover methodology was applied to a cohort of 6645 European CKD patients undergoing haemodialysis and prescribed ESAs. Ninety-day ESA exposure periods were defined, haemoglobin (Hb) response was calculated using the last 30 days of one period and the first 30 days of the next, and periods were classified based on a median ESA dose (80.8 IU/kg/week) and a 10 g/dL Hb threshold. Clinical, dialysis and laboratory data from patients' first hyporesponsive 'case' period was compared with the preceding responsive 'control' period using conditional logistic regression. A similar approach was applied to hyporesponsiveness reversal.

Results Of the patients, 672 experienced hyporesponsiveness periods with preceding responsive periods; 711 reversed to normality from hyporesponsiveness periods. Transition to hyporesponsiveness was associated with hospitalization, vascular access changes or worsening inflammation, with these factors accounting for over two-thirds of transitions. Findings were largely insensitive to alternative ESA doses and Hb thresholds. Continued hospitalization, catheter insertion and uncontrolled secondary hyperparathyroidism were associated with a lack of regain of responsiveness.

Conclusions Transition to hyporesponsiveness is linked to the development of conditions such as hospitalization events, vascular access issues or episodes of systemic inflammation. However, a third of hyporesponsive episodes remain unexplained. © 2015 The Authors. *Pharmacoepidemiology and Drug Safety* Published by John Wiley & Sons Ltd.

KEY WORDS—anaemia; CKD; erythropoietin; Europe; haemodialysis; hyporesponsiveness; pharmacoepidemiology

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INTRODUCTION

Mammalian kidneys fulfill a major endocrine function in producing erythropoietin to maintain a stable haemoglobin (Hb)-dependent oxygen transport capacity and red blood cell (RBC) mass, compensating,

through erythropoiesis, for the daily ongoing loss of erythrocytes (normally ~1% per day¹); the hormonal secretion is inversely proportional to oxygen availability to the renal cortex, so that an effective feedback loop is established.² Erythropoietin production decreases with declining kidney function,^{3,4} meaning that severe anaemia is a frequent complication in chronic kidney disease patients, where insufficient compensatory erythropoiesis occurs in the face of increased erythrocytes loss⁵ and a shortened RBC half-life.^{6,7} Prior to the mid-1980s, renal anaemia was managed with regular blood transfusions, which increased patients' infection risk, induced progressive

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iron overload and decreased their chances of a successful kidney transplant due to allosensitization; advances in recombinant DNA technology following the isolation and cloning of the gene for human erythropoietin in 1983 mean that erythropoiesis-stimulating agents (ESAs), along with iron, now form the cornerstone of the treatment of anaemia in chronic kidney disease (CKD) patients.⁸

The lack of response to ESA therapy (ESA hyporesponsiveness), which occurs in a small, but important minority of CKD patients, has important clinical and economic implications. ESA doses are titrated according to changes in Hb level, and specific Hb targets encourage progressive increases in ESA dosing in patients with poor response below-target haemoglobin concentrations, leading to a skewed distribution of ESA doses in dialysis patients and hence costs: approximately 50% of total ESA costs are spent on the 15% of patients requiring the highest dosage.⁹ Observational studies showed that patients requiring the highest doses of ESA therapy relative to haemoglobin response experience poorer outcomes.¹⁰ *Post hoc* analyses of clinical trial data^{11–13} demonstrated a positive correlation between hyporesponsiveness and adverse outcomes, with the most hyporesponsive patients experiencing the highest rate of death and/or cardiovascular events. Several smaller studies have provided additional evidence of the clinical importance of ESA hyporesponsiveness in haemodialysis patients.^{14–17} Many of these studies were cross-sectional and examined the relationship between ESA hyporesponsiveness and outcomes at a particular point in time. Common to all these studies, it was impossible to discriminate between a causal link between higher ESA doses and poorer outcomes or whether the effect was simply due to confounding-by-indication.

Risk factors for ESA hyporesponsiveness have been well described over the last two decades and include iron insufficiency,^{18,19} vascular access changes,^{15,20} hospitalization,^{15,21} inflammation,^{22–26} malnutrition,^{23,27} along with a number of less frequent causes such as blood loss, haemolysis, bone marrow disorders, haemoglobinopathies and neutralizing antibodies to ESAs.^{28–31} These studies have focused on baseline predictors for hyporesponsiveness and hence have been unable to distinguish between factors stably associated with certain patients and risk factors immediately preceding the development of hyporesponsiveness. In fact, given the life-time of RBCs⁷ and the time-lag between a change in ESA dose and haemoglobin response,³² there are relevant time intervals that published analyses have not accounted for to date.

The present study aimed to gain a fresh perspective on factors driving ESA hyporesponsiveness by applying case-crossover methodology³³ to examine transient factors associated with patients becoming hyporesponsive to ESA therapy in a large European haemodialysis cohort. We hypothesized that pathophysiological changes within the patients gave rise to periods of ESA hyporesponsiveness; the case-crossover methodology, which has been used extensively in the field of pharmacoepidemiology^{34–36} and seeks to address the ‘why now?’ rather than the ‘why me?’ questions,³⁷ is best suited to examine the effect of such transient exposures on outcomes of interest. Furthermore, we introduced a time-lag approach to account for changes in ESA dose and subsequent haemoglobin response.³² Finally, we examined factors associated with recovering from periods of hyporesponsiveness.

SUBJECTS AND METHODS

Study population

The Analyzing Data, Recognizing Excellence, and Optimizing Outcome (ARO) research initiative has been described previously.³⁸ Briefly, 8963 haemodialysis patients in 134 Fresenius Medical Care facilities in 2005 and 2006 were selected randomly from nine European countries and from Turkey. AROi was designed as an open cohort to recruit a heterogeneous mix of incident and prevalent dialysis patients and to maintain a fixed period of follow-up (approximately 15 000 person-years); patients leaving the study through death or censoring were replaced with randomly selected patients from the same facility. Patients’ demographic characteristics and clinical history were captured on admission, whilst detailed information on dialysis, laboratory testing, medications and clinical outcomes were captured throughout follow-up.

