



*Original Contribution*

## STAGE-RELATED CHANGES IN IRON METABOLISM AND ANAEMIA IN PATIENTS WITH OVARIAN CANCER

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### ABSTRACT

Ovarian cancer is one of the most frequent and lethal malignancies in women, and is often diagnosed at an advanced stage due to the lack of specific early symptoms. Anaemia is a common complication in these patients. It has a multifactorial aetiology - absolute or functional iron deficiency, associated with inflammation, blood loss or chemotherapy.

The present study analyzed serum iron, transferrin and haemoglobin in 60 women with ovarian cancer at different FIGO stages. The results showed that with advancing stage, there is a significant decrease in serum iron and transferrin, while lower haemoglobin levels are statistically insignificant. A positive correlation was found between iron and haemoglobin, while the relationship between transferrin and iron as well as between haemoglobin and transferrin varied depending on the stage.

In early stages (I and II) anaemia was less than 50% and was mainly due to absolute iron deficiency, while in stage III 68% of patients were anaemic due to functional iron deficiency, caused by inflammation and increased expression of transferrin receptors in the tumour. The systemic inflammatory response, mediated by IL-6 and hepcidin, leads to intracellular iron retention and reduced serum concentration, which limits erythropoiesis.

The conclusion is that monitoring of serum iron, transferrin and haemoglobin provides valuable information about the metabolic and inflammatory status of patients and can serve as an accessible biochemical marker for assessing prognosis and systemic inflammation in ovarian carcinoma.

**Keywords:** ovarian carcinoma, transferrin, iron, anaemia, haemoglobin.

### INTRODUCTION

Ovarian carcinoma is one of the most common and highly lethal malignant diseases in women, representing a leading cause of mortality among gynecological neoplasms (1).

The disease is often diagnosed at an advanced stage due to the lack of specific symptoms in the early phases, which significantly worsens prognosis and reduces survival.

Anaemia is a problem that frequently accompanies patients with ovarian carcinoma. It has a multifactorial aetiology and may result from blood loss, chemotherapy, or alterations in iron metabolism, functional iron deficiency, oxidative stress, and inflammation (2,3).

Haemoglobin is among the haematological parameters with most commonly reduced values in oncological patients. It is an indicator of oxygen transport in the blood and also an important indirect marker of systemic iron metabolism.

In patients with FIGO stage III and IV disease, significantly lower haemoglobin levels are often observed. This may occur not only due to absolute iron deficiency but also to functional iron deficiency, in which iron is present in tissues but cannot be utilized for erythropoiesis (4). Haemoglobin correlates positively with serum iron, as low iron levels directly limit heme synthesis and, consequently, haemoglobin production. An inverse correlation is frequently observed between haemoglobin and inflammatory markers (CRP, IL-6), further supporting the link between anaemia and inflammation in ovarian carcinoma. Anaemia

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and low haemoglobin levels are commonly associated with poorer prognosis and reduced patient survival (5).

In recent years, increasing attention has been paid to the biochemical and metabolic changes accompanying tumour progression, including disturbances in iron metabolism - an essential trace element for cellular proliferation and redox processes (6,7).

Disorders of serum iron, transferrin, and haemoglobin frequently accompany malignant diseases and may be associated with anaemia of chronic disease (8). In this type of anaemia, serum iron and haemoglobin levels are reduced, while transferrin levels may vary depending on disease stage and activity. In the context of ovarian carcinoma, these changes may reflect not only the systemic inflammatory response but also the metabolic activity of the tumour itself (9). This can lead to a paradoxical condition characterized by low serum iron in the presence of intracellular iron accumulation within tumour tissues.

