

Oral iron for anaemia in non-dialysis chronic kidney disease. The relationship between hepcidin-25, iron metabolism and inflammation.

Carmen Caldararu^{1*}, Grigore Dogaru¹, Dorin Ionut Tarta¹, Adina Hutanu^{2,3}, Maria T Dogaru⁴, Emilian Carasca⁵, Mirela Liana Gliga¹

¹Department of Nephrology, University of Medicine and Pharmacy of Targu Mures, Romania

²CCAMF of University of Medicine and Pharmacy of Targu Mures, Romania

³Department of Laboratory Medicine, University of Medicine and Pharmacy of Targu Mures, Romania

⁴Department of Pharmacology, University of Medicine and Pharmacy of Targu Mures, Romania

⁵Department of Internal Medicine, University of Medicine and Pharmacy of Targu Mures, Romania

Abstract

Background: Hepcidin and inflammatory markers can differentiate patients with chronic kidney disease who respond to oral iron therapy.

Objectives: To assess if the answer to iron oral therapy is influenced by hepcidin or by inflammation and to describe the relationship between hepcidin, inflammation and anaemia in patients with non-dialysis chronic kidney disease.

Methods: Non-randomized prospective study on a population of non-dialysis chronic kidney disease and anaemia attending a tertiary care hospital. Eligible patients were over 18 years, with no treatment for anaemia and estimated Glomerular Filtration Rate (eGFR) < 60 ml/min/1.73 mp at least 6 months before enrolment. Exclusion criteria: bleeding, malignancy, infection, systemic or hepatic disease, immunosuppression, MCV higher than 100 fl, dialysis and renal transplantation. All patients received a four-week treatment with oral iron (Ferrous sulfate) at a dose of 105 mg once daily. Iron metabolism parameters, serum hepcidin-25, IL-6, TNF- α , Erythropoietin (EPO), high-sensitivity CRP were measured at baseline and after four weeks of oral iron therapy.

Results: Four weeks of oral iron therapy significantly improved haemoglobin values ($p < 0.05$). Serum hepcidin values were significantly higher after four weeks of oral iron compared with baseline. Hepcidin-25 was positively correlated with ferritin, serum iron, TSAT and TNF-alpha. A negative correlation between hepcidin-25, eGFR and serum EPO was demonstrated. In multivariate regression analysis, TNF- α was the parameter that best predicted hepcidin values.

Conclusion: Our results agree that oral iron treatment is effective in raising haemoglobin in non-dialysis CKD patients after four weeks of treatment. Hepcidin seems to be more sensitive than ferritin to confirm the improvement in iron utilization. TNF- α was the best predictor of hepcidin values in our patients.

Keywords: Hepcidin-25, Non-dialysis chronic kidney disease, Anaemia, Inflammation.

Accepted on February 1, 2017

Introduction

Chronic Kidney Disease (CKD) has emerged as an important public health issue because of its pandemic dimensions, the significant comorbidity and the financial burden imposed on medical systems worldwide [1-4].

Anaemia is a common complication in chronic kidney disease, the existing evidence supporting worsening anaemia with decreased glomerular filtration rate [5].

We know today that CKD anaemia is a multifactorial process where lack of erythropoietin, bone marrow suppression by uremia, blood losses in haemodialysis circuit, gastrointestinal bleeding, malnutrition, inflammation and other factors may be involved.

Iron metabolism is also impaired in CKD and due to the peculiarities of the disease; both functional iron deficiency as well as absolute iron deficiency can complicate the management of anaemia [6,7].

The diagnosis of absolute or functional iron deficiency is particularly challenging in patients with CKD [8,9]. Chronic kidney disease is recognized as a proinflammatory state.

The marker most frequently used for differentials between absolute and functional iron deficiency, ferritin, is also an acute phase reactant. Given that ferritin is increased in inflammation, its values should be interpreted with caution in chronic kidney disease.

Hepcidin, a recently discovered protein produced mainly in the liver, is a key regulator of iron homeostasis. By binding and inducing internalization and degradation of ferroportin, hepcidin decrease intestinal iron absorption and iron release from deposits, so the net effect is the reduction of plasma iron available for erythropoiesis [10,11].

Since the discovery, continuous efforts have been made to understand the role of hepcidin in chronic kidney disease anaemia and to assess if hepcidin can be used as a more reliable marker than ferritin and haemoglobin.

A good understanding of the relationship hepcidin-inflammation-anaemia may also provide help in understanding which category of CKD patients will respond to oral iron therapy and where it is needed intravenous iron because there are on-going controversies whether iron supplementation in non-dialysis CKD patients must be made orally or intravenous. Also, it will be possible to understand which patients and how will respond to erythropoiesis stimulating agent therapy.

In this setting, the primary objective of our study was to assess if in non-dialysis CKD patients iron oral therapy is influenced by hepcidin or by inflammation. The secondary objective was to describe the relationship between hepcidin, inflammation and anaemia in patients with non-dialysis chronic kidney disease.

Material and Methods

Non-randomized prospective interventional single center study on a population of non-dialysis chronic kidney disease and anaemia attending a tertiary care hospital

The study protocol was approved by the institutional ethics committee and was conducted according to the Declaration of Helsinki. All patients were informed about the stages of the study and signed an informed consent for participation in the research and for plasma sample collection.

Eligible patients were over 18 years, with no treatment for anaemia at least 6 months before enrolment, estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m² and stable creatinine values eight weeks before enrolment.

Anaemia was defined according to KDIGO Clinical Practice Guideline for Anaemia in Chronic Kidney Disease as haemoglobin concentrations lower than 13.0 g/dl in males and lower than 12.0 g/dl in females. Oral iron supplementation was indicated if TSAT is < 30% and ferritin is < 500 ng/ml [12].

Exclusion criteria: bleeding, malignancy, infection, systemic or hepatic disease, immunosuppression, vitamin B12 deficiency, MCV higher than 100 fl, dialysis and renal transplantation, any anaemia treatment six months before the screening visit, unstable serum creatinine concentrations.

