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The role of inflammation, iron, and nutritional status in cancer-related anemia: results of a large prospective observational study

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Running head: Role of inflammation and malnutrition in CRA

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Abstract

Anemia in cancer patients is often considered a side effect of cancer therapy; however, it may occur before any antineoplastic treatment (cancer-related anemia). This study aimed to evaluate cancer-related anemia prevalence of in a large cohort of cancer patients and whether inflammation and malnutrition were predictive of its development and severity. The present study included 888 patients with cancer at different sites between May 2011 and January 2014. Patients were assessed at diagnosis before any cancer treatment. The prevalence of anemia according to the main clinical factors (tumor site, stage and performance status) was analyzed. In each patient markers of inflammation, iron metabolism, malnutrition and oxidative stress as well as the modified Glasgow Prognostic Score, a combined index of malnutrition and inflammation, were assessed and their role in predicting hemoglobin level was evaluated. The percentage of anemic patients was 63% with the lowest hemoglobin levels in the most advanced cancer and compromised performance status. Hemoglobin differed by tumor site and was lowest in ovarian cancer patients. Hemoglobin negatively correlated with inflammatory markers, hepcidin, ferritin, erythropoietin and reactive oxygen species, and positively correlated with leptin, albumin, cholesterol and antioxidant enzymes. In multivariate analysis, stage, interleukin-6 and leptin were independent predictors of hemoglobin. Furthermore, hemoglobin was inversely dependent on modified Glasgow Prognostic Score. In conclusion, cancer-related anemia is a multifactorial problem with immune, nutritional and metabolic components that affect its severity. Only a detailed assessment of cancer-related anemia pathogenesis may enable clinicians to provide a safe and effective individualized treatment.

Key words: cancer-related anemia, inflammation, leptin, hepcidin, Glasgow Prognostic Score

Introduction

Quality of life represents the main objective of any oncological approach[1] and anemia is one of the most frequently reported problems in patients with cancer,[2] which is associated with decline in patient Performance Status (PS), cognitive function, energy-activity levels,[3] and decreased survival.[4] Anemia is often considered a side effect of cancer therapy; however, many patients are already anemic before the start of any treatment. In our view, anemic cancer patients can be divided into two major groups: those with Hb in the normal range prior to medical treatment (often receiving adjuvant cancer therapy after surgery, with at most microscopic tumor burden); here, the occurrence of anemia during cancer therapy should be considered a specific side effect of treatment; and those with preceding cancer-related anemia (CRA) (often undergoing cancer therapy for clinically detectable tumors): here, CRA may be a consequence of the chronic inflammation present in advanced cancer patients.[5]

Several pieces of evidence attribute a central role in the etiopathogenesis of CRA to inflammatory mediators.[5] Indeed, proinflammatory cytokines induce changes in the proliferation of erythroid progenitors, erythropoietin (EPO) production, and survival of circulating erythrocytes.[6] This inflammatory state is characterized by elevated plasma C-reactive protein (CRP) levels, weight loss with hypoalbuminemia, and erythropoietin-resistant anemia.[5] Plasma CRP reflects interleukin (IL)-6 levels[7] that also modulate the concentration and biological activity of hepcidin,[8] and other acute-phase proteins[9] that may induce serious hematological, nutritional, and metabolic disorders.[10] The identification of hepcidin has enabled a better understanding of the relationship between immune system, iron homeostasis, and anemia of chronic inflammatory diseases.[11] Hepcidin, the synthesis of which by the liver is strongly induced by IL-6,[8] inhibits duodenal absorption of iron and blocks iron release from macrophages.[12] The release of proinflammatory cytokines in cancer patients is often associated with increased production of reactive oxygen species (ROS), either as a component of immune response or consequence of increased metabolism.[13] ROS, in turn, may inhibit erythropoiesis.[14] Inflammation can interfere also with nutritional status, which in turn may

induce anemia.[15,16]

Therefore, in cancer patients, it is essential to analyze all these factors that cause anemia, particularly before beginning any form of therapy that may worsen anemia.

The aim of this work is to document the prevalence of anemia in a large cohort of patients with solid tumors before any exposure to antineoplastic treatment, and to assess the possible correlation between Hb levels and the commonly used indices of inflammation, malnutrition, and metabolic stress. We hypothesized that inflammation and malnutrition are independent predictors for the development and severity of anemia and that a better knowledge of CRA may allow its most adequate treatment.

Methods

The study was a prospective observational trial performed accordingly with Helsinki declaration after approval by Local Institutional Ethics Committee. Between May 2011 and January 2014, 888 consecutive patients with histologically confirmed solid cancer at different sites referring to Departments of Obstetrics and Gynecology, Sirai Hospital, Carbonia, and Medical Oncology at "N.S. Bonaria" Hospital, San Gavino, "Nuova Casa di Cura", Decimomannu, and "A. Businco" Hospital, Cagliari, Italy, were enrolled. Table 1 reports participant clinical characteristics. Patients were assessed at diagnosis before receiving any cancer treatment. Exclusion criteria were: evidence of infections, chronic inflammatory disease, active bleeding, hemolysis, renal insufficiency, hypothyroidism; known history of hematologic disorders (including hemoglobinopathies), family history of thalassemia or hemocromatosis; treatment with EPO, i.v. iron or blood transfusion in the previous 12 weeks; current iron, vitamin B12 or folate supplementation.

Anemia was defined according to our laboratory population-based normal ranges as Hb <13.0 g/dl for males and <12.0 g/dl for females. Karnofsky PS (KPS) was categorized in four prognostic classes.[17] Blood samples were obtained at eight a.m. after overnight fasting since serum hepcidin and iron showed similar circadian changes. In accordance with Ganz and colleagues,[18] the eight

a.m. fasting hepcidin concentrations were more consistent compared to other times of the day. After blood samples centrifugation, serum was stored at -80°C until analysis. In all patients Hb and chronic inflammation (CRP, fibrinogen, IL-6, IL-1β, TNFα), iron metabolism (iron, ferritin, transferrin, hepcidin), EPO, nutritional (albumin, leptin, cholesterol, HDL, LDL, triglycerides) and oxidative stress (ROS, glutathione peroxidase, GPx, and superoxide dismutase, SOD) parameters were measured. The formula for expected EPO was: 2.5 x (140-Hb g/l).[19] Since leptin is highly dependent from body mass index (BMI), leptin/BMI ratio was reported. The modified Glasgow prognostic score (mGPS) was calculated as follows: 2, both elevated CRP (≥10 mg/l) and low albumin (<3.5 g/dl); 1, elevated CRP only; 0, normal CRP (<10 mg/l).[20]

Laboratory variables assays

Routine analysis of Hb, CRP, fibrinogen, serum iron, transferrin, ferritin, triglycerides, cholesterol, HDL, and LDL was performed. Proinflammatory cytokines, EPO and leptin were detected by commercially available enzyme-linked immunosorbent assays (ELISA) (DRG Instruments GmbH, Marburg, Germany, for IL-6, TNFα, EPO; Immunotech SA, Marseille, France for IL-1β; DSL Inc., Webster, Texas for leptin). The variation's coefficient was <5%. Hepcidin was measured by a competitive ELISA using a commercial kit (DRG Instruments GmbH, Marburg, Germany),[21] recently considered a "well-performing method" in a "Round Robin" study.[22] Intra- and inter-assay variations were 4.4% and 9.7%, respectively. ROS were determined by FORT test (Callegari, Parma, Italy). GPx and SOD were measured using a commercial kit (Ransod; Randox Lab, Crumlin, United Kingdom). More details have been previously described.[23]

Statistical Analysis

Differences between two groups were compared by t-test. Multiple groups were compared by analysis of variance with Tukey's post-hoc test and polynomial contrast for trend where indicated.

Pearson (or Spearman) correlation analysis was performed using Bonferroni's correction for multiple comparisons. Significant relationships were included in multivariate linear regression analysis. Results were significant for $p<0.05$. Computations were performed using SPSS version 17.0 (SPSS, Chicago, IL).

