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Calcium antagonists, digoxin, calcaemia and anaemia in heart failure.

Satı Sena Yildiz[1], Didem Dikerdem[1], Ali Ramazan Benli[2], Pinar Kalem[3*], Mehmet Ali Cikrikcioglu[1]

[1]Department of Internal Medicine, Bezmialem Vakif University, Faculty of Medicine, Fatih, Istanbul, Turkey

[2]Department of Family Medicine, Karabuk University, Faculty of Medicine, Karabuk, Turkey

[3]Department of Family Medicine, Ahi Evran University, Faculty of Medicine, Kirsehir, Turkey

Abstract

Objective: To reveal that Calcium Antagonist (CA) use is associated with lower haemoglobin (Hb) and digoxin use is associated with higher Hb in Heart Failure (HF).
Method: 223 chronic HF patients in acute decompensation phase were included in the study. Patients with comorbidities leading to anemia and those receiving blood transfusion or antianaemic treatment were excluded. Patients were classified into two groups as anemic and non-anemic groups. Two groups were compared retrospectively with demographics, clinical findings, medication use, echocardiography findings, complete blood count and biochemistry. Different independent variables between two groups were subjected to Multivariate Binary Logistic Regression Analysis (MBLRA) under the dependent variable anemia. Multivariable linear Regression Analysis (RA) was also performed with the dependent variable of Hb.
Results: MBLRA results showed that anemia was seen less frequently in digoxin users, whereas it was more frequent in the following conditions: CA use, chronic renal failure, lower AST, lower LDL cholesterol, lower triglyceride, lower Transferring Saturation Rate (TSR). RA results also showed that lower LDL cholesterol, lower eGFR, lower transferrin saturation rate, lower corrected calcium, female gender and CA use were associated with lower Hb; whereas, digoxin use was associated with higher Hb.
Conclusions: Haemoglobin levels were found higher in digoxin users. CA use, lower corrected calcium and lower AST were associated with lower Hb in heart failure. These findings have not been reported so far.

Keywords: Heart failure, Anemia, Digoxin, Calcium antagonists, Calcium.

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Introduction

Anemia in chronic heart failure is a commonly observed comorbidity. It is thought to be caused by proinflammatory and neurohormonal activation and by renal dysfunction which frequently accompanies heart failure (cardiorenal syndrome), and is also considered as a chronic disease anemia. Reduced erythropoietin release, urinary erythropoietin loss, erythropoietin resistance, reduced iron absorption, and reduced iron mobilization from iron stores are the most advocated pathophysiologic mechanisms in describing the anemia associated with heart failure [1,2]. Anemia associated with Chronic Kidney Disease (CKD) resembles the anemia observed in chronic heart failure in many aspects. The common characteristics are presence of EPO resistance, EPO deficiency, frequent association of CKD and heart failure (cardio-renal syndrome), EPO therapy can be used successfully in the anemia co-existing with heart failure (though not a routine practise). Both ACE inhibitors and Angiotensin Receptor Blockers (ARB's) lead to lower haemoglobin levels both in heart failure and in CKD [1-6].

In order that EPO can stimulate the differentiation and proliferation of erythroblasts, it is imperative that it should increase free calcium concentration within the erythroblast. EPO was shown *in vitro* to increase intracellular free calcium concentration in healthy individuals through calcium influx from extracellular fluid to BFU-E derived erythroblast cells; whereas nifedipine impedes the calcium influx and blocks the effect of EPO. Therefore, in EPO stimulated cells, calcium ion (Ca^{2+}) influx rather than intracellular Ca^{2+} mobilization is responsible for increased intracellular free calcium [7]. In CKD patients who have EPO resistance and reduced EPO release, Calcium Antagonist (CA) use could additionally impedes EPO activity, and thereby diminishes haemoglobin (Hb) and haematocrit (Htc) levels. Our study also showed that CA users had lower haemoglobin compared to patients not using CAs among stage 2-5 CKD patients not receiving renal replacement therapy, EPO and vitamin D treatment [8]. Similarly, chronic heart failure patients using CA are expected to have lower haemoglobin levels than those not using CAs. However, digoxin use may be associated with higher haemoglobin

through potentiation of EPO activity by increasing the calcium ion concentration within the erythrocytes in chronic heart failure patients with EPO deficiency or EPO resistance, just as it increases the calcium ion concentration within the cardiomyocytes by blocking the NaK-ATPase enzyme on the cellular membrane [9]. To test the hypothesis that CA use is associated with lower haemoglobin and digoxin use is associated with higher haemoglobin in chronic heart failure, we classified chronic heart failure patients in acute decompensation phase into two groups; anemic and non-anemic patients. Then we compared these two groups according to demographic characteristics, medical history, clinical findings, echocardiographic findings, regular medication use, Complete Blood Count (CBC) and detailed biochemistry analysis (Table 1).

