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Vitamin D and iron levels correlate weakly with hepcidin levels in postoperative patients with digestive neoplasms undergoing open abdominal surgery

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Abstract. – OBJECTIVE: Patients with digestive neoplasms have both iron deficiency and chronic inflammatory anemia with hepcidin upregulation, which may be aggravated in the postoperative period. Vitamin D impacts hepcidin levels. We aimed to investigate the correlations between vitamin D and iron status vs. hepcidin levels in patients with digestive tumors undergoing open abdominal surgery.

PATIENTS AND METHODS: This prospective observational study was performed during 2016-2018 in a University teaching hospital. After obtaining the Ethical Approval and the patients' informed consent, 30 adult patients with digestive tumors were included. Hepcidin, vitamin D and iron levels were measured in the first 24 hours after surgery.

RESULTS: We observed a negligible/weak correlation between serum iron and hepcidin levels in the first 24 hours after surgery, with a correlation coefficient of 0.24 and a weak/low correlation between hepcidin and vitamin D levels, with a correlation coefficient of 0.37.

CONCLUSIONS: The correlations between vitamin D and hepcidin levels, as well as between hepcidin and serum iron levels, are weak. Inter-individual variability in iron-hepcidin-vitamin D regulation might be wide and other regulatory mechanisms might also play important roles in inflammatory anemia modulation in the perioperative period.

Key Words:
Hepcidin/iron status in surgical patients.

Introduction

Patient blood management is a multimodal approach for the treatment of perioperative anemia and is important in the perioperative care of the surgical patients. Over 60% of the surgical patients with digestive tumors are anemic[1]. In these patients, anemia management deserves special consideration, both anemia and red cells transfusion having increased risk of morbidity and mortality. While anemia contributes to cardio-vascular perioperative morbidity due to decreased oxygen-carrying capacity, transfusions are linked to immune suppression, with higher risks of infections and cancer recurrence. Patient blood management is desirable in order to improve patient safety[2]. The recommendations in most patient blood management programs are to adopt restrictive transfusion strategies while avoiding cellular hypoxia. Complementary methods would be the use of perioperative iron administration. Any other method that would ameliorate iron status and that would lower transfusion rates, would be of interest, especially if associated with a good safety profile.

Hepcidin, which mediates anemia of inflammation, might be an important target for perioperative blood management. Hepcidin levels are upregulated in inflammatory conditions, such as neoplastic disease, inflammation, and sepsis[3,4]. Hepcidin is the key regulator involved in the intracellular sequestration of iron which characterizes acute and chronic inflammatory states. Hepcidin blocks ferroportin, the iron exporter, preventing iron release from cells and leading to iron restriction anemia[4-6]. In patients with chronic inflammatory, conditions like chronic kidney disease on hemodialysis, vitamin D supplementation reduces hepcidin levels and transfusion requirements[7,8]. Hepcidin levels decrease in response to vitamin D administration in healthy volunteers, and also in critically ill patients[9,10].

Patients with digestive neoplastic diseases display both iron deficiency and iron-sequestration

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anemia due to chronic inflammation, while surgery adds elements of acute inflammation. The effects of vitamin D administration upon iron status in patients with digestive malignant tumors and their transfusion requirements have not yet been evaluated in studies similar to the aforementioned patient populations. The impact of individual vitamin D levels on hepcidin levels in patients with digestive neoplasia might be of interest. Are patients with vitamin D deficiency at increased perioperative risk due to the need for higher transfusion rates? Do patients with higher serum levels of vitamin D demonstrate lower hepcidin levels and are they protected from the requirements of transfusion-associated complications? The relation between vitamin D status and hepcidin levels in the perioperative period, as well as the relation between iron status and hepcidin levels, have not been extensively investigated in this patient category.

The aim of this study is to describe the correlation between Vitamin D and iron status *vs.* hepcidin levels in postoperative patients with digestive neoplasms undergoing open abdominal surgery.