Iron is a key component of haemoglobin and participates in numerous cellular enzymatic systems. Its role in normal physiology is associated with oxygen transport, DNA synthesis, and maintenance of redox homeostasis (10). However, intracellular iron accumulation may exert pro-oxidant effects by promoting the generation of free radicals via the Fenton reaction, which can damage DNA, lipids, and proteins - processes associated with carcinogenesis (11). In addition, the concept of ferroptosis (iron-dependent cell death mediated by lipid peroxidation) has gained increasing importance as a therapeutic approach in ovarian carcinoma, further supporting the role of iron in tumour pathophysiology (12).

For this reason, the body maintains strict control over free iron levels, primarily through the hepcidin-ferroportin system and the proteins transferrin and ferritin. Tumour growth and the inflammatory response increase IL-6 and other cytokines, stimulating hepatic hepcidin production. Hepcidin inhibits iron export from macrophages and enterocytes (via ferroportin), leading to reduced serum iron availability for erythropoiesis. This mechanism is part of the pathogenesis of anaemia associated with inflammation and certain malignancies (13,14). In advanced stages of the disease, this tendency usually becomes more pronounced, as inflammatory and tumour burden increase, while concomitant malnutrition and anorexia further

limit iron intake. Some studies indicate that low serum iron levels may correlate with higher tumour activity and unfavourable prognosis (15).

Transferrin is the main iron transport protein in the blood, delivering iron ions to cells via receptor-mediated uptake. Its levels are regulated by hepatic synthesis and reflect both overall iron status and inflammatory activity. In inflammatory and neoplastic diseases, hypotransferrinemia is observed as a result of decreased hepatic synthesis and increased consumption by tumour cells (8).

In patients with ovarian carcinoma, reduced transferrin levels are frequently observed in advanced stages, associated with decreased hepatic synthesis in the setting of systemic inflammation (7). In early stages, however, a compensatory increase in transferrin may be detected as a response to reduced serum iron levels and activation of iron-binding capacity (3). This explains why some patients in FIGO stages I–II may exhibit higher transferrin levels despite moderately reduced serum iron. In later stages, with progressive deterioration of liver function and increased catabolism, transferrin levels decrease, which is characteristic of a negative acute-phase protein. Low transferrin levels in women with ovarian carcinoma are associated with functional iron deficiency (low serum iron and often reduced transferrin levels). Moreover, hypotransferrinemia may also have prognostic value (6).

Correlation analysis between serum iron and transferrin usually demonstrates an inverse relationship - when iron levels are low, the body increases transferrin synthesis to facilitate iron transport; however, in chronic inflammation and advanced disease, this regulatory mechanism becomes impaired (6).

Assessment of serum iron, transferrin, and haemoglobin in patients with ovarian carcinoma may provide valuable information on metabolic disturbances associated with tumour progression and contribute to understanding the pathophysiological mechanisms of anaemia in this disease. Furthermore, changes in these parameters across different FIGO stages may have diagnostic and prognostic significance, reflecting the degree of systemic inflammation, nutritional status, and tumour activity.

## AIM

The aim of the present study is to analyse serum iron, transferrin, and haemoglobin levels in

women with ovarian carcinoma across different FIGO stages and to investigate possible relationships among these parameters.

## MATERIALS AND METHODS

The study included 60 women with histopathologically confirmed ovarian carcinoma. The patients were operated on and hospitalized at the Gynecology Clinic of St. Marina University Hospital, Pleven, during the period January 2020–November 2020. From every patient venous blood was collected from v. *brachialis* in the morning (iron levels are highest in the morning) in two Greiner Vacuette test tubes – a serum tube without anticoagulant and a tube containing K2EDTA. After centrifugation at 3500 rpm for 15 minutes, the separated serum (without hemolysis) was used for quantitative determination of iron and transferrin, while the K2EDTA sample was used for hemoglobin measurement. All laboratory analyses were performed in the Clinical Laboratory of St. Marina University Hospital, Pleven.

Serum iron concentrations were determined using a colorimetric ferrozine method on a Beckman Coulter AU 480 biochemical analyzer. Transferrin levels were measured by an immunoturbidimetric method on the same analyzer. Hemoglobin was determined using a non-cyanide method on a Medonic M32 hematology analyzer.