Data preparation

Data preparation, conducted in SAS for Unix (version 9.1.3; SAS, Cary, NC, USA) and reproduced independently by a second programmer, was restricted to non-United Kingdom patients (medication data were unavailable for this country) prescribed ESAs. Darbepoetin alfa doses were converted to epoetin alfa equivalents using a 1:200 dose conversion ratio.³⁹ Patients’ follow-up times (defined as study start [cohort entry] until censoring by transplantation, loss to follow-up or death) were divided into consecutive 90-day periods of weight-adjusted weekly exposure to

ESAs (henceforth ‘ESA exposure periods’; Figure 1). For each ESA exposure period, summary data on hospitalization, dialysis and laboratory parameters were captured for each patient (Supporting Information, Table S1). Response to ESA, operationally defined by mean haemoglobin levels, was evaluated for the last 30 days of one ESA exposure and the first 30 days of the next (henceforth the ‘haemoglobin observation periods’). This time frame was chosen according to the observed time-lag to elicit a response in Hb concentration to ESAs and the reduced RBC half-life in haemodialysis patients.^{7,32,40} Together, these data were used to define response to ESAs according to a coding algorithm (Box 1). In the final step of data generation, each patient’s follow-up time was considered sequentially. Where patients’ first period of ESA hyporesponsiveness was immediately preceded by an ESA response period, these time periods were coded as the ‘case’ and ‘control’ periods, respectively, and subsequent follow-up was ignored. Similarly, for the reversibility analyses, patients’ first period of ESA response was coded as the case period if it was preceded by a period of ESA hyporesponsiveness.

Statistical analyses

All statistical analyses were performed in SAS for Windows (version 9.2; SAS, Cary, NC, USA) and were reproduced independently by a second statistician. Initially, case and control periods were compared by univariate analyses, with conditional odds ratios and 95% confidence intervals (CIs) calculated for each explanatory variable. Stepwise conditional logistic

regression was then applied to obtain maximum likelihood estimates of the effect of exposures on the outcome whilst controlling for potential confounding. Variables significant at the 10% level on univariate analysis were considered for entry into the multivariate model, whereas a likelihood ratio test significance level of 5% was required for retention. Population Attributable Risk Fractions – in this study, the proportion of transitions to hyporesponsiveness that would not have occurred in the absence of a particular risk factor – were calculated for factors independently associated with transition to hyporesponsiveness, as described for case-crossover studies by Spurling and Vinson.⁴¹ Missing values, coded as such in the explanatory data, were excluded from the univariate analysis but included in the multivariate analysis. Where required, correlations between explanatory variables were assessed visually and by calculating Pearson’s correlation coefficients.

Sensitivity analyses

For the main analysis, an ESA cut-off based on the median weight-adjusted weekly ESA dose (80.8 IU/kg/week) was applied and compared with a haemoglobin threshold of 10 g/dL. To test the robustness of the findings to variations in hyporesponsiveness definition, additional analyses were conducted examining combinations of the above criteria with an upper quartile of weight-adjusted weekly ESA dose (140.4 IU/kg/week) and a haemoglobin threshold of 9 g/dL. Furthermore, due to known associations between ESA hyporesponsiveness and gender,^{42,43} the main analyses (median ESA cut-off; haemoglobin

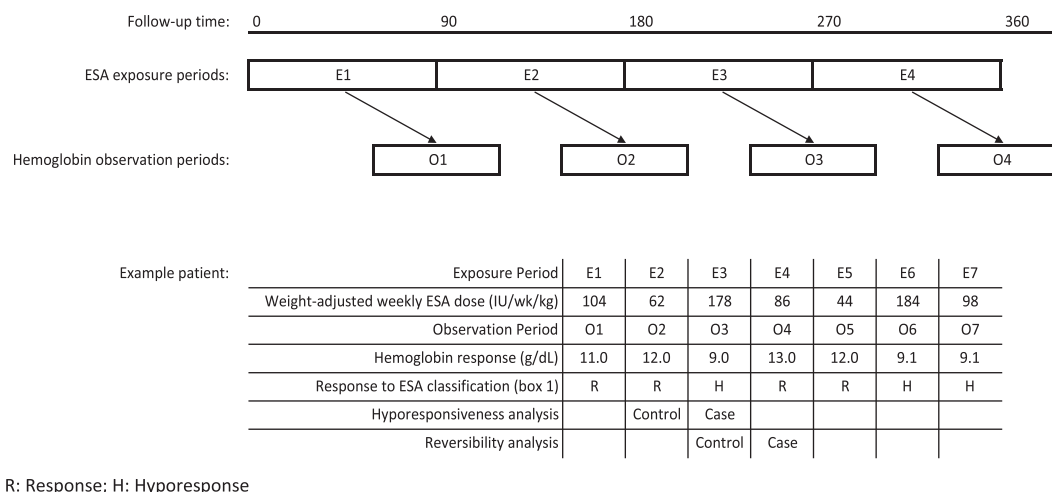


Figure 1. Schematic diagram of ESA exposure (E) and haemoglobin observation (O) periods for a hypothetical patient, with subsequent classification of ESA response in the main analysis

Box 1 Classification of patients' ESA response

- ❖ Haemoglobin value available in observation period:
 - > Haemoglobin above threshold under investigation:
 - Define as 'Responsive' regardless of the ESA value
 - > Haemoglobin below threshold under investigation:
 - ESA value available:
 - Define as 'Hyporesponsive' if ESA dose > threshold under investigation
 - Define as 'Undertreated' if ESA < threshold under investigation
 - ESA value unavailable:
 - Define period as 'Undefined responsiveness—missing ESA'

- ❖ Haemoglobin value unavailable in observation period:
 - > ESA value available :
 - Define as 'Undefined responsiveness—missing haemoglobin'
 - > ESA value unavailable:
 - Define as 'Undefined responsiveness—missing both'

- ❖ ESA exposure period less than 90 days:
 - Define as 'Short period'

10 g/dL) were performed separately for male and female patients. All sensitivity analyses were limited to univariate analysis.