Patients and Methods

The Ethical approval for this prospective study was obtained from the "Iuliu Hatieganu" University of Medicine and Pharmacy (The Research Ethics Committee, Approval No. 434/24.112016) and from the Clinical Emergency County Hospital Cluj-Napoca (Approval No. 21833/24.10.2016).

Participants

Adult patients, aged 18-80 years, undergoing open abdominal surgery for digestive neoplasms (liver, esophageal, gastric and colon malignant tumors) were included. Written informed consent was obtained from the patient. Exclusion criteria were: chronic inflammatory conditions (chronic kidney disease, hematologic, and rheumatic/autoimmune disease), morbid obesity (BMI over 40 kg/m^2), pregnancy and lactation, hypercalcemia (total calcium> 10.6 mg/dL, serum ionized calcium> 5.4mg/dL), tuberculosis, sarcoidosis, nephrolithiasis, recent history of vitamin D supplementation or erythropoietin treatment.

Data Collection and Laboratory Dosing

Six mL of blood was drawn from each patient for laboratory dosing in the first 24 hours after surgery for baseline values. Full blood count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), serum iron, ferritin, transferrin, C reactive protein (CRP), and fibrinogen were recorded. Transferrin saturation (TSAT%) was calculated then as the ratio between serum iron (μg/dL) and transferrin concentration (mg/dL) multiplied by a constant of 70.9[11]. TSAT below 20%, transferrin lower than 12 ng/mL and ferritin lower than 100 ng/mL reflect absolute iron deficiency[12,13]. Blood samples were centrifuged at 3000 rotations/minute and serum was stored at -20°C for a maximum of 30 days. Hepcidin and vitamin D levels were determined. Hepcidin dosing was performed using a standard sandwich high-sensitivity enzyme-linked assay (ELISA) (Human Hepcidin PicoKine®, Boster Biological Technology, Pleasanton, Canada). Vitamin D dosing was done using a standard immunoenzymetric assay: 25(OH) Vitamin D Total ELISA (DIAsource ImmunoAssays®, Louvain-la-Neuve, Belgium). Other laboratory analyses were performed *via* automated dosing using standard hospital methods.

Data Analysis

Continuous data are presented as mean and 95% confidence intervals in parentheses. Correlations (regression analysis with linear correlation equations) were performed to evaluate the association between each continuous data (quantitative variables) and Pearson correlation coefficient to evaluate the strength of association was calculated with Excel® (Microsoft Office).

Results

Recruitment and Baseline Values

A total number of 30 patients were included in the study. From these, 19 presented colorectal adenocarcinoma, 2 patients had esophageal adenocarcinoma, one liver hepatocarcinoma, and 8 had gastric adenocarcinoma. Baseline demographic and laboratory data, including hepcidin, vitamin D, and serum iron concentrations are presented (Table I). All patients had underlying comorbidities at enrollment, including diabetes mellitus, coronary artery disease, and hypertension.

Only three men had hemoglobin value above 13 g/dL and 5 women had values higher than 12 g/dL, so that only 8 patients were considered as

Table I. Baseline characteristics and laboratory data (mean values and 95% confidence intervals in parentheses).

	Mean ± Standard deviation
Age (years)	63 ± 8
Body mass index (kg/m^2)	25.15 ± 5.08
Urea (mg/dL)	34.55 ± 17.32
Creatinine (mg/dL)	0.85 ± 0.24
Hemoglobin (g/dL)	10.97 ± 1.52
Hematocrit (%)	33.53 ± 4.09
Leukocytes (109/L)	11398 ± 3387
Platelets (109/L)	222433 ± 68635
MCV (fL)	84.73 ± 7.62
MCH (pg)	27.76 ± 3.42
MCHC (g/dL)	32.68 ± 1.41
Fibrinogen (mg/dL)	425.57 ± 85.83
Ferritin (ng/mL)	149.65 ± 130.97
Transferrin (mg/dL)	194.73 ± 37.62
Serum iron (µg/dL)	19.03 ± 13.43
TSAT %	7.27 ± 5.55
CRP (mg/dL)	11.68 ± 4.88
Hepcidin (ng/mL)	101.78 ± 89.96
Vitamin D (ng/mL)	42.36 ± 11.71