Materials and Methods

Study design

This study is designed as a retrospective, cross sectional and observational study.

Study population

From Oct 2009 to Jan 2011, 223 adult acute heart failure patients of any etiology with systolic or diastolic dysfunction who were consecutively admitted and treated in the internal medicine clinic, cardiology clinic, coronary care unit or intensive care unit of Vakif Gureba (VG) Training and Research Hospital and Bezmialem Vakif University Faculty of Medicine were included in the study. Diagnostic criteria used for AHF were in accordance with the guidelines of European Society of Cardiology, and included the following:

1. Underlying heart disease.
2. At least 2 symptoms or signs of heart failure (dyspnoea, orthopnoea, rales, elevated jugular venous pressure).
3. Chest x-ray consistent with pulmonary congestion.
4. Worsening symptoms and signs within 7 d in chronic heart failure patients [10].

Among the patients meeting these criteria, only NYHA Class III-IV patients with chronic heart failure were enrolled.

In subjects with serum creatinine level ≥ 1.3 mg/dL, Chronic Renal Failure (CRF) diagnosis was established based on history and/or reduced kidney size in ultrasonography and/or elevated parathormone, elevated phosphorous and decreased calcium levels. Estimated Glomerular Filtration Rate (eGFR) was calculated according to MDRD formula [11]. Acute coronary syndrome, cardiogenic shock, renal replacement treatment, COPD in exacerbation period, malign solid tumours, chemotherapy or radiotherapy administration, hematologic malign diseases, myelodysplastic syndrome, haemolytic anemias, portal hypertension, collagen tissue diseases, chronic liver diseases, chronic immunosuppressive treatment, hyperthyroidism, hypothyroidism, pituitary and surrenal hormonal dysfunction, chronic and acute infections, decubitus ulcers, diabetic foot, recurrent epistaxis, meno-metrorrhagia, marked persistent haematuria, hematemesis, melena and hematochezia anamnesis, gastrectomy, vegetarian diet, and major surgery, blood transfusion or anti-anemic treatment within the last 6 months were reasons for exclusion.

Detailed information on regular drug use during minimum 3 months prior to inclusion in the study, disease history, personal history and family history were obtained from the hospital records of each patient.

Patients with haemoglobin values <12 g/dL (women) and <13 g/dL (men) were considered as anemic acute heart failure patients [12].

Laboratory

Samples: Blood samples for complete biochemistry, thyroid hormones, Erythrocyte Sedimentation Rate (ESR), sensitive C-Reactive Protein (sCRP) and HBsAg, anti-HCV, HIV 1-2, and urine samples for urine-analysis were obtained at admission or early in the next morning. Hemogram, pro-Brain Natriuretic Peptide (ProBNP), d-Dimer, and when needed cardiac troponins were measured at admission.

Measurements

Haemograms were measured with Abbot Cell Dyne 3700 apparatus (22-parameter haemogram). Biochemistry tests were done with Roche Modular P 800 apparatus (Roche Diagnostics, Indianapolis, USA). ProBNP was measured with Siemens Dimation XP and Plus (Siemens Healthcare Diagnostic Inc., Deerfield, Germany) and D-dimer with Siemens Stratus CS. Thyroid hormones were measured using Elecsys kits with Modular apparatus (Roche).

Chest x-ray of each patient and an ECG of each patient (which had been repeated when required) were reviewed. For the assessment of left ventricular diastolic and systolic function, all patients underwent transthoracic 2-dimensional echocardiography and Doppler imaging using Hewlett-Packard Sonos 2500 apparatus (Hewlett-Packard, Andover, Massachusetts, USA). Standard imaging was performed in the left lateral decubitus position. Images were obtained using a 2.5-3.5 MHz transducer in the parasternal and apical views.