RESULTS

Study population

Between 01 January 2005 and 31 December 2006, 11 153 patients were recruited from 134 Fresenius Medical Care facilities in nine European countries. Patients recruited from centers where the majority of data on key dialysis parameters (i.e. actual blood flow or dialysis adequacy [Kt/V]) were missing were excluded ($N=1352$), and patients from the UK ($N=838$) were also excluded as information on medication was unavailable, leaving 8963 patients. Data were sequentially limited further to patients prescribed ESAs ($N=8393$; 93.6%), where the standardized weekly dose was greater than zero (8271; 98.5%) and where follow-up was ≥ 90 days duration ($n=6645$; 80.3%). The baseline characteristics of this population are shown in Table S2 in the Supporting Information.

These patients contributed 45 027 ESA exposure periods (range two to nine periods, median 8), with 9964 person-years of exposure accrued. The final ESA exposure period was excluded for 6600 patients as it was less than 90 days in length (range 1–89 days; median 10 days), leaving 38 427 observation periods. Applying a median ESA dose cut-off (80.8 IU/kg/wk) and a haemoglobin threshold of

10 g/dL, over three quarters of ESA exposure periods were followed by periods of haemoglobin response (29,076; 75.7%). Almost five percent resulted in periods of hyporesponsiveness (1795; 4.7%), whilst slightly fewer periods of undertreatment were observed (1582; 4.1%). It was not possible to assign a responsiveness status for over 15% of periods (missing haemoglobin 2264 (5.9%); missing ESA 605 (1.6%); missing both 3105 (8.1%)) and the proportion of patients with at least one unassigned responsiveness status increased with follow-up length (Supporting Information, Figure S1). Accordingly, whilst 2656 (40.0%) and 69 (1.0%) patients were responsive and hyporesponsive, respectively, throughout their follow-up, these patients tended to have shorter follow-up periods. Six hundred and seventy-two patients experienced periods of hyporesponsiveness with preceding periods of response, 711 experienced periods of response with preceding periods of hyporesponsiveness and 471 patients experienced both.

Risk factors for hyporesponsiveness

In the univariate analysis, periods of hyporesponsiveness were more likely to occur following periods of hospitalization or changes in vascular access (Table 1). Where the type of vascular access change was considered, a change from an arteriovenous (AV) fistula or a graft to a catheter was associated with an increased risk, whereas a change in the

Table 1. Risk factors for development of ESA hyporesponsiveness in a cohort of European Haemodialysis patients ($N=672$) [univariate and multivariate analysis]

Parameters	Period		Univariate		Multivariate	
	Case	Control	OR* [95%CI [†]]	<i>p</i> -value	OR [95%CI]	<i>p</i> -value
Vascular access change (vs. none)						
No change	630 (93.8)	657 (97.8)	1		— [‡]	
Any change	42 (6.3)	15 (2.2)	3.08 [1.65, 5.75]	<0.001	—	
No change	655 (97.5)	664 (98.8)	1		—	
Catheter to fistula or graft	17 (2.5)	8 (1.2)	2.12 [0.92, 4.92]	0.079	—	
No change	637 (94.8)	663 (98.7)	1		—	
Fistula or graft to catheter	35 (5.2)	9 (1.3)	3.89 [1.87, 8.09]	<0.001	—	
Vascular access category (vs. no change) [§]						
No change	630 (93.8)	657 (97.8)	— [‡]		1	
Catheter to fistula or graft	17 (2.5)	8 (1.2)	—		1.50 [0.45, 4.99]	0.021
Fistula or graft to catheter	35 (5.2)	9 (1.3)	—		2.96 [1.36, 6.46]	
Dialysis adequacy (Kt/V)						
Q1 (low)	115 (18.0)	110 (17.4)	1			
Q2	171 (26.8)	154 (24.4)	1.09 [0.71, 1.65]	0.122		
Q3	147 (23.0)	175 (27.7)	0.74 [0.46, 1.20]			
Q4 (high)	205 (32.1)	192 (30.4)	1.09 [0.65, 1.84]			
Interdialytic weight change [kg]						
<0 (i.e. weight loss)	33 (6.1)	20 (3.6)	1.49 [0.75, 2.98]	0.013		
0–1	126 (23.3)	121 (21.9)	1			
2–3	184 (34.0)	168 (30.4)	1.02 [0.69, 1.51]			
3–4	130 (24.0)	167 (30.2)	0.60 [0.39, 0.92]			
≥4 (high weight gain)	68 (12.6)	77 (13.9)	0.63 [0.37, 1.09]			
Hospitalization (vs. none)						
None	506 (75.3)	584 (86.9)	1		1	
Any	166 (24.7)	88 (13.1)	3.17 [2.18, 4.61]	<0.001	2.95 [1.99, 4.36]	<0.001
C-Reactive Protein [mg/L]						
Q1 (low)	95 (20.9)	104 (24.1)	1		1	
Q2	98 (21.6)	91 (21.1)	1.20 [0.74, 1.95]	0.004	1.31 [0.84, 2.05]	0.012
Q3	102 (22.5)	126 (29.2)	1.01 [0.57, 1.80]		1.10 [0.67, 1.80]	
Q4 (high)	159 (35.0)	110 (25.5)	2.44 [1.30, 4.57]		2.02 [1.20, 3.38]	
Ferritin [μg/L]						
<100	40 (6.6)	52 (8.8)	1		1	
100–<500	271 (44.9)	295 (49.8)	1.26 [0.67, 2.37]	0.005	1.52 [0.84, 2.76]	0.006
500–799	148 (24.5)	133 (22.5)	2.19 [1.07, 4.49]		2.21 [1.14, 4.31]	
≥800	144 (23.9)	112 (18.9)	3.05 [1.36, 6.85]		3.46 [1.64, 7.27]	
TSAT [%]						
<20	297 (67.5)	300 (69.9)	1.19 [0.81, 1.74]	0.38		
≥20	143 (32.5)	129 (30.1)	1			
Serum albumin [g/dL]						
Q1 (high)	71 (12.7)	95 (17.0)	1			
Q2	156 (27.9)	164 (29.4)	1.63 [0.93, 2.85]	0.026		
Q3	139 (24.9)	126 (22.6)	2.26 [1.20, 4.24]			
Q4 (low)	193 (34.5)	173 (31.0)	2.78 [1.40, 5.52]			
Cholesterol [mmol/L]						
Q1 (low)	167 (32.8)	153 (30.4)	1.57 [0.85, 2.88]	0.031		
Q2	126 (24.8)	114 (22.6)	1			
Q3	115 (22.6)	134 (26.6)	0.56 [0.34, 0.93]			
Q4 (high)	101 (19.8)	103 (20.4)	0.62 [0.32, 1.20]			
Calcium [mg/dL]						
<8.4	103 (15.7)	109 (17.0)	0.77 [0.50, 1.18]	0.177		
8.4–9.5	374 (56.8)	342 (53.4)	1			
>9.5	181 (27.5)	190 (29.6)	0.76 [0.53, 1.08]			
Phosphate [mg/dL]						
<3.5	130 (20.2)	114 (18.0)	1.44 [0.96, 2.18]	0.071		
3.5–5.5	354 (55.0)	346 (54.6)	1			
>5.5	160 (24.8)	174 (27.4)	0.78 [0.55, 1.11]			
PTH [pg/mL]						
<75	99 (21.2)	93 (20.1)	1.34 [0.68, 2.64]	0.028		
75–<150	115 (24.6)	103 (22.2)	1.54 [0.90, 2.64]			