After an initial assessment consisted in a complete physical examination and in evaluating medical history, eligible patients were included in the study.

Treatment

All patients received a four-week treatment with oral iron with commercially- available ferrous sulfate at a dose of 325 mg (105 mg of iron) once daily before breakfast.

Over the study period no patient has been treated with other iron preparations, erythropoiesis stimulating agents or blood transfusion. Repeated administration of proton pump inhibitors or drugs whose administration would modify iron absorption was avoided [13].

Patients who were not adherent on oral iron therapy or who did not complete study visits were excluded from the statistical analysis.

Laboratory data

Blood samples were collected using standardized techniques after an overnight fast, between 8:00 and 9:00 in the morning, within 24 h after the last dose of oral iron.

Serum iron, Total Iron Bind Capacity (TIBC), haemoglobin, haematocrit, total protein, and fibrinogen were assayed by standard laboratory methods. Mean Corpuscular Volume (MCV) was measured as part of the complete blood count.

Blood samples for determination of hepcidin and inflammatory parameters were centrifuged within 1 h of collection, divided in aliquots and serum was stored at -80°C.

Inflammatory parameters (hrCRP, IL-6, TNF- α), hepcidin 25 and EPO assays were done by the same person, at the same time. Iron metabolism parameters, hepcidin-25, erythropoietin, TNF- α , IL-6, hsCRP were evaluated at baseline and after 4 weeks of treatment.

Tumor Necrosis Factor (TNF)- α , IL-6, Erythropoietin (EPO), B12 vitamin was assessed using a solid phase chemiluminescent enzyme immunometric assay on an automated immunoanalyzer (Siemens Germany, Immulite 1000). Two patients had IL-6 and TNF- α values below 2 ng/ml, respectively 4 ng/ml (the lower limit of sensitivity for the method), in the statistics we used values 2 ng/ml, respectively 4 ng/ml. Analytical sensitivity was 2 pg/ml for IL-6 assay, 1.7 pg/ml for TNF- α , 0.24 mIU/ml for EPO. EPO and TNF- α measurement were missing for 2 patients at the beginning of the study.

Plasma ferritin concentrations and hepcidin-25 were measured using immunoenzymatic colorimetric methods (ELISA Novatec Immundiagnostica GmbH, Dietzenbach, Germany and

Oral iron for anaemia in non-dialysis chronic kidney disease. The relationship between hepcidin-25, iron metabolism and inflammation

DRG® Hepcidin 25 bioactive ELISA, DRG International, Inc., USA) according to manufacturer’s indication.

Analytical sensitivity was 0.135 ng/ml for hepcidin-25 and 0.04 ng/ml for serum ferritin.

Transferrin Saturation (TSAT) was calculated as $TSAT = (Fe / TIBC) \times 100$.

Serum creatinine values were assessed with Abbott Architect kinetic, Jaffe method. Estimated Glomerular Filtration Rate (eGFR) was calculated using 4-variable Modification of Diet in Renal Disease equation [14].

Statistical analysis

Statistical analysis was realized using GraphPad Prism 6 Software. Baseline characteristics were assessed with standard descriptive statistics. Data were presented as mean ± standard deviation for normally distributed measurements and as median (minimal-maximal value) for non-normally distributed measurements.

Differences between visits were evaluated with paired Student’s t-test for Gaussian distribution and with Wilcoxon test for a non-Gaussian distribution. Unpaired Student’s t-test (or Mann-Whitney test for a non- Gaussian distribution) was used to assess the differences between parameters in patients with low versus high ferritin.

Correlations between quantitative variables were assessed with Pearson test for data with Gaussian distribution and with nonparametric Spearman test for variables with non-Gaussian distribution.

Normality of distribution was investigated with d’ Agostino-Pearson normality test. Log10 transformation was made in the case of variables with non-normal distribution.

A value of p<0.05 was considered significant.

Results/Observations

Out of 500 patients presented in the Nephrology Department of County Clinic Hospital Targu Mures between February 2015-June 2016, 40 consecutive patients with CKD met the inclusion criteria. Of those, only 30 have agreed to participate in the study (have signed the informed consent). Three patients had very high hsCRP values at baseline. Chauvenet test (on the elimination of inadequate data) was applied, upon which that value outliers were removed from the sample [15].

Ten patients (37.03%) had estimated GFR lower than 15 ml/min/1.73 mp (stage 5 CKD), eight patients (29.62%) had eGFR between 15 and 30 ml/min/1.73 mp (stage 4 CKD), and 9 had eGFR between 30 and 60 ml/min/1.73 mp (stage 3 CKD).

Fifteen woman and twelve men completed the study. Age distribution was 59.37 ± 13.47 years. Twelve patients had diabetes mellitus (44.44%) and 21 patients (77.77%) had Body Mass Index (BMI) higher than 25 kg/mp.

Table 1 summarizes the distribution of haemoglobin, TSAT, ferritin, hepcidin, erythropoietin, IL6 and TNF-alpha at baseline and after oral iron therapy for all patients.

After four weeks of oral iron there was significantly improvement of haemoglobin values (p<0.05).

TIBC was significantly lower and TSAT and MCV were significantly higher after treatment (p<0.05) (Table 1).

Serum hepcidin values were significantly higher after four weeks of oral iron compared with baseline.

Table 1. The distribution of iron metabolism parameters, hepcidin, erythropoietin, IL6 and TNF-α at baseline and after oral iron therapy for all patients.

