PRO-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

WILMER RAMÍREZ CARMONA

ABORDAGEM MULTIFATORIAL PARA DIAGNÓSTICO E TERAPÊUTICA FARMACOLÓGICA EM PROCESSOS CARCINOGÊNICOS

Presidente Prudente - SP
2021

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Defesa de Dissertação apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências da Saúde.

Orientador:
Prof. Dr. Leonardo de Oliveira Mendes

Presidente Prudente - SP
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Presidente Prudente, 23 de abril de 2021.

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DEDICATÓRIA

Desejo dedicar esta dissertação especialmente à minha família porque sempre tive o seu apoio incondicional, aos meus professores, que me ensinaram e acolheram durante os dois anos do mestrado no #teamunoeste, o que me tornou um melhor profissional. À minha esposa "Bea", parceira dos meus sonhos.

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“A felicidade existe na terra e é conquistada por meio do exercício prudente da razão, do conhecimento da harmonia do universo e da prática constante da generosidade.” (José Martí)

LISTA DE SIGLAS

AR	- Androgen receptor
CaP	- Prostate cancer
CAPES	- Coordination for the Improvement of Higher Education
HE	- Hematoxylin-eosin
IL	- Interleukin
MNU	- N-methyl-N-nitrosourea
NOS	- Newcastle-Ottawa scale
PDL	- Dorsolateral prostate
PV	- Ventral prostate
SF	- Serum ferritin
TNF	- Tumor necrosis factor
TNM	- Tumor-node-metastasis

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SUMÁRIO

INTRODUÇÃO GERAL

O câncer é uma das doenças crônicas mais incidentes no mundo[1], segundo o Observatório Global do Câncer da Organização Mundial de Saúde a incidência em 2018 foi de 18,1 milhões de casos, com mortalidade de 9,6 milhões[2]. Os dados são mais impactantes quando se avaliam os números totais de incidência no Brasil, onde os mais frequentes são os cânceres de próstata, mama, pulmão e cólon retal[3].

Dessa maneira impõe-se a necessidade de esforços na prevenção e diagnóstico precoce da doença, sendo importante desenvolver e aprimorar métodos diagnósticos eficazes, bem como novas terapias que ajudem a melhorar a qualidade de vida. Para atingir esse objetivo as pesquisas têm focado na descoberta de biomarcadores, moléculas importantes para o diagnóstico precoz e que auxiliam no acompanhamento da progresão da doença. Moléculas como a ferritina sérica estão sendo pesquisadas para este fim, tanto para processos inflamatórios[4,5] como no diagnóstico e prognóstico de tumores[6–8].

Além da busca de biomarcadores, as terapias também vêm sendo aprimoradas no tratamento do câncer, desde a remoção cirúrgica, quimioterapia, radioterapia, imunoterapia, terapias combinadas até terapias alternativas[9,10]. No caso do câncer de próstata são utilizadas terapias antiandrogênicas e quimioterápicas, atuando nos receptores de andrógenos, visto que esse tipo de câncer é, em grande parte, dependente de hormônios. Tais receptores não são as únicas vias de sinalização presentes no câncer prostático, fato este que favorece o aparecimento do câncer de próstata resistente à castração[11]. São necessários novos medicamentos e terapias combinadas que sejam mais eficazes e seguras[12].

Ainda há muito a melhorar na compreensão, diagnóstico precoce e tratamento dessa doença. Os avanços feitos no combate ao câncer nos últimos anos são indiscutíveis, mas esforços maiores precisam ser realizados para melhorar a qualidade e aumentar a sobrevida do paciente, com trabalhos que foquem na identifição de biomarcadores eficazes e de drogas capazes de superar a resistência aos medicamentos de alguns tipos de tumores. Para atingir esse objetivo é imprescindível o estudo de biomarcadores de maior especificidade, que junto às novas terapias, permitiram o melhor direcionamento no manejo eficiente do câncer.