(Continues)

Table 1. (Continued)

Parameters	Period		Univariate		Multivariate	
	Case	Control	OR* [95%CI [†]]	p-value	OR [95%CI]	p-value
150–300	112 (23.9)	108 (23.3)	1			
300–<600	78 (16.7)	96 (20.7)	0.82 [0.50, 1.37]			
600–<800	24 (5.1)	15 (3.2)	2.59 [0.76, 8.81]			
>800	40 (8.5)	48 (10.4)	0.33 [0.10, 1.11]			

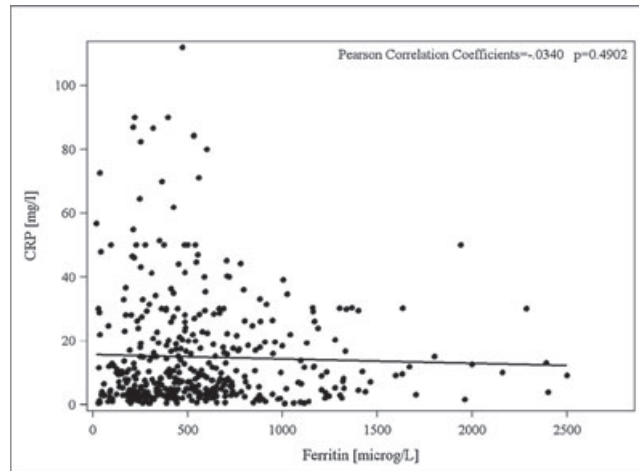
*Conditional odds ratio.

[†]Confidence interval.[‡]Not estimated in this analysis.[§]Where patients experienced both changes 'graft/fistula to catheter' takes precedence over 'catheter to graft/fistula'; quartiles thresholds are provided in Table S1 in the Supporting Information.

opposite direction had no effect on the likelihood of hyporesponsiveness. Increasing levels of ferritin and C-reactive protein and decreasing levels of

serum albumin increased the likelihood of hyporesponsiveness. During 'case' hyporesponsive periods, ferritin was not correlated with CRP, and only weakly correlated with serum albumin (Figure 2). Overall, interdialytic weight gain appeared to reduce the risk, but only a single strata was significant. When considered in a multivariate analysis, vascular access change, hospitalization, CRP and ferritin were associated with a transition to ESA hyporesponsiveness. Of these factors, high ferritin levels made the greatest overall contribution to transitioning to hyporesponsiveness, with Population Attributable Risk Fractions for the 500–799 and ≥ 800 $\mu\text{g/L}$ strata of 13.4% and 17.0%, respectively. High CRP levels (upper quartile) also contributed greatly (17.7%), as did hospitalization (16.3%), whilst a vascular access change from a graft or fistula to a catheter made a small contribution (3.5%). Combined, these factors accounted for 67.9% of all transitions to hyporesponsiveness in this study.

a) Ferritin vs. CRP



b) Ferritin vs. serum albumin

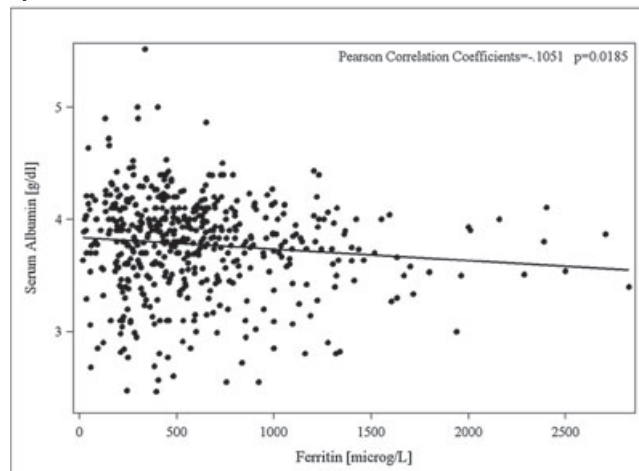


Figure 2. Relationship between (a) ferritin and CRP and (b) ferritin and serum albumin in 'case' ESA hyporesponsive periods in a cohort of European haemodialysis patients. Data trimmed at the 1% and 99% levels

