Prediction of Resistance to Erythropoiesis Stimulating Agent Therapy in Hemodialysis Patients

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Abstract. *Background and objective: The present standard of treatment of renal anemia is erythropoiesis stimulating agents (ESA) and intravenous (IV) iron preparations. Although the majority of hemodialysis (HD) patients have a good response to treatment with ESA, up to 25% of patients can be resistant to treatment with ESA, which has an important clinical and economic meaning: the studies indicate the relation between the resistance to ESA and worse clinical outcomes, increased cardiovascular morbidity and general mortality. Besides, ESA therapy is expensive and leads to enormous costs for health care systems. Therefore, methods on how to reduce the resistance to ESA and avoid unnecessary ESA consumption in the clinical practice are very necessary. The aim of this work was to provide prognostic factors for ESA resistance based on easily obtainable clinical parameters and routine laboratory markers, which allows accurate identification of HD patients at risk of ESA resistance.*

Materials and methods: The prospective study was conducted in all Kaunas city outpatient HD centers from January 1, 2010, to December 31, 2015. The study group consisted of 301 patients ill with the final stage of chronic kidney disease (CKD) who underwent outpatient HD procedures at least for 6 months before the inclusion into the study. Aiming to evaluate the demand for ESA depending on the degree of anemia, we calculated the erythropoietin resistance index (ERI) defined as a weekly dose of ESA for a kilogram of body weight (IU/kg/week) divided by Hb concentration (g/dL).

Results: During multivariate binary logistic regression analysis the most significant factors predicting resistance to ESA were female sex, BMI < 20 kg/m^2, cumulative iron dose > 3450 mg/year, TSAT < 22.5%, ferritin < 402.3 μg/L, phosphorus > 1.78 mmol/L, albumin < 39.6 g/L, CRP > 4.8 mg/L and the number of hospital days due to infection per year > 13.5. Diagnosis of diabetes mellitus was associated with a better response to ESA.

Conclusions: We suggest that routinely obtained data can be used in clinical practice to stratify patients according to the risk of ESA resistance, which may help to assign appropriate treatment strategies.

Introduction

Renal anemia is one of the principal chronic kidney disease (CKD) complications which develop due to the reduced erythropoietin production in the kidneys, inhibiting the effect of uremia on erythropoiesis, deficit in iron and folates, secondary hyperparathyroidism, shorter duration of existence of erythrocytes, and loss of blood during hemodialysis. Chronic anemia manifests itself in tiredness, reduced physical power, worsened attention concentration, symptoms of chest angina, and worse quality of the patients' life. Besides, anemia is one of the principal risk factors of morbidity with cardiovascular diseases and mortality from them in hemodialysis (HD) patients, and cardiovascular diseases is the most frequent cause of death of these patients. Thus, effective anemia correction for HD patients is especially important. The present standard of treatment of renal anemia is erythropoiesis stimulating agents (ESA) and intravenous (IV) iron preparations. Although the majority of HD patients have a good response to treatment with ESA, up to 25% of patients can be resistant to treatment with ESA [1–4], and that has and important clinical and economic meaning: studies indicate the relation between the resistance to ESA and worse clinical outcomes, increased cardiovascular morbidity and general mortality [5,6]. Besides, ESA therapy is expensive and leads to enormous costs for health care systems [7]. Therefore, methods on how to reduce the resistance to ESA and avoid unnecessary ESA consumption in the clinical practice are very necessary. They would both improve the clinical outcomes of HD patients and have a big economic effect.

Several factors have been described to promote ESA resistance in HD patients, namely, inflammation, malnutrition, secondary hyperparathyroidism, deplete iron stores, and vitamin D deficiency [7–9]. The aim of this work was to provide prognostic factors for ESA resistance based on easily obtainable clinical parameters and routine laboratory markers, which allows accurate identification of HD patients at risk of ESA resistance.

Materials and Methods

The prospective study was conducted at the Department of Nephrology of the Lithuanian University of Health Sciences after receiving the permissions of Kaunas Regional Biomedical Research Ethics Committee (minutes No. BE-2-40) and State Data Protection Inspectorate (No. 2R-7566).

The prospective study was conducted in all Kaunas city outpatient HD centers (Detoxification Department of Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Hemodialysis Department of Kaunas Clinical Hospital, limited liability company (LLC) *B.Braun Avitum*, LLC *Diaverum klinikos*) from January 1, 2010, to December 31, 2015. The study group consisted of 301 patient with end-stage renal disease (ESRD) who underwent outpatient HD procedures at least for 6 months before the inclusion into the study. A period of 6 months is considered to be sufficient aiming at the establishment of the maintaining dose of ESA from the beginning of treatment with HD. The patients were involved in the study and their data were collected in 2 stages: from January 1, 2010, to December 31, 2010, and from January 1, 2014, to December 31, 2014. After the collection of the data, the general analysis of the data of all patients was made.

For all the investigated patients, 3 times per week a standard bicarbonate HD of 3–4-hour duration was applied. Routine care of HD patients and prescription of medicines during the study were taken following the recommendations confirmed by the Lithuanian Ministry of Health. For all the patients, a study questionnaire was filled in. From the outpatient cards of the patients (form No. 025/a), the demographic data, information on the cause of ESRD, beginning of treatment with HD, vascular access, HD regimen, anamnesis of kidney transplantation, concurrent diseases, anamnesis of cardiovascular diseases and echocardiographic parameters (cardiac ultrasound for HD patients is performed once per year routinely; we evaluated mass of the left ventricle myocardium, index of myocardium mass, and ejection fraction), and markers of B and C hepatitis. During the observation of the patients, we also prospectively collected the data on their hospitalization (number, causes of hospitalizations, and number of hospital days).

According to everyday practice in Lithuania, routine tests for outpatient HD patients are performed once per month, and following the results of the tests, renal anemia treatment with ESA is corrected. The amount of ferritin is tested every 3 months and, according to it, the treatment with IV iron preparations is corrected. A transferrin saturation (TSAT) test is not included in the algorithm of renal anemia diagnosis and treatment in Lithuania; therefore, as a matter of routine, it is not conducted for all HD patients. According to the established internal order, it is conducted only in certain HD centers (LLC *Diaverum klinikos*, LLC *B.Braun Avitum*) or was expediently conducted during the period of our study (Department of Detoxification of Kauno Klinikos). We evaluated the parameters related to an HD procedure once per month on the day of plan tests (dialyzer, Kt/V, "dry" weight, and blood pressure before HD).

The specific documentation on the treatment of renal anemia with ESA and IV iron preparations was available in all Kaunas outpatient HD centers. For the patients treated with darbepoetin alfa, its dose was translated into international units (1 μg = 200 IU). Aiming to evaluate the demand for ESA depending on the degree of anemia, we calculated the erythropoietin resistance index (ERI) defined as a weekly dose of ESA for a kilogram of body weight (IU/kg/week) divided by Hb concentration (g/dL). We calculated the ERI every month during the whole 12 months of patient observation, and then we counted the ERI average during the period of the observed year. Taking into consideration the meanings of ERI average quartiles, the patients were divided into 4 groups: $Q_1 < 4.14$; Q_2 4.14–7.54; Q_3 7.55–12.63 and $Q_4 > 12.63$. The patients of ERI Q_4 group were evaluated as resistant to ESA. By applying the corresponding statistical methods, we compared the patients having a good response to ESA and resistant to ESA (ERI Q_4). Aiming to analyze the patients' data and evaluate the demand for ESA, taking into consideration the status of nutrition of HD patients, and employing the classification of the nutritional status of World Health Organisation (WHO), we divided the patients into 4 groups: patients of insufficient nutrition (BMI < 18.5 kg/m^2), normal nutrition (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2) and having obesity (BMI $\geqslant$ 30 kg/m^2). For the evaluation of the nutrition and inflammation state, the data on the quantities of urea, creatinine, cholesterol, albumin, total protein, C–reactive protein (CRP) in the blood serum and the averages of the year of observation of these parameters were evaluated. The left ventricular mass index (LVMI) was evaluated taking into consideration the patients' sex (for men > 130 g/m^2; for women > 112 g/m^2, following the *Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging*, 2015) [10].