REFERÊNCIAS

1. Fane M, Weeraratna AT. How the ageing microenvironment influences tumour progression. Nat Rev Cancer. 2020 Feb;20(2):89–106.
2. Mattiuzzi C, Lippi G. Current Cancer Epidemiology. J Epidemiol Glob Health. 2019 Dec;9(4):217–22.
3. Araujo LH, Baldotto C, Castro G de J, Katz A, Ferreira CG, Mathias C, et al. Lung cancer in Brazil. J Bras Pneumol publicacao Of da Soc Bras Pneumol e Tisilogia. 2018;44(1):55–64.
4. Elimam H, Abdulla AM, Taha IM. Inflammatory markers and control of type 2 diabetes mellitus. Diabetes Metab Syndr. 2019;13(1):800–4.
5. Pitchika A, Schipf S, Nauck M, Dörr M, Lerch MM, Felix SB, et al. Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: Results from the prospective SHIP study. Diabetes Res Clin Pract. 2020 May;163:108149.
6. Gao Y, Wang J, Zhou Y, Sheng S, Qian SY, Huo X. Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for Colorectal Cancer. Sci Rep. 2018 Feb;8(1):2732.
7. Lian M, Zhang C, Zhang D, Chen P, Yang H, Yang Y, et al. The association of five preoperative serum tumor markers and pathological features in patients with breast cancer. J Clin Lab Anal. 2019 Jun;33(5):e22875.
8. Beale AL, Penney MD, Allison MC. The prevalence of iron deficiency among patients presenting with colorectal cancer. Color Dis Off J Assoc Coloproctology Gt Britain Irel. 2005 Jul;7(4):398–402.
9. Gotwals P, Cameron S, Cipolletta D, Cremasco V, Crystal A, Hewes B, et al. Prospects for combining targeted and conventional cancer therapy with immunotherapy. Nat Rev Cancer. 2017 May;17(5):286–301.
10. Gorbet M-J, Ranjan A. Cancer immunotherapy with immunoadjuvants, nanoparticles, and checkpoint inhibitors: Recent progress and challenges in treatment and tracking response to immunotherapy. Pharmacol Ther. 2020 Mar;207:107456.
11. Vlachostergios PJ, Puca L, Beltran H. Emerging Variants of Castration-Resistant Prostate Cancer. Curr Oncol Rep. 2017 May;19(5):32.
12. Gonçalves BF, de Campos SGP, Fávaro WJ, Brandt JZ, Pinho CF, Justulin LA, et al. Combinatorial Effect of Abiraterone Acetate and NVP-BEZ235 on Prostate Tumor Progression in Rats. Horm Cancer. 2018;1–13.

ARTIGO 1

ARE SERUM FERRITIN LEVELS A RELIABLE CANCER BIOMARKER? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract

Although serum ferritin (SF) has been shown in several studies to be a potential cancer biomarker, the results are inconsistent. Herein, a systematic review was performed to investigate the clinical SF levels in different types of tumors in order to verify the role of SF levels as a biomarker for cancer diagnosis. The search was performed using the PubMed/Medline, Cochrane Library, and Scopus databases. Observational studies comparing SF levels between healthy adults and patients with cancer were included. The meta-analysis was carried out according to the inverse variance and random effects model. The standardized mean differences (SMDs) were assessed at 95% confidence intervals (CIs). We found that SF was higher in patients with cancer (SMD 3.07; CI 1.96,4.17), especially for head and neck cancer (SMD 3.88; CI 0.42,7.34), lung cancer (SMD 1.72; CI 0.67,2.78), pancreatic cancer (SMD 6.79; CI 5.66,7.91), and renal cell carcinoma (SMD 1.77; CI 0.48,3.05). Moreover, in the advanced stages (Stages III and IV), ferritin levels were higher than in healthy adults (SMD 4.89; CI 2.72,7.06, and SMD 8.40; CI 6.99,9.82, respectively). SF acts as a biomarker for pancreatic cancer, renal cell carcinoma, lung cancer, and head and neck cancer and is a sensitive biomarker for the detection of advanced stages of tumors.

Keywords: Carcinoma, Renal Cell; Head and Neck Neoplasms; Iron-Binding Proteins; Lung Neoplasms; Pancreatic Neoplasms.