Predictive factors for reversal of hyporesponsive status

The analyses examining factors predictive of reversal of hyporesponsive status mirrored the hyporesponsiveness analysis to an extent, in that hospitalization was inversely associated with transitioning from hyporesponsive to responsive (i.e. remaining in hospital increased the likelihood of remaining hyporesponsive; Table 2). Similarly, a change in vascular access from an AV fistula or a graft to a catheter was associated with remaining hyporesponsive. Conversely, no association was observed with levels of ferritin or serum albumin. Instead, reversibility was weakly associated with cholesterol concentrations and strongly associated with levels of parathyroid hormone. Of these factors, only hospitalization, cholesterol and parathyroid hormone were independently associated with a lack of reversibility from a hyporesponsive state.

Sensitivity analyses

Varying the definition of ESA response with different combinations of ESA exposure cut-off and/or haemoglobin thresholds had little effect on the findings (Table 3). Results were generally consistent across the different analyses in terms of the magnitude and direction of the conditional odds ratios generated, but it is possible that the smaller sample size in the final analysis (upper quartile of ESA dose; 10 g/dL haemoglobin response) may have reduced precision. Findings did not differ by gender (data not shown).

DISCUSSION

Although there have been previous studies investigating ESA hyporesponsiveness in haemodialysis patients, to our knowledge, this is the first time that case-crossover methodology has been applied to identify factors associated with transition to this status and also the first time that a time-lag model has been applied to account for the inevitable delay between changes in ESA dose and subsequent haemoglobin response. Furthermore, no previous studies have examined hyporesponsiveness reversibility in detail. Finally, this study is the first attempt to calculate Population Attributable Risk Fractions in a European haemodialysis cohort, examining factors independently associated with transition to hyporesponsiveness.

The findings from previous studies on ESA hyporesponsiveness, which focused on between-patient comparisons, have the potential to be influenced by underlying prognostic differences that exist between comparison groups. In case-crossover studies, however, patients act as their own control, and the subsequent within-patient comparisons afford a unique opportunity to investigate factors associated with the transition to/from a hyporesponsive state whilst largely eliminating confounding-by-indication. The fact that risk factors for hyporesponsiveness identified in this study concur with previous studies (vascular access changes,^{15,20} hospitalization,^{15,21} inflammation,^{22–26} malnutrition^{23,27}) strengthens the consistency of our analysis. The fact that these are independent risk factors for poorer outcomes is indicative of a worsening clinical state, suggesting that ESA hyporesponsiveness is a risk *marker* rather than a risk *factor*.

The association between high CRP, low serum albumin values and ESA hyporesponsiveness in the current study confirms the major role of inflammation in hyporesponsiveness.^{23,24,27,44} Conversely, iron deficiency—a long-recognized ESA hyporesponsiveness determinant^{18,19}—was not identified as a risk factor.

Instead, higher ferritin values were associated with hyporesponsiveness, and no relationship with TSAT was apparent. Ferritin and TSAT are highly modified by inflammation,⁴⁵ however, potentially diminishing their roles as markers of iron metabolism and/or predictors of ESA responsiveness.^{46,47} Thus, high ferritin values in our study are more likely to be indicative of inflammation, rather than as a marker of iron load and/or availability for erythropoiesis. Alternatively, the medium- to long-term characteristics of iron deficiency may not be captured by the shorter term case-crossover study design employed. The total contribution of recognized risk factors amounted to 68%, meaning that approximately one-third of factors are unexplained.

In the same patient cohort, factors predicting reversibility of ESA hyporesponsiveness were also examined. On univariate analysis, vascular access change, changing vascular access from a fistula or graft to a dialysis catheter, and any hospitalization all predicted an approximately 60% reduction in the chances of achieving reversibility. On multivariate analysis, only the association with hospitalization remained independent. Because such an approach has to our knowledge not previously been conducted, we cannot compare these findings with those of others. However, because they largely mirror those from the hyporesponsiveness analysis, they provided additional methodological validation for the observed links.

We applied a novel method of defining ESA responsiveness based on the median dose threshold, despite the existence of a number of methods for defining this disease status.^{13,48} Previous methods^{13,48} were not applicable in the current study as the case-crossover methodology necessitates a dynamic method of defining response over time and hence cannot rely on baseline measurements. A definition based on the ratio of ESA dose to haemoglobin levels was not considered, as such metrics do not consider the magnitude of the haemoglobin response. The sensitivity analyses using different cut-offs for ESA dose (median dose 80.8 IU/kg per week; upper quartile dose 140.4 IU/kg per week) and haemoglobin response (cut-offs 9 and 10 g/dL) confirmed the robustness of the approach and did not alter the overall results and conclusions. The time-lag employed in our definition may be impractical in routine clinical practice. It advocates, however, for monitoring ESA responsiveness in a time-dependent manner through available methods including measurement of the reticulocyte count, new reticulocyte indices⁴⁹ or ESA responsiveness index.¹⁴

There were, however, a number of limitations inherent in these analyses. We considered only patients' first observed period of hyporesponsiveness, despite

Table 2. Risk factors for ESA hyporesponsiveness reversibility in a cohort of European Haemodialysis patients ($N=711$) [univariate and multivariate analysis]