	Baseline	After treatment	p
Haemoglobin (g (%))	10.51 ± 1.23	10.95 ± 1.51	<0.05
TSAT (%)	21.65 ± 7.28	25.03 ± 8.22	<0.05
Hepcidin 25 (ng/ml)	30.36 (6.81-197.30)	30.60 (5.80-205.70)	<0.05
Ferritin (ng/ml)	106.5 ± 81.36	122.9 ± 94.93	NS
TIBC (µg/dl)	273.0 (155.0-556.0)	230.0 (144.0-347.00)	<0.05
hsCRP (mg/l)	5.03 (0.32-16.64)	5.45 (0.31-44.13)	NS
eRFG (ml/min/1.73 mp)	21.46 ± 13.00	22.09 ± 13.16	NS
Il-6 (pg/ml)	3.35 (2.00-27.60)	3.80 (2.00-9.40)	NS
TNF- α (pg/ml)	10.20 (6.50-23.90)	10.10 (4.10-23.10)	NS
BMI (kg/mp)	28.16 ± 4.02	28.31 ± 4.23	NS
EPO (mIU/ml)	9.3 (3.80-32.20)	8.7 (4.40-22.90)	NS
Serum iron (µg/dl)	57.41 ± 22.23	57.67 ± 20.15	NS
MCV (fl)	86.27 ± 7.38	87.98 ± 6.39	<0.05

Mean ± SD for normally distributed data, median (minimal-maximal value) for non-normally distributed data

Table 2. Distribution of iron metabolism parameters, hepcidin, erythropoietin, IL6 and TNF-α at baseline and after oral iron therapy in the low ferritin group at baseline (ferritin ≤ 100 ng/ml).

	Baseline mean ± SD	After treatment mean ± SD	p
Haemoglobin	10.42 ± 1.24	10.92 ± 1.63	NS
TSAT (%)	18.95 ± 7.87	22.88 ± 6.91	NS
Hepcidin (ng/ml)	15.35 (6.81-135.80)	17.05 (5.80-205.70)	NS
TIBC (µg/dl)	285.00 (215.0-556.0)	245.00 (189.0-344.0)	<0.05
hsCRP (mg/l)	4.29 (0.99-15.96)	4.69 (0.82-19.42)	NS
eRFG (ml/min/1.73 mp)	23.13 ± 15.21	23.13 ± 15.93	NS
Il-6 (pg/ml)	3.35 (2.00-27.65)	3.80 (2.00-9.40)	NS
TNF-α (pg/ml)	10.28 ± 4.70	10.68 ± 4.59	NS
BMI (kg/mp)	28.74 ± 5.08	28.86 ± 5.36	NS

EPO (mIU/ml)	12.21 ± 5.15	10.49 ± 4.41	NS
Serum iron (µg/dl)	53.00 ± 21.91	58.29 ± 18.58	NS
Ferritin	48.80 (2.76-98.69)	49.07 (3.56-296.40)	NS

Mean ± SD for normally distributed data, median (minimal-maximal value) for non-normally distributed data

Table 3. Distribution of iron metabolism parameters, hepcidin, erythropoietin, IL6 and TNF-α at baseline and after oral iron therapy in the high ferritin group at baseline (ferritin>100 ng/ml).

	Baseline	After treatment	p
Haemoglobin	10.63 (7.27-11.90)	10.80 (7.65-14.57)	NS
TSAT (%)	24.57 ± 5.52	27.35 ± 9.13	NS
Hepcidin (ng/ml)	39.12 (14.50-197.30)	48.10 (15.19-198.60)	NS
TIBC (µg/dl)	248.8 ± 54.10	212.2 ± 58.18	<0.05
hsCRP (mg/l)	5.28 (0.32-16.64)	5.45 (0.31-44.13)	NS
eRFG (ml/min/1.73 mp)	19.67 ± 10.43	20.98 ± 12.74	NS
IL-6 (pg/ml)	3.97 ± 1.54	4.68 ± 2.27	NS
TNF-α (pg/ml)	13.20 ± 5.48	13.20 ± 5.43	NS
BMI (kg/mp)	27.83 ± 2.63	27.71 ± 2.63	NS
EPO (mIU/ml)	6.60 (3.80-32.20)	7.3 (4.7-22.90)	NS
Serum iron (µg/dl)	62.92 ± 23.72	57.0 ± 22.46	< 0.05
Ferritin	158.80 (107.30-324.50)	176.70 (58.07-304.30)	NS

Mean ± SD for normally distributed data, median (minimal-maximal value) for non-normally distributed data

We did not found significant differences for ferritin, serum iron, eGFR, EPO values, hsCRP, IL-6, and TNF-alpha when compared values at baseline and at the end of treatment (Table 1).

Because in CKD patients serum ferritin<100 ng/ml is considered to reflect absolute iron deficiency [16], a subgroup analysis was made for patients with baseline ferritin values lower or equal with 100 ng/ml (14 patients) versus patients with ferritin higher than 100 ng/ml (13 patients).

Patients in the low ferritin group had no significant changes in haemoglobin, ferritin, serum iron, TSAT, hepcidin, hsCRP, IL-6, TNF-α and EPO values by the end of the study (four weeks of oral iron treatment) (Table 2).

TIBC values were significantly lower after oral iron treatment in the low ferritin group (Table 2).

In the high ferritin group, serum iron and TIBC were significantly lower at the end of treatment (Table 3). All other

parameters showed no significant differences between baseline and the end of treatment (Table 3).

Table 4. Distribution of iron metabolism parameters, hepcidin, erythropoietin, IL6 and TNF-alpha at baseline in the low versus high ferritin group (ferritin ≤ 100 ng/ml vs. ferritin>100 ng/ml).