Aiming to establish the threshold meanings of the variables, and predicting resistance to ESA (ERI Q_4), we applied the ROC analysis. The univariate and multivariate binary logistic regression analysis was also conducted.

The statistical data analysis was made employing the software package of data accumulation and analysis SPSS 22.0 (*Statistical Package for Social Science*

22 for Windows). Under nonhomogenous groups, the groups were randomized in the way of random selection. $P < 0.05$ was chosen as the significance level when checking the statistical hypotheses.

Results

The mean age (± standard deviation) of the subjects of our study (n = 301) was 63.2 ± 15.6 years, and 51.2% of them were men. The causes of ESRD of the patients were interstitial nephritis (29.2%, n = 88), vascular pathology (18.3%, n = 55), chronic glomerulonephritis (14.6%, n = 44), diabetes mellitus type 2 (10.3%, n = 31), diabetes mellitus type 1 (8.3%, n = 25), polycystic kidney disease (9.3%, n = 28), systemic diseases (4.7%, n = 14), and other reasons (5.3%, n = 16). The mean dialysis duration until the involvement into the study was 40.06 ± 41.25 months. Besides, 8.0% of the patients had kidney transplantation in the past and 2.0% of the patients still had a standing not eliminated nonfunctioning transplant. The majority of the patients (85.0%) had AVF as a vascular access. The average dialysis time was 683.32 ± 76.44 minutes per week and the mean spKt/V was 1.45 ± 0.18. For 20.8% of the patients, during all the 12 months of observation, high-flux membranes were used; for the rest of the patients, low-flux membranes were used or the type of them was changed in the course of a year. For 15 patients (5.0%), treatment with ESA was not prescribed during the course of the whole year. Another 107 patients (35.5%) used short action ESA. In 16 patients (5.3%), short action ESA was changed to darbepoetin alfa during the course of the observed year, and 163 patients (54.2%) took darbepotein alfa during the course of the whole year. For 85.3% of the patients who used ESA, IV was prescribed. The mean ESA weekly dose was 6941.65 ± 5032.62 IU/week (99.43 ± 74.32 IU/kg/week), and the mean ERI was 9.88 ± 8.11 IU/kg/week/g 100 mL. We compared the data obtained during the study in the patient group resistant to ESA (ERI Q_4) and in the group with a good response to ESA. During the evaluation of demographic data and the data related with hemodialysis procedure (age, sex, kidney transplantation present in the past, percent of the patients having a nonfunctioning transplant, duration of dialysis until inclusion into the study, time of dialysis (minutes per week), percent of the patients who used high-flux membranes for all the 12 months, average ultrafiltration, spKt/V, percent of patients having AVF), we did not establish statistically significant differences in the groups. Clinical and laboratory data of the patients who participated in the study, their comparison in groups of patients resistant to ESA and with a good response to ESA are presented in Table 1.

During the comparison of HD patients resistant to ESA and those who had a good response to ESA, we established that although the patients resistant to ESA received a statistically significantly higher ESA dose, they were more anemic, as they had a significantly lower Hb concentration, lower Ht and a lower number of erythrocytes. Besides, erythrocytes of the patients resistant to ESA were more hypochromic – their MCH was statistically significantly lower. The number of thrombocytes between the groups did not differ significantly. During the evaluation of iron metabolism, we established that the patients resistant to ESA had a significantly lower TSAT and ferritin, although the cumulative iron dose received during a year was significantly higher. During the evaluation of the inflammatory and nutrition status of the patients, we established that the patients resistant to ESA had statistically significantly lower BMI, albumin, total protein and higher CRP. The concentrations of urea, creatinine and cholesterol among the groups did not differ statistically significantly. During the evaluation of calcium-phosphorus metabolism, we established that the concentration of phosphorus was statistically significantly higher in the group of patients resistant to ESA. The amounts of PTH, calcium, corrected calcium and alkaline phosphatase between the groups did not differ statistically significantly. During the evaluation of concurrent diseases, we established that in the group of the patients resistant to ESA, there were statistically significantly fewer patients with diabetes mellitus (DM) in comparison with the group of the patients having a good response to ESA (12.9 vs. 26%; $P = 0.022$). The frequency of ischemic cardiac disease (67.6 vs. 66.5%; $P = 0.865$), arterial hypertension (88.4 vs. 85.9%; $P = 0.584$), cardiac insufficiency (84.5 vs. 81.3%; $P = 0.541$), oncological disease (10.0 vs. 5.7%; $P = 0.246$), viral liver disease (25.7 vs. 21.5%; $P = 0.681$), ulcer/erosion of intestine detected during the course of the investigated year (40.3 vs. 34.3%; $P = 0.460$), blood disease (4.3 vs. 2.3%; $P = 0.388$), chronic infectious/inflammatory disease (22.9 vs. 16.3%; $P = 0.212$), chronic obstructive pulmonary disease (COPD) (7.1 vs. 7.0%; $P = 0.962$), brain and peripheral vascular disease (14.3 vs. 29.8%; $P = 0.066$), and other concurrent diseases (67.1 vs. 66.6%; $P = 0.922$) between the groups did not differ statistically significantly.

The patients resistant to ESA had significantly more hospitalizations and hospital days during the observed year. In this group, there were also more patients who were hospitalized at least once during the course of the year (68.6 vs. 52.4%; $P = 0.016$). The evaluation of the hospital days according to causes showed that they had significantly more hospital days due to infection (7.12 ± 15.90 vs. 2.02 ± 5.79; $P = 0.013$), anemia correction (0.93 ± 2.84 vs. 0.02 ± 0.33; $P = 0.012$) and other causes (2.75 ± 7.58 vs. 1.56 ± 5.45; $P = 0.043$), and did not have hospital days due to DM complications

Table 1. Comparison of the data of patients resistant to ESA and having a good response to ESA