Parameters	Periods		Univariate		Multivariate	
	Case	Control	OR* [95%CI [†]]	<i>p</i> -value	OR [95%CI]	<i>p</i> -value
Vascular access change (vs. none)						
No change	686 (96.5)	669 (94.1)	1		— [‡]	
Any change	25 (3.5)	42 (5.9)	0.45 [0.24, 0.85]	0.014	—	
No change	693 (97.5)	689 (96.9)	1		—	
Catheter to fistula or graft	18 (2.5)	22 (3.1)	0.81 [0.43, 1.53]	0.517	—	
No change	699 (98.3)	682 (95.9)	1		—	
Fistula or graft to catheter	12 (1.7)	29 (4.1)	0.39 [0.20, 0.79]	0.009	—	
Dialysis adequacy (Kt/V)						
Q1 (low)	125 (18.3)	133 (19.6)	1			
Q2	181 (26.5)	161 (23.8)	1.30 [0.86, 1.94]	0.317		
Q3	181 (26.5)	176 (26.0)	1.14 [0.71, 1.82]			
Q4 (high)	197 (28.8)	207 (30.6)	0.89 [0.51, 1.53]			
Interdialytic weight change [kg]						
<0 (i.e. weight loss)	30 (5.2)	33 (5.8)	0.83 [0.44, 1.56]	0.329		
0–1	145 (25.0)	132 (23.0)	1			
2–3	173 (29.8)	198 (34.6)	0.78 [0.54, 1.12]			
3–4	141 (24.3)	132 (23.0)	1.03 [0.68, 1.58]			
≥4 (high weight gain)	92 (15.8)	78 (13.6)	1.18 [0.70, 1.98]			
Hospitalization (vs. none)						
None	592 (83.3)	529 (74.4)	1		1	
Any	119 (16.7)	182 (25.6)	0.45 [0.32, 0.62]	<0.001	0.45 [0.32, 0.63]	<0.001
C-Reactive Protein [mg/L]						
Q1 (low)	87 (18.6)	91 (19.7)	1			
Q2	87 (18.6)	98 (21.3)	0.93 [0.54, 1.60]	0.434		
Q3	132 (28.2)	113 (24.5)	1.41 [0.78, 2.52]			
Q4 (high)	162 (34.6)	159 (34.5)	1.21 [0.66, 2.21]			
Ferritin [μg/L]						
<100	72 (11.2)	62 (10.0)	1			
100–<500	278 (43.2)	273 (44.1)	0.80 [0.45, 1.45]	0.841		
500–799	140 (21.7)	144 (23.3)	0.76 [0.38, 1.52]			
≥800	154 (23.9)	140 (22.6)	0.86 [0.39, 1.90]			
Transferrin Saturation (TSAT) [%]						
<20	283 (57.6)	273 (60.3)	1.15 [0.81, 1.63]	0.425		
≥20	208 (42.4)	180 (39.7)	1			
Serum albumin [g/dL]						
Q1 (high)	87 (14.0)	82 (13.8)	1			
Q2	164 (26.4)	154 (25.8)	0.97 [0.61, 1.54]	0.337		
Q3	150 (24.2)	133 (22.3)	0.91 [0.53, 1.58]			
Q4 (low)	220 (35.4)	227 (38.1)	0.67 [0.38, 1.20]			
Cholesterol [mmol/L]						
Q1 (low)	222 (40.1)	172 (33.1)	1.46 [0.89, 2.40]	0.099	1.78 [1.19, 2.67]	0.013
Q2	125 (22.6)	133 (25.6)	1		1	
Q3	118 (21.3)	119 (22.9)	0.77 [0.47, 1.25]		1.02 [0.67, 1.54]	
Q4 (high)	89 (16.1)	96 (18.5)	0.60 [0.32, 1.14]		0.93 [0.55, 1.58]	
Calcium [mg/dL]						
<8.4	109 (16.0)	128 (18.7)	0.74 [0.50, 1.10]	0.115		
8.4–9.5	377 (55.2)	375 (54.7)	1			
>9.5	197 (28.8)	183 (26.7)	1.29 [0.90, 1.85]			
Phosphate [mg/dL]						
<3.5	142 (20.8)	151 (22.1)	0.88 [0.60, 1.28]	0.281		
3.5–5.5	360 (52.6)	369 (54.0)	1			
>5.5	182 (26.6)	163 (23.9)	1.27 [0.91, 1.78]			
PTH [pg/mL]						
<75	115 (23.2)	100 (20.8)	1.21 [0.64, 2.29]	0.032	0.96 [0.58, 1.61]	0.044
75–<150	106 (21.4)	111 (23.1)	0.98 [0.60, 1.61]		0.80 [0.52, 1.24]	
150–<300	124 (25.0)	106 (22.1)	1		1	
300–<600	96 (19.4)	85 (17.7)	0.76 [0.43, 1.34]		0.81 [0.50, 1.30]	
600–<800	24 (4.8)	30 (6.3)	0.26 [0.10, 0.71]		0.46 [0.22, 0.95]	
>800	31 (6.3)	48 (10.0)	0.16 [0.05, 0.51]		0.26 [0.12, 0.58]	

*Odds ratio.

†Confidence interval.

‡Not estimated in this analysis; quartiles thresholds are provided in Table S1 of the Supporting Information.