	Baseline, Ferritin ≤ 100 ng/ml	Baseline, Ferritin>100 ng/ml	p
Haemoglobin	10.51 (8.62-12.55)	10.63 (7.27-11.90)	NS
TSAT (%)	18.95 ± 7.84	23.86 ± 5.11	NS
Hepcidin (ng/ml)	25.79 ± 33.31	51.20 ± 46.29	<0.0001
TIBC (µg/dl)	285.0 (215.9-556.0)	272.0 (155.9-327.9)	NS
hsCRP (mg/l)	0.67 ± 0.39	0.55 ± 0.45	NS
eRFG (ml/min/1.73 mp)	23.36 ± 16.17	20.02 ± 10.54	NS
IL-6 (pg/ml)	3.35 (2.00-27.60)	3.30 (2.00-6.00)	NS
TNF-α (pg/ml)	9.30 (4.00-23.00)	10.55 (7.10-23.80)	NS
BMI (kg/mp)	30.38 (20.05-38.29)	28.14 (21.30-38.29)	NS
EPO (mIU/ml)	11.15 (5.50-23.40)	6.60 (3.80-32.20)	<0.05
Serum iron (µg/dl)	53.00 ± 21.91	62.92 ± 23.72	NS

Mean ± SD for normally distributed data, median (minimal-maximal value) for non-normally distributed data

Hepcidin-25 values were significantly higher and EPO values were significantly lower in the group with ferritin>100 ng/ml compared with the group with ferritin ≤ 100 ng/ml (Table 4).

In the low ferritin group, hepcidin-25 was positively correlated with ferritin, serum iron, TSAT and TNF-alpha (Table 5). A negative correlation between hepcidin-25 and serum EPO was demonstrated (Table 5).

hsCRP showed negative correlation with serum iron and TSAT (Table 5). TNF-alpha was negatively correlated with EPO levels (Table 5).

In the high ferritin group, hepcidin-25 showed negative correlation with EPO values and no correlation with ferritin (Table 6).

For the entire group serum hepcidin-25 showed significant positive correlation with ferritin and TNF-α. Hepcidin-25 was negatively correlated with eGFR and EPO values (Table 7).

No correlations between hepcidin-25 and haemoglobin or IL-6 were demonstrated at baseline (Table 7).

In a multivariate regression model using ferritin, TNF-α and endogenous erythropoietin as independent variables, TNF-α was the parameter that best predicted hepcidin values in our patients (R=0.90, R square=0.81, coefficient s 0.63, p<0.05).

Oral iron for anaemia in non-dialysis chronic kidney disease. The relationship between hepcidin-25, iron metabolism and inflammation

Table 5. Correlations between studied parameters in patients with ferritin ≤ 100 ng/ml at baseline.

	Hgb	Fer	IL-6	TNF- α	EPO	HEP-25	hsPCR	eGFR	Fbg	TSAT	Iron	Glu	BMI
Hgb		0.45	-0.16	-0.09	0.39	0.04	-0.09	0.15	-0.1	0.27	0.37	-0.06	0.14
Fer			0.05	0.27	-0.19	0.59 [®]	-0.25	0.17	0.37	0.72 [®]	0.59 [®]	-0.13	0.07
IL-6				-0.26	0.21	-0.21	0.32	0.16	-0.24	-0.28	-0.21	-0.47	-0.3
TNF- α					-0.77 [®]	0.81 [®]	0.1	-0.13	0.52	0.25	0.23	-0.03	0.09
EPO						-0.74 [®]	0	0.17	-0.28	-0.22	-0.12	0.23	0.14
HEP-25							-0.24	-0.24	0.31	0.61 [®]	0.55 [®]	-0.28	0.14
hsPCR								-0.1	0.33	-0.57 [®]	-0.65 [®]	-0.03	0.19
eGFR									-0.12	0.02	0.07	-0.26	0.23
Fbg										-0.11	-0.31	0.4	0.4
TSAT											0.91 [®]	-0.1	0.03
Iron												-0.19	-0.01
Glu													0.61 [®]
BMI													

Table 6. Correlations between parameters in patients with ferritin > 100 ng/ml at baseline.

	Hgb	Fer	IL-6	TNF- α	EPO	HEP-25	hsPCR	eGFR	Fbg	TSAT	Iron	Glu	BMI
Hgb		0.31	-0.38	-0.58	0.4	-0.21	-0.12	0.52	-0.18	0.17	0.47	0.35	0.45
Fer			0.2	-0.37	-0.41	0.33	0.1	0.38	0.18	-0.33	-0.21	-0.32	0.51
IL-6				0.12	-0.13	0.3	0.71 [®]	-0.15	0.43	-0.38	-0.36	-0.09	0.35
TNF- α					-0.26	0.4	0.42	-0.28	0.19	-0.06	-0.4	-0.29	0
EPO						-0.67 [®]	0.11	0.6	-0.49	0.51	0.66 [®]	-0.36	0.09
HEP-25							0.15	-0.24	0.79 [®]	0.42	-0.46	-0.11	0.21
hsPCR								-0.03	0.57	-0.43	-0.41	-0.1	0.68 [®]
eGFR									-0.61	0.54	0.59 [®]	0	0.33
Fbg										-0.72 [®]	-0.81 [®]	0.25	0.43
TSAT											0.79 [®]	0.37	-0.21
Iron												0.31	-0.11
Glu													0.15
BMI													

Table 7. Correlations between parameters for the whole group at baseline.

	Hgb	Fer	IL-6	TNF- α	EPO	HEP-25	hsPCR	eGFR	Fbg	TSAT	Iron	Glu	BMI
Hgb		0.31	-0.23	-0.17	0.25	0	-0.11	0.27	-0.1	0.23	0.43 [®]	0.11	0.22
Fer			-0.02	0.28	-0.41 [®]	0.68 [®]	-0.19	0.04	0.44 [®]	0.61 [®]	0.40 [®]	-0.13	0.03
IL-6				-0.2	0.03	-0.12	0.65 [®]	-0.02	0.23	-0.32	-0.36	-0.23	-0.15
TNF- α					-0.49 [®]	0.62 [®]	0.26	-0.27	0.4	0.21	-0.04	-0.15	0.03
EPO						-0.61 [®]	0.15	0.26	-0.42 [®]	-0.14	0.12	0.26	0.06
HEP-25							0.17	-0.38 [®]	0.57 [®]	0.05	-0.08	-0.18	-0.27

hsPCR	-0.14	0.44 [®]	-0.50 [®]	-0.56 [®]	-0.09	0.09
eGFR		-0.35	0.12	0.27	-0.16	0.29
Fbg			-0.14	-0.47 [®]	0.27	0.24
TSAT				0.84 [®]	0.04	0
Iron					0.03	0
Glu						0.47 [®]
BMI						

Discussion

CKD is considered a pro-inflammatory state. There are many explanations for this status, from the disease that causes kidney damage (diabetes, connective tissue diseases, glomerulonephritis secondary to viral diseases, etc.), to uremic toxins and dialysis treatment (bio incompatible dialysis membrane, repeated peritonitis in peritoneal dialysis) or endothelial dysfunction and atherosclerosis which are common in kidney failure [17,18].