Left Ventricular End-Diastolic (LVEDD) and End-Systolic (LVESD) Diameters were determined with M-mode echocardiography under two-dimensional guidance in the parasternal long-axis view, according to the recommendations of the American Society of Echocardiography [13]. Left Ventricular Ejection Fraction (LVEF), Left Ventricular End-Diastolic Volume (LVEDV) and Left Ventricular End-Systolic Volume (LVESV) were calculated from apical four-chamber views, according to the modified Simpson's rule.

Pulsed-Wave (PW) Doppler was performed in the apical 4-chamber view to obtain mitral inflow indices to assess LV filling according to the recommendations of the American Society of Echocardiography [14]. Measurements of mitral inflow include the peak early filling (E-wave) and late diastolic

filling (A-wave) velocities, the E/A ratio, Deceleration Time (DT) of early filling velocity.

Pulsed-wave TDI was performed in the apical views by placing a 3 mm sample volume at the lateral, septal mitral annulus. Peak systolic (s), early (e') and late (a') diastolic myocardial velocities were recorded. Several cardiac cycles were evaluated and the best three consecutive ones were analyzed and averaged. Systolic dysfunction was defined as LVEF <45%, and diastolic dysfunction as E/A<1 and/or E/E'>10 and/or EDT<140 ms.

Other laboratory tests: When needed, coloured Doppler and B mode ultrasonography, CT, MRI, endoscopy, further blood and urine analyses, bone marrow aspiration and/or biopsy, direct Coombs test, faecal occult blood test, blood smear and tissue biopsies were performed in order to diagnose and treat comorbidities accompanying AHF.

Statistics

Numerical variables are presented as means with standard deviation, and nominal variables in ratios. Patients were classified into 2 groups as anemic and non-anemic, based on the dependent variable anemia. Nominal independent variables were compared using chi-square test between the anemic and non-anemic groups.

One sample Kolmogorov-Smirnov test was done to see if the continuous independent (numerical) variables were normally distributed. Normally distributed independent continuous variables were compared with Student's t-test, whereas non-normally distributed independent continuous variables were compared with Mann-Whitney U-test among the groups. Significant independent nominal variables and independent continuous variables between two groups were subjected to Multivariate Binary Logistic Regression Analysis (MBLRA) under the dependent variable of anemia.

The resultant variables after MBLRA were considered as independent variables accounting for anemia. These independent variables are presented as OR, 95% CI and p value. The overall extent of accountability of these independent variables obtained through MBLRA analysis for anemic patients was presented.

Bivariate correlations were sought between certain continuous nominal variables. Multivariable Linear Regression Analysis (RA) was also performed with the dependent variable of haemoglobin to show the association of CA use with lower haemoglobin and association of digoxin use with higher haemoglobin, after controlling for other factors that may be related with haemoglobin levels in heat failure. A two-tailed p value of <0.05 was considered as statistically significant (Table 2).

Ethics

The study was approved by Vakif Gureba Training and Research Hospital ethics committee.

Results

There were 223 patients included in our study, 106 of them were female, 117 were male, and mean age was 69.22 ± 11.21 y, with an age range of 23-91 y. 52.9% of the patients were anemic. Anemic and non-anemic groups were compared according to gender, age and BMI. 60.99% of the patients had systolic and 39.01% had diastolic heart failure. Systolic and diastolic heart failure were observed in similar rates in the anemic and non-anemic groups. The mean Ejection Fraction (EF) value was 33.16 ± 10.13 in systolic heart failure patients and 55.61 ± 11.15 in diastolic heart failure patients. Systolic and diastolic heart failure sub-groups in the anemic group were similar to those in the non-anemic group in terms of EF. 37.7% of the patients had hypertension related heart failure, 41.7% had ischemic, 10.8% had valvular, and 9.8% had non-ischemic heart failure. There was no significant association between the cause of heart failure and anemia.

Arterial hypertension (systolic/diastolic ≥ 140/85 mmHg), previous valvular surgery, DM and CRF were significantly more frequent in the anemic group.