Feature	All the patients (n = 301)	Patients treated with ESA (n = 286)	Patients having good response to ESA (n = 215) ERI 1st, 2nd, 3rd quartiles	Patients resistant to ESA (n = 71) ERI 4th quartile	*P* value
Hematologic parameters					
Erythrocytes ($\times 10^{12}$/L)	3.54 ± 0.39	3.53 ± 0.39	**3.57 ± 0.36**	**3.39 ± 0.44**	**0.001**
Hemoglobin (g/L)	107.33 ± 9.2	106.72 ± 8.89	**108.76 ± 7.28**	**100.62 ± 10.34**	**< 0.001**
Hematocrit (%)	33.24 ± 3.24	33.08 ± 3.2	**33.60 ± 2.9**	**31.50 ± 3.62**	**< 0.001**
MCV (fL)	94.38 ± 6.82	94.35 ± 6.94	94.64 ± 6.96	93.57 ± 6.49	0.276
MCH (pg)	30.55 ± 2.77	30.52 ± 2.83	**30.77 ± 2.89**	**29.83 ± 2.30**	**0.020**
Iron metabolism					
Cumulative iron dose (in mg per year)	2447.13 ± 1203.19	2470.49 ± 1171.5	**2308.20 ± 1105.07**	**2966.10 ± 1371.71**	**0.001**
Ferritin (μg/L)	348.38 ± 136.23	349.76 ± 134.01	**359.19 ± 131.99**	**320.92 ± 136.94**	**0.044**
[a]Transferrin saturation (%)	30.27 ± 11.52	30.36 ± 11.5	**31.89 ± 11.43**	**26.43 ± 11.18**	**0.008**
Inflammatory and nutritional status					
BMI (kg/m^2)	26.30 ± 6.21	26.04 ± 5.87	**26.47 ± 5.93**	**24.73 ± 4.79**	**0.035**
Albumin (g/L)	37.89 ± 3.71	37.83 ± 3.70	**38.22 ± 3.58**	**36.65 ± 3.92**	**0.002**
Total protein (g/L)	66.97 ± 4.46	66.91 ± 4.43	**67.40 ± 4.30**	**65.46 ± 4.74**	**0.004**
CRP (mg/L)	12.45 ± 17.23	12.54 ± 17.47	**10.51 ± 14.98**	**18.73 ± 22.29**	**0.005**
Calcium - phosphorus metabolism					
Phosphorus (mmol/L)	1.74 ± 0.41	1.74 ± 0.40	**1.70 ± 0.37**	**1.84 ± 0.51**	**0.041**
Taking of ESA and mean doses					
Patients taking darbepoetin alfa for all the 12 mths., %	52.2	56.1	**51.7**	**68.6**	**0.006**
ESA used IV, %	81.8	85.3	85.1	85.9	0.869
ESA dose (IU/week)	6595.72 ± 5133.25	6941.65 ± 5032.62	**4590.54 ± 2425.3**	**13848.69 ± 4378.7**	**< 0.001**
ESA dose (IU/kg/week)	94.48 ± 75.61	99.43 ± 74.32	**63.71 ± 32.49**	**204.26 ± 64.74**	**< 0.001**
ERI (IU/kg/week/g 100 mL)	9.38 ± 8.19	9.88 ± 8.11	**5.96 ± 3.20**	**21.1 ± 7.4**	**< 0.001**
Concurrent diseases					
Diabetes mellitus, %	23.3	22.8	**26.0**	**12.9**	**0.022**

ERI values are divided into quartiles: Q_1 < 4.14; Q_2 4.14–7.54; Q_3 7.55–12.63; Q_4 > 12.63.
The quantitative variables are presented as average ± standard deviation and the categorical variables in percentage.
a: n = 162;
BMI, body mass index; CRP, C reactive protein; ESA, erythropoiesis stimulating agents; ERI, erythropoietin resistance index; *P* value, significance level.

(0.0 vs. 0.91 ± 4.64; $P = 0.005$). The frequency of hospital days due to complications of vascular access (1.18 ± 3.42 vs. 0.93 ± 3.78; $P = 0.708$), cerebrocardiovascular causes (2.51 ± 6.58 vs. 2.40 ± 8.72; $P = 0.841$), bleeding (0.90 ± 3.80 vs. 0.28 ± 1.51; $P = 0.208$) among the groups did not differ statistically significantly.

Aiming to compare the patients having the best (ERI Q_1) and the worst (ERI Q_4) response to ESA, we evaluated the data of the patients in each ERI quartile separately. We established that the patients of the ERI Q_4 group were significantly younger in comparison with the ERI Q_1 group patients (61.2 ± 14.6 vs. 68.1 ± 12.5 years, accordingly) and their BMI was significantly lower (24.73 ± 4.79 vs. 28.17 ± 6.02 kg/m^2, accordingly). In the ERI Q_4 group, statistically fewer patients had AVF as a vascular access for performing HD procedure (79.7% vs. 93.3%, accordingly) in comparison with the ERI Q_1 group. Among the patients of ERI Q_4, there were statistically significantly fewer patients with DM, for account of DM type 2, in comparison with the ERI Q_1 group (10.0 vs. 22.5%, accordingly). During the evaluation of cardiovascular parameters, we additionally established that the patients of the ERI Q_4 group had significantly higher diastolic blood pressure (DBP) in comparison with the ERI Q_1 group (78.41 ± 11.79 vs. 73.28 ± 9.22 mm Hg, accordingly). In the ERI Q_4 group, there were significantly more patients having a markedly increased left ventricular mass index (LVMI) (63.6% vs. 46.5%, accordingly). Although the amount of used antihypertensive medication in ERI quartile groups did not differ significantly, in the ERI Q_1 group, there was the least percentage of patients (35.3%) who took ACEI/ARB, and that significantly differed from the other 3 quartile groups of the patients. In terms of the laboratory data, the patients of the ERI

Q_4 group had statistically significantly smallest Hb concentration, Ht, number of erythrocytes, MCH, TSAT, concentrations of total protein and albumin, the highest CRP and phosphorus concentration. Although the patients of the ERI Q_4 group received the biggest cumulative iron dose (mg/year) in comparison with other 3 patient groups, the concentrations of ferritin among ERI quartile groups did not differ statistically significantly.

In order to analyze the patient data and evaluate the demand for ESA taking into consideration the nutritional status of HD patients, we evaluated the patient data in 4 groups: small weight patients (BMI < 18.5 kg/m^2), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2) and obese patients (BMI $\geqslant$ 30 kg/m^2). The patients in these groups did not differ statistically significantly according to age and sex. In the group of obese patients, there was the biggest percentage of patients with DM (42.2%; in the patient group selected randomly 47.8%) and DM type 2 (39.1%; in the patient group selected randomly 41.2%), and that was statistically significantly more in comparison with other 3 patient groups. The frequency of other concurrent diseases (ischemic cardiac disease, arterial hypertension, cardiac insufficiency, oncologic disease, viral liver disease, ulcer/erosion of the intestine detected in the course of the investigated year, blood disease, chronic infectious/inflammatory disease, COPD, brain and peripheral vascular disease) did not differ statistically significantly among the groups. Although in the group of small weight patients, the least share of patients had AVF as a vascular access (60%) in comparison with the patients of other 3 groups, their spKt/V was the highest (1.61 ± 0.21). The hematologic parameters between small weight, normal nutrition and obese patients did not significantly differ; however, for the patients of small weight, in spite of good dialysis quality (the highest spKt/V), a significantly higher ESA dose for a kilogram of body weight was necessary in order to achieve the same Hb level in comparison with other patient groups, and their ERI was the highest of all. The parameters of iron metabolism (MCV, MCH, ferritin, and TSAT) among the groups did not differ statistically significantly. The evaluation of the parameters of inflammatory/nutrition condition showed that the patients of small weight had the lowest concentration of total protein and the patients of normal nutrition had the least quantity of CRP and cholesterol; however, after randomizing the groups, we did not establish a significant difference among these parameters. The concentrations of albumin, urea, and creatinine also did not differ significantly among the groups. The frequency of hospitalizations and the number of hospital days per year also were not significantly different among the groups (Table 2).