Anaemia is a common condition in chronic kidney disease patients, most of the studies agreeing that the risk of anaemia as well as its degree increases with decreasing glomerular filtration rate. McClellan et al. demonstrated that 47.7% of 5222 predialysis CKD patients had anaemia and a recent analysis from United States calculated a prevalence of anaemia of 8.4% in CKD stage 1 and of 53.4% in CKD stage 5 [5,19].

In CKD patients, true iron deficiency is common due to increased iron losses (digestive losses in uremic gastropathy, frequent phlebotomies, etc.) but also from reduced Fe absorption and availability for erythropoiesis.

Deteriorating renal function is followed not only by the decline in production of erythropoietin but also by an increased inflammatory response due to increase production or decreased clearance of pro-inflammatory cytokines.

In these conditions, CKD patients are prone to develop functional iron deficiency, situation characterized by increased iron stores but with the impossibility of using them for erythropoietic demands, with high serum ferritin [20].

Given the confounding variables in diagnosing CKD anaemia and the fact that some patients are non-responders to high doses of erythropoietin therapy, the relationship anaemia-inflammation-chronic kidney disease is considered a subject of great importance to medical practice.

In this setting, the discovery of hepcidin, the major regulator of iron absorption, a protein which is feedback regulated by iron serum concentration, by erythropoiesis but also by inflammation (via IL-6 and the IL-6/Janus kinase 2 (JAK2)-Signal Transducer and Activator of Transcription 3 (STAT3) pathway), led to questions about its involvement in iron metabolism in patients with chronic kidney disease [21,22].

The response is difficult because CKD population is heterogeneous: five stages of disease with features related to each stage, different types of renal replacement treatments, and patients with comorbidities that influence each other. In addition, surveillance of patients for longer periods of time in the same stage of the disease is often difficult to achieve due to the evolving nature of disease.

Previous works helped us to understand some of the peculiarities of the relationship hepcidin-anaemia in chronic kidney disease, but most were cross sectional and were performed in patients with kidney substitution or who are known to have been treated with iron or erythropoiesis stimulating agents.

Few analyses were performed prospectively on patients who were not receiving treatment for anaemia on the beginning of the study.

Our study has aimed to prospectively analyse the role of hepcidin in the response to oral iron therapy in non-dialysis CKD patients without treatment for anaemia at least 6 months before enrolment.

The secondary objective of the study was to describe the relationship between hepcidin, markers of inflammation and anaemia in patients with non-dialysis chronic kidney disease.

After four weeks of oral iron treatment there was an improvement of haemoglobin, MCV, TSAT and TIBC, with no differences in serum iron, ferritin, inflammatory markers (IL-6, TNF- α , fibrinogen and hsCRP).

Patients with CKD are at risk for developing iron deficiency which is a major cause of hypo responsiveness to erythropoiesis-stimulating agents. In terms of iron therapy, the method of administration (oral versus i.v.) and the optimal dose are important issues for anaemia management in CKD, both from a medical standpoint, as well regarding the costs imposed by health care systems.

There is no consensus on whether oral or intravenous iron should be indicated as first-line therapy to treat anaemia in pre-dialysis chronic kidney disease [23].

Both forms of administration show advantages and disadvantages in terms of safety management, stability of haemoglobin after iron discontinuation and cost of treatment [24-26].

Oral iron for anaemia in non-dialysis chronic kidney disease. The relationship between hepcidin-25, iron metabolism and inflammation

Our results agree that oral iron treatment is effective in raising haemoglobin values in non-dialysis CKD patients, but whether oral iron is better than intravenous iron is an issue that needs more studies for a convincing answer.

Patients in the lower ferritin group had lower hepcidin and higher EPO values on baseline comparing with patients in the higher ferritin group. TIBC decreased significantly after four weeks of treatment even if serum iron were not significantly increased.

Although from a theoretical perspective this biochemical profile with increased EPO and low hepcidin is best suited for a good response to iron therapy, we did not reveal statistically significant differences concerning haemoglobin levels before and after treatment in these patients, but the result is likely to have been influenced by the small number of cases.

In the high ferritin group, serum iron was statistically lower after four weeks of oral iron therapy compared with baseline values.

The failure to increase serum iron following oral iron loading in this group suggests impaired iron absorption.

One explanation for our results may be related to daily administration of iron because according to Moret's research, consecutive-day iron administration results in decreased iron availability and that the fractional iron absorption is greater for low iron doses, this behavior being explained by serum hepcidin [27].

Comparative analysis showed that hepcidin was significantly higher and EPO was significantly lower in high versus low ferritin group. Therefore, we believe that decreased serum iron after iron loading is explained by impaired absorption motivated by higher hepcidin values in this group.

Excess of hepcidin can impair intestinal iron absorption and macrophage iron recycling leading to anaemia of chronic disease [28].

Relationship between hepcidin and endogenous erythropoietin

The relationship between hepcidin and endogenous erythropoietin it is not completely understood.

In a group of patients with anaemia and combined renal and heart failure, it was demonstrated that hepcidin levels predicted long-term bone marrow response to exogenous EPO, the authors concluding that hepcidin values seems to reflect iron load and response to EPO rather than inflammation and EPO resistance [29].