Results

Evaluation of Hb levels and anemia incidence

At diagnosis, mean Hb was 11.6 g/dl (11.8 g/dl in males and 11.4 g/dl in females) with a percentage of anemic patients of 63.4% [Table 2]. Mean Hb was significantly different among tumor site categories. Specifically, ovarian cancer patients had the lowest Hb, while breast cancer patients were the least anemic, accounting for gender [Table 3]. Hemoglobin was also significantly different among stage and PS categories. The percentage of anemic patients was significantly higher in the most advanced stage and compromised PS [Table 3]. As anemia seems to be a characteristic of advanced stages, we compared stage III-IV patients with stage I-II patients. We found that advanced stage patients exhibited significantly lower Hb compared with early stage patients ($P<0.05$). Moreover, the percentage of anemic subjects was higher in stage III-IV compared with stage I-II patients ($P <0.05$).

Laboratory parameters of chronic inflammation, iron metabolism, nutritional status, and oxidative stress

Levels of inflammatory, iron metabolism, nutritional and oxidative stress parameters are reported in Table 2. Comparing the non anemic with the anemic cancer patients, we showed significant difference in inflammatory, iron metabolism, nutritional and oxidative stress parameters between the two groups [Supplementary Table S1].

Comparing the parameters of chronic inflammation, iron metabolism, nutritional status, and

oxidative stress according to stage, we found that CRP, fibrinogen, IL-6, TNFα, IL-1β, ferritin, hepcidin, EPO and ROS were significantly higher in stage III-IV than stage I-II patients. In contrast, iron, leptin, triglyceride, and GPx were significantly lower in stage III-IV patients compared with early stage patients [Supplementary Table S2].

Additionally, comparing the patients at the same stage with and without anemia we found that: patients at stage I did not show any difference in laboratory parameters on the basis of the presence of anemia; patients at stage II showed a significant difference in serum levels of serum iron, ferritin, leptin, albumin and colesterol (total cholesterol and HDL) according to the anemic status, thus suggesting a potential role for nutritional-related factors; patients at stage III showed a significant difference of serum levels of CRP, IL-6, EPO, iron, hepcidin and leptin between anemic and non anemic patients; patients at stage IV had a significant difference in all inflammatory parameters as well as nutritional, iron and oxidative stress parameters in relation to the anemic status [Supplementary Table S3].

In order to verify whether the mediators of chronic inflammation, iron metabolism, nutritional status, and oxidative stress may differ according to tumor sites, we performed a detailed comparison of all laboratory parameters between different cancer types. The results showed that: inflammatory parameters CRP and IL-6 were significantly different among cancer types, with the ovarian cancer patients showing the highest value and the breast cancer patients the lowest ones; serum iron, ferritin and hepcidin were significantly different with the lower GI cancer patients showing the lowest levels of these parameters in comparison to other sites. This suggests a major role for iron deficiency in the pathogenesis of CRA in colorectal cancer patients [Supplementary Table S4].

Correlation of Hb levels with clinical and laboratory parameters

Hemoglobin was negatively correlated with stage and PS. A significant positive relationship was found between Hb and BMI. Among markers of chronic inflammation, iron metabolism, nutritional status, oxidative stress, Hb was significantly negatively correlated with CRP, Fbg, IL-6, TNFα, IL-

1β, ferritin, hepcidin, EPO and ROS, whereas it was positively correlated with serum iron, transferrin, leptin, albumin, total cholesterol, HDL, GPx, and SOD [Table 4]. As supplementary data we assessed also the correlation between Hb and laboratory parameters of chronic inflammation, iron metabolism, nutritional status, and oxidative stress in the different categories of stage, PS and tumor sites. This analysis showed that patients at advanced stage (III and IV) had the same significant correlations found in the overall population, whilst in the early stage (I and II) patients Hb was positively significantly correlated with serum levels of EPO, iron, transferrin saturation, ferritin as well as leptin, albumin, total cholesterol and HDL [Supplementary Table S5]. The correlation between Hb and laboratory parameters in the different categories of PS was superimposable to that found in the overall population [Supplementary Table S6]. The correlations between Hb and other laboratory parameters in the different cancer sites are reported in Supplementary Table S7: the main results emerging form this analysis are the positive significant correlation between Hb and serum ferritin in patients with GI cancers, differently from patients with other cancer sites where Hb was inversely significantly related with serum ferritin. These data suggest a role for iron-deficiency in CRA pathogenesis in GI cancer patients.

Since it is known that in conditions of chronic inflammation and oxidative stress, as observed in our patients, the EPO synthesis can be blunted by proinflammatory cytokines,[24] we have added the evaluation of the expected EPO value in comparison to Hb levels.[25] Indeed, in different anemic status EPO levels increase proportionally to the severity of anemia (decrease of Hb levels).[26] Then, the observed/predicted EPO ratio has been correlated with the main indexes of inflammation and oxidative stress. In the present study we observed “inappropriately” low EPO levels in comparison to the expected values for the degree of anemia in accordance to findings reported in literature in patients with anemia of chronic disease.[5] The observed EPO/expected EPO ratio was negatively related with serum levels of CRP, Fbg, IL-6, and ROS and it was positively related to GPx and SOD [Supplementary Table S8].

Multivariate regression analysis showed that stage (β coefficient=-0.537; 95% CI: -0.971 to -0.071;

P =0.004), IL-6 (β coefficient=-0.831; 95% CI:-0.146 to -0.029, *P* =0.023), and leptin (β coefficient=0.745; 95% CI: 1.694 to 0.050; *P* =0.015) were independent predictive variables of Hb. Then, we tested whether both IL-6 and leptin correlated to each other and with the other laboratory markers of inflammation, iron metabolism, nutritional status, and oxidative stress. Single regression analysis showed that: IL-6 and leptin were inversely related to each other (p<0.001); IL-6 was positively correlated with CRP (*P* <0.001), Fbg (*P* =0.009), IL-1 (*P* <0.001), hepcidin (*P* =0.003), and ROS (*P* =0.034), whilst it was inversely related to EPO ratio (p=0.035), total cholesterol (*P* =0.017), albumin (*P* =0.020), and GPx (*P* <0.001); leptin ratio was negatively correlated with CRP (*P* =0.036), IL-1 (*P* =0.027), and ROS (*P* =0.036), while positively related to EPO ratio (p=0.029), serum iron (*P* =0.006), albumin (*P* =0.045), total cholesterol (*P* =0.015), and GPx (*P* =0.009).

Evaluation of laboratory parameters according to Hb levels

Anemic patients were also divided on the basis of Hb values ≤ or >10 g/dl, which is the threshold for starting ESA treatment for chemotherapy-induced anemia according to international guidelines.[27] Patients with Hb≤10.0 g/dl had significantly higher CRP, fibrinogen, IL-6, ferritin, hepcidin, EPO and ROS than patients with Hb>10 g/dl. Conversely, leptin, albumin, total cholesterol, HDL, and GPx were significantly lower in patients with Hb≤10.0 g/dl compared with patients with Hb>10 g/dl [Supplementary Table S9].

Additionally, after patient stratification into quartiles of Hb, polynomial contrast for trend showed that: CRP, IL-6, EPO, hepcidin, and ROS were progressively higher with decreasing quartiles of Hb; serum iron, leptin, total cholesterol, HDL, and GPx were progressively lower with decreasing quartiles of Hb [Table 5]. In an alternative analysis comparing Hb levels according to quartiles of laboratory parameters, we found similarly that Hb was progressively lower with decreasing quartiles of serum iron, transferrin saturation, leptin, total cholesterol, HDL, and GPx, whereas it was progressively lower with increasing quartiles of CRP, IL-6, IL-1β, EPO, ferritin, hepcidin, and

ROS [Supplementary Table S10].