All participants provided written informed consent. Disease staging was performed according to FIGO, resulting in the formation of three groups:

Stage I – 19 patients;

Stage II – 19 patients;

Stage III – 22 patients;

Inclusion criteria: age  $\geq 18$  years; histologically confirmed ovarian carcinoma.

Exclusion criteria: anaemia of other origin, chronic inflammatory diseases, hepatic or renal insufficiency, current parenteral iron therapy, or blood transfusion.

The present study does not include a control group of healthy individuals. To assess deviations in the studied parameters, reference intervals provided by the manufacturers of the reagents and equipment used were employed.

Statistical analysis was performed using methods appropriate to the nature of the studied processes and data. The study data were processed using STATGRAPHICS, SPSS, and Microsoft Excel for Windows. A significance level of  $p < 0.05$  was accepted for rejection of the null hypothesis.

To control for the probability of a Type I error when performing multiple comparisons, a Bonferroni correction was applied. Accordingly, the level of statistical significance was adjusted to  $p < 0.017$ . Boxplot diagrams were used to visualize the data distribution and for comparisons between the groups.

## RESULTS

The reference intervals (**Table 1**) for the measured parameters were those provided by the manufacturers of the reagent kits used for the laboratory analyses.

**Table 1.** Reference intervals according to the manufacturers of the working reagents.

| Parameter   | Reference / cut off value |
|-------------|---------------------------|
| transferrin | 2-3.6 g/L                 |
| serum iron  | 10.7- $\mu$ mol/L         |

Our results showed that 28% of the patients ( $n = 17$ ) had transferrin levels outside the reference range, while 60% ( $n = 36$ ) had serum iron levels below the reference range.

According to the World Health Organization (WHO), anemia in *non-pregnant women* is defined when hemoglobin levels are below 120 g/L. In our study group 45 % of the patients ( $n=27$ ) have such values. The data are present in **Table 2** and **Table 3**.

**Table 2.** Descriptive statistical characteristics of the measured parameters in the studied group of women with ovarian carcinoma.