To establish the threshold values of the factors predicting resistance to ESA (ERI Q_4), ROC analysis was applied. It was established that the prognosis indicators of the biggest sensitivity and specificity resistance to ESA were: MCH < 29.7 pg, ferritin concentration < 402.3 μg/L, TSAT < 22.5%, concentration of total protein < 68 g/L, concentration of albumin < 39.6 g/L, BMI < 20 kg/m^2, cumulative iron dose > 3450 mg/year, CRP > 4.8 mg/L, phosphorus concentration > 1.78 mmol/L, frequency of hospitalizations per year > 2.5, number of hospital days per year > 3, hospital days due to infection > 13.5, and hospital days due to other causes > 1. The univariate logistic regression analysis showed that TSAT < 22.5% had the biggest prognotic value of resistance to ESA, OR 5.236; 95% CI 2.47–11.12; $P < 0.001$. The data are presented in Table 3.

We used the multivariate logistic regression analysis to look for models significantly predicting resistance to ESA (ERI Q_4). During the first stage, we evaluated the demographic and clinical factors such as sex, age, BMI, presence or not of DM, vascular access for performing an HD procedure, use of ACEI/ARB, and a cumulative iron dose per year. In the first model, DM, BMI, and a cumulative iron dose per year remained significant factors predicting resistance to ESA (Model 1).

In the second stage, we included laboratory parameters which were significant in univariate analysis into multivariate logistic regression analysis. In the second model, BMI, sex and TSAT remained significant factors predicting resistance to ESA (Model 2).

In the third stage, we added statistically significant data on the hospitalizations/hospital days of the patients in the course of the observed year to the second model. The same factors remained statistically significant: BMI, sex, and TSAT (Model 3).

Taking into consideration all the regressors that significantly had predicted resistance to ESA in univariate logistic analysis and after evaluating their multi-colinearity, we arrived at the following models significantly predicting ERI Q_4 (Model 4–7).

During multivariate binary logistic regression analysis, the most significant factors predicting resistance to ESA were female sex, BMI < 20 kg/m^2, cumulative iron dose > 3450 mg/year, TSAT < 22.5%, ferritin < 402.3 μg/L, phosphorus > 1.78 mmol/L, albumin < 39.6 g/L, CRP > 4.8 mg/L and the number of hospital days due to infection per year > 13.5. DM diagnosis was associated with a better response to ESA.

Discussion

During this scientific study, the factors which statistically significantly worsened the response to treatment with ESA were established for the first time in Lithuania. As we cannot apply the defini-

Table 2. Comparison of clinical and laboratory data of the patients and demand for ESA taking into consideration the body mass index

Feature	BMI < 18.5 kg/m² n = 15	BMI 18.5–24.9 kg/m² n = 129 (n = 27)	BMI 25–29.9 kg/m² n = 93 (n = 24)	BMI ⩾ 30 kg/ m² n = 64 (n = 23)	F / χ^2; *P* value
Demographic data and data related with hemodialysis procedure					
Patients with DM, %	**0[a,c,e] (0)[a]**	**19.4 (14.8)[b]**	**19.6 (12.5)[d]**	**42.2 (47.8)**	$\chi^2 = 19.140^*$; [a,c,e] < 0.05 ($\chi^2 = 16.040^*$; [a,b,d] < 0.05)
Patients with DM type 2, %	**0[a,c,e] (0)[a]**	**3.9 (3.7)[b]**	**14.1 (13.6)[d]**	**39.1 (41.2)**	$\chi^2 = 54.933^*$; [a,c,e] < 0.05 ($\chi^2 = 50.753^*$; [a,b,d] < 0.05)
spKt/V	**1.61 ± 0.21[a] (1.61 ± 0.21)[a,e,c]**	**1.47 ± 0.17[e,b] (1.46 ± 0.11)[b]**	**1.42 ± 0.18[c] (1.42 ± 0.19)**	**1.39 ± 0.18 (1.39 ± 0.13)**	F = 7.158; [a,b,c,e] < 0.05 ($\chi^2 = 12.082$; [a,b,c,e] < 0.05)
Vascular access (AVF), %	**60.0[a,c,e] (60.0)[a,c,e]**	**84.4 (92.6)**	**88.2 (79.2)**	**87.5 (95.7)**	$\chi^2 = 8.440^*$; [a,c,e] < 0.05 ($\chi^2 = 10.796^*$; [a,c,e] < 0.05)
Use of ESA and mean doses					
ESA dose (IU/week)	6965.55 ± 5008.62 (6965.6 ± 5008.62)	6475.8 ± 5025.17 (5388.1 ± 4651.1)	7029.3 ± 5292.38 (8557.2 ± 5899.7)	6120.4 ± 5208.63 (5313.4 ± 5446.7)	$\chi^2 = 1.674$; 0.643 ($\chi^2 = 6.268$; 0.099)
ESA dose (IU/kg/week)	**146.07 ± 103.36[a] (146.07 ± 103.36)[a]**	**106.42 ± 81.75[b] (90.24 ± 77.42)**	**91.07 ± 67.11[c] (107.19 ± 75.16)[d]**	**63.27 ± 52.56[d] (54.41 ± 53.27)**	$\chi^2 = 20.129$; [a,b,c,d] < 0.05 ($\chi^2 = 12.038$; [a,d] < 0.05)
ERI (IU/kg/ week/g 100 mL)	**14.65 ± 11.86[a] (14.65 ± 11.86)[a]**	**10.67 ± 8.97[b] (8.98 ± 8.48)**	**8.98 ± 7.17[c] (10.6 ± 8.06)[d]**	**6.15 ± 5.39[d] (5.41 ± 5.86)**	$\chi^2 = 18.700$; [a,b,c,d] < 0.05 ($\chi^2 = 11.593$; [a,d] < 0.05)
Hematologic parameters					
Erythrocytes ($\times 10^{12}$/L)	3.44 ± 0.47 (3.44 ± 0.47)	3.54 ± 0.43 (3.5 ± 0.38)	3.55 ± 0.35 (3.55 ± 0.38)	3.55 ± 0.36 (3.61 ± 0.42)	F = 0.343; 0.794 ($\chi^2 = 1.429$; 0.699)
Hemoglobin (g/L)	106.08 ± 8.74 (106.08 ± 8.34)	107.03 ± 9.66 (107.53 ± 9.14)	107.21 ± 9.11 (106.51 ± 10.99)	108.39 ± 8.67 (108.29 ± 10.29)	F = 0.422; 0.737 ($\chi^2 = 0.755$; 0.860)
Hematocrit (%)	32.13 ± 3.81 (32.13 ± 3.81)	33.16 ± 3.17 (33.45 ± 2.77)	33.03 ± 2.77 (32.96 ± 3.03)	33.94 ± 3.76 (33.57 ± 3.45)	$\chi^2 = 2.757$; 0.431 ($\chi^2 = 1.485$; 0.686)
Inflammatory and nutritional status					
Total protein (g/L)	**63.86 ± 5.18[a]** (63.86 ± 5.18)	66.74 ± 4.35 (65.95 ± 5.39)	66.98 ± 4.48 (66.03 ± 4.05)	**68.04 ± 4.20** (67.98 ± 4.19)	F = 3.810; [a] < 0.05 ($\chi^2 = 5.568$; 0.135)
CRP (mg/L)	17.38 ± 25.65 (17.38 ± 25.65)	**11.42 ± 18.86[b, f]** (12.61 ± 23.58)	**13.17±18.62** (11.31±12.29)	**12.36±11.49** (10.56±9.71)	χ^2=9.707; [b,f]<0.05 (χ^2=0.759; 0.859)
Cholesterol (mmol/L)	4.77 ± 1.29 (4.77 ± 1.29)	**4.69 ± 1.11[b]** (4.82 ± 1.1)	4.88 ± 1.05 (4.85 ± 1.03)	**5.18 ± 1.0** (5.08 ± 0.97)	F = 2.901; [b] < 0.05 ($\chi^2 = 1.075$; 0.783)