Evaluation of Hb levels according to mGPS categories

Considering these results, we assessed whether in the clinical practice a simple tool, such as the mGPS, which evaluates the correlation between inflammation (CRP) and nutritional status (albumin levels), correlates with Hb and is able to predict it. Hemoglobin was significantly different between mGPS categories (P<0.001). In detail, post-hoc test showed that Hb was significantly lower in the highest *vs* the other mGPS categories. Moreover, in a linear regression model mGPS category was a significant negative predictor of Hb (β coefficient=-1.176; P<0.001) [Figure 1].

Moreover, mGPS was positively correlated with Fbg (P <0.001), IL-6 (P <0.001), TNF-α (P<0.001), IL-1 (P=0.001), hepcidin (P =0.016), EPO (p=0.034), ROS (P=0.047), and inversely related with serum iron (P<0.001), leptin (P=0.024), total cholesterol (P<0.001), HDL (P<0.001), and GPx (P=0.006).

Discussion

More than 30% of cancer patients have CRA at the time of diagnosis[28] and thus before starting any antineoplastic treatment. CRA has been associated with more advanced stages[28] and has biological and hematological features similar to those observed in chronic inflammatory disease-related anemia.[26] However, only rare studies have correlated CRA with markers of inflammation.[23,29] Moreover, if we consider that in cancer patients the state of chronic inflammation is associated with serious eating disorders and with profound changes in energy metabolism, which by itself are capable of inducing anemia, the literature on this subject has been even more lacking.

In the present study, we confirmed in 888 cancer patients at diagnosis that the percentage of anemic patients was high, with significantly lower Hb in the most advanced stages and compromised PS. Mean Hb was significantly different among tumor site categories: in particular,

ovarian cancer patients had the lowest levels, as previously observed.[28] Furthermore, we confirmed that the lowest Hb levels were associated with the highest values of inflammatory markers. The multivariate analysis demonstrated that IL-6, in particular, was a strong predictor of Hb. Of note, IL-6 was in turn positively correlated with other markers of inflammation (CRP, Fbg, IL-1), hepcidin, ferritin, oxidative stress (ROS), and inversely related to EPO, nutritional (cholesterol, leptin, albumin) and antioxidant (GPx) parameters. It is known that proinflammatory cytokines impair erythropoietin (EPO) production, the proliferation and differentiation of erythroid progenitors and shorten the survival of circulating erythrocytes.[5] In our study, the EPO production was not optimal for the level of anemia as previously demonstrated.[6] Importantly, high cytokine levels are also associated with "functional iron deficiency" (FID).[30] This condition is mediated by hepcidin, a liver-derived peptide regulated by IL-6 and included among the inflammatory (type II) acute-phase proteins.[31] Increased hepcidin levels have been detected in patients with anemia associated with chronic inflammatory diseases, such as inflammatory bowel disease, chronic kidney disease, and hematological cancer.[32-35] Our data showed that, particularly in advanced cancer patients, hepcidin was inversely correlated with Hb. High hepcidin was associated with higher ferritin, lower serum iron, and lower transferrin saturation levels. Accordingly, in a recent paper Shu et al.,[36] showed that in cancer patients with anemia of chronic disease, hepcidin was positively correlated with IL-6 and inversely with serum iron, differently from cancer patients with iron-deficiency anemia, where hepcidin decreased with decreasing quartiles of serum iron. In contrast, Durigova et al.,[37] in a population of early breast cancer undergoing adjuvant chemotherapy demonstrated a positive correlation between hepcidin and hemoglobin levels concluding that baseline low hepcidin levels were predictive of onset of severe anemia.

The profile of iron metabolism seems to be quite different in the various tumor sites assessed by us, where the colorectal cancer patients showed, in the presence of comparable inflammatory status, lower hepcidin and ferritin levels than other tumor sites thus suggesting a role of iron deficiency in the pathogenesis of CRA in this subgroup as already reported in literature.[38]

Motivated by the evidence of iron-restricted erythropoiesis, multiple clinical trials demonstrated that, in patients with CRA, i.v. iron improves the response rate to ESAs, reduces the time to target hemoglobin levels, decreases ESA requirements, and is more effective than oral iron.[39] However, to date long-term data on the role of iron supplementation in managing CRA are not available and it has been reported that iron supplementation may act very differently in various cancers.[39,40] In particular, the use of i.v. iron, in the presence of FID, has been recently debated.[41,42] Accordingly, in a previous randomized clinical trial we suggested the need to choose the route of iron administration as a function of the patient clinical characteristics, and showed that oral lactoferrin, in anemic advanced cancer patients undergoing chemotherapy, reduces ferritin levels, and supports the efficacy of ESAs, similarly to i.v. iron.[43]

Cancer growth and associated inflammation also induce changes in energy metabolism and food intake (cancer-anorexia), potentially contributing to anemia. These conditions leads to severely altered glycaemia, albumin, and cholesterol levels, with consequent reduced circulating leptin levels.[44] Generally, in advanced cancer patients leptin levels are inversely correlated with proinflammatory cytokines and stage, independent from BMI.[45] Our results show that leptin, albumin, and cholesterol, along with BMI, were positively correlated with Hb. Of relevance, here we found that leptin, beside IL-6 and stage of disease, was an independent predictive factor of Hb levels. Unfortunately, to date, the close correlation between nutritional status and anemia in cancer patients has been studied very little, in contrast to patients with chronic renal failure, where this condition has been widely demonstrated and, as a result, the need for a proper nutritional support, especially in patients candidates for the use of ESAs,[46] is well established.

It is also known that any inflammatory condition characterized by high cytokines levels is associated with high ROS levels, the synthesis of which is considered an integral part of the inflammatory response, and also an evidence of metabolic deregulation induced by cytokines.[47] In the present paper, ROS were negatively correlated, while GPx and SOD were positively correlated with Hb. ROS are able to inhibit the EPO synthesis.[16] Moreover, oxidative stress *per se* increases

the fragility of red blood cells and decreases the rate of erythroid maturation, as well as erythrocyte lifespan. A recent paper[48] demonstrated *in vitro* that sustained levels of H_2O_2, similar to inflammatory conditions, are sufficient to activate hepcidin transcription in hepatocytes via increased STAT-3 phosphorylation. The authors further found that H_2O_2 acts synergistically with IL-6 in inducing hepcidin, thus suggesting another mechanism through which oxidative stress contributes to ACD.[48]

To strengthen our results we compared anemic and non anemic patients, confirming that anemia was significantly associated with the highest levels of CRP, inflammatory cytokines, EPO, ferritin, hepcidin and ROS levels, and with the lowest levels of serum iron, transferrin, leptin, albumin, lipid profile parameters and antioxidant enzymes. Moreover, to increase the power of analysis, we divided anemic patients according to Hb quartiles and further confirmed that increasing severity of anemia was associated with progressively increased CRP, cytokines, ferritin, EPO and ROS and decreased leptin, cholesterol, and GPx levels. These results highlight that CRA is a multifactorial problem with immune, nutritional, and metabolic components, all of which can affect the onset and severity of anemia.

In the attempt to give to practicing physicians a simple tool to evaluate the correlation between Hb levels and the immune-metabolic status, we used the mGPS, which evaluates the correlation between inflammation (CRP) and nutritional status (albumin levels), correlating it with Hb. Our results showed that the highest mGPS score was predictive of lower Hb levels, in agreement with data obtained from patients with chronic kidney failure, in whom the “malnutrition-inflammation score” was associated with anemia.[49,50] In turn, GPS in our paper was positively correlated with IL-6, hepcidin, EPO and ROS and inversely related to leptin and GPx levels, thus demonstrating its ability to reflect the complex inflammatory, nutritional/metabolic, and oxidative status of cancer patients.

The main limitations of our study included the difficulty in assessing so many different parameters in patients as well as the fact that the patients were enrolled from a small number of

centers of a limited geographical area and were heterogeneous with respect to tumor site and stage of disease.