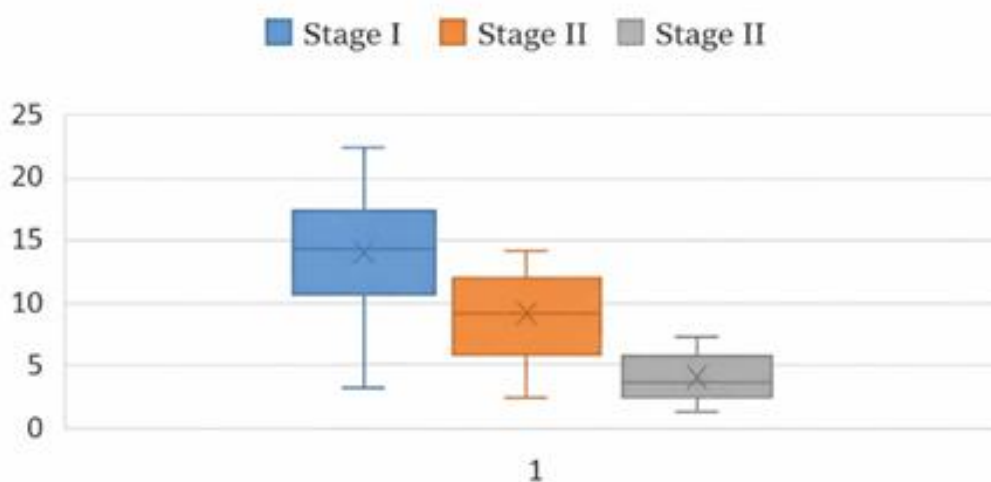
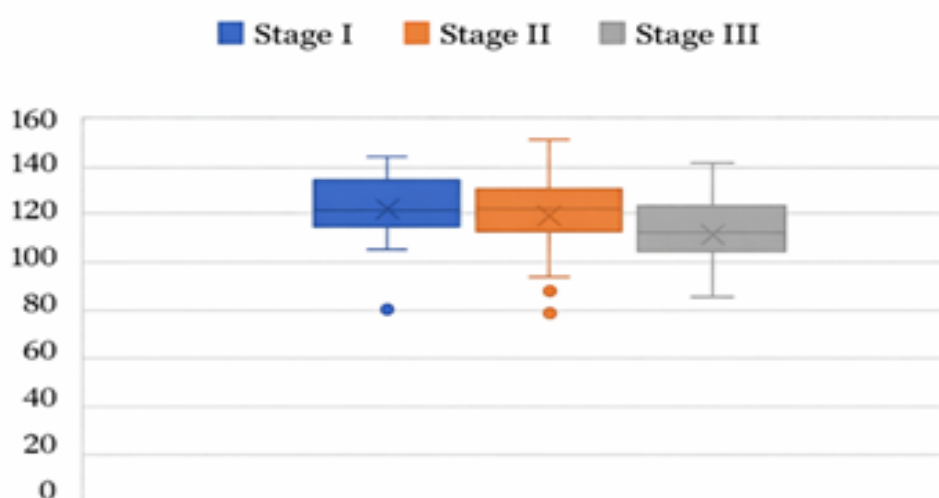
| Parameter              | Characteristics   |               |      |      |
|------------------------|-------------------|---------------|------|------|
|                        | Mean $\pm$ SD     | 95 % CI       | Min  | Max  |
| Serum iron $\mu$ mol/l | 8,21 $\pm$ 4,9    | 6,94 - 9,48   | 1,2  | 20,1 |
| Transferrin g/l        | 2,31 $\pm$ 0,58   | 2,16 - 2,46   | 0,49 | 3,41 |
| Haemoglobin g/l        | 120,6 $\pm$ 16,63 | 116,3 - 124,9 | 79   | 153  |

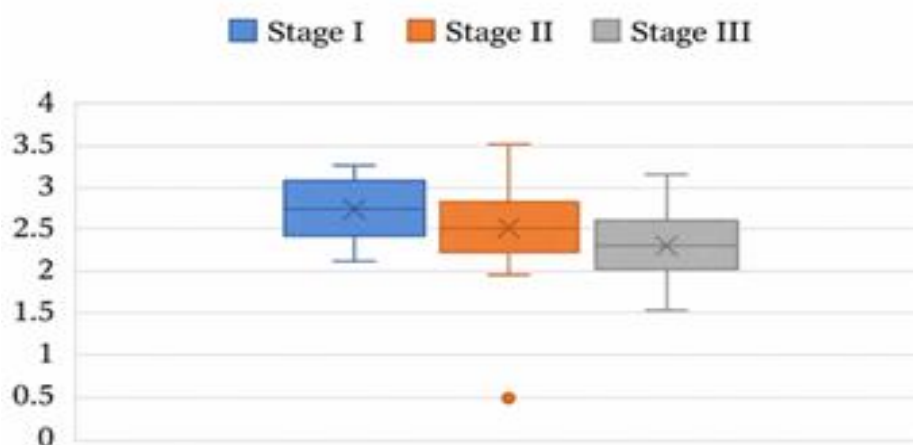
**Table 3.** Levels of statistical significance of the measured parameters according to FIGO stage.

| Parameter                       | Stage I<br>n=19 | Stage II<br>n=19 | Stage III<br>n=22 | Level of<br>statistical<br>significance p |
|---------------------------------|-----------------|------------------|-------------------|-------------------------------------------|
| Serum iron<br>$\mu\text{mol/l}$ | 12,73           | 8,41             | 3,99              | < 0.00001                                 |
| Transferrin g/l                 | 2,64            | 2,26             | 2,08              | 0.0066                                    |
| Haemoglobin g/l                 | 126             | 122              | 115               | 0.1074                                    |

The results of our study demonstrate that with advancing stage, all three parameters decrease. For serum iron and transferrin, these changes are statistically significant, whereas the change in haemoglobin concentration does not reach statistical significance.