Introduction

Iron is an essential nutrient for several bodily functions. It is recycled principally through phagocytosis of erythrocytes by macrophages[1], and the remaining is absorbed by enterocytes in the small intestine, where gastric juice is essential in this process[2]. In the enterocytes, it is stored in ferritin and transported to the plasma by transferrin. This process is mediated by ferroportin and hepcidin; together, these are the main regulators of iron metabolism at systemic levels[3]. Intracellular iron is used by the mitochondria to produce heme groups, which are indispensable for hemoglobin, cytochromes, and other enzymes[4,5].

In general, ferritin present in the cell nucleus captures free iron and protects DNA. However, when abnormal synthesis of heme groups overcomes the storage capacities of ferritin, iron accumulates in the mitochondria, increasing the production of oxygen free radicals resulting from the Fenton reaction[6] and activating signaling pathways related to cancer growth and proliferation[7]. In addition, tumor-associated macrophages are undoubtedly the main source of serum ferritin (SF) through its synthesis and secretion, contributing to the metabolism of cancer cells by stimulating proliferation, angiogenesis, and immunosuppression[3].

In pancreatic tumors, for example, SF levels act as an efficient inflammatory biomarker, which increases as a result of the action of inflammatory mediators such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, which activate the nuclear factor-kappa B pathway, thus improving the pathological activity of the *KRAS* oncogene[8]. Meanwhile, SF levels are contradictory in colorectal cancer with a sensitivity of 10.39%, which is considered low for a tumor biomarker regardless of tumor stage[9].

Although the mechanisms by which SF levels are altered in cancer are poorly understood, we hypothesized an interaction between cancer signaling pathways and modifications in iron metabolism[10], raising the possibility of SF levels as a biomarker in different types of cancer.

We performed a meta-analysis to investigate clinical SF levels in different types of tumors to verify the role of SF levels as a biomarker for cancer diagnosis.

Materials and Methods

Protocol and registration

The systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol[11], and it was submitted to PROSPERO (CRD42020207284).

Eligibility criteria

The PECOS question was structured as follows: Are there differences between (P) adults with (E) cancer without any previous treatment and (C) healthy adults regarding their (O) SF levels based on (S) observational studies? Second, we investigated whether ferritin levels could be altered according to disease progression. Studies were selected based on the following eligibility criteria: a) studies with adults (older than 18 years of age) comparing SF levels between healthy individuals (not reporting treatment that could alter SF levels) and patients with cancer (not receiving previous treatment for this condition); b) groups of healthy adults without history of chronic or acute diseases, local or general infection or inflammation, or benign or pre-malignant lesions; c) studies written in English, Spanish, and Portuguese; and d) studies published before March 2020.

Information sources and search

The literature review was carried out by two independent researchers (WRC and BDF) in the PubMed/Medline, Cochrane Library, and Scopus databases according to the eligibility

criteria, and any disagreement was resolved by consensus with help from a third researcher (LOM). In addition, the researchers performed a manual search using references. The MeSH terms used were: “Adult,” “Patients,” “Ferritins,” and “Neoplasms,” and the search strategy is described in Supplement 1.

Study selection

Two independent researchers (WRC and BDF) identified and screened the manuscripts according to the title, abstract, language, type of study, and other eligibility criteria. In the second phase, the full text of the articles was reviewed, and articles were eliminated when they did not meet the criteria.

Data collection process

A form was generated to evaluate the eligibility of the studies for inclusion in the review according to the criteria and to facilitate the rapid determination of the studies to be excluded. The data collection process was manual and independent, and the extracted data were compared to verify the collection process. Any disagreements between the researchers were resolved by group consensus with the assistance of a third researcher (LOM).

Data items

Country, study design, number of participants, age, sex, and race of exposure and control groups, selection process and characteristics of case and control, principal results, limitations reported by the authors, conflict of interests, and funding were collected in addition to SF levels at different TNM stages, cancer type, and control groups.

Risk of bias in individual studies

The bias risk criteria of the Newcastle–Ottawa scale (NOS)[12] were used to assess the quality of the observational studies included in the review and meta-analysis by two independent researchers (WRC and BDF). Regarding the risk of bias, individual studies were assessed as having low risk (≥ 7 score) or high risk (< 7 score)[13].