The present findings suggest that a careful evaluation of cancer patients should be performed before starting treatment for anemia: in addition to including ESAs, the treatment of CRA should include the correction of nutritional deficiencies, personalized iron supplementation[42,43,51] and, in some cases, antioxidant treatment.[52]. Moreover, our results suggest that patients with different cancer types and at various stages may have different factors influencing the pathogenesis and severity of anemia and therefore need specific individualized mechanism-based treatment of CRA. For example, patients with gastrointestinal tumors showing, as observed in our study, lower levels of serum iron, ferritin and hepcidin may have more benefit from iron supplementation in comparison with patients with different tumor sites. Additionally, recent findings that anti-IL6 mAb, administered in advanced cancer patients with different tumor types,[53,54] achieves a significant Hb increase associated with CRP decrease, suggests that this or other anti-cytokine or anti-inflammatory treatments may prove useful in the treatment of CRA.

Therefore, on the basis of our data, we recommend that the evaluation of anemia in cancer patients should be performed over and above the current standard testing for blood loss, iron deficiency, and vitamin B12 or folate nutritional deficiency, and should include the assessment of CRP, albumin (ideally associated as in the GPS) and, where available, the analysis of the oxidative stress status, which is a feasible and simple test with the currently available methodologies. In conclusion, a detailed understanding of the pathogenesis of CRA should enable clinicians to provide effective individualized treatment thus supporting a much better quality of life for patients.

Authorship and disclosure

AM designed the study, collected and analysed data, and wrote the paper; CMa performed the research, collected and analysed data, contributed reagents or analytical tools, and wrote the paper;

GG, designed the study, performed the research, recruited patients, analysed and collected data; CMu, LT, MCC, CF, and IO performed the research, recruited patients, collected and analysed data; AB contribute to the study design, collected and analyzed data; TG designed the study and critically reviewed the paper.

No conflict of interest has to be declared

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Table 1. Patient clinical characteristics

	Patients		
	Total	**Males**	**Females**
N	888	450	438
Mean age, y (range)	65.1±11 (42-78)	66.6±9.8 (42-75)	63.8±11.9 (44-78)
Mean weight, Kg	59.64±15.47	59.2±11.7	59.9±17.4
Mean height, cm	158.24±7.3	159.2±11.7	155±6.4
BMI	23.83±6.28	22.2±3.7	24.8±7.2
Tumor sites	***N* (%)**	***N* (%)**	***N* (%)**
Ovary	178(20)	0 (0)	178 (41)
Breast	133(15)	2 (0.5)	131 (30)
Lung	178 (20)	123(27.3)	55 (12)
Prostate	53(6)	53 (11.8)	0 (0)
Upper Gastrointestinal	142 (16)	118 (26.2)	24 (5)
Colorectal	115 (13)	85 (18.9)	30 (7)
Head and neck	62 (7)	46 (10.2)	16 (4)
Bladder	27 (3)	23 (5.1)	4 (1)
Tumor stage	***N* (%)**	***N* (%)**	***N* (%)**
I	15 (2)	7(2)	8 (2)
II	130 (15)	60 (13)	70 (16)
III	113 (12)	62 (14)	51 (12)
IV	630 (71)	321 (71)	309 (70)
KPS	***N* (%)**	***N* (%)**	***N* (%)**
100-80	280 (32)	139 (31)	141 (32)
50-70	303 (34)	151 (34)	152 (34)
30-40	208 (23)	110 (24)	98 (23)
0-20	97 (11)	50 (11)	47 (11)

Abbreviations: BMI, body mass index; KPS, Karnofsky Performance Status

Table 2. Evaluation of Hb and laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress in 888 cancer patients

Parameters	Total (***N***=888)	Males (***N***=450)	Females (***N***=438)
Hb, g/dl	11.6±1.9	11.8±2.04	11.4±1.67
Anemic, %	63.4	66.7	60
Chronic Inflammation			
CRP, mg/l	13.1±8.3	11.5±6.9	14.5±9.7
Fbg, mg/dl	436.2±142.9	431.9±141.8	441±145
IL-6, pg/ml	21.3±18.8	22.6±16.7	20.6±15.9
TNF-α, pg/ml	30.7±20.4	28.3±18.3	31.8±18.4
IL-1 β, pg/ml	26.6±23	30.8±25.3	24±21.7
Iron Metabolism			
Serum observed EPO, mIU/ml	26.1±18	25.9±13.8	27.1±7
Observed/expected EPO ratio	0.49±0.34	0.5±0.3	0.48±0.35
Serum iron, g/dl	58.5±38.4	59.1±41.5	57.4±33.9
Transferrin, mg/dl	213±64.9	199.1±55.9	223±71.3
Transferrin saturation, %	26±5	28±6	23±4
Ferritin, ng/ml	326.3±253	379.2±256	240±224
Hepcidin, ng/ml	93±33	80.7±43.47	91±54.9
Nutritional status			
Leptin/BMI ratio, ng/ml	0.5±0.4	0.3±0.2	0.4±0.5
Albumin, g/dl	3.79±0.77	3.67±0.80	3.9±0.7
Cholesterol, mg/dl	179.2±46.1	170±46	188±44.6
HDL, mg/dl	49.2±18.1	46.5±19.7	52.5±15.5
LDL, mg/dl	105±33.9	101.5±32.8	111.3±35.7
Triglycerides, mg/dl	135.3±85.6	127.9±63.5	143.3±104.8
Oxidative stress			
ROS, FORT U	399.3±113.4	383.1±107.7	411±116.7
SOD, U/ml	103.4±45.5	113.1±49.4	95.5±40.8
GPx, U/l	7025±2913	7230±3114	6874±2767

Anemia is defined by Hb values <13.2 g/dl for males and <12.0 g/dl for females according to population-based normal range at our laboratory. Data are reported as mean ±SD. Abbreviations: Hb, hemoglobin; CRP, C-reactive Protein; Fbg, Fibrinogen; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin;

BMI, body mass index; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table 3. Mean Hb levels and percentage of anemic patients according to tumor sites, stage of disease and ECOG PS

	Total			Males		Females	
Clinical parameter (*N*)	**Hb (g\dl) Mean±SD**	**Anemic pts (%)***	***P***	**Hb (g\dl) Mean±SD**	**Anemic pts (%)**	**Hb (g\dl) Mean±SD**	**Anemic pts (%)**
Tumor site							
Ovary (178)	10.9±1.8	67.9	**0.013**	N.V.	0	10.98±0.18	67.9
Breast (133)	12.1±0.2	35.5		N.V.	0	12.13±0.22	35.5
Lung (178)	11.6±0.2	78.8		11.51±0.25	87	12.05±0.35	63.6
Prostate (53)	11.7±0.4	80		11.84±0.44	80	N.V.	0
Upper GI (142)	11.4±0.2	77.5		11.33±0.27	90	11.5±0.34	77
Lower GI (115)	11.7±0.3	40.9		12.01±0.34	46	10.9±0.31	51
Head and neck (62)	12.2±0.4	69		12.46±0.45	72	11.3±0.77	50
Bladder (27)	11.2±0.46	63.6		11.16±0.48	81	10.8±1.2	65
Stage							
I (15)	13.±0.9	13.3	**0.017**	13.6±0.2	14.3	12.3±0.68	12.5
II (130)	12.5±0.6	19.3		12.8±0.92	20	12.3±0.81	18.6
III (113)	12.1±0.3	33		11.8±0.43	41.9	12.0±0.42	21.6
IV (630)	11.5±0.1	79.2		11.1±0.14	85	11.2±0.12	72
KPS							
100-80 (280)	12.5±2.0	44	**0.014**	12.1±0.56	53	12.3±0.28	40
50-70 (303)	11.5±1.4	64		11.8±0.29	68	11.3±0.21	57
30-40 (208)	11.0±1.6	76		11.2±0.30	75	10.8±0.29	78
0-20 (97)	10.2±2.4	91.7		10.1±0.81	90	9.5±0.57	95

Anemia is defined by Hb values < 13.2 g/dl for males and <12.0 g/dl for females according to population-based normal range at our laboratory. Significance was calculated by ANOVA test. Abbreviations: Hb, hemoglobin; KPS, Karnofsky Performance Status.