After applying the Bonferroni correction, the statistically significant differences in serum iron and transferrin between the individual stages were maintained, whereas no statistically significant difference was found for haemoglobin.

**Figure 1.** Boxplot of serum iron levels by stage**Figure 2.** Boxplot of hemoglobin levels by stage



**Figure 3.** Boxplot of transferrin levels by stage

Boxplot analysis clearly visualized the trend of a progressive decrease in serum iron, transferrin, and haemoglobin as the disease stage advanced. The results are presented

graphically in **Figure 1**, **Figure 2** and **Figure 3**. To identify relationships between individual parameters, Pearson's correlation coefficient was used (**Table 4**).

**Table 4.** Correlation analysis between the studied parameters in ovarian carcinoma.

| N=60        |                | Transferrin | Iron     | Haemoglobin |
|-------------|----------------|-------------|----------|-------------|
| Transferrin | Pearson        | 1           | 0.21     | 0.008       |
|             | Sig. (2tailed) |             | <0.00001 | <0.00001    |
| Iron        | Pearson        | 0.21        | 1        | 0.39        |
|             | Sig. (2tailed) | <0.00001    |          | <0.00001    |
| Haemoglobin | Pearson        | 0.008       | 0.39     | 1           |
|             | Sig. (2tailed) | <0.00001    | <0.00001 |             |

A statistically significant positive correlation was established between:

- transferrin and iron;
- haemoglobin and iron.

Applying the same statistical methods within each stage yielded the following results (**Table 5**):

Our results indicate that in early stages (I–II), anaemia ranges from 26% to 37%. In the

advanced stage (III) anaemia is present in 68% of patients. With disease progression, there is an increase in the proportion of women with serum iron levels below 10.7  $\mu\text{mol/L}$  and in Stage III 91% of the women are with iron levels below the lower reference limit. With advancing disease stage, the percentage of patients with reduced transferrin levels also increased, from 5% to 41%.

**Table 5.** Percentage distribution of patients with pathological deviations in the studied parameters by ovarian carcinoma stage.

| Parameter                     | Stage I<br>n=19 | Stage II<br>n=19 | Stage III<br>n=22 |
|-------------------------------|-----------------|------------------|-------------------|
| Iron < 10.7 $\mu\text{mol/l}$ | 26 % (n=5)      | 58 % (n= 11)     | 91 % (n=20)       |
| Transferrin < 2.0 g/l         | 5 % (n=1)       | 37 % (n=7)       | 41 % (n=9)        |
| Haemoglobin < 120 g/l         | 26 % ( n= 5)    | 37 % (n=7)       | 68 % (n= 15)      |

By examining the correlations between the studied parameters across the individual stages, the following relationships were identified:  
In stage I (data are present in **Table 6**):  
a statistically significant positive correlation between:

- iron and haemoglobin.
- negative correlation between:
- haemoglobin and transferrin
  - iron and transferrin

**Table 6.** Correlation analysis of the studied parameters in Stage I.

| N=19               |                | Transferrin | Iron     | Haemoglobin |
|--------------------|----------------|-------------|----------|-------------|
| <b>Transferrin</b> | Pearson        | 1           | - 0.0088 | - 0.117     |
|                    | Sig. (2tailed) |             | <0.00001 | <0.00001    |
| <b>Iron</b>        | Pearson        | - 0.0088    | 1        | 0.50        |
|                    | Sig. (2tailed) | <0.00001    |          | <0.00001    |
| <b>Haemoglobin</b> | Pearson        | - 0.117     | 0.50     | 1           |
|                    | Sig. (2tailed) | <0.00001    | <0.00001 |             |

In stage II (**Table 7**):  
a statistically significant positive correlation between:  
• iron and haemoglobin.