N = number of patients, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, CRP = C reactive protein, TSAT% = transferrin saturation.

not anemic. All patients had serum iron concentrations below 60 µg/dL. Almost all patients had TSAT below 20%, except one patient. None of the patients had ferritin below 12 ng/mL. No patient presented absolute iron deficiency anemia. Thus, 22 from our 30 patients presented both markers of iron deficiency anemia, as well as increased levels for the inflammatory markers, characteristic for inflammatory anemia. Fibrinogen levels were more strongly correlated with CRP levels compared to leukocytes values and hepcidin (Figure 1A, B, C). Only one patient had baseline vitamin D levels below 20 ng/mL (severe vitamin D deficiency). None of our elective surgical patients received red packed cells transfusions during hospital stay.

Correlations

We observed a negligible/weak correlation between serum iron and hepcidin levels in the first 24 hours after surgery, with a correlation coefficient of 0.24 (Figure 2).

We observed a weak/low correlation between hepcidin and vitamin D levels in the first 24 hours after surgery, with a correlation coefficient of 0.37 (Figure 3).

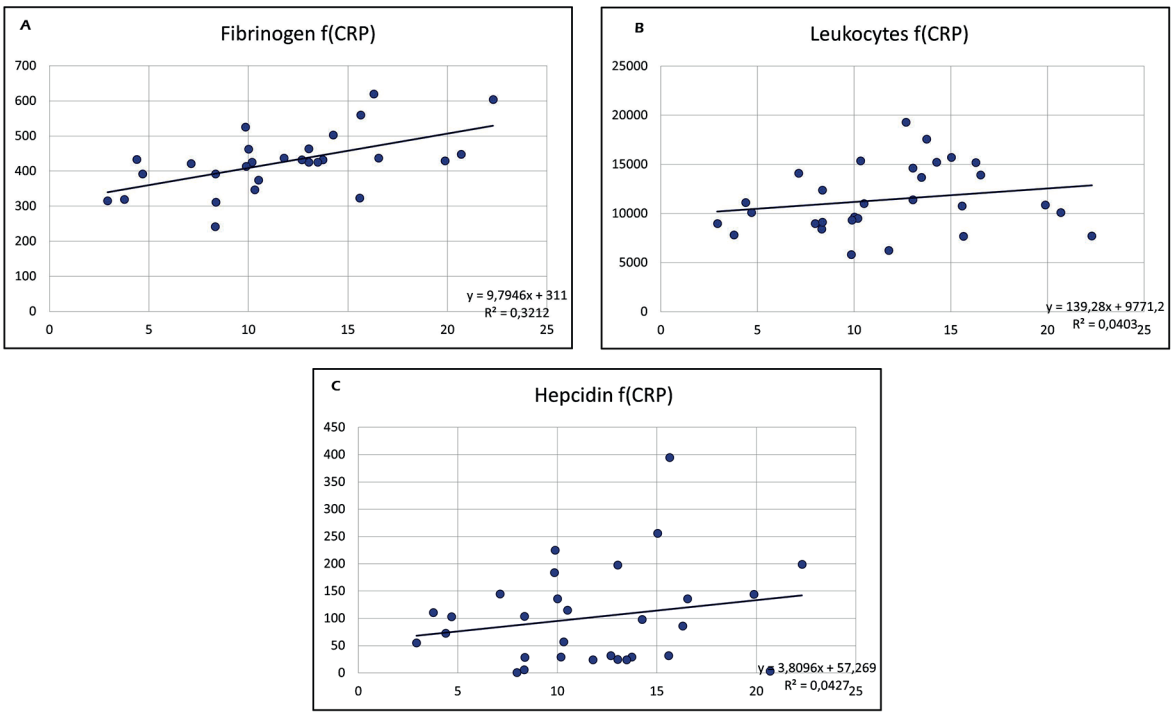

Figure 1. A, Fibrinogen and C-reactive protein (CRP) correlation. **B,** Leukocytes and C-reactive protein (CRP) correlation. **C,** Hepcidin and C-reactive protein correlation.