Table 4. Correlation of Hb levels with clinical parameters (stage, ECOG PS, BMI) and markers of chronic inflammation, iron metabolism, nutritional status and oxidative stress in patients with cancer at different sites

Parameters	Pearson	*P*
Clinical		
Stage	-0.156	**0.002****
KPS	-0.321	**0.001****
BMI	0.225	**0.003****
Chronic inflammation		
CRP	-0.399	**<0.001****
Fbg	-0.150	**0.003****
IL-6	-0.223	**0.002****
TNF-α	-0.223	**0.002****
IL1β	-0.266	**0.006****
Iron metabolism		
Serum observed EPO	-0.481	**0.041**
Serum iron	0.414	**<0.001****
Transferrin	0.316	**<0.001****
Transferrin saturation	0.218	**0.042**
Ferritin	-0.164	**0.031**
Hepcidin	-0.459	**0.021***
Nutritional status		
Leptin	0.479	**0.003***
Albumin	0.416	**<0.001****
Total Cholesterol	0.452	**<0.001****
HDL	0.457	**<0.001****
LDL	0.271	0.057
Triglycerides	0.054	0.593
Oxidative stress		
ROS	-0.240	**0.007****
SOD	0.268	**0.002****
GPx	0.255	**0.003***

Results are considered significant for $P<0.05$* and highly significant for $p<0.01$**. Abbreviations: KPS, Karnofsky Performance Status; BMI, body mass index; Fbg, Fibrinogen; CRP, C-reactive Protein; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin, ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table 5. Evaluation of laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress in the population of anemic cancer patients according to quartiles of Hb levels

	Hb quartiles					
	<9.8 g/dl (*N*=126)	**9.8-11.0 g/dl** (*N*=144)	**11.1-11.8 g/dl** (*N*=152)	**>11.9 g/dl** (*N*=141)	***P***	***Test for trend***
Parameters						
Chronic Inflammation						
CRP	18.2±9.4	10.1±11.7	7±10.6	3.4±4.9	**<0.001**	**<0.001**
Fibrinogen	491±168	443±140	426±158	422±132	0.091	0.139
IL-6	29.2±13.9	19.1±12.9	17±8.00	17.00±9.4	**0.042**	**0.006**
TNF-α	24.8±16.5	21.1±8.5	19.9±9.8	9.6±10.8	0.157	0.058
IL1β	27.5±14.7	31.5±21.7	17.4±15.8	17.8±7.6	0.196	0.071
Iron metabolism						
Serum EPO	45±16.8	39±17.3	27.2±12.2	13.1±3.4	**0.030**	**0.018**
Serum iron	38.4±32.8	53.9±47.3	57.2±38.2	63.1±33.4	**0.030**	**0.018**
Transferrin	180.3±55.9	195.9±65.1	206.1±58	211.3±45.7	0.160	0.103
Transferrin saturation	21±8	27±11	28±9	29±10	0.054	0.051
Ferritin	432.9±282.6	381.1±238	375.3±282.7	241±225.4	**0.046**	0.422
Hepcidin	129.1±26.3	88.1±48.9	65.3±37.1	56.6±15.1	**0.017**	**0.033**
Nutritional status						
Leptin	0.2±0.1	0.36±0.47	0.5±0.6	0.6±0.32	**0.036**	**0.026**
Albumin	3.2±0.8	3.8±0.7	3.7±0.8	3.6±0.9	0.197	0.198
Total cholesterol	140.5±18.8	162.8±35.6	171.56±43.1	187.3±54.5	**0.007**	**0.001**
HDL	31.6±11.9	38.6±10.9	51.8±21.5	53.6±22.6	**0.036**	**0.007**
LDL	85.8±16.9	102.9±34.1	105.1±38.7	100.1±41.3	0.753	0.384
Triglycerides	123.1±55.6	123.7±49	112.5±51.9	133.8±80.7	0.920	0.796
Oxidative stress						
ROS	469±133.8	403.2±124.5	381.8±107.5	366.3±69	**0.015**	**0.040**
SOD	89.8±42.4	91.1±43.9	106.9±50.7	120.3±53.4	0.069	0.094
GPx	5457.9±1989.8	6554.6±2207.7	7395.2±2861.4	8536±3366.7	**0.009**	**0.002**

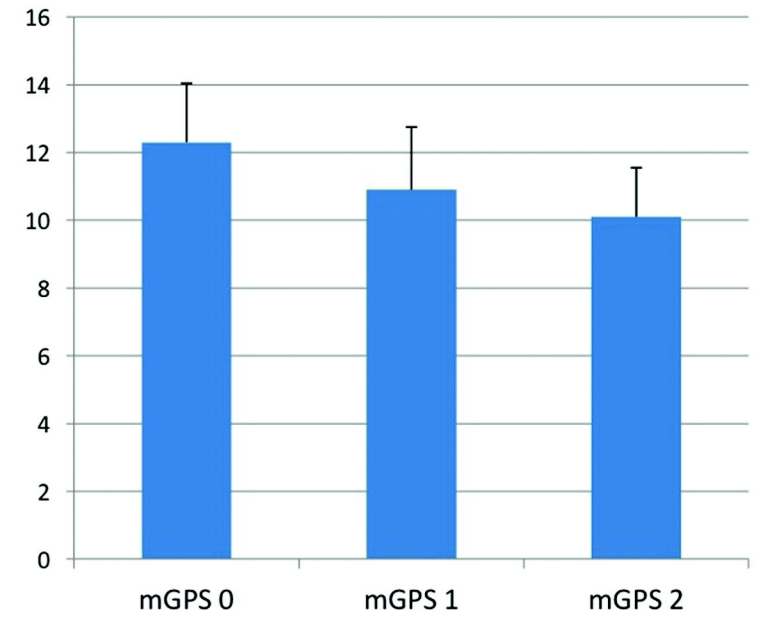
Groups are compared by ANOVA test. Results are considered significant for $P \leq 0.05$. Significant p-values are reported in bold. Abbreviations: CRP, C-reactive protein; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Figure legend

Figure 1. Evaluation of Hb levels according to mGPS categories. A) Bars represent mean ±SD. Mean Hb levels were significantly different between mGPS categories (ANOVA test; $P<0.001$). The post-hoc test showed that Hb level was lower in the highest *vs* the other mGPS categories (mGPS 0 *vs.* mGPS 1 category, $P<0.001$; mGPS 0 *vs.* mGPS 2, $P<0.001$; mGPS 1 *vs.* mGPS 2, $P=0.045$). B) Scatter plots with linear regression line (black line) showing that mGPS category is a significant negative predictor of Hb level (dependent variable) (β coefficient=-1.176; $P<0.001$). Abbreviations: mGPS, modified Glasgow Prognostic Score, Hb, hemoglobin

Figure 1.

A.


mean Hb levels (g/dl)
16
14
12
10
8
6
4
2
0
mGPS 0
mGPS 1
mGPS 2

B.

