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ORIGINAL ARTICLE

Changes of serum prohepcidin, iron status and zinc-protoporphyrin in a random group of patients with malignant diseases

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Summary

Purpose: *To investigate the changes in the serum levels of prohepcidin (pHp) and markers of iron homeostasis for gathering more data on the pathogenesis of anemia in malignancies.*

Methods: *In 84 patients with advanced solid malignant tumors, but without iron or vitamin B12 and folate deficiency anemia, we measured serum pHp levels and common markers of iron status, erythropoiesis and inflammation. Two months later the same tests were repeated to determine possible changes in the levels of the measured parameters.*

Results: *The first blood sample characterized the group with a moderately low hemoglobin (Hb) and high CRP levels, suggesting anemia in some patients. Two months later higher levels of serum iron (sFe), total iron binding capacity (TIBC), transferrin saturation, ferritin and zinc-protoporphyrine (ZPP) in erythrocytes were found, along with lower pHp and high sensitivity C-reactive protein (hs-CRP). The correlation coefficient (R) between the values of iron-containing substances and pHp were low (R=0.244). Allocations by sex, Hb concentration and pHp showed that the changes in each group were similar, keeping the trend of increased sFe, ferritin and ZPP, decreased hs-CRP and pHp, at a stable hematological state.*

Conclusion: *Because of low correlation between sFe and pHp, it seems more likely that the positive two-month change of iron-containing substances in the serum of the studied patients is a result of treatment, impaired liver function or malignant intoxication, rather than of decreased pHp.*

Key words: anemia, ferritin, iron binding capacity, malignancy, prohepcidin, zinc-protoporphyrin

Introduction

It is known that some patients with malignant diseases develop a moderate anemia resembling the anemia of inflammation or of chronic disease [1-3]. The etiology of such a normocytic, normochromic anemia is not well understood, but evidence is now available that the rise of pro-inflammatory cytokines and CRP affects iron turnover, lowering serum iron without clinically manifested iron deficiency [1,4]. On the other hand pro-inflammatory substances stimulate hepatocytes to produce prohepcidin, the precursor of the more active hepcidin. The latter binds and degrades the membrane iron-transporter ferroportin, leading to interruption of the intestinal absorption of iron and its release by macrophages, resulting in low serum iron and hypoproliferative anemia [5-9].

Possibly most cases of anemia in patients with malignant diseases have complex etiology depending on the level of tissue damage, intensity of inflammation and regulatory capability of the liver to avoid disturbance of iron homeostasis, for example via hepcidin production. If it is so, the irreversible evolution of the malignant disease despite treatment, would give a chance to reveal some trends in the pathogenesis of that kind of anemia.

With the aim to gather more data on the change in the level of the factors involved in the pathogenesis of this common complication in malignancy we measured the concentration of serum pHp, some markers of hemopoiesis and iron status in patients with malignant diseases.

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Methods

Study population

The eligible patients were selected from all persons with advanced solid malignant tumors consecutively admitted to the Clinic of Oncology and Hematology, University Hospital, Plovdiv, Bulgaria, from March to August, 2009.

Exclusion criteria

Positive medical history for kidney, heart and liver disease, recent infections or antibacterial treatment, blood transfusion or erythropoietin administration, known causes for iron, vitamin B12 and folate deficiency with or without relevant treatment, mean corpuscular volume of red blood cells < 75 fl, to remove patients with potential thalassemia or iron deficiency anemia and > 95 fl to remove patients with vitamin B12 and folate deficiency anemia.

A random group of 84 patients was formed - 56 women, aged 26-77 years (average 53.5± 11.0 standard deviation/SD) and 28 men aged 27-79 years (average 60.1± 11.1 SD).

The primary tumors' locations were as follows:

32 cases of HER negative breast cancer

28 cases after operation for gastric, colon and rectal cancer

10 cases with non small cell lung cancer (NSCLC) after pneumonectomy

14 cases with prostate cancer after orchidectomy, all with bone metastases.

Study design

Venous blood (5 cm^3 with anticoagulant and 5 cm^3 without anticoagulant) was taken in a vaccutainer system from all eligible patients on the morning next to the day of admission (first sample). Approximately two months later (63 days±16 SD for women, and 67.5 days ±18.3 SD for men) eligible patients were admitted for the next planned hospitalization. Then a second blood sample was taken. The difference between the results for any analyzed parameter was defined as "change" (second sample minus first sample) and presented as percent increase (+) or decrease (-). Hb concentration < 120 g/l for women and < 130 g/l for men was used to define patients with anemia, according to the Word Health Organization criteria. During the study period all patients continued to receive treatment for their cancer.

Laboratory analysis

Within 6 hours from blood sampling, an automated analyzer Sysmex SE-9500, RAM-I (Japan) was used for the determination of > 30 hematological parameters. However, only the results of Hb, mean corpuscular volume of red blood cells (MCV), number of erythrocytes (RBC), reticulocytes (Ret), leucocytes (WBC) and platelets (PLT) were considered as more informative.