As for regular medication use during minimum 3 months before admission to the hospital, digoxin use was more frequent in the non-anemic group, whereas Calcium Antagonist (CA) use was insignificantly more frequent in the anemic group (Table 3).

Corrected calcium levels were insignificantly lower in the anemic group. Absolute blood lymphocyte count, eGFR, albumin, LDL cholesterol, triglyceride, serum AST, serum ALT, serum indirect bilirubin, Transferrin Saturation Rate (TSR) and FT3 were lower in the anemic group; whereas Erythrocyte Sedimentation Rate (ESR), d-dimer, proBNP and serum creatinine were higher in the anemic patients.

Thus, totally 19 independent variables were found to be significantly different between the anemic and non-anemic groups and MBLRA was performed with these variables (excluding creatinine and including corrected calcium level and CA use, consequently 20 variables in total) under dependent variable of anemia.

As the result of MBLRA, 7 independent variables were found to be associated with anemia in AHF, independent from each other.

Anaemia prevalence was higher in the following conditions:

1. Lower serum AST (OR: 1.02), (95% CI: 1.005-1.035), (p=0.010).
2. Lower LDL cholesterol (OR: 1.015), (95% CI: 1.004-1.028), (p=0.008).
3. Lower triglyceride (OR: 1.008), (95% CI: 1.001-1.014), (p=0.015).
4. Lower transferrin saturation rate (OR: 1.053), (95% CI: 1.021-1.085), (p=0.001).
5. Chronic renal failure (OR: 2.89), (95% CI: 1.284-6.494), (p=0.010).

6. CA use (OR: 3.029), (95% CI: 1.211-7.578), (p=0.018).

Table 1. *Demographics and laboratory results of patients with acute heart failure in anaemic and non-anaemic groups. NND: Non-Normal Distribution; ND: Normal Distribution.*

Parameters	Anaemic group		Non-anaemic group		Normal range	P value	Type of distribution
	Mean	sd	Mean	sd			
Age (y)	70	11.11	68.35	11.3		0.274	ND
BMI, kg/m^2	26.08	5.31	27.01	6.4	18.5-24.9	0.267	NND
Ejection fraction, proportion of 1	42.37	14.67	40.58	15.06	>0.50	0.347	NND
proBNP, pmol/L	1545.57	3127.47	918.39	1686.36	<14.8	0.017	NND
D-Dimer, nmol/L	9.16	8.97	6.43	8.35	<3	0.001	NND
sCRP, nmol/L	22.1	21.33	18.86	25.14	0.00-4.76	0.188	NND
ESR, mm/h	38.23	26.03	28.13	21.69	5-15	0.003	NND
TSH, uIU/mL	2.06	1.76	1.95	1.78	0.53-3.59	0.48	NND
FT3, pmol/L	3.39	0.99	3.74	1.19	3.54-7.70	0.013	ND
FT4, pmol/L	17.89	3.99	17.63	5.02	43099	0.867	NND
Glucose, mmol/L	7.93	4.16	7.94	3.8	3.9-6.1	0.521	NND
HbA1C, proportion of total hemoglobin	0.0654	0.0137	0.0701	0.0214	<0.06	0.428	NND
Uric acid, µmol/L	453.87	171.91	428.89	153.47	142.8-339.1	0.259	ND
Albumin, g/L	37.6	5.2	39.4	5.9	35-50	0.004	NND
Natrium, mEq/L	138.72	4.88	138.79	5.11	135-155	0.671	NND
Magnesium, mmol/L	0.78	0.14	0.76	0.11	0.649-1.048	0.702	ND
Kalium, mEq/L	4.52	0.74	4.53	0.6	3.50-5.50	0.878	ND
Corrected serum calcium, mmol/L	2.25	0.19	2.28	0.2	2.10-2.68	0.07	ND
Phosphorous, mmol/L	1.27	0.35	1.21	0.28	0.87-1.45	0.276	NND
CK, U/L	119.71	226.95	101.79	124.01	29-168	0.944	NND
Creatinine, µmol/L	140.56	93.7	94.59	33.59	<106.08	0.001	NND
eGFR (MDRD), ml/min	56.98	32.49	75.21	40.35	>90	0.001	ND
Triglyceride, mmol/L	1.29	0.73	1.58	0.83	<2.26	0.001	NND
LDL, mmol/L	2.3	0.85	2.85	1.04	1.55-3.89	0.001	NND
HDL, mmol/L	0.98	0.35	1.07	0.37	>1.68	0.092	ND
Direct bilirubin, µmol/L	4.79	4.45	4.62	4.45	0-4.28	0.967	NND
Indirect bilirubin, µmol/L	6.84	4.79	8.38	4.96	3.42-16.25	0.003	NND
AST, U/L	26.14	26.31	39.52	76.25	15-38	0.001	NND
ALT, U/L	26.03	41.44	35.07	65.61	10-50	0.001	NND
ALP, U/L	95.42	62.13	93.06	36.79	40-129	0.259	NND
GGT, U/L	47.91	64.42	49.23	49.47	8-61	0.07	NND
LDH, U/L	456.19	263.46	470.8	217.95	240-480	0.258	NND
White blood cell count, no × 10^9/L	8.784	3.906	8.692	2.776	4.6-10.2	0.524	NND
Absolute lymphocyte count, no/L × 10^9	1.399	0.73	1.643	0.836	0.6-3.4	0.02	ND