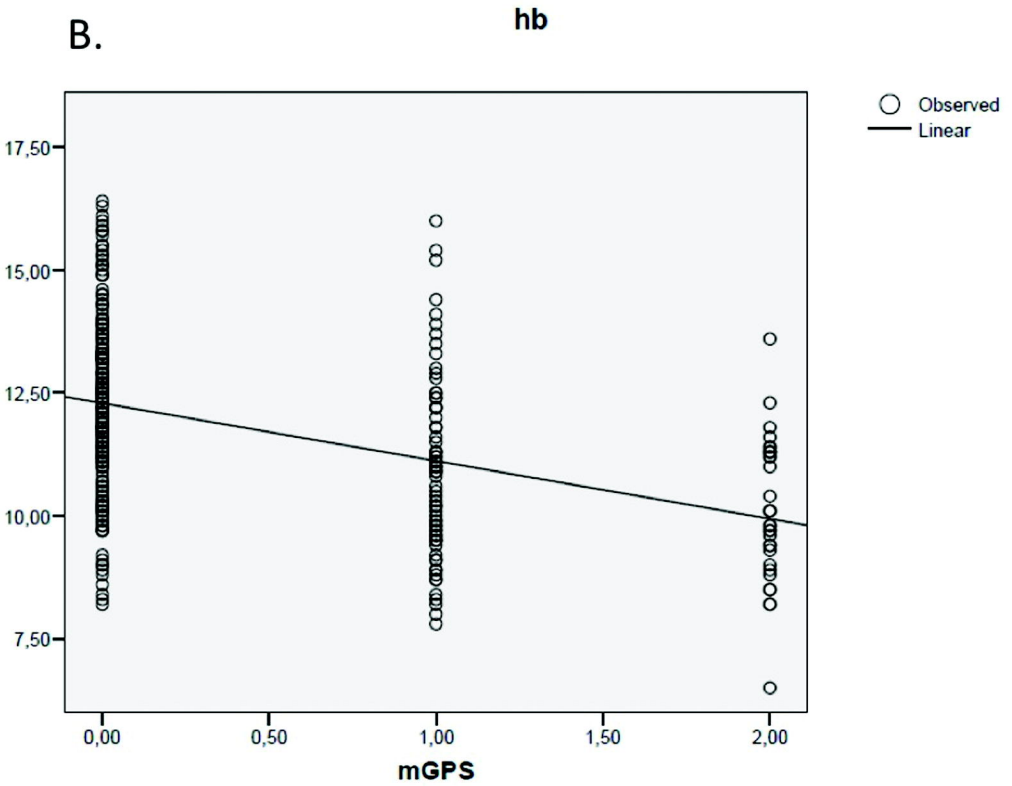

hb
Observed
Linear
17,50
15,00
12,50
10,00
7,50
0,00
0,50
1,00
1,50
2,00
mGPS

Table S1. Evaluation of laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress and in 888 patients with cancer according to anemic status

	Non anemic cancer patients (325)	Anemic cancer patients (563)	P value
Chronic Inflammation			
CRP, mg/l	4.1±0.8	14±1.1	<0.001
Fbg, mg/dl	380±109	452±169	0.020
IL-6, pg/ml	14.0±7.8	25.3±9.0	0.028
TNF-α, pg/ml	20±9	29±12	0.018
IL-1 β, pg/ml	18±8	37±18	0.009
Iron metabolism			
Serum observed EPO, mUI/ml	22±12	38.7±18	0.008
Observed/expected EPO ratio	0.9±0.8	0.4±0.3	0.020
Serum iron, g/dl	71±55	53±41	0.048
Transferrin, mg/dl	239±77	214±57	<0.001
Transferrin saturation,%	27±11	24±6	0.182
Ferritin, ng/ml	228±58	436±177	<0.001
Hepcidin, ng/ml	56±19	109±47	0.033
Nutritional status			
Leptin, ng/ml	0.7±0.3	0.4±0.1	0.026
Albumin, g/dl	4.1±0.6	3.5±0.5	<0.001
Cholesterol, mg/dl	194±63	170±46	0.015
HDL, mg/dl	55±15	41±19	0.001
LDL, mg/dl	107±39	100±30	0.063
Triglycerides, mg/dl	145.6±27	125±69	<0.001
Oxidative stress			
ROS, FORT U	370±180	410±113	0.036
SOD, U/ml	118±59.4	88±42	<0.001
GPx, U/l	8670±2350	7210±1228	<0.001

Results are considered significant for $P<0.05$ as calculated by Student's t test in comparison to non anemic patients. Abbreviations: CRP, C-reactive Protein; Fbg, Fibrinogen; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S2. Evaluation of laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress and in 888 patients with cancer according to stage of disease

	Stage I-II	Stage III-IV
Chronic Inflammation		
CRP, mg/l	1.2±0.8	13.5±1.3§
Fbg, mg/dl	327±68	439±142§
IL-6, pg/ml	5.9±3.8	28.9±20.5§
TNF-α, pg/ml	10.1±4.8	40.2±21.4§
IL-1 β, pg/ml	10.8±5.3	30±17§
Iron metabolism		
Serum observed EPO, mUI/ml	13.7±5.3	37.2±11.1
Observed/expected EPO ratio	1.01±0.6	0.44±0.39
Serum iron, g/dl	73.4±28.8	57.8±38.5
Transferrin, mg/dl	228±44.9	212±73
Transferrin saturation,%	28±14	23±5
Ferritin, ng/ml	325±253	485±354§
Hepcidin, ng/ml	55±6	105±51§
Nutritional status		
Leptin, ng/ml	0.4±0.5	0.2±0.03§
Albumin, g/dl	4.1±0.63	3.7±0.7
Cholesterol, mg/dl	196±56.8	176±45.2
HDL, mg/dl	55±12.6	47.2±19.5
LDL, mg/dl	113.3±22.7	103.8±37.1
Triglycerides, mg/dl	213.2±88.3	129.1±61.7§
Oxidative stress		
ROS, FORT U	345±38	403±113§
SOD, U/ml	138±59.4	88±42
GPx, U/l	9560±2435	7019±2928§

§ $P<0.05$ in comparison to stage I-II cancer patients. Abbreviations: CRP, C-reactive Protein; Fbg, Fibrinogen; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S3. Evaluation of laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress and in 888 patients with cancer according to stage of disease and anemic status

	Stage I		Stage II		Stage III		Stage IV	
Chronic Inflammation	No anemia (13)	Anemia (2)	No anemia (105)	Anemia (25)	No anemia (76)	Anemia (37)	No anemia (131)	Anemia (499)
CRP, mg/l	1.1±0.04	1.2±0.1	1.1±0.8	1.3±0.7	5.6±1	10±2.1*	13±2.7	20±4.2*
Fbg, mg/dl	318±156	345±174	339±47	352±122	394±125	425±230	429±132	490±155*
IL-6, pg/ml	2.1±0.4	2.6±0.5	5.6±2.8	6.8±3.5	12.4±7.6	18±8.1*	22±5.7	35±11*
TNF-α, pg/ml	9.8±1.4	10.2±1.9	9.7±5.6	12±6.7	16.1±7.9	18±11	38±9	45±21*
IL-1 β, pg/ml	9±4.1	10±3.2	11.8±3.4	14±6.9	21±6.4	22±5.6	31±14	35±16*
Iron metabolism								
Serum observed EPO, mUI/ml	16±6	10±8	18±8	16±14	20±8	38.9±10*	31.2±7	51±15*
Observed/expected EPO ratio	1.1±0.4	0.9±0.3	1.0±0.6	0.8±0.6	0.9±0.8	0.5±0.3*	0.7 ±0.5	0.3±0.1*
Serum iron, g/dl	80.7±32	63±20	89±38	73±31*	66±37	50±24*	58±26	42±25*
Transferrin, mg/dl	250±32	250±44	251±136	260±57	230±91	205±84	220±63	190±61
Transferrin saturation,%	30±6	25±5	35±7	28±3	29±2	24±9	26±6	22±5
Ferritin, ng/ml	196±53	208±44	349±153	280±1*65	340±218	386±245	434±264	518±285*
Hepcidin, ng/ml	44±12	49±17	51±19	55±23	67±15	90.8±29*	87.7±25	138±24*
Nutritional status								
Leptin, ng/ml	0.7±0.2	0.6±0.04	0.7±0.2	0.5±0.2*	0.47±0.2	0.36±0.3*	0.4±0.2	0.2±0.05*
Albumin, g/dl	4.3±0.5	3.8±0.9	4.4±0.2	3.7±0.5*	4±0.6	3.6±0.4	3.7±0.8	3.1±0.7*
Cholesterol, mg/dl	185±37	161±25	261±37	163±43*	193±37	180±47	181±52	151±38*
HDL, mg/dl	54±11	51±16	64±22.6	48±21*	58±14	47±18	51±18	35±19*
LDL, mg/dl	111±14	100±25	121±34	113±45	108±27	99±61	98±40	95±33
Triglycerides, mg/dl	218±32	214±52	219±137	203±61	123±66	113±31	110±65	108±53
Oxidative stress								
ROS, FORT U	310±33	340±54	345±58	365±76	390±125	370±134	410±160	480±123*
SOD, U/ml	125±35	133±41	146±49	126±79	108±41	92±37	88±22	78±42
GPx, U/l	9750±3240	9320±2890	9890±2560	9230±3190	8960±1130	7580±1260	8450±2170	6210±1328*