The quantitative variables are presented as average ± standard deviation and the categorical variables in percentage.
Number of degrees of freedom = 3; In the brackets, the data of patients selected at random are presented.
P < 0.05 BMI < 18.5 kg/m² vs. BMI ⩾ 30 kg/ m²;
P < 0.05 BMI 18.5–24.9 kg/m² vs. BMI ⩾ 30 kg/ m²;
P < 0.05 BMI < 18.5 kg/m² vs. BMI 25–29.9 kg/ m²;
P < 0.05 BMI 25–29.9 kg/m² vs. BMI ⩾ 30 kg/ m²
P < 0.05 BMI < 18.5 kg/m² vs. BMI 18.5–24.9 kg/ m²;
P < 0.05 BMI 18.5–24.9 kg/m² vs. BMI 25–29.9 kg/ m²;
Fisher criterion (F) – employed when the distribution of quantitative value is normal;
Chi quadrate criterion (χ^2) following Kruskal-Wallis test – employed when the distribution of quantitative value is abnormal;
*Chi quadrate criterion (χ^2) – employed during the evaluation of difference between the qualitative values;
BMI, body mass index; DM, diabetes mellitus; AVF, arteriovenous fistula; ESA, erythropoiesis stimulating agents;
ERI, erythropoietin resistance index; CRP, C reactive protein; *P* value, significance level.

tions of the resistance to ESA indicated in the global recommendations [1,11], due to the limitations of the algorithm of renal anemia treatment valid in Lithuania [12], for the evaluation of resistance to ESA taking into consideration the ERI quartiles, we selected the patients belonging to the ERI Q_4 group considering that they are resistant to ESA (ERI > 12.63 IU/kg/week/g 100 mL). In this group, the mean ESA dose was 204.26 ± 64.74 IU/kg/ week. In the HD patients' sample, the ERI Q_4 group patients were reliably younger in comparison with the ERI Q_1 group patients. This corresponds to the data of the study described by Davit T. Gilbertson [13] indicating that black-skinned patients younger than 40 whose renal function insufficiency cause was not DM or polycystic kidney disease needed higher ESA doses. However, according to the data of Juan M. Lopez-Gomez and co-authors, older HD patients had reliably bigger ERI (*P* < 0.008) [4]. In Scott P. Sibbel's study [14], patients with established chronic resistance to ESA were reliably younger in comparison with patients with diagnosed acute resistance to ESA and with patients having a good response to ESA. Sex and race in this study did not differ statistically significantly between the groups. The study we have conducted revealed that female sex was related with higher ERI and in the multivariate logistic regression analysis significantly

Table 3. Values prognosticated by ROC tests and their characteristics and the data of univariate logistic regression analysis taking into consideration the groups of the investigated patients (ERI Q1-Q3 and ERI Q4.)

Factor/ Its threshold value	Area under ROC curve (%)	**Sensitivity/ Specificity (%)**	**ERI Q_1-Q_3/ ERI Q_4. (%)**	***P* value**	**ERI Q_4 gr. OR [95 % CI]**
BMI < 20 kg/m^2	57.7	44.4/77.7	22.3/44.4	0.005	2.793 [1.362–5.729]
Albumin < 39.6 g/L	56.7	31.1/85.3	14.7/31.1	0.002	2.634 [1.443–4.811]
Protein < 68 g/l	60.9	75.7/44.6	55.4/75.7	0.003	2.51 [1.365–4.617]
CRP > 4.8 mg/L	63.2	71.6/46.2	55.8/71.6	0.008	2.169 [1.228–3.833]
TSAT < 22.5%	**69.2**	54.5/81.4	18.6/54.5	< 0.001	**5.236 [2.466–11.117]**
Ferritin < 402.3 µg/L	59.1	81.4/38.3	61.7/81.4	0.002	2.724 [1.404–5.283]
MCH < 29.7 pg	61.7	52.9/68.8	31.2/52.9	0.001	2.483 [1.406–4.383]
Cumulative iron dose > 3450 mg/ year	63.9	37.3/86.2	13.8/37.3	< 0.001	3.705 [1.894–7.245]
Phosphorus > 1.78 mmol/L	60.7	56.8/61.8	38.2/56.8	0.006	2.121 [1.245–3.614]
Frequency of hospitalizations per year > 2.5	63.1	31.4/90.2	9.8/31.4	< 0.001	4.234 [2.153–8.327]
Hospital days per year > 3	62.5	61.6/58.0	42.0/61.6	0.004	2.216 [1.291–3.805]
Hospital days due to infection > 13.5	56.1	19.7/94.8	5.2/19.7	< 0.001	4.504 [1.91–10.623]
Hospital days due to other causes > 1	55.7	29.9/83.1	16.9/29.9	0.021	2.092 [1.11–3.945]

OR, odds ratio; CI, confidence interval; *P* value, significance level; ROC, receiver operating characteristic curve; BMI, body mass index; CRP, C reactive protein; TSAT, transferrin saturation.

Model 1

Clinical factor	OR [95% CI]	*P* value
DM (nondiabetic vs. diabetic patients)	**2.943 [1.009–8.586]**	**0.048**
BMI < 20 kg/m^2	**3.598 [1.418–9.131]**	**0.007**
Sex (woman vs. man)	1.921 [0.910–4.057]	0.087
Age (years)	0.988 [0.973–1.020]	0.764
Vascular access (CVC vs. AVF)	1.196 [0.457–3.651]	0.715
ACEI/ARB (uses vs. does not use)	1.874 [0.885–3.965]	0.101
Cumulative iron dose > 3450 mg/year	**4.843 [2.097–11.186]**	**< 0.001**
Constant	0.042; *P* = 0.001	

Model **Nagelkerke R^2 = 0.254**, general prognosis percent **79.5.**
OR, odds ratio; CI, confidence interval; *P* value, significance level; BMI, body mass index;
CVC, central vein catheter; AVF, arteriovenous fistula;
ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers.

prognosticated resistance to ESA. This corresponds to the data of Lopez-Gomez and Gilbertson [4, 13]. Takahiro Kuragano and co-authors [15] have also established that female sex was a significant factor prognosticating high ERI and have explained that by the different mechanism of iron release from the reticuloendothelial cells among men and women. However, the data of the studies conducted by Andreas Schneider [7] and Vincenzo Panichi [16] have indicated that the patients belonging to ERI Q_4 were older and mostly men.