After four weeks of oral iron, hepcidin values in the high ferritin group were still significantly higher compared with low ferritin group, but statistical differences between EPO values no longer maintain (data not shown). This means that, despite high levels of hepcidin, the group with higher ferritin reacts to iron loading.

Relationship between hepcidin and estimated GFR

In our patients, we found a weak negative correlation between hepcidin and estimated GFR.

The relationship between hepcidin and GFR is still debated. Some authors demonstrated an inverse association between GFR and hepcidin in CKD patients [30-32] but a high number of studies prove no association [33-35].

Even if the assumption of raising hepcidin with decreasing GFR seems correct given the decreased clearance of hepcidin in conditions of low glomerular filtration, the results so different from an author to another are easily explained by the large differences in terms of populations studied and the methods to determine hepcidin (different percentages of diabetics or obese, systemic diseases as the cause of kidney failure, different rates of patients with end-stage chronic kidney disease, various comorbidities, patients previously treated for anaemia).

Relationship between hepcidin and ferritin

The positive correlation between hepcidin-25 and ferritin demonstrated in our study is reported by most authors and is normal given that hepcidin regulation is influenced by iron deposits [32,36].

Circulating serum ferritin is an indicator of iron stores in the liver and reticulo-endothelial system, used with one major limitation: it is an acute phase protein, i.e. will generate high levels in inflammatory syndromes.

Interestingly, in our study the positive correlation between the two parameters is demonstrated for the entire group and for the low ferritin group, but not in patients with high ferritin.

Relationship between hepcidin and endogenous erythropoietin

We don't find correlations between hepcidin-25 and haemoglobin neither for the entire group, even after dividing the patients according to the ferritin values. We think that the result seems plausible because the suppressor effect on hepcidin is directly influenced by the erythropoietic activity and by storage iron and not by anaemia.

Studies are fully disagreeing on the correlation between hepcidin-25 and haemoglobin in CKD. Some authors found positive correlation; others have found negative correlation or lack of correlation between hepcidin and haemoglobin [37-40].

We believe that large variations between authors are due to the differences between studied patients, with different grades of inflammation, different stages of kidney disease and different treatments for anaemia.

Relationship between hepcidin and IL-6

Previous experimental and clinical studies showed that IL-6 induces hepcidin mRNA expression and that IL-6 is the

necessary and sufficient cytokine for the induction of hepcidin in inflammation [21,41].

Somewhat unexpectedly, we found no correlation between hepcidin-25 and IL-6, for the entire group, and even after separating patients according to ferritin values.

If our result is logical for patients with low ferritin, it is a little difficult to understand it in patients with high ferritin level at which a positive correlation between hepcidin-25 and hsCRP was also demonstrated.

This lack of correlation with IL-6 has been previously demonstrated in other patient groups with presumed inflammation, such as those with breast cancer [42], primary myelofibrosis [43] but also in CKD [31,44].

Relationship between hepcidin and TNF- α

Another unexpected finding in our study is that hepcidin-25 was positively correlated with TNF- α for the entire group and for the patients with low ferritin, with a stronger correlation for the low ferritin group. Moreover, multivariate regression showed that TNF- α was the parameter that best predicted hepcidin values in our patients.

A positive correlation between hepcidin and TNF alpha is somewhat unusual because TNF- α was shown to suppress hepcidin mRNA levels in the Hep3B human hepatoma cell line [21].

There is evidence that TNF- α can contribute to anaemia of chronic disease by iron sequestration in the spleen and by reduced duodenal iron transfer by modifying Divalent Metal Transporter 1 (DMT1) and Iron-Regulated protein 1 (IREG1) function [45,46].

A positive correlation hepcidin-TNF- α was found especially in studies those targeting CKD patients with diabetes nephropathy and insulin resistance [47-49].

We assume that the high rate of diabetes and obesity in the low ferritin group can explain our results (7 of 12 patients in the group with low ferritin versus 3 of 12 in the group with high ferritin, results not shown). Insulin resistance has not been studied in our patients, so this correlation cannot be confirmed.

Regarding the negative relationship between EPO and TNF- α found in our patients, pro-inflammatory cytokines were shown to trigger the suppression of renal erythropoietin production even if the mechanism of TNF- α involved in EPO regulation remains unclear [50].

Relationship between hepcidin and BMI

We have not found correlations between hepcidin and BMI even if 77.77% of patients had BMI over 25 kg/mp. Obesity is a metabolic inflammatory state and adipose tissue is very active in cytokine secretion, especially IL-6 who is a well-known activator of hepcidin expression. Recent studies have shown that hepcidin expression is increased in patients with

severe obesity and that the response of oral iron therapy is smaller in obese patients [51,52].

Relationship between hepcidin and hsCRP

In our study, hepcidin-25 was positively correlated with hsCRP in the whole group. We consider this result as further evidence that the relationship hepcidin, anaemia, iron metabolism is mediated by inflammation, even if the latter it is not clinically evident.

In a cross-sectional analysis of 2221 chronic kidney disease patients' non-requiring dialysis, Conchol et al. demonstrate that anaemia is related to inflammation and that inflammation is present starting with early stages of CKD [53].

The strength of our study is the fact that it is a prospective analysis in patients without treatment for anaemia for 6 months before inclusion in the study.

The limitations of our analysis are the small group size, the low number of patients in each category of GFR, ELISA hepcidin assay, the estimation of glomerular filtration rate and Jaffe assay for serum creatinine.

In conclusion, four weeks of oral iron therapy improved haemoglobin, TIBC, TSAT and MCV values, and raised hepcidin-25 without statistical changes in ferritin and serum iron.

In a population of non-dialysis chronic kidney disease patients, hepcidin levels were shown to be positively correlated with TNF-alpha and fibrinogen and inversely correlated with EPO serum values.

Even though our study did not demonstrate a predictive ability of hepcidin in the response to iron therapy, hepcidin seems to be more sensitive than ferritin to confirm the improvement in iron utilization.