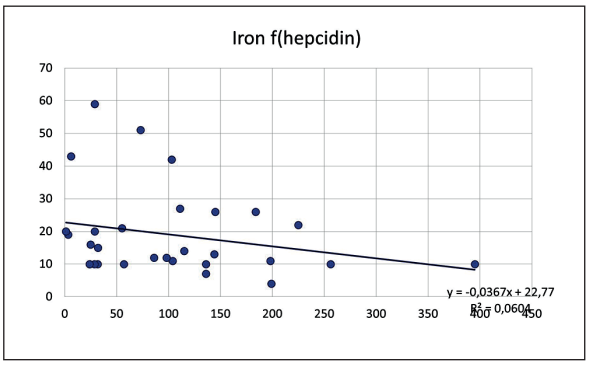

Figure 2. Iron and hepcidin correlation.

Discussion

Hepcidin is the master regulator of iron metabolism, and hence, a modulator of anemia in states of inflammation. Hepcidin is an acute phase protein synthetized in the liver which acts as an hyposideremic hormone[14]. It binds to ferroportin (an iron exporter) and prevents the release of iron from the cells: prevents the absorption of dietary iron from enterocytes and prevents iron release from macrophages, where it is stored[3,5]. Thus, the effect of hepcidin would be iron sequestration, lowering the serum iron concentrations. The beneficial result would be a low availability of iron for bacterial growth and less oxidative stress. The detrimental result is the limited possibility for the synthesis of new hemoglobin molecules and the occurrence of anemia. The upregulation of hepcidin, as a pro-inflammatory biomarker, characterizes both acute and chronic inflammatory conditions. Excess values of the iron regulating hormone hepcidin causes intracellular sequestration of iron and decreases the availability of iron for erythropoiesis, leading to the anemia of inflammation. Anemia is frequent in the cancer surgical population and is associated with increased transfusion rates and worse outcomes[15].

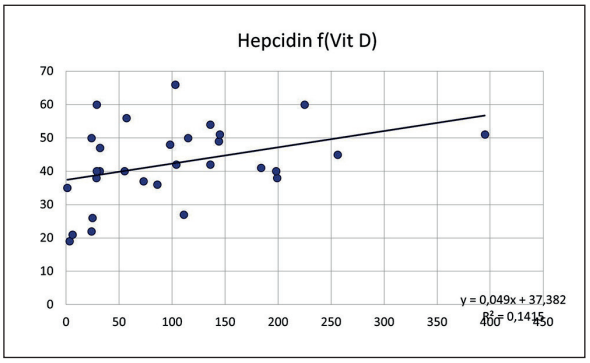

Figure 3. Hepcidin and vitamin D correlation.

In well-defined patient populations with chronic anemia of inflammation, the administration of oral vitamin D supplements lowered hepcidin values, improved iron status profiles and lowered transfusion requirements[7,8]. However, in patients with digestive cancers, no study evaluated the benefit of vitamin D administration on transfusions and patient blood management programs do not recommend the evaluation of vitamin D status simultaneously with iron status preoperatively. Also, the relation between endogenous individual levels of vitamin D and hepcidin values has not been evaluated. The relationship between vitamin D and iron status has not been extensively investigated so far in surgical patients. Vitamin D levels might have an impact on hepcidin serum levels in patients with digestive tumors undergoing open abdominal surgery and could be integrated in patient blood management programs. Patients with digestive tumors might display nutritional vitamin D deficiency. Our hypothesis was that vitamin D levels might be important for iron status management in the perioperative period through the hepcidin link. Higher vitamin D levels might lower hepcidin levels and increase iron availability. Vitamin D directly suppresses hepcidin mRNA transcription and has been shown to support erythropoiesis[7]. Also, vitamin D has anti-inflammatory and immune-regulating properties[16].