* $p<0.05$ as assessed by ANOVA test. Abbreviations: CRP, C-reactive Protein; Fbg, Fibrinogen; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S4. Evaluation of laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress and in 888 patients with cancer according to tumor site

	Ovary (178)	Breast (133)	Lung (178)	Prostate (53)	Upper GI (142)	Lower GI (37 (115)	Head and neck (62)	Bladder (27)
Chronic Inflammation								
CRP, mg/l	13.6±2.4	6.2±1.3	10.3±1.5	8.5±1.6	6.3±1.2	6.1±1	8.9±3.5	7.5±1.2*
Fbg, mg/dl	466±155	390±80	483±158	424±160	386±163	390±100	433±197	450±135
IL-6, pg/ml	37±16	25±10	52±23	22.5±1.4	26±5	26±7.5	18±3	25±5*
TNF-α, pg/ml	33±6	25±8	27±9	26±6.7	33±11	17±5.3	19±4	23±16
IL-1 β, pg/ml	21±9.9	18±8	39±11	19±9.8	25±6	15±7	16.9±3	12±3.9
Iron metabolism								
Serum observed EPO, mUI/ml	40.3±18	23.8±14	33.3±16	30.3±13	31±12	37±17	20.7±5.6	28±11
Observed/expected EPO ratio	0.3±0.1	0.8±0.4	0.5±0.36	0.9±0.4	0.4±0.3	1.1±0.6	1.1±0.4	0.4±0.1
Serum iron, g/dl	66±19	74±34	55±15	71±12	51±36	52±37	84±47	54±25*
Transferrin, mg/dl	202±67	259±67	181±47	286±39	45±62	252±63	246±41	189±45
Transferrin saturation,%	23±3	27±3	30±5	25±3	21±7	20±6	29±12	28±2
Ferritin, ng/ml	420±157	262±129	418±182	339±97	137±62	102±65	334±113	302±162*
Hepcidin, ng/ml	68±39	38±16	62±29	44±13	38±19	34±12	40±18	55±28*****
Nutritional status								
Leptin, ng/ml	0.4±0.3	0.7±0.4	0.5±0.3	0.4±0.3	0.2±0.1	0.6±0.3	0.2±0.06	0.2±0.08
Albumin, g/dl	3.6±0.8	4.2±0.5	3.5±0.8	4.1±0.7	3.8±0.6	3.6±0.9	4.3±0.1	3.7±0.8
Cholesterol, mg/dl	178±46	200±45	177±55	185±22	160±33	175±59	162±24	173±48
HDL, mg/dl	48±15	56±12	48±23	60±17	39±14	34±16	48±10	44±18
LDL, mg/dl	91±40	125±26	107±49	101±23	96±22	114±45	95±32	94±23
Triglycerides, mg/dl	126±51	158±32	116±35	111±63	135±87	159±76	125±82	115±86
Oxidative stress								
ROS, FORT U	429±109	377±98	409±124	430±113	351±100	352±113	425±126	336±85
SOD, U/ml	98±35	133±41	89±29	116±79	94±36	124±61	78±32	98±42
GPx, U/l	8450±324	9420±290	6980±2610	9830±2190	7190±1870	10540±2305	6800±1230	9219±1280

*$p<0.05$ as assessed by ANOVA test. Abbreviations: CRP, C-reactive Protein; Fbg, Fibrinogen; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S5. Correlation of Hb levels with markers of chronic inflammation, iron metabolism, nutritional status and oxidative stress among cancer patients at different stage of disease

	Stage I-II		Stage III-IV	
Parameters	**Pearson**	***P***	**Pearson**	***P***
Chronic inflammation				
CRP	-0.164	0.631	-0.338	**<0.001**
Fbg	-0.250	0.369	-0.250	**0.001**
IL-6	-0.118	0.145	-0.410	**<0.001**
TNF-α	-0.177	0.717	-0.264	**0.026**
IL1β	-0.266	0.169	-0.266	**0.036**
Iron metabolism				
Serum observed EPO	-0.981	**<0.001**	-0.364	**0.011**
Serum iron	0.414	**0.012**	0.494	**<0.001**
Transferrin	0.116	0.087	0.316	**0.010**
Transferrin saturation	0.418	**0.022**	0.318	**0.012**
Ferritin	0.294	**0.041**	-0.384	**0.016**
Hepcidin	-0.159	0.121	-0.309	**0.001**
Nutritional status				
Leptin	0.540	**0.021**	0.479	**0.021**
Albumin	0.414	**0.002**	0.371	**0.002**
Total Cholesterol	0.547	**0.001**	0.368	**0.001**
HDL	0.397	**0.041**	0.359	**0.011**
LDL	0.162	0.701	0.150	0.342
Triglycerides	0.164	0.238	0.113	0.317
Oxidative stress				
ROS	-0.140	0.070	-0.340	**0.006**
SOD	0.268	0.230	0.268	**0.036**
GPx	0.155	0.130	0.355	**0.002**

Results are considered significant for $P<0.05$. Abbreviations: Fbg, Fibrinogen; CRP, C-reactive Protein; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin, ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S6. Correlation of Hb levels with markers of chronic inflammation, iron metabolism, nutritional status and oxidative stress according to performance status

	KPS 100-70		KPS 40-0	
Parameters	**Pearson**	***P***	**Pearson**	***P***
Chronic inflammation				
CRP	-0.393	**0.015**	-0.420	**<0.001**
Fbg	-0.350	**0.010**	-0.380	**0.003**
IL-6	-0.609	**<0.001**	-0.584	**<0.001**
TNF-α	-0.223	**0.038**	-0.496	**0.043**
IL1β	-0.296	**0.016**	-0.266	**0.022**
Iron metabolism				
Serum observed EPO	-0.318	**0.018**	-0.284	**0.021**
Serum iron	0.575	**<0.001**	0.334	**0.033**
Transferrin	0.260	**0.047**	0.216	0.018
Transferrin saturation	0.318	**0.012**	0.395	**0.002**
Ferritin	0.464	**<0.001**	-0.385	**0.001**
Hepcidin	-0.545	**<0.001**	-0.409	**<0.001**
Nutritional status				
Leptin	0.428	**<0.001**	0.490	**<0.001**
Albumin	0.492	**0.001**	0.352	**0.033**
Total Cholesterol	0.422	**0.001**	0.364	**0.018**
HDL	0.486	**0.003**	0.307	**0.044**
LDL	0.052	0.779	0.181	0.065
Triglycerides	0.167	0.206	0.164	0.084
Oxidative stress				
ROS	-0.304	**0.007**	-0.340	**0.005**
SOD	0.368	**0.030**	0.289	**0.016**
GPx	0.355	**0.034**	0.355	**0.004**

Results are considered significant for $P<0.05$. Abbreviations: KPS, Karnofsky Performance Status; Fbg, Fibrinogen; CRP, C-reactive Protein; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin, ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S7. Correlation of Hb levels with markers of chronic inflammation, iron metabolism, nutritional status and oxidative stress according to tumor site