According to our data, in the ERI Q_4 group, there were significantly fewer patients with DM in comparison with the remaining patients (12.9 vs. 26%; $P = 0.022$). When every ERI quartile was evaluated separately, in the ERI Q_4 group there were significantly fewer patients with DM in comparison with ERI Q_1, Q_2 and Q_3 groups. During the evaluation

Model 2

Clinical and laboratory factor	OR [95% CI]	*P* value
DM (nondiabetic vs. diabetic patients)	4.175 [0.893–19.511]	0.069
BMI < 20 kg/m²	**4.695 [1.335–16.517]**	**0.016**
Sex (woman vs. man)	**4.864 [1.379–17.152]**	**0.014**
Age (years)	0.970 [0.937–1.005]	0.095
Vascular access (CVC vs. AVF)	1.568 [0.442–5.565]	0.487
ACEI/ARB (takes vs. does not take)	1.135 [0.377–3.417]	0.822
Cumulative iron dose > 3450 mg/year	2.699 [0.684–10.657]	0.156
MCH < 29.7 pg	2.127 [0.675–6.698]	0.197
Ferritin < 402.3 µg/L	2.261 [0.635–8.054]	0.208
TSAT < 22.5%	**4.670 [1.389–15.704]**	**0.013**
Albumin < 39.6 g/L	0.762 [0.201–2.893]	0.690
Protein < 68 g/L	0.864 [0.245–3.045]	0.820
CRP > 4.8 mg/L	2.242 [0.579–8.679]	0.242
Phosphorus > 1.78 mmol/L	2.917 [0.861–9.885]	0.086

Model **Nagelkerke R^2 = 0.589,** general prognosis percent **81.8.**
OR, odds ratio; CI, confidence interval; *P* value, significance level;
BMI, body mass index; CVC, central vein catheter; AVF, arteriovenous fistula;
ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers;
TSAT, transferrin saturation; CRP, C reactive protein.

Model 3

Clinical, laboratory factors and data of hospitalizations	OR [95% CI]	*P* value
DM (nondiabetic vs. diabetic patients)	3.099 [0.693–13.849]	0.139
BMI < 20 kg/m²	**4.496 [1.235–14.417]**	**0.003**
Sex (woman vs. man)	**4.140 [1.254–13.673]**	**0.020**
Age (years)	0.965 [0.930–1.002]	0.061
Vascular access (CVC vs. AVF)	1.209 [0.316–4.629]	0.782
ACEI/ARB (takes vs. does not take)	1.117 [0.365–3.420]	0.847
Cumulative iron dose > 3450 mg/year	2.523 [0.646–9.855]	0.183
MCH < 29.7 pg	2.107 [0.645–6.886]	0.217
Ferritin < 402.3 µg/L	2.329 [0.638–8.499]	0.201
TSAT < 22.5%	**5.268 [1.528–18.159]**	**0.009**
Albumin < 39.6 g/L	1.8 [0.607–5.355]	0.288
Protein < 68 g/L	1.3 [0.503–4.365]	0.188
CRP > 4.8 mg/L	1.740 [0.485–6.244]	0.395
Phosphorus > 1.78 mmol/L	2.604 [0.780–8.698]	0.120
Frequency of hospitalizations per year > 2.5	1.552 [0.238–10.127]	0.646
Hospital days per year > 3	1.371 [0.365–5.153]	0.640
Hospital days due to infection > 13.5	1.151 [0.155–8.549]	0.891
Hospital days due to other causes > 1	1.818 [0.431–7.669]	0.416

Model **Nagelkerke R^2 = 0.607**, general prognosis percent **84.3.**
OR, odds ratio; CI, confidence interval; *P* value, significance level;
BMI, body mass index; CVC, central vein catheter; AVF, arteriovenous fistula;
ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers;
TSAT, transferrin saturation; CRP, C reactive protein.

of every type of DM separately, we established that in the ERI Q_4 group there were significantly fewer patients with DM type 2 than in ERI Q_1 (10 vs. 22.5 %; $P < 0.05$). The DM diagnosis was a significant factor prognosticating less resistance to ESA in the multivariate logistic regression analysis as well. Such results can be explained by the fact that the patients with DM had a statistically significantly higher BMI (29.19 ± 7.71 vs. 25.41 ± 5.4; $P < 0.001$) in comparison with the patients without DM, and the BMI in the sample of the HD patients we studied was in reverse correlation with ERI. The hypothesis is

Model 4

Factor	OR [95% CI]	*P* value
BMI < 20 kg/m²	**4.695 [1.335–16.517]**	**0.016**
Sex (woman vs. man)	**3.258 [1.184–8.964]**	**0.022**
Vascular access (CVC vs. AVF)	0.997 [0.315–3.153]	0.996
ACEI/ARB (takes vs. does not take)	0.839 [0.306–2.296]	0.732
Age (years)	0.989 [0.957–1.021]	0.482
TSAT < 22.5%	**5.649 [1.964–16.249]**	**0.001**
Phosphorus > 1.78 mmol/L	**3.483 [1.229–9.869]**	**0.019**
Albumin < 39.6 g/L	1.803 [0.607–5.355]	0.288
Cumulative iron dose > 3450 mg/year	3.527 [1.093–11.386]	0.035
Constant	0.055; $P = 0.014$	

Model **Nagelkerke $R^2 = 0.398$;** general prognosis percent **81.2.**

Model 5

Factor	OR [95% CI]	*P* value
Albumin < 39.6 g/L	**2.024 [1.063–3.854]**	**0.032**
Ferritin < 402.3 µg/L	**3.200 [1.525–6.714]**	**0.002**
Phosphorus > 1.78 mmol/L	1.757 [0.971–3.181]	0.063
Hospital days due to infection > 13.5	4.874 [1.874–12.674]	0.001
Constant	0.054; $P < 0.001$	

Model **Nagelkerke $R^2 = 0.265$,** general prognosis percent **79.1.**

Model 6

Factor	OR [95% CI]	*P* value
Albumin < 39.6 g/L	**2.485 [1.327–4.656]**	**0.004**
Ferritin < 402.3 µg/L	**2.754 [1.395–5.436]**	**0.004**
Phosphorus > 1.78 mmol/L	**1.774 [1.009–3.118]**	**0.047**
Constant	0.065; $P < 0.001$	

Model **Nagelkerke $R^2 = 0.254$;** general prognosis percent **75.4.**

Model 7

Factor	OR [95% CI]	*P* value
BMI < 20 kg/m²	**2.148 [1.015–4.543]**	**0.046**
CRP > 4.8 mg/L	**2.060 [1.136–3.736]**	**0.017**
Phosphorus > 1.78 mmol/L	**1.842 [1.057–3.210]**	**0.031**
Constant	0.138; $P < 0.001$	

Model **Nagelkerke $R^2 = 0.247$**, general prognosis percent **75.0.**

that the fat tissue may autonomously modulate the response to ESA via autocrine regulation: leptin, higher in overweight patients, has shown to stimulate erythropoiesis [9]. The BMI of the patients with DM type 2 was 32.84 ± 7.37 kg/m², i.e., significantly higher than that of the patients without DM ($P < 0.001$). The BMI average of the patients with DM type 1 was 23.38 ± 3.55 kg/m² and did not significantly differ from those who were not ill with DM. Among the patients with BMI ⩾ 30 kg/m², patients with DM accounted for 42.2%; and after the randomization of the groups, in the group of patients with BMI ⩾ 30 kg/m², the patients with DM accounted for 47.8%; that was significantly more than in the other 3 BMI groups ($P = 0.001$). When evaluating DM types separately, in the group of patients with BMI ⩾ 30 kg/m², the patients with DM type 2 accounted for 39.1% (after the randomization of the groups 41.2 %), which was statistically significantly more in comparison with the other 3 BMI groups ($P < 0.001$). According to the data of Gilbertson [13], Kuragano [15], and Lopez-Gomez [4], the relation between DM and ERI has not been established. In the study conducted by Almudena Vega, the ERI Q_4 group patients were also younger, and had lower BMI; however, the relation between ERI and DM has not been proved as well [9].