- negative correlation between:
- haemoglobin and transferrin
  - iron and transferrin

**Table 7.** Correlation analysis of the studied parameters in Stage II.

| N=19               |                | Transferrin | Iron     | Haemoglobin |
|--------------------|----------------|-------------|----------|-------------|
| <b>Transferrin</b> | Pearson        | 1           | - 0.58   | - 0.41      |
|                    | Sig. (2tailed) |             | <0.00001 | <0.00001    |
| <b>Iron</b>        | Pearson        | - 0.58      | 1        | 0.13        |
|                    | Sig. (2tailed) | <0.00001    |          | <0.00001    |
| <b>Haemoglobin</b> | Pearson        | - 0.41      | 0.13     | 1           |
|                    | Sig. (2tailed) | <0.00001    | <0.00001 |             |

In stage III (**Table 8**):  
a statistically significant positive correlation between:

- iron and haemoglobin.
- haemoglobin and transferrin
- iron and transferrin

**Table 8.** Correlation analysis in Stage III.

| N=22               |                | Transferrin | Iron     | Haemoglobin |
|--------------------|----------------|-------------|----------|-------------|
| <b>Transferrin</b> | Pearson        | 1           | 0.29     | 0.31        |
|                    | Sig. (2tailed) |             | <0.00001 | <0.00001    |
| <b>Iron</b>        | Pearson        | 0.29        | 1        | 0.199       |
|                    | Sig. (2tailed) | <0.00001    |          | <0.00001    |
| <b>Haemoglobin</b> | Pearson        | 0.31        | 0.199    | 1           |
|                    | Sig. (2tailed) | <0.00001    | <0.00001 |             |

## DISCUSSION

The present study found that the prevalence of anaemic syndrome in patients with ovarian carcinoma increases as the disease stage advances, being below 50% in the early stages (I–II) and reaching 68% in stage III. This indicates that anaemia progresses alongside the tumour process and likely reflects both the increasing inflammatory burden and the dysregulation of iron metabolism.

The observed progressive decrease in serum iron and transferrin, combined with a relatively less pronounced change in haemoglobin, suggests a transition from absolute to functional iron deficiency as the disease progresses. This trend is further supported by the increasing percentage of patients with low iron and transferrin values in the later stages.

Correlation analysis further revealed the dynamics of these processes. In the early stages, a maintained positive correlation between serum iron and haemoglobin is observed, reflecting relatively preserved erythropoiesis. As the stage advances, a shift in the interrelationships between parameters occurs; in stage II, stronger negative correlations with transferrin are established. This change reflects the transition from a relatively compensated iron balance to functional dysregulation, typical of neoplastic progression (16,17). The results in stage II confirm that with the progression of ovarian carcinoma, a redirection of iron metabolism from erythropoiesis to tumour tissue occurs, which may carry not only diagnostic but also potential prognostic significance (13).

In stage III, we observe a simultaneous decrease in all parameters with positive interrelationships between them. This reflects a deepening of the systemic dysregulation of iron metabolism. The positive correlations between the three parameters in our sample reflect a common pathophysiological mechanism: the degree of inflammation-mediated disruption of iron metabolism is directly linked to the severity of the anaemia. Similar dependencies have been reported in previous studies of patients with ovarian carcinoma, where anaemia correlates with inflammatory activity and impaired iron metabolism (17).

From a clinical perspective, these changes are of significant importance, as anemia and iron metabolism disorders are associated with poorer functional status, reduced therapy tolerance,

and an unfavorable prognosis. This highlights the necessity of monitoring these parameters as part of the routine assessment of patients with ovarian carcinoma.

## Clinical Implications

The results obtained have important clinical implications for the diagnosis and monitoring of patients with ovarian carcinoma. The observed progressive decrease in serum iron, transferrin, and hemoglobin as the stage advances indicates that these parameters can serve as accessible and informative biomarkers for assessing systemic inflammation and metabolic status. Distinguishing between absolute and functional iron deficiency is essential for choosing a therapeutic approach, as the inappropriate administration of iron-containing preparations may be ineffective in the presence of inflammation-mediated anemia.