The blood serum for clinical chemistry analysis was separated immediately after phlebocentesis. sFe and TIBC were measured by means of Ferozin test (FluritestFe-FZ, Biocon, No 4551, UK) on clinical chemistry analyzer Konelab 60i (Thermo Electron Co, USA), and the transferrin saturation was calculated as sFe/TIBC × 100. hs-CRP was determined by Konelab 60i analyzer using an immuno-turbidimetric test (No 98 1798, Thermo Fisher Scientific, USA). ZPP was measured in lysed erythrocytes by using ProtoFluor-Z Hematoflluorimeter (Helena Laboratories, Inc, USA). The results were estimated by the ratio between fluorescence due to ZPP and absorbance due to heme, and expressed as ZPP mol / heme mol. Serum pHp was determined by a competitive immunotest (New ELISA DRG Hepcidin Prohormone EIA-4644, DRG International, Inc., USA). Serum concentration of ferritin, vitamin B12 (B12) and folate were measured by fluorogenic MEIA-kit (No 3C79-66 and No 3C81-20) on AXZIM immunological analyzer (all from Abbott Laboratories USA).

During the period of analysis no pitfalls in intralaboratory quality control and National external quality assessment scheme were documented. The results obtained were analyzed in relation to sex, anemic state, pHp concentration and tumor location.

Statistical analysis

Excel software was used to calculate averages, SDs, the probability associations between averages of identical analyses (Student's t-test, one-tailed or two-tailed distribution), and the degree of association between two variables (Pearson's correlation coefficient).

Results

The averages of the results obtained from the first blood sampling were within the corresponding reference intervals, with the exception of the lower Hb and higher hs-CRP. At the end of the two-month period a clear increase of sFe, TIBC, ZPP and ferritin, decrease of pHp and hs-CRP, at relatively stable state of the other measured substances, could be seen (Table 1).

Table 1. Average (±SD) values of both samples (n= 84)

Studied parameters (units)	*First sample*	*Second sample*	*Change %*	*p-value*
Hb (g/l)	122 (14.4)	122 (16.7)	0.5	NS
RBC (T / l)	4.43 (0.553)	4.31 (0.607)	–2.8	<0.01
MCV (fl)	84.5 (6.98)	85.4 (8.00)	1.1	NS
WBC (G / l)	6.35 (2.38)	6.62 (3.01)	4.2	NS
PLT (G / l)	281 (100)	282 (110)	0.4	NS
Ret (T / l)	7.26 (5.80)	9.09 (6.73)	25.1	<0.05
hs-CRP (mg / l)	9.76 (14.2)	8.67 (12.5)	–11.1	NS
pHp (µg / l)	64.7 (26.8)	50.3 (39.7)	–22.2	<0.005
sFe (µmol / l)	13.2 (4.64)	15.1 (5.68)	14.4	<0.005
TIBC (µmol / l)	56.7 (9.11)	63.1 (9.30)	11.3	<0.0001
Saturation (%)	23.7 (8.58)	24.3 (9.31)	2.2	NS
ZPP (µmol/mol heme)	60.7 (23.2)	73.6 (32.7)	21.2	<0.005
Ferritin (mg / l)	102 (136)	137 (226)	33.6	<0.05
B12 (pmol / l)	220 (170)	221 (154)	0.2	NS
Folate (nmol / l)	23.3 (7.46)	23.4 (11.0)	0.6	NS

NS: non significant. For other abbreviations see text

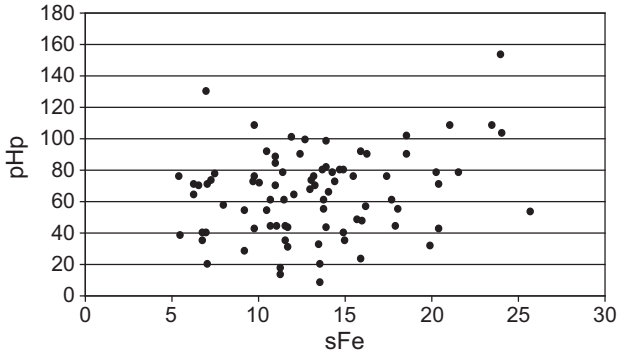

Figure 1. Scatter plot of concentration of sFe against pHp from the first sampling (r = 0.244).

Although the analyzed parameters in the second sample remained within the reference intervals, the decrease of pHp and the increase of iron-containing substances and ZPP in RBC can not be neglected. However, the correlation between decreased pHp and increased sFe was low and positive (Figure 1), instead of an expected higher and negative relationship.

The correlations between the other studied substances and pHp concentrations and iron-containing substances are shown in Table 2.

The impact of age on the sFe and pHp was very low (R=0.00524 and 0.11735, respectively), as well as the relationship of the number of the days between the two samplings (R=0.01158 and 0.2113, respectively).

Looking for other potential causes for the changes obtained, we allocated the results by sex, Hb and pHp concentration.