Table 3. Risk factors for ESA hyporesponsiveness in a cohort of European haemodialysis patients when applying different cut-offs for ESA dose and haemoglobin (univariate analysis)

ESA cut-off [UI/kg/week]:	Conditional odds ratio [95% confidence intervals]			
	Median [80.8]		Upper quartile [140.4]	
	10	9	10	9
Hb cut-off [g/dL]:				
<i>N</i>	672	301	359	181
Vascular access change (vs. none)				
No change	1	1	1	1
Any change	3.08 [1.65, 5.75]	3.40 [1.25, 9.22]	2.20 [1.04, 4.65]	2.25 [0.69, 7.31]
Catheter to fistula or graft				
No change	1	1	1	1
No change	2.12 [0.92, 4.92]	1.75 [0.51, 5.98]	1.57 [0.61, 4.05]	2.00 [0.50, 8.00]
Fistula or graft to catheter				
No change	1	1	1	1
Fistula or graft to catheter	3.89 [1.87, 8.09]	3.75 [1.24, 11.30]	2.71 [1.14, 6.46]	1.75 [0.51, 5.98]
Dialysis adequacy (Kt/V)				
Q1 (low)	1	1	1	1
Q2	1.09 [0.71, 1.65]	0.65 [0.34, 1.23]	1.04 [0.56, 1.91]	0.63 [0.26, 1.55]
Q3	0.74 [0.46, 1.20]	0.58 [0.28, 1.21]	0.67 [0.33, 1.34]	0.69 [0.26, 1.84]
Q4 (high)	1.09 [0.65, 1.84]	0.78 [0.35, 1.76]	0.84 [0.39, 1.80]	0.63 [0.22, 1.85]
Interdialytic weight change [kg]				
<0 (i.e. weight loss)	1.49 [0.75, 2.98]	0.92 [0.37, 2.30]	0.54 [0.19, 1.55]	1.42 [0.42, 4.75]
0-1	1	1	1	1
2-3	1.02 [0.69, 1.51]	0.52 [0.28, 0.97]	0.84 [0.49, 1.44]	0.85 [0.38, 1.87]
3-4	0.60 [0.39, 0.92]	0.89 [0.45, 1.77]	0.77 [0.43, 1.40]	1.20 [0.50, 2.90]
≥4 (high weight gain)	0.63 [0.37, 1.09]	0.78 [0.35, 1.76]	0.85 [0.40, 1.80]	1.26 [0.46, 3.46]
Hospitalization (vs. none)				
None	1	1	1	1
Any	3.17 [2.18, 4.61]	2.41 [1.47, 3.96]	2.81 [1.71, 4.62]	2.62 [1.38, 4.96]
C-Reactive Protein [mg/L]				
Q1 (low)	1	1	1	1
Q2	1.20 [0.74, 1.95]	1.68 [0.69, 4.11]	1.53 [0.75, 3.15]	1.57 [0.46, 5.33]
Q3	1.01 [0.57, 1.80]	2.34 [0.87, 6.24]	0.94 [0.40, 2.20]	1.43 [0.42, 4.90]
Q4 (high)	2.44 [1.30, 4.57]	4.13 [1.35, 12.61]	2.04 [0.81, 5.14]	2.93 [0.75, 11.38]
Ferritin [µg/L]				
<100	1	1	1	1
100-500	1.26 [0.67, 2.37]	1.54 [0.60, 3.96]	1.38 [0.64, 2.98]	1.30 [0.45, 3.73]
500-799	2.19 [1.07, 4.49]	2.86 [0.95, 8.64]	1.92 [0.77, 4.78]	3.15 [0.80, 12.39]
≥800	3.05 [1.36, 6.85]	4.36 [1.24, 15.29]	3.66 [1.25, 10.67]	8.55 [1.57, 46.53]
TSAT [%]				
<20	1.19 [0.81, 1.74]	1.71 [0.89, 3.31]	1.60 [0.92, 2.80]	2.12 [0.92, 4.92]
≥20	1	1	1	1
Serum albumin [g/dL]				
Q1 (high)	1	1	1	1
Q2	1.63 [0.93, 2.85]	1.20 [0.53, 2.73]	1.62 [0.70, 3.75]	0.33 [0.09, 1.18]
Q3	2.26 [1.20, 4.24]	1.26 [0.51, 3.12]	2.00 [0.78, 5.15]	0.44 [0.12, 1.66]
Q4 (low)	2.78 [1.40, 5.52]	1.61 [0.62, 4.18]	2.36 [0.89, 6.26]	0.81 [0.22, 3.07]
Cholesterol [mmol/L]				
Q1 (low)	1.57 [0.85, 2.88]	2.23 [0.91, 5.46]	1.55 [0.67, 3.59]	2.50 [0.79, 7.95]
Q2	1	1	1	1
Q3	0.56 [0.34, 0.93]	0.60 [0.27, 1.32]	0.55 [0.27, 1.15]	0.42 [0.14, 1.21]
Q4 (high)	0.62 [0.32, 1.20]	0.42 [0.14, 1.27]	0.88 [0.34, 2.31]	0.43 [0.10, 1.86]
Calcium [mg/dL]				
<8.4	0.77 [0.50, 1.18]	1.06 [0.59, 1.93]	0.75 [0.42, 1.35]	1.64 [0.77, 3.45]
8.4-9.5	1	1	1	1
>9.5	0.76 [0.53, 1.08]	0.65 [0.37, 1.14]	0.79 [0.48, 1.29]	0.55 [0.26, 1.19]
Phosphate [mg/dL]				
<3.5	1.44 [0.96, 2.18]	2.35 [1.20, 4.61]	1.13 [0.60, 2.13]	1.88 [0.70, 5.03]
3.5-5.5	1	1	1	1
>5.5	0.78 [0.55, 1.11]	0.50 [0.27, 0.93]	0.83 [0.50, 1.38]	0.52 [0.23, 1.16]
PTH [pg/mL]				
<75	1.34 [0.68, 2.64]	1.73 [0.67, 4.43]	2.63 [0.89, 7.78]	2.95 [0.81, 10.82]

(Continues)

Table 3. (Continued)