Platelets, no × 10^9/L	239.0746	98.5726	234.0198	86.0061	142-424	0.758	NND
Hemoglobin, g/L	104.4	16.1	138.7	11.4	127-181	0.001	ND
Hematocrit, proportion of 1	0.321	0.051	0.412	0.039	0.377-0.537	0.001	ND
MCV, fL	83.69	8.65	86.82	7.14	80.0-97.0	0.004	NND
Red cell distribution width, proportion of 1	0.1801	0.1276	0.1595	0.0479	0.116-0.148	0.004	NND
Folic acid, nmol/L	20.05	9	20.64	7.77	10.88-84.30	0.154	NND
Vitamin B12, pmol/L	407.53	335.99	364.38	352.24	140.9-489.2	0.17	NND
Ferritin, pmol/L	364.08	396.26	404.33	399.83	29.21-337.05	0.102	NND
Transferrin saturation rate, proportion of 1	0.1713	0.1287	0.2321	0.1117	0.15-0.4	0.001	NND

Table 2. *Nominal variables between anaemic and non-anaemic groups.*

Parameters	**All patients n: 223**	**Anaemic group n: 118**	**Non-anaemic group n: 105**	**P value**
Gender, female/male, n	106/117	59/59	47/58	0.434
Systolic heart failure, n	136	72	64	0.992
Diastolic heart failure, n	87	46	41	0.992
Hypertensive heart failure, n	84	44	40	0.901
Ischemic heart failure, n	93	56	37	0.065
Non-ischemic heart failure, n	24	9	15	0.109
Valvular heart failure, n	22	9	13	0.235
Mitral regurgitation, n	149	76	73	0.418
Aortic valve disease, n	59	26	33	0.112
Tricuspide regurgitation, n	117	59	58	0.434
Previous coronary by-pass operation, n	51	35	16	0.252
Pleural effusion and/or ascites, n	69	41	28	0.193
Hypertension, n	170	100	70	0.036
Hypotension, n	23	13	10	0.714
Previous valvular surgery, n	17	5	12	0.043
Chronic renal failure, n	67	49	18	<0.001
Acute renal failure, n	11	6	5	0.912
Atrial fibrillation, n	107	56	51	0.868
COPD, n	61	32	29	0.933
Diabetes mellitus, n	111	68	43	0.013

Table 3. *Medicines patients used regularly during at least 3 months prior to hospitalization.*

Medicine	**All patients n=223**	**Anaemic group n=118**	**Non-anaemic group n=105**	**P value**
Warfarin, n	28	12	16	0.254
Acetyl salicylic acid, n	106	62	46	0.193

Calcium antagonists, n	56	35	21	0.097
ACE inhibitors, n	74	41	33	0.6
Angiotensin receptor blockers, n	51	30	21	0.254
Statins, n	29	16	13	0.794
Beta blockers, n	107	54	53	0.482
Spironolactone, n	77	38	39	0.439
Diuretics, n	174	93	81	0.764
Digoxin, n	95	37	58	0.001

Anemia prevalence was less in the following condition:

1. Digoxin use (OR: 3.633), (95% CI: 1.68-7.858), (p=0.001).

In our study, these 7 independent variables after MBLRA accounted for 82.6% of all our anemia cases. Chi-square value of our model was 59.86 and Nagelkerke R square was 0.363.