Monitoring these parameters dynamically can aid in assessing disease progression and treatment response, as well as in identifying patients at increased risk of developing clinically significant anaemia. This, in turn, could improve the individualization of treatment and supportive care, including the timely initiation of corrective measures for anemia, aiming to enhance quality of life and therapeutic outcomes.

## CONCLUSION

Alterations in serum iron, transferrin, and haemoglobin levels in women with ovarian carcinoma reflect the complex interplay between tumour growth, the inflammatory response, and systemic iron metabolism. With advancing disease stage, a progressive decrease in all three parameters is observed.

Reductions in haemoglobin and serum iron in ovarian carcinoma may have clinical value for assessing patients' overall status and prognosis. Anaemia is associated with poorer tolerance to chemotherapy, lower functional status, and reduced survival. On the other hand, changes in transferrin may serve as an additional indicator of inflammatory activity and hepatic function.

When considered in combination, these parameters may serve as accessible biochemical markers for evaluating systemic inflammation, nutritional status, and prognosis in patients with ovarian carcinoma, supporting their inclusion in routine laboratory assessment.

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## REFERENCES

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA: a cancer journal for clinicians*, 74(1):12-49, 2024.
2. Basak T, Kanwar RK. Iron imbalance in cancer: Intersection of deficiency and overload. *Cancer Medicine*, 11(20):3837-53, 2022.
3. Ludwig H, Müldür E, Endler G, Hübl W. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Annals of oncology*, 1;24(7):1886-92, 2013.
4. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood*. 3;133(1):40-50, 2019.
5. Foley, O.W., Vega, B., Roque, D., Hinchcliff, E., Marcus, J., Tanner, E.J. and Barber, E.L., Characterization of pre-operative anemia in patients undergoing surgery by a gynecologic oncologist and association with post-operative complications. *International Journal of Gynecological Cancer*, 33(11), pp.1778-1785, 2023.
6. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nature Reviews Cancer*, 13(5):342-55, 2013.
7. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. *Nature Reviews Immunology*, 15(8):500-10, 2015.
8. Weiss G, Goodnough LT. Anemia of chronic disease. *New England Journal of Medicine*, 10;352(10):1011-23, 2005.
9. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. *Biochemical Journal*, 15;434(3):365-81, 2011.
10. Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics*, 6(4):748-73, 2014.
11. Toyokuni S. Iron and carcinogenesis: from Fenton reaction to target genes. *Redox Report*, 1;7(4):189-97, 2002.
12. Li L, Qiu C, Hou M, Wang X, Huang C, Zou J, Liu T, Qu J. Ferroptosis in ovarian cancer: a novel therapeutic strategy. *Frontiers in oncology*, 11:665945, 2021.
13. Torti SV, Torti FM. Iron and cancer: 2020 vision. *Cancer research*, 15;80(24):5435-48, 2020.
14. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, 17;306(5704):2090-3, 2004.
15. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nature genetics*, 46(7):678-84, 2014.
16. Ivanova TI, Klabukov ID, Krikunova LI, Poluektova MV, Sychenkova NI, Khorokhorina VA, Vorobyev NV, Gaas MY, Baranovskii DS, Goryainova OS, Sachko AM. Prognostic value of serum transferrin analysis in patients with ovarian cancer and cancer-related functional iron deficiency: a retrospective case-control study. *Journal of Clinical Medicine*, 12;11(24):7377, 2022.
17. Macciò A, Madeddu C, Gramignano G, Mulas C, Tanca L, Cherchi MC, Floris C, Omoto I, Barracca A, Ganz T. The role of inflammation, iron, and nutritional status in cancer-related anemia: results of a large, prospective, observational study. *Haematologica*, 100(1):124, 2015.

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