We found weak associations between vitamin D and iron levels *vs.* hepcidin levels in our modest cohort of 30 surgical patients. This suggests that other factors might play important roles in inflammatory anemia modulation in the perioperative period, along with hepcidin-vitamin D complex regulation. Other iron regulators might be involved, including fibroblast growth factor, parathyroid hormone, iron regulatory proteins, and matriptase[6,17].

This study describes the relation between vitamin D and hepcidin levels in patients with digestive tumors, immediately after surgery, together with the relation between hepcidin levels and postoperative iron status. Studies[7,8] including other patients with inflammatory conditions proved the benefit of vitamin D administration on iron status and transfusion requirements by providing a safe alternative. We wondered whether the levels of vitamin D in the perioperative setting are correlated with hepcidin levels

and iron status. Patients with digestive tumors could be offered a safe adjuvant therapy that might lower transfusion requirements. Are patients with vitamin D deficiency and digestive tumors at increased postoperative transfusion risk? Larger studies are needed to evaluate this hypothesis. Only one patient in our cohort had vitamin D deficiency and none of the patients received transfusions.

There are several limitations to our study. First, the patient sample is modest, and we included only cross-sectional values, not dynamic measurements in time. Definitive answers regarding the impact of vitamin D levels or supplementation on transfusion requirements and iron status could be obtained by including larger patient numbers and performing interventional studies with different dosing regimens. Our study shows that endogenous vitamin D levels might be less important for the transfusion requirements in the perioperative period. Regular determination of vitamin D levels dosing are not suggested by our data as there is no strong link between vitamin D levels and hepcidin/iron levels. Still, it could be of interest to investigate the impact of vitamin D supplementation on iron status and transfusion requirements. Our data highlights the weak correlation between static levels, which does not exclude the effect of increasing vitamin D levels by using supplements on hepcidin levels variations. Vitamin D supplementation is easy to perform and could be easily integrated in patient blood management guidelines if found effective for the improvement of iron status and lowering of transfusion rates.

We chose a category of surgical patients with inflammatory iron-restriction anemia, in whom blood management and perioperative transfusion issues are of major importance for the clinical outcome. This well-defined patient category displays anemia due to chronic illness or inflammatory anemia, as well as iron deficient anemia due to poor nutritional status and occult blood loss, or a combination of the above[15]. Anemia caused by chronic disease, also known as anemia of inflammation, is prevalent in patients with cancer[18]. After major abdominal surgery, an acute inflammatory stress response is added. The results were not influenced by iron, erythropoietin or vitamin D administration. Our patients presented baseline low iron concentrations due to both iron deficiency and excess hepcidin levels as a result of inflammation. Anemia of chronic disease is aggravated in acute inflammatory stress responses. The inflammatory status of the patients was assessed using ferritin, CRP, and fibrinogen levels. Iron deficient anemia *vs.* inflammatory anemia may be difficult to discriminate. However, the aim of our study was not to differentiate between these two conditions, but to investigate the relation between vitamin D levels, hepcidin and iron status parameters in the early postoperative period.

Conclusions

The correlations between vitamin D and hepcidin levels, as well as between hepcidin and serum iron levels, after major abdominal surgery in patients with digestive tumors, are weak. Thus, regular preoperative evaluation of vitamin D levels in patient blood management bundles cannot be recommended based on our data. Interindividual variability in iron-hepcidin-vitamin D regulation might be wide and other regulatory mechanisms might also play important roles in inflammatory anemia modulation in the perioperative period.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Declaration of funding interests: these data represent a pilot analysis for No. 4466/2019 "Iuliu Hatieganu" University of Medicine and Pharmacy.

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