There was a moderate bivariate positive correlation between corrected calcium levels and haemoglobin (r=0.213, p=0.001). There was no correlation between corrected calcium levels and eGFR or proBNP.

After performing RA, the following independent variables were found to be unrelated with haemoglobin levels: age (p=0.956), BMI (p=0.969), diabetes mellitus (p=0.879), hypertension (p=0.965), previous valvular surgery (p=0.906), ACE inhibitor use (p=0.922), ARB use (p=0.926), absolute lymphocyte count (p=0.885), FT3 (p=0.934), proBNP (p=0.875), albumin (p=0.855), AST (p=0.963), ALT (p=0.955), and triglyceride (p=0.769).

Table 4. *Multivariable linear Regression Analysis (RA) with the dependent variable Hb.*

Independent Variables	B	SE	Beta	t	p value
Female gender	-0.864	0.279	-0.191	-3.095	0.002
Digoxin use	0.837	0.295	0.177	2.835	0.005
CA use	-1.017	0.331	-0.191	-3.07	0.002
Corrected calcaemia	0.482	0.173	0.17	2.784	0.006
eGFR	0.009	0.004	0.157	2.464	0.015
LDL cholesterolemia	0.016	0.004	0.261	4.284	0.001
R square: 313; Adjusted R square: 288; F: 12.250.					

Variables associated with lower haemoglobin levels were these factors: female gender, CA use, lower eGFR, lower LDL cholesterol, TSR and lower corrected calcium. However, digoxin use was associated with higher haemoglobin (Table 4).

Discussion

Relations of some of the independent variables seen in MBLRA and RA to anemia are already well-known. Therefore, these are not going to be discussed here.

We had mentioned before that the increase of free intracellular calcium ion by EPO is necessary in order to stimulate the differentiation and proliferation of erythroid precursor cells and this was achieved through calcium ion influx from extracellular fluid, not by the mobilization of intracytoplasmic calcium ions [7]. Interestingly, though EPO causes calcium influx into the erythroblasts through voltage independent calcium channels and nifedipin is known to be a voltage dependent calcium channel blocker, nifedipin impeded calcium influx to erythroblasts and blocked the EPO activity *in vitro* [7-15].

Another *in vitro* study reported that nifedipin inhibited the differentiation of pluripotent stem cell into cardiomyocytes by altering calcium ion influx into the cell at the early stages of differentiation. This is because calcium ion influx into the stem cell activates Ca^{+2} signalling pathway, and this contributes to numerous physiological cellular pathways [16].

It was reported in an *in vitro* study that burst forming unit-erythrocyte (BFU-E) and colony forming unit-erythrocyte (CFU-E) derived erythroblasts of continuous ambulatory peritoneal dialysis patients with anemia exhibited less proliferation and had lower intracytoplasmic free calcium before rHuEPO therapy, compared to controls. *In vitro* addition of calcium to the medium surrounding the erythroblasts was shown to increase intracellular free calcium concentration and proliferation of erythroblasts and this process was potentiated by active vitamin D. When the same patients received rHuEPO treatment, the patients' anemia improved and intracellular calcium concentrations in their erythroblasts turned back to normal levels [17].

In a cross-sectional clinical study involving 625 haemodialysis patients, CA users were shown to have lower Hb levels and consumed higher amounts of EPO, compared to non-user [5]. We also showed that CA using patients had lower haemoglobin compared to non-user patients in our previous study-accepted but not published-conducted on stage 2-5 CKD patients not receiving renal replacement therapy, EPO and vitamin D treatment [8].

CA users also had lower Hb than CA non-users in our heart failure study population. CA use by individuals without CKD or chronic HF may not cause a reduction in Hb and Hct, since there is no EPO resistance and no limitation on EPO release. In

patients with CKD or chronic HF who have reduced EPO release and EPO resistance, CA use may additionally impede EPO activity, and thereby further diminish Hb and Htc levels.