Chronic Inflammation	Ovary	Breast	Lung	Prostate	Upper GI	Lower GI	Head and neck	Bladder
CRP, mg/l	-0.636*	-0.391*	-0.379*	-0.585*	-0.371*	-0.321*	-0.468*	-0.399*
Fbg, mg/dl	-0.367*	-0.311*	-0.285*	0.532*	0.154	-0.166	-0.271	-0.110
IL-6, pg/ml	-0.418*	-0.360*	-0.560*	0.682*	-0.388*	-0.419*	-0.767*	-0.354*
TNF-α, pg/ml	-0.342*	-0.222	-0.509*	0.604*	-0.140	-0.620*	-0.406*	-0.074
IL-1 β, pg/ml	-0.373*	0.108	-0.362*	0.558*	-0.250	-0.350*	-0.340*	-0.284*
Iron metabolism								
Serum observed EPO, mUI/ml	-0.957*	-0.959*	-0.650*	-0.540*	-0.670*	-0.261*	-0.371*	-0.410*
Observed/expected EPO ratio	0.452*	0.812*	0.673*	0.420*	0.638*	0.501*	0.961*	0.520*
Serum iron, g/dl	0.326*	0.373*	0.378*	0.394*	0.278*	0.474*	0.326*	0.210*
Transferrin, mg/dl	0.168	0.062	0.483*	0.127	0.356*	0.221	0.336*	0.140
Transferrin saturation,%	0.198	0.145	0.250*	0.150	0.450*	0.341*	0.633*	0.310*
Ferritin, ng/ml	-0.431*	0.023	-0.315*	-0.256*	0.711*	0.596*	-0.736*	-0.478*
Hepcidin, ng/ml	-0.601*	-0.300*	-0.443*	-0.340*	0.281	-0.367*	0.159	-0.370*
Nutritional status								
Leptin, ng/ml	0.335*	-0.298	0.452*	0.520*	0.324*	0.140	0.450*	0.382*
Albumin, g/dl	0.651*	0.513*	0.698*	0.434*	0.380*	0.201	0.587*	0.387*
Cholesterol, mg/dl	0.454*	0.560*	0.396*	0.116	0.525*	0.098	0.472*	0.240*
HDL, mg/dl	0.505*	0.311*	0.509*	0.119	0.096	0.065	0.380*	0.326*
LDL, mg/dl	0.373*	0.045	0.076	0.056	0.094	0.049	0.048	0.122
Triglycerides, mg/dl	0.026	0.207	0.104	0.098	0.007	0.010	0.101	0.010
Oxidative stress								
ROS, FORT U	-0.306*	-0.320*	-0.540*	-0.374*	-0.265*	0.320*	-0.570*	-0.349*
SOD, U/ml	0.240*	0.080	0.245*	0.155	0.024	0.150	0.250*	0.364*
GPx, U/l	0.354*	0.350*	0.386*	0.350*	0.550*	0.320*	0.382*	0.507*

*$P<0.05$ calculated with Spearman correlation test. Abbreviations: CRP, C-reactive Protein; Fbg, Fibrinogen; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S8. Correlation of observed/expected EPO ratio with markers of chronic inflammation, and oxidative stress in patients with cancer at different sites

Parameters	Pearson	*P*
Chronic inflammation		
CRP	-0.500	**0.035***
Fbg	-0.500	**0.035***
IL-6	-0.357	**0.045***
TNF-α	-0.223	0.052
IL1β	-0.266	0.060
Oxidative stress		
ROS	-0.440	**0.007****
SOD	0.368	**0.002****
GPx	0.455	**0.003***

Results are considered significant for $P<0.05$* and highly significant for $p<0.01$**. Abbreviations: Fbg, Fibrinogen; CRP, C-reactive Protein; IL, Interleukin; TNF, Tumor Necrosis Factor; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S9. Evaluation of laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress in the population of anemic cancer patients according to Hb levels (Hb ≥ 10 g/dl vs Hb <10 g/dl)

	Hb categories		
Parameters	**Hb≥10.0 gr/dl**	**Hb<10.0 gr/dl**	***p value***
Chronic inflammation			
CRP	6.2±10.5	17±18.4	**<0.001**
Fbg	422±132	492±170	**<0.001**
IL-6	16.7±10.5	26.6±13.2	**0.013**
TNF-α	17.3±9.5	24.1±14.4	0.066
IL-1β	21.5±16.4	27.7±17.2	0.310
Iron metabolism			
Serum EPO	23.1±7.8	38±14.3	**0.035**
Serum iron	63.1±38.1	39.9±34.7	0.075
Transferrin	220.4±65.3	183±55.4	0.883
Transferrin saturation	21±6	24±5	0.051
Ferritin	444.2±359.55	637.0±680.5	**<0.001**
Hepcidin	84.33±38.54	121.1±53.77	**0.032**
Nutritional status			
Albumin	3.9±0.7	2.9±0.8	**0.001**
Leptin	0.6±0.2	0.4±0.3	**0.019**
HDL	51.7±17.6	31.6±11.94	**0.003**
LDL	107.3±35.1	85.8±16.9	0.148
Total cholesterol	185.5±46.7	141.7±18.8	**<0.001**
Triglycerides	135.2±89.9	125.9±54.9	0.691
Oxidative stress			
ROS	381.2±103.5	474±126.2	**<0.001**
SOD	106.1±45.5	92.6±46	0.666
GPx	7402±3003	5447±1925	**0.002**

Abbreviations: BMI, body mass index; Fbg, Fibrinogen; CRP, C-reactive Protein; IL, Interleukin; TNF, Tumor Necrosis Factor;EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.

Table S10. Evaluation of Hb levels in the population of anemic cancer patients according to quartiles of the laboratory parameters of chronic inflammation, iron metabolism, nutritional status and oxidative stress

	Hb mean levels (g/dl) according to quartiles of different parameters (from the lowest to the highest value)					
	1	**2**	**3**	**4**	***P***	***Test for trend***
Chronic Inflammation						
CRP	11.1±0.9	10.9±1.1	10.5±1.4	10.1±1.1	**<0.001**	**<0.001**
Fibrinogen	10.9±1.1	10.4±1.4	10.8±1.2	10.5±1.2	**0.031**	0.125
IL-6	10.9±0.8	10.7±1.2	10.5±1.3	10.2±1.3	**0.019**	**0.019**
TNF-α	10.7±1.4	10.6±1.2	10.7±1.1	10.3±1	0.290	0.176
IL1β	11.1±1.1	10.4±1.1	10.3±1.0	10.2±1.1	**0.033**	**0.011**
Iron Metabolism						
Serum EPO	11.3±0.6	10.8±1.2	10.5±1.3	10.2±1.3	**0.029**	**0.021**
Serum iron	10.1±1.3	10.6±1.3	10.8±1.3	11.1±1.0	**0.001**	**<0.001**
Transferrin	10.2±1.2	10.4±1.3	11.3±1.3	10.6±1.1	**0.006**	**0.055**
Transferrin saturation	9.5±0.2	10.1±0.6	10.7±1.1	11.3±1.5	**0.003**	**0.034**
Ferritine	10.4±1.1	10.7±1.3	11.2±1.1	10.7±1.2	0.195	0.250
Hepcidin	10.8±0.7	10.5±0.9	10.6±0.5	10.1±0.3	**0.046**	**0.036**
Nutritional status						
Leptin	10.0±1.2	10.7±0.9	10.7±1.3	10.9±0.7	**0.012**	**0.023**
Albumin	9.9±1.3	10.8±1.2	10.4±1.6	11±1.1	0.243	0.119
Total cholesterol	10.2±1.3	10.4±1.4	10.6±1.3	11.6±0.7	**0.007**	**0.001**
HDL	9.8±1.5	10.9±1.0	10.6±1.4	11.6±1.0	**0.054**	**0.015**
LDL	11.1±1.2	10.0±1.1	11±1.3	11.3±1.2	0.183	0.470
Triglycerides	11±1.4	10.8±1.3	9.9±1.1	11±1.2	**0.049**	0.625
Oxidative stress						
ROS	10.8±0.8	11.1±1.3	10.2±0.9	10.4±1.1	**0.035**	**0.035**
SOD	10.6±1.2	10.0±1.2	10.9±0.9	11.1±1.2	**0.017**	**0.050**
GPx	10.5±1.1	10.0±1.1	10.7±1.0	11.4±0.9	**<0.001**	**<0.001**

Groups are compared by ANOVA test. Results are considered significant for $p \leq 0.05$. Significant p-values are reported in bold. Abbreviations: CRP, C-reactive protein; IL, Interleukin; TNF, Tumor Necrosis Factor; EPO, erythropoietin; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, Glutathione Peroxidase.