Other comorbidity factors did not have influence on the resistance to ESA in our sample of HD patients. In Jiacong Luo's [17] study, among the patients resistant to ESA, there were statistically significantly more patients ill with an oncological disease (3.1 vs. 1.9%), COPD (5.0 vs. 3.8%), heart failure (14.5 vs. 12.0%), ischemic heart disease (7.6 vs. 7.0%), arterial hypertension (35.2 vs. 32.4%), and they had more episodes of bleeding from the digestive tract per year (1.5 vs. 1.0%). However, the frequency of DM between the groups did not differ. In Jarome Rossert's study [18], older age, higher BMI, taking of ACEI/ARB and DM as the cause of ESRD were related with a greater demand for epoetin. Gilbertson's study has revealed that heart failure, bleeding from the digestive tract, and oncological disease were significantly related with resistance to ESA [13]. According to his data, greater frequency of hospitalizations per year and hospitalizations due to infections were also statistically significantly related with resistance to ESA. In Lopez-Gomez's study, during the first observation month 19% of patients were hospitalized due to cardiovascular reasons and the ERI of the hospitalized patients was significantly greater ($P = 0.04$). Another 10.1% of patients were hospitalized due to other causes, and their ERI was also significantly greater ($P< 0.001$). 5 % of patients during the first month of observation had the signs of acute infection, and their ERI was significantly greater than that of the remaining patients ($P < 0.001$). The patients with an oncological disease diagnosed irrespective of its localization and clinical course had higher ERI than the rest of the patients ($P < 0.001$) [4]. According to our data, with the help of the ROC test, we established threshold regressor values: the factors of biggest sensitivity and specificity prognosticating the ESA resistance were as follows: the frequency of hospitalization > 2.5 per year; > 3 hospital days per year; > 13.5 hospital days due to infection; and > 1 hospital day due to other causes per year. In the multivariate logistic analysis, among the most significant factors prognosticating resistance to ESA, the number of hospital days due to infection per year (> 13.5) remained.

In Luo's [17] study, patients resistant to ESA, as in our data, were also younger; in this group, there were more women and, in performing HD, CVC was a more frequent vascular access than AVF. The patients studied by Sibbel [14] with chronic (21.7%) or acute (23.8%) resistance to ESA established were more often dialyzed through CVC than the patients having a good response to ESA (14%). According to our data, the ERI of the patients whose vascular access was CVC was also significantly higher in comparison with the patients having AVF ($P = 0.027$), but in the logistic regression analysis, this factor was insignificant in prognosticating the resistance to ESA.

Hypertension has been indicated as one of the very frequent (manifesting itself in more than 10 persons of 100) side effects in taking ESA. In our HD patients' sample, ERI Q_4 group patients who had the highest ESA dose administered also had a reliably bigger DBP in comparison with ERI Q_1 group patients. Although the amount of taken antihypertensive medication among ERI quartiles' groups did not differ statistically significantly, in the group of the best response to ESA (ERI Q_1), the patients took the least ACEI/ARB in comparison with the patients of the other 3 quartiles' groups. However, in the logistic regression analysis, the taking of ACEI/ARB was not a significant factor prognosticating the resistance to ESA. Kuragano and co-authors, like Salman Mallick and Schneider [7, 15, 19], have established that the taking of ACEI/ARB was a significant factor prognosticating high ERI; however, not all the studies have confirmed these results: Wolfgang Winnicki's study has indicated that the long-term taking of a high dose of lisinopril did not influence the resistance to ESA [20].

Iron deficiency is doubtlessly one of the most important causes of resistance to ESA. Besides absolute iron deficiency, functional iron deficiency is typical of HD patients [21–26]. Our data coincide with Kamyar Kalantar-Zadeh's and Adam E. Gaweda's data [27,28] and confirm that iron deficiency, which is indicated by low MCH, TSAT and ferritin, is related to a poorer response to ESA. In our sample, in the group of patients resistant to ESA, the ferritin level, TSAT, and MCH were statistically significantly lower, although the cumulative iron dose obtained per year was statistically significantly higher in comparison with the patient group of a good response to ESA. Kuragano and co-authors [15] have established that a big IV iron dose was a significant factor prognosticating high ERI. A higher iron dose per month was related to the resistance to ESA in Gilbertson's study as well [13]. In Alberto Rosati's study, the cumulative iron dose/kilogram body weight of 2 years was significantly higher in ERI Q_4 patients' group, and it also directly correlated with ERI ($r = 0.07$; $P < 0.0001$) [29]. Francesco Locatelli [30] has described a study revealing that the median of the ferritin level was significantly lower in the group of patients resistant to ESA; however, the dose of iron in the groups did not differ (median 250 mg/mth.). In the study conducted by Maria Do Sameiro-Faria [8], the ferritin level between the groups did not differ and TSAT was lower in the patients' group of a poorer response to ESA. In Luo's study [17], the ferritin level was significantly greater in the patients' group resistant to ESA, TSAT was lower, and the patients resistant to ESA received more IV iron during the observation period. Iain A. Gillespie [31] has also indicated that a high ferritin level is related to resistance to

ESA; however, he does not reveal the relation to TSAT. Ferritin and TSAT are the factors immensely affected by inflammation and that potentially reduces their role as biomarkers of iron metabolism and the factors prognosticating the resistance to ESA. Thus, in the opinion of the authors, a high ferritin level in their study was an indication more of an inflammation than of iron resources and their accessibility for erythropoiesis.

During the evaluation of every ERI quartile separately, the ferritin level in them did not differ reliably; however, the cumulative iron dose received per year in the ERI Q_4 group was the largest in comparison with ERI Q_1, Q_2 and Q_3 patients' groups. TSAT and MCH in the ERI Q_1 group were significantly greater in comparison with the ERI Q_4 group. As in the ERI Q_4 group, the ferritin level, although reliably less in comparison with the group of a good response to ESA, was > 100 μg/L, and TSAT was > 20 %, we cannot state that the cause of the resistance to ESA in the sample we studied is absolute iron deficiency. However, with reference to our data indicating normal iron resources, lower TSAT and lower MCH in the ERI Q_4 patients' group, we can judge that functional iron deficiency, described as a state when iron resources are sufficient, but sufficient iron mobilization to the bone marrow necessary for maintaining adequate erythropoiesis is not possible, can be the cause of resistance to ESA. In logistic regression analysis, ferritin, TSAT and cumulative iron dose per year remained significant factors prognosticating the resistance to ESA. According to the method of ROC analysis, we established the threshold values of these factors: TSAT < 22.5%, ferritin level < 402.3 μg/L, cumulative iron dose > 3450 mg per year were the factors of the biggest sensitivity and specificity prognosticating the resistance to ESA. Following Lithuanian algorithm of anemia treatment for HD patients, the level of ferritin in the blood serum must be maintained within the limits of 200–500 μg/L. Gaweda's [28] study has indicated that the best response to ESA was when the ferritin level was 350–500 μg/L, and after its increase (> 500 μg/–), the response to ESA was worsening. The authors explained that by the malnutrition-inflammation syndrome which inhibits erythropoiesis. During our study, the established threshold ferritin level value when performing ROC analysis was rather high, i.e., 402.3 μg/L, due to which one can assume that in order to "to bypass" the functional iron deficiency bigger iron resources ought to be maintained. Nonetheless, with reference to the data of logistic regression analysis, we can state that a more significant factor in prognosticating the resistance to ESA was TSAT; therefore, it would be meaningful to include it into our algorithm of renal anemia treatment. The DRIVE (Dialysis Patients' Response to Intravenous Iron with Elevated Ferritin) study [32] has indicated that for the patients having a high level of ferritin (> 800 μg/L), the use of ferric gluconate reduced the demand for ESA. The authors state that under resistance to ESA and sufficient iron resources, bigger IV iron dose could assist in "bypassing" the functional iron deficiency. According to the data of our study, although the ERI Q_4 group patients received most IV iron per year, their ferritin level according to the recommendations valid at present was sufficient; however, TSAT remained the lowest in comparison with the other 3 ERI quartiles' groups. This enables us to think that iron is "blocked" in the resources and cannot be used effectively for erythropoiesis. The meta-analysis of the clinical studies of random samples indicated that although the use of IV iron increased the Hb level and reduced the demand for blood transfusion, it can be related to a greater risk of infection (RR 1.33; 95 % CI 1.10–1.64) than when taking oral iron preparations or not taking them at all [33]. Studies have not proved that the reduction of an ESA dose by administering for treatment big IV iron doses would be related to better survival of HD patients having sufficient iron resources [15].