ESA cut-off [UI/kg/week]:	Conditional odds ratio [95% confidence intervals]			
	Median [80.8]		Upper quartile [140.4]	
	10	9	10	9
Hb cut-off [g/dL]:				
<i>N</i>	672	301	359	181
75–<150	1.54 [0.90, 2.64]	1.23 [0.55, 2.73]	3.03 [1.32, 6.98]	1.42 [0.51, 3.92]
150–300	1	1	1	1
300–<600	0.82 [0.50, 1.37]	1.43 [0.62, 3.26]	0.92 [0.46, 1.85]	1.49 [0.56, 3.95]
600–<800	2.59 [0.76, 8.81]	1.67 [0.38, 7.39]	0.83 [0.18, 3.91]	1.10 [0.19, 6.37]
>800	0.33 [0.10, 1.11]	0.39 [0.06, 2.40]	0.20 [0.05, 0.88]	0.36 [0.05, 2.72]

Quartiles thresholds are provided in Table S1 in the Supporting Information.

the fact that several had multiple periods of hyporesponsiveness. Similarly, only a single preceding ‘control’ period of response was considered even though many patients experienced multiple preceding response periods. The inclusion of additional periods may have facilitated the analyses as additional statistical power provided may have improved precision. Alternatively, a single hyporesponsiveness period might increase the risk of further periods of hyporesponsiveness, and this could confound any such analyses. Furthermore, the inclusion of multiple control periods would assume that each was independent of the next, which may not be the case. Earlier analyses (data not shown), considering multiple periods of hyporesponsiveness and utilizing all available control periods, gave similar findings to those in the current study but with attenuated effect estimates, suggesting one or both of the above scenarios was the case. On a related matter, we cannot establish in this mixed incident/prevalent cohort if those patient on ESA therapy at study start had not experienced periods of ESA hyporesponsiveness prior to entering the cohort and, hence, whether their characteristics would differ from those patients experiencing a hyporesponsive event for the first time. A study focusing on ESA-naïve patients would be able to rule out such left censoring but might be less feasible in the haemodialysis setting as renal anaemia commences in CKD before the need for renal replacement therapy.

Study outcomes were defined on the basis of contiguous periods of ESA exposure and haemoglobin response data, and therefore, the reported prevalence of hyporesponsiveness in this cohort will undoubtedly represent an underestimation due to missing data. Such misclassification may have affected the findings if the availability of data for either parameter is related to the condition under investigation. For example, if a patient is hospitalized elsewhere due to factors precipitating hyporesponsiveness, then results of laboratory testing undertaken at this time might not be captured

at a tertiary dialysis center. Data on both ESA dosing and haemoglobin levels were missing for over half the periods with missing data, suggesting an association between lack of capture for these variables. Missingness increased with length of follow-up, however, and therefore by capturing and investigating the first period of hyporesponsiveness as described above, we may have minimized the potential for bias. Explanatory variables, whilst comprehensive, were not exhaustive, and therefore, other unmeasured factors may contribute to hyporesponsiveness but were not assessed in the current study.

The effect of commonly prescribed medications was not included in the final analysis. Initial investigations revealed a near-universal ‘protective effect’, in that medication use appeared to be associated with a decreased risk of ESA hyporesponsiveness. It was hypothesized that this reflected the withdrawal of long-term medication in those patients who experienced the acute conditions that precipitated ESA hyporesponsiveness. Subsequent comparisons of hyporesponsive periods (medications coded ‘0’) with preceding periods of responsiveness (medications coded ‘1’) revealed an inverse association with their use. Similarly, this was the rationale for not introducing prescribed dialysis parameters (session frequency, dialysis dose, etc.) in the analysis.

In summary, we have applied a novel methodology to investigate factors predicting hyporesponsiveness to ESA therapy, as well as reversibility of a hyporesponsiveness episode, and have identified a number of factors using this approach. The major factors identified were vascular access changes, hospitalizations and underlying inflammation and malnutrition. Because vascular access changes and hospital admissions are usually unavoidable and causes of inflammation are unpredictable, it would appear, therefore, that clinicians are fairly powerless to prevent the major causes of hyporesponsiveness to ESA therapy.

Clearly, hematinic deficiencies and some causes of blood loss are indeed reversible and should continue to be excluded in any patients becoming hyporesponsive to ESA therapy. Chronic inflammation remains a major cause of hyporesponsiveness to ESA therapy, and investigation for an underlying reversible cause should be mandatory in any patient developing ESA resistance. Treatment of infectious causes is obvious when identifiable; whether strategies to reduce inflammatory mediators are beneficial in the absence of infection remain unproven.⁵⁰ Finally, one-third of hyporesponsive events cannot be explained by the risk factors assessed in this study, and thus, a means of assessing ESA hyporesponsiveness in a time-dependent manner may be helpful.

CONFLICT OF INTEREST

I.M.D. has received speakers' fees, honoraria and consultancy fees from several ESA and IV iron manufacturers, including Affymax, AMAG, Amgen, Ortho Biotech, Pharmacosmos, Roche, Takeda and Vifor Pharma. He has received consultancy fees and reimbursements for travel and accommodation expenses from Amgen related to the ARO initiative. K.U.E. has received speaker's fees and consultancy fees from several ESA and IV iron manufacturers, including Affymax, Amgen, Bayer, Johnson & Johnson, Roche and Vifor Pharma and a research grant from Amgen. He has received consultancy fees and reimbursements for travel and accommodation expenses from Amgen related to the ARO initiative. I. A. G., M. F. and S. R. are full-time Amgen employees. V. J. is a contractor to Amgen.

KEY POINTS

- Hyporesponsiveness to erythropoiesis-stimulating agents (ESAs) has important clinical and economic consequences for the treatment of renal anaemia. Previous research has focused on baseline predictors of ESA hyporesponsiveness.
- Case-crossover methodology was applied for the first time to identify factors associated with transition to an ESA hyporesponsive state and its reverse.
- The factors associated with transitioning to ESA hyporesponsiveness are similar to those identified through baseline analyses, suggesting that ESA hyporesponsiveness is an indicative marker of a worsening clinical state. Response to ESA therapy should be monitored in a continuous time-dependent manner.

ETHICS STATEMENT

All ethical and regulatory obligations concerning the use of patient data were met at each participating FMC site.

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