Oral iron is inexpensive and has less serious adverse events compared with intravenous iron. Even if evidences exist that subclinical inflammation is present in early stages of kidney disease which may influence the response to therapy, we think that in chronic kidney disease patients without renal substitution, oral iron therapy must be tried.

Currently, despite the large number of studies, we know little about the factors involved in the response to iron therapy in patients with CKD. A better understanding of the relationship chronic kidney disease-anaemia-hepcidin-inflammation in the "real world" is a key factor for a better anaemia management. For this reason, more extensive prospective analyses are needed.

Acknowledgements

This work was supported by the Universitatea de Medicina si Farmacie Targu Mures under Grant No 639/16.01.2015. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Wyld M, Lee C, Zhuo X, White S, Shaw J, Morton R, Chadban S. Cost to government and society of chronic kidney disease stage 1-5: a national cohort study. *Intern Med J* 2015; 45: 741-747.
2. United States Renal Data System. Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. National Institutes of Health. National Institute of Diabetes and Digestive and Kidney Diseases. Bethesda MD 2013.
3. Honeycutt AA, Segel JE, Zhuo X, Hoerger TJ, Imai K. Medical costs of CKD in the Medicare population. *J Am Soc Nephrol* 2013; 24: 1478-1483.
4. Hoerger TJ, Simpson SA, Yarnoff BO, Pavkov ME, Ríos Burrows N. The future burden of CKD in the United States: a simulation model for the CDC CKD initiative. *Am J Kidney Dis* 2015; 65: 403-411.
5. Stauffer ME, Fan T. Prevalence of anaemia in chronic kidney disease in the United States. *PLoS One* 2014; 9: e84943.
6. Babitt JL, Lin HY. Mechanisms of anaemia in CKD. *J Am Soc Nephrol* 2012; 23: 1631-1634.
7. Eschbach J. The anaemia of chronic renal failure: Pathophysiology and the effects of recombinant erythropoietin. *Kidney Int* 1989; 35: 134-148.
8. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood* 2010; 116: 4754-4761.
9. Camaschella C. Iron-deficiency anaemia. *N Engl J Med* 2015; 372: 1832-1843.
10. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Annu Rev Nutr* 2006; 26: 323-342.
11. Ganz T. Hepcidin-a peptide hormone at the interface of innate immunity and iron metabolism. *Curr Top Microbiol Immunol* 2006; 306: 183-198.
12. Kidney Disease: Improving Global Outcomes (KDIGO) Anaemia Work Group. KDIGO Clinical Practice Guideline for Anaemia in Chronic Kidney Disease. *Kidney Int* 2012; 279-335.
13. Campbell NR, Hasinoff BB. Iron supplements: a common cause of drug interactions. *Br J Clin Pharmacol* 1991; 31: 251-255.
14. Levey A, Coresh J, Greene T, Stevens L, Zhang Y, Hendriksen S, Kusek JW. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; 145: 247-254.
15. Costescu MR. Identifying data affected by aberrant errors. Applied program, *Revista Informatica Economica* 2008, 1: 52-55
16. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol* 2006; 1: 4-8.
17. Bergstrom J, Lindholm B, Lacson E, Owen W, Lowrie E, Glassock R, Ikizler TA, Wessels FJ, Moldawer LL, Wanner C, Zimmermann J. What are the causes and consequences of the chronic inflammatory state in chronic dialysis patients? *Semin Dial* 2000; 13: 163-175.
18. Akchurin OM, Kaskel F. Update on inflammation in chronic kidney disease. *Blood Purif* 2015; 39: 84-92.
19. McClellan W, Aronoff SL, Bolton WK, Hood S, Lorber DL. The prevalence of anemia in patients with chronic kidney disease. *Curr Med Res Opin* 2004; 20: 1501-1510.
20. Malyszko J, Mysliwiec M. Hepcidin in anemia and inflammation in chronic kidney disease. *Kidney Blood Press Res* 2007; 30: 15-30.
21. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S. IL-6 mediates hypoferrremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004; 113: 1271-1276.
22. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X. The gene encoding the iron regulatory peptide hepcidin is regulated by anaemia, hypoxia, and inflammation. *J Clin Invest* 2002; 110: 1037-1044.
23. Phan O, Ivanovski O, Nikolov IG, Joki N, Maizel J. Effect of oral calcium carbonate on aortic calcification in apolipoprotein E-deficient (apoE^{-/-}) mice with chronic renal failure. *Nephrol Dial Transplant* 2008; 23: 82-90.
24. Pisani A, Riccio E, Sabbatini M, Andreucci M, Del Rio A. Effect of oral liposomal iron versus intravenous iron for treatment of iron deficiency anaemia in CKD patients: a randomized trial. *Nephrol Dial Transplant* 2015; 30: 645-652.
25. Shepshelovich D, Rozen-Zvi B, Avni T, Gafer U, Gafer-Gvili A. Intravenous versus oral iron supplementation for the treatment of anemia in ckd: an updated systematic review and meta-analysis. *Am J Kidney Dis* 2016; 68: 677-690.
26. Agarwal R, Kusek JW, Pappas MK. A randomized trial of intravenous and oral iron in chronic kidney disease. *Kidney Int* 2015; 88: 905-914.
27. Moretti D, Goede J, Zeder C, Jiskra M, Chatzinakou V, Tjalsma H, Melse-Boonstra A, Brittenham G, Swinkels DW, Zimmermann M. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood* 2015; 126: 1981-1989.
28. Weinstein D, Roy C, Fleming M, Loda M, Wolfsdorf J, Andrews N. Inappropriate expression of hepcidin is associated with iron refractory anaemia: implications for the anemia of chronic disease. *Blood* 2002; 100: 3776-3781.
29. van der Putten K, Jie K, van den Broek D, Kraaijenhagen R, Laarakkers C, Swinkels D. Hepcidin-25 is a marker of the response rather than resistance to exogenous erythropoietin in chronic kidney disease/chronic heart failure patients. *Eur J Heart Failure* 2010; 12: 943-950.
30. Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G. Hepcidin-a potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol* 2009; 4: 1051-1056.