In both sexes the two-month interval led to similar trends - decrease of pHp, increase of ZPP and iron-containing substances. Intra-group differences appeared in almost all parameters, but the only significant one was the higher WBC number in men ($p<0.05$). With Hb cut-off values 120 g/l for women and 130 g/l for men 40 patients were classified as "without anemia" and 44 patients as "with anemia". The two-month interval led in both groups to similar decrease of pHp and increase of TIBC, ZPP, ferritin and sFe. Besides the expected 20% lower Hb and RBC intra-group differences showed that patients with anemia had higher PLT number ($p<0.01$), ZPP concentration ($p<0.005$) and lower pHp ($p<0.035$) than patients without anemia. Patients with anemia, in contrast to patients without anemia, expressed more increase of sFe and transferrin saturation ($p<0.02$), but non significant drop of hs-CRP ($p>0.05$). With pHp

Table 2. Coefficients of correlation (R) between the studied parameters (n = 84)

Parameters	*R*	*Parameters*	*R*	*Parameters*	*R*
Hb/RBC	0.769	RBC/sFe	0.207	sFe/TIBC	0.238
Hb/sFe	0.211	RBC/TIBC	0.331	sFe/Ferritin	0.087
Hb/TIBC	0.128	RBC/Ferritin	–0.227	sFe/ZPP	–0.073
Hb/Ferrit	–0.255	RBC/ZPP	–0.471	sFe/pHp	–0.034
Hb/ZPP	–0.447	RBC/pHp	0.011	ZPP/pHp	–0.011
Hb/pHp	–0.028	TIBC/Ferritin	–0.074	Ferritin/ZPP	0.351
TIBC/ZPP	–0.327	Ferritin/pHp	0.099	TIBC/pHp	0.103

For abbreviations see text

Table 3. Two-month changes as % increase or decrease of the studied parameters

Parameters	*Men (n=27)*	*Women (n=57)*	*Without anemia (n=40)*	*With anemia (n=44)*	*High pHp (n=57)*	*Low pHp (n=27)*
Hb	–0.0	0.8	0.2	0.8	–0.1	1.4
RBC	–3.5	–2.3	–4.0	–1.6	–3.2	–1.8
MCV	1.2	1.1	1.00	1.3	0.6	2.1
WBC	3.6	4.6	1.4	8.1	6.2	0.2
PLT	4.2	4.6	0.3	1.0	–4.2	10.0**
Ret	38.5	19.6	27.0	23.8	6.7	69.6*
hs-CRP	–22.2	–1.2	24.9	–239	38.7	7.2
pHp	–26.5	–20.2	–16	–29	0.2	–83.7***
sFe	11.5	15.8	1.7	31.5**	9.25	27.8
TIBC	13.4	10.3	11.0	11.6	13.3	7.3
Saturation	2.4	4.5	–9.5	18.1**	–4.6	20.4**
ZPP	7.7	28.5	24.8	18.8	17.5	28.9
Ferritin	20.5	43.9	15.7	44.5	31.3	41.1
B12	–3.9	2.0	–3.4	3.4	–5.6	14.9
Folate	–0.0	0.5	–3.8	4.9	1.2	–3.0

*p<0.05; **p< 0.02; ***p<0.001
For abbreviations see text

cut-off value 40 μg/l 57 patients were defined as having "high" and 27 as having "low" pHp. During the two months the high pHp group showed some rise of TIBC, ZPP and ferritin (p>0.05), but no change in pHp concentration. In contrast, the low pHp group displayed increase of PLT, Ret, and modest rise of iron-containing substances, transferrin saturation and iron (p>0.05), but steep decrease in pHp (p<0.001) (Table 3).

Discussion

The studied patients formed a heterogeneous group and their only common characteristic was the presence of advanced solid tumor. The first blood sample characterized the group by a moderately low Hb and high hs-CRP concentrations, suggesting anemia in malignancy [1-3]. The second sample, taken two months later, showed lower pHp concentration, higher iron-containing substances and raised ZPP in RBC. This finding corresponds well with the current concept for the regulative role of pHp in iron homeostasis [6,8], and an acceptable correlation between pHp and iron would be expected [9]. However, the correlations between pHp and iron-containing substances, calculated from the results obtained, are too low and not convincing. The results by sex, anemia and pHp concentration show that the trend of increasing iron-containing substances and decreasing pHp persists, despite the finding of increased pHp in colorectal tumors [10].

At the same time the hematological state of patients remains relatively stable. The raised sFe, transferin saturation, reticulocyte number and the decreased pHp that can improve the iron deficiency at the level of bone marrow, offer a chance for better erythropoiesis. However, the increased ZPP is a sign for suppressed erythroid progenitor cells [11,12], the increased ferritin indicates higher iron content in stores, and favorable change in Hb concentration is not yet visible. On the other hand, the excess of iron works as a double-edged sword creating additional risk for the patients by many mechanisms, including the generation of reactive oxygen species [13,14].

The 2-month interval is likely short enough to rule out a potential direct suppressive effect of a specific cytotoxic treatment, and therefore we do not discuss such a potential influence on iron homeostasis. The results obtained do not support the idea that the increase of iron and the other changes of iron homeostasis in these patients is a direct result of regulatory decrease of pHp. A more likely explanation may be that the decreased pHp is a passive result of impaired liver function, a degree of malignant intoxication, and potential positive treatment effect or faster hydrolysis to hepcidin.

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