Data analysis

SF level differences between the cancer and healthy groups were evaluated as the primary outcome. Moreover, we analyzed the differences in SF levels among TNM stages (stages I to IV), and between TNM stages and control groups. The data were transformed into the same units of measurement (ng/ml) and compared by pooling according to the type of cancer. Studies with a high risk of bias (according to the NOS) were excluded from the meta-analysis.

The meta-analysis was carried out according to Inverse Variance as a statistical method, and the random-effects model was used as the analysis model to evaluate the standardized mean differences (SMD) at 95% confidence intervals (CI). The chi-square test and I^2 statistic were used to assess the heterogeneity among the studies ($p<0.05$). The overall effect was assessed using the Z statistic at a 5% significance level. The meta-analysis was performed using Review Manager 5.3. In addition, publication bias was assessed by Egger's regression test (at least 10 studies) with the statistical software program R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria), and $p<0.10$ was taken as statistical evidence of the presence of small study effects and potential publication bias[14].

Results

Study selection

A total of 2,608 articles from the PubMed/Medline database, 218 from the Cochrane Library, and 4,951 from the Scopus database were evaluated. After duplicates were removed

and according to the eligibility criteria 80 articles were assessed and them, 48 were excluded. The principal reasons for the exclusion were: absence of control groups identified as healthy adults with no history of chronic or acute disease; SF levels not provided for each group or type of cancer evaluated; and several type of cancer assessed or with any previous treatment. The list of excluded articles and their causes was described in the Supplement 2. Thus, 32 articles were included in the systematic review, and 22 articles were included in the quantitative synthesis (meta-analysis). The exclusion of articles from the meta-analysis was due to the high risk of bias (n = 5) and absence of standard deviation (n = 5) *[Figure 1 near here]*.

Study characteristics

The evaluated studies were from 10 different countries: Austria (n = 2), China (n = 6), Greece (n = 2), India (n = 4), Iran (n = 1), Italy (n = 4), Japan (n = 1), Poland (n = 2), Turkey (n = 6), and USA (n = 4). A total of 4,952 adults were included in the studies, them 2,609 as case and 2,342 as control. The patients were affected with breast cancer[15–17](n = 3), colorectal cancer[18,19](n = 2), gastric cancer[20](n = 1), head and neck cancer[21–26](n = 6), hepatocellular carcinoma[27–29](n = 3), lung cancer[30–35](n = 6), malignant melanoma[36](n = 1), mesothelioma[37](n = 1), myeloma multiple[38](n = 1), ovarian cancer[39,40](n = 2), pancreatic cancer[41,42](n = 2), prostate cancer[43](n = 1), and renal cell carcinoma[44–46](n = 3). Both the cases and the controls had no comorbidities, no history of infections in the last 6 months, no anemia, or liver disease. In addition, they did not have blood transfusions in the last 6 months, nor iron supplementation or use of medications that could interfere with the values of ferritin and iron metabolism. Controls were selected from community volunteers, hospital staff and blood donors. In a single study, the controls were outpatient patients, and in three of them there was no description of how they were selected.

The age and sex of the subjects were matched between the groups, except in Alexandrakis *et al.,* and Maxim & Veltri studies, where the ages of the cases were significantly higher in relation to the controls. Furthermore, in Maxim & Veltri study, the proportion of men with cancer was higher compared to control group where the female sex predominated. Only 8 studies showed smoking habits among the participants, 5 of them managed to control this confounding factor with homogeneity between the groups. The identification of SF levels and the characteristics of studies included were described in the Supplement 3.

Risk of bias of studies

Five studies showed a high risk of bias according to NOS. The main biases in the selection of cases and control groups were the inadequate definition of the case (n = 2), low representativeness of the cases (n = 1), no description in the control selection (n = 3) or these were hospital controls (n = 1), and no adequate description of the medical history for controls (n = 5). In addition, the main deficiency to the comparability was found in confounding factors such as age and sex that were not matched between the groups (n = 3). The results of risk of bias of all studies were presented in the Supplement 3.