31. Ashby D, Gale D, Busbridge M, Murphy K, Duncan N, Cairns T, Taube DT, Bloom SR, Tam FWK, Chapman RS, Maxwell PH, Choi P. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. *Kidney Int* 2009; 75: 976-981.
32. Mercadal L, Metzger M, Haymann JP, Thervet E, Boffa JJ. The relation of hepcidin to iron disorders, inflammation and haemoglobin in chronic kidney disease. *PLoS One* 2014; 9: e99781.
33. Uehata T, Tomosugi N, Shoji T, Sakaguchi Y, Suzuki A, Kaneko T, Okada N, Yamamoto R, Nagasawa Y, Kato K, Isaka Y, Rakugi H, Tsubakihara Y. Serum hepcidin-25 levels and anaemia in non-dialysis chronic kidney disease patients: a cross-sectional study. *Nephrol Dial Transplant* 2012; 27: 1076-1083.
34. Wagner M, Ashby DR, Kurtz C, Alam A, Busbridge M. Hepcidin-25 in diabetic chronic kidney disease is predictive for mortality and progression to end stage renal disease. *PLoS One* 2015; 10: e0123072.
35. Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M. Hepcidin, iron status, and renal function in chronic renal failure, kidney transplantation, and haemodialysis. *Am J Hematol* 2006; 81: 832-837.
36. Bratescu L, Barsan L, Garneata L, Stanciu A, Lipan M, Hildegard Stancu S, Mircescu G. Effects of additional iron doses on hepcidin-25 level in haemodialysis patients without evident iron deficiency. *Int Urology Nephrology* 2014; 46: 1005-1012.
37. Takasawa K, Takaeda C, Maeda T, Ueda N. Hepcidin-25, mean corpuscular volume, and ferritin as predictors of response to oral iron supplementation in hemodialysis patients. *Nutrients* 2014; 7: 103-118.
38. Mogadam R, Nemati A, Amani F, Ghorbanihaghjo A, Argani H, Bashardoust B. Association between hepcidin, haemoglobin level and iron status in stage 4 chronic kidney disease patients with anaemia. *J Pak Med Assoc* 2015; 65: 354-357.
39. Atkinson MA, Kim JY, Roy CN, Warady BA, White CT. Hepcidin and risk of anemia in CKD: a cross-sectional and longitudinal analysis in the CKiD cohort. *Pediatr Nephrol* 2015; 30: 635-643.
40. Sabau A, Valeanu M, Bolosiu H, Craciun A. Evaluation of serum hepcidin variation in patients with rheumatoid arthritis according to anaemia profile and its correlation with disease activity. *Revista Romana de Medicina de Laborator* 2013; 21: 17-27.
41. Villarroel P, Le Blanc S, Arredondo M. Interleukin-6 and lipopolysaccharide modulate hepcidin mRNA expression by HepG2 cells. *Biol Trace Elem Res* 2012; 150: 496-501.
42. Ciniselli C, deBortoli M, Taverna E, Varinelli L, Pizzamiglio S, Veneroni S, Bonini C, Orlandi R, Verderio P, Bongarzone I. Plasma hepcidin in early-stage breast cancer patients: no relationship with interleukin-6, erythropoietin and erythroferrone. *Expert Rev Proteom* 2015; 12: 695-701.
43. Pardanani A, Finke C, Abdelrahmen R, Lasho T, Tefferi A. Associations and prognostic interactions between circulating levels of hepcidin, ferritin and inflammatory cytokines in primary myelofibrosis. *Am J Hematol* 2013; 88: 312-316.
44. Kuragano T, Shimonaka Y, Kida A, Furuta M, Nanami M. Determinants of hepcidin in patients on maintenance haemodialysis: role of inflammation. *Am J Nephrol* 2010; 31: 534-540.
45. Laftah AH, Sharma N, Brookes MJ, McKie AT, Simpson RJ. Tumour necrosis factor alpha causes hypoferraemia and reduced intestinal iron absorption in mice. *Biochem J* 2006; 397: 61-67.
46. Johnson D, Bayele H, Johnston K, Srai S, Sharp P. Tumour necrosis factor alpha regulates iron transport and transporter expression in human intestinal epithelial cells. *FEBS Lett* 2004; 573: 195-201.
47. Ashourpour M, Djalali M, Djazavery A, Eshraghian M, Taghdir M, Saedisomeolia A. Relationship between serum ferritin and inflammatory biomarkers with insulin resistance in a Persian population with type 2 diabetes and healthy people. *Int J Food Sci Nutr* 2010; 61: 316-323.
48. Oh DJ, Kim HR, Lee MK, Woo YS. Profile of human β^2 -defensins 1,2 and proinflammatory cytokines (TNF- α , IL-6) in patients with chronic kidney disease. *Kidney Blood Press Res* 2013; 37: 602-610.
49. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes* 1994; 43: 1271-1278.
50. Faquin WC, Schneider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. *Blood* 1992; 79: 1987-1994.
51. Bekri S, Gual P, Anty R, Luciani N, Dahman M. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; 131: 788-796.
52. Sanad M, Osman M, Gharib A. Obesity modulate serum hepcidin and treatment outcome of iron deficiency anaemia in children: A case control study. *Italian Journal of Pediatrics* 2011; 37: 34-39.
53. Chonchol M, Lippi G, Montagnana M, Muggeo M, Targher G. Association of inflammation with anaemia in patients with chronic kidney disease not requiring chronic dialysis. *Nephrol Dial Transplant* 2008; 23: 2879-2883.

*Correspondence to

Carmen Caldararu

Department of Nephrology

University of Medicine and Pharmacy of Targu Mures

Romania