Lower corrected calcium level was also associated with lower Hb after RA. There was a positive correlation between corrected calcium and haemoglobin. Higher calcium may have a clinically positive influence on haemoglobin levels and potentiate the effect of EPO. There was no correlation between corrected calcium and eGFR; and no correlation existed between corrected calcium and proBNP, either. In other words, the positive effect of calcium on haemoglobin seems to be independent of the severity of renal impairment or heart failure. These results overlap with the experiment in which addition of calcium into surrounding medium of erythroblast enhanced erythroblast proliferation under suboptimal EPO presence in the *in vitro* study mentioned above [17].

Studies conducted on patients on haemodialysis treatment have found that CA use did not result in EPO resistance [18,19]. These studies were conducted to find out whether ACE inhibitors or angiotensin receptor blockers (ARBs) caused EPO resistance or not. Patients who were not on antihypertensive treatment and who used CAs were taken as controls in these studies, and it was concluded that ACE inhibitors and ARBs caused EPO resistance, but CAs did not. However these studies included few CA using patients; 20 subjects in one study and 10 subjects in the other [18,19]. Saudan et al. divided the haemodialysis patients into 5 groups based on antihypertensive medication use, and these 5 groups were found to be similar in terms of EPO resistance (Group 1: ACE inhibitors, Group 2: ARBs, Group 3: ACE inhibitor plus ARB, Group 4: other antihypertensive medications, Group 5: no antihypertensive treatment) [20]. In this study, duration of antihypertensive medications before enrolment in the study is not clear and the numbers of patients in the "other antihypertensive medications" group were using CAs is also not mentioned.

Digoxin users had higher Hb levels compared to patients not using digoxin in our study. It is known that digoxin strongly inhibits Na/K-adenosine triphosphatase (Na/K-ATPase) found on the cell membrane and increases calcium concentration within the cardiomyocyte [9]. In the same manner, it might exhibit an anti-anemic effect by inhibiting the Na/K-ATPase on the cell membrane of erythrocyte precursors, thereby increasing calcium concentration within the erythroblast under conditions of EPO resistance or EPO deficiency. It is notable that, in the setting of EPO resistance or EPO deficiency, factors increasing or decreasing Ca ion influx into the cell through any mechanism can increase or decrease EPO activity as a consequence. An *in vitro* study supports that digoxin use may be associated with higher Hb levels in clinical heart failure. Digoxin was shown to stimulate *in vitro* murine erythroid stem cell colony formation in suboptimal EPO concentrations [21]. The positive correlation between corrected calcium and Hb, and anemia being more frequent in CA users and being less frequent in digoxin users may be explained by a unique mechanism in this manner.

Serum AST and ALT enzyme activities were lower in anemic patients compared with non-anemic patients. Pyridoxine is a co-factor required for AST and ALT enzyme activities [22]. In end stage renal failure patients, lower levels of AST and ALT activities were found to be associated with pyridoxine deficiency compared to controls [23]. In a study examining deficiencies of B group vitamins in heart failure, pyridoxine deficiency was found in 38% of heart failure patients, and in 19% of the control group (p=0.02) [24]. It is classical knowledge that pyridoxine deficiency is a cause of anemia. Therefore, lower AST may indicate the presence of anemia originating at least partially from pyridoxine deficiency in patients with AHF.

The MBLRA provided an explanation for more than 80 percent of anemic patients in the study. In addition, RA also confirmed our study results.

Limitations to our study are that serum digoxin levels and serum erythropoietin levels could not be determined because of the retrospective design. Cross-sectional design was another limitation. Trials should be conducted to reach a definitive conclusion in the future. We hope that our study may draw attention to this topic.

The most recent guideline recommends not using CAs in patients with current or prior symptoms of heart failure and reduced EF (risk ≥ benefit, level of evidence: A) [25]. On the contrary, digoxin use reduces mortality and hospitalization in heart failure [26]. The effect of CAs is opposite to effects of digoxin on anemia in heart failure and anemia in heart failure is a predictor of mortality [1,2]. As such, our study findings overlap with the current literature on heart failure treatment.

In conclusion, CA use, lower calcium and lower serum AST are related with anemia in heart failure. Digoxin use may have a positive effect on anemia in heart failure.

Conflict of Interest

We declare that there is no conflict of interest.

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*Correspondence to

Pinar Kalem

Department of Family Medicine

Ahi Evran University

Faculty of Medicine

Kirsehir

Turkey