The nutritional and inflammatory state is very important in evaluating the clinical state of HD patients. In the population of HD patients, the deficiency of protein energy and inflammation are very much related and through mediators IL-6 and TNF-alfa can influence the resistance to ESA. Our data confirm the above: bigger BMI was related with a better response to ESA. The mean ERI of small weight patients was 14.65 ± 11.86, and that of normal weight patients was 10.67 ± 8.97, that of overweight patients was 8.98 ± 7.17 and that of obese patients was 6.15 ± 5.39 IU/kg/week/g 100 mL ($P < 0.05$). This corresponds to the data of Lopez-Gomez and co-authors [4]. Although these data can be interpreted as a mathematical artifact (as the body weight is used in the formula according to which ERI is calculated), we believe that this relation is clinically significant and can be a part of "reverse epidemiology" typical of HD patients. In making a logistic regression analysis, BMI in the HD patients' sample we studied was a significant factor prognosticating ERI. In Do Sameiro-Faria's [8] study, BMI and albumin quantity in the blood serum were lower and the quantities of CRP and IL-6 were higher in the group of a poor response to ESA. However, not all the studies confirm this relation: in Gilbertson's study, BMI had no influence [13], and in Sibbel's study, [14] the patients with chronic resistance to ESA established had a higher mean BMI (29.1 kg/m^2) in comparison with other patients' groups. Although the albumin level in the blood serum is considered to be a marker of the nutrition state, a low albumin level is also a bio-marker of the inflammatory state.

In our study, the lowest mean serum albumin was in the patients' group resistant to ESA ($P = 0.002$). Thus, our data have confirmed that hipoalbuminemia was related to a worse response to ESA and could be related to the malnutrition-inflammation syndrome. Kuragano and co-authors [15] have also established that a low serum albumin level was a significant factor prognosticating high ERI. The total protein level was also reliably lower in the patients' group resistant to ESA. According to the data of our study, the cholesterol level in the blood serum did not differ statistically significantly in the patients' groups of a good response and resistance to ESA; however, in Lopez-Gomez and co-authors' study, ERI was reliably lower under higher cholesterol amount [4]. Our study indicated that the ERI Q_4 group patients had the highest CRP level in the blood serum and it was an independent prognostic factor of resistance to ESA in logistic regression analysis. Many studies have revealed the relation between increased inflammatory indicators and reduced response to ESA [34,35]. According to Brian D. Bradbury's data, the patients whose CRP value was prevailing in the upper quartiles were older, had CVC, lower albumin, Hb levels, and lower TSAT, needed higher ESA doses [35]. On the other hand, the results of Panichi and co-authors' study [16] have not confirmed the relation between ESA resistance and increased CRP. In their study, IL-6 and TNF-α have directly correlated with a bigger demand for ESA of HD patients.

The relation between high CRP, a small quantity of albumin and resistance to ESA confirms the important role of inflammation [8,36,37]. We established that a higher concentration of inflammatory biomarkers and lower concentration of the indicators reflecting nutrition which are collectively called the malnutrition-inflammation complex are independent factors prognosticating bigger ERI.

Although according to our data the PTH level in the blood serum of the studied patients' groups did not differ statistically significantly, the phosphorus level in the blood serum was statistically significantly higher among the patients resistant to ESA than among the patients typically with a good response to ESA. In the studies described by Lopez-Gomez and co-authors [4] and Kuragano [15], the relation between ERI and PTH has not been established as well.

According to the data of literature, the deficiency of vitamin D is an important factor for the development of resistance to ESA. During the observation of HD patients, the reverse correlation between $25(OH)D_3$ and ERI has been established and the use of vitamin D reduced anemia and demand for ESA [38]. Although we did not evaluate the vitamin D level in our study, a higher phosphorus level in the patients' group resistant to ESA could partially explain this mechanism: the deficiency of PTH and phosphates stimulates the synthesis of a biologically active vitamin D form, whereas during hyperphosphatemia synthesis is switched to the synthesis of biologically inert molecule 24,25-$(OH)_2$-vitamin D_3 [39]. According to our data, a higher phosphorus level among the patients' group resistant to ESA enables us to think about the possible deficiency of vitamin D and the resistance to ESA possibly conditioned thereof. Phosphorus as a prognostic factor of ESA resistance was significant in logistic regression analysis and the value of the phosphorus level of the supreme sensitivity and specificity established in the ROC test while prognosticating the resistance to ESA was > 1.78 mmol/L. The calcium and the corrected calcium level in the sample of HD patients in ERI quartiles we studied did not differ; however, Kuragano and co-authors [15] have established that low serum calcium quantity was a significant factor prognosticating high ERI.

Some authors indicate that less spKt/V is related to higher ESA doses [17, 28, 40]. Our results did not confirme that. The mean spKt/V of our patients was 1.45 ± 0.18 and did not correlate with ERI. In the HD patients' group of small weight (BMI < 18.5 kg/m^2), the biggest ERI in comparison with the other 3 BMI groups was established in spite of the highest spKt/V. This corresponds to the data of Lopez-Gomez and co-authors [4].

During our study, we established the prognostic models of resistance to ESA based on easily accessible clinical indicators and usual laboratory tests. Generalizing the data, we can judge that accurate evaluation of iron metabolism, improvement of the patients' nutrition status and reduction of inflammation, regulation of calcium and phosphorus metabolism can play an important role in aiming to avoid the resistance to ESA and improve the prognoses of HD patients.

Conclusions

We provided prognostic models for ESA resistance based on easily obtainable clinical parameters, data of hospitalizations and routine laboratory markers, which allow accurate identification of HD patients at risk of ESA resistance. The resistance to ESA was most significantly prognosticated by the following: female sex, BMI < 20 kg/m^2, cumulative intravenous iron dose > 3450 mg/year, TSAT < 22.5%, ferritin concentration < 402.3 μg/L, phosphorus concentration > 1.78 mmol/L, albumin concentration < 39.6 g/L, CRP concentration > 4.8 mg/L and > 13.5 hospital days due to infection per year. Diagnosis of diabetes mellitus was associated with a better response to ESA.

We suggest that routinely obtained data can be used in clinical practice to stratify patients according to the risk of ESA resistance, which may help to assign appropriate treatment strategies.

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