Meta-analysis

In the global comparing, the SF was higher in patient with cancer (SMD 3.07; CI 1.96,4.17; $p<0.01$; I^2 99%) with considerable heterogeneity among subgroups ($p<0.01$). SF levels were shown as a biomarker for head and neck cancer (SMD 3.88; CI 0.42,7.34; $p<0.01$; I^2 99%; n=4), lung cancer (SMD 1.72; CI 0.67,2.78; $p<0.01$; I^2 94%; n=4), pancreatic cancer (SMD 6.79; CI 5.66,7.91; $p<0.01$; I^2 0%; n=2), and renal cell carcinoma (SMD 1.77; CI 0.48,3.05; $p<0.01$; I^2 94%; n=3). Despite the low number of studies (n = 1), hepatocellular carcinoma, mesothelioma and ovarian cancer showed SF levels as possible biomarkers (SMD

17.76; CI 16.21,19.31; p<0.01, SMD 1.36; CI 0.92,1.81; p<0.01, and SMD 3.58; CI 3.00,4.17; p<0.01 respectively). On the other hand, breast cancer had no differences between the groups and in patients affected by prostate or colorectal cancer were observed lower SF levels compared to healthy subjects (SMD -4.47; CI -5.25, -3.70; p<0.01, and SMD -3.83; CI -4.65, -3.02; p<0.01 respectively) *[Figure 2 near here]*.

The behaviour of SF levels between cancer stages leads to similar results between stage I and II (SMD -0.66; CI -1.61,0.28; p=0.17; I^2 81%), without statistical difference between the early stages (Stage I and II) of disease progression compared to healthy control (SMD 4.60; CI -2.21,11.41; p=0.19; I^2 99%, and SMD 5.60; CI -1.31,12.51; p=0.11; I^2 99% respectively). However, in the advanced stages (Stage III and IV) the SF levels were higher than healthy adults (SMD 4.89; CI 2.72,7.06; p<0.01; I^2 93%, and SMD 8.40; CI 6.99,9.82; p<0.01; I^2 61% respectively). Despite the heterogeneity among subgroups (p<0.01), the results showed an increasing in ferritin levels according with the disease progression. In the renal cell carcinoma, this increase was more evident and marked from the stage I of cancer, already showing differences in relation to control group (Stage I: SMD 0.83; CI 0.02,1.63; p=0.04, Stage II: SMD 3.08; CI 1.76,4.40; p<0.01, Stage III: SMD 4.43; CI 2.83,6.04; p<0.01, and Stage IV: SMD 7.53; CI 5.53,9.52; p<0.01). SF levels (ng/ml) Meta-analysis comparing TNM stages and control group were presented in Supplement 4. *[Table 1 near here]*.

Publication bias

The funnel plot analysis again emphasized the high variability between the type of cancer resulted an asymmetry graphic, which undermines the regression analysis. The Egger's test showed publication bias (p< 0.0001), may be due to variability among studies[14] *[Figure 3 near here]*.

Discussion

In the present meta-analysis was demonstrated for first time SF levels in various cancers and tumor stages. The results showed that the SF levels in patient affected by cancer were higher to healthy adults. In addition, this levels were increased with the disease progression. Herewith, the SF acted as biomarker for pancreatic cancer, renal cell carcinoma, lung cancer, and head and neck cancer, and also it was a sensitive biomarker in the detection of advanced stages of tumor.

Despite the lack of knowledge regarding all aspects of the role of SF in the malignant, a hypotheses have been raised that concern the fusion of macrophages to the tumor cells[47]. Macrophages engulf erythrocytes at the end of their life cycle, and subsequently secrete ferritin into the circulation. In the association macrophages-tumor cells, the mitochondria of tumor cells activate a state of massive iron metabolism and ferritin secretion, which could be explain the high levels in cancer patients[48].

The mitochondria play an important role in cancer development involving several functions including redox control[49]. Therefore, cancer cells accumulate high levels of iron and ROS to promote their metabolic activity and tumor growth[50]. Tumor cells increase iron intake through protein dysregulation of iron metabolism, changing several pathways of cell death[51]. These processes can lead to an understanding of the relationship between iron and cancer, and the need for greater amounts of iron in the advanced stages of the disease, corresponding to greater SF levels.

On the other hand, the literature addresses the relationship between cancer and inflammation, where the tumor environment expresses inflammatory chemical mediators[52] that in turn can interfere with iron metabolism proteins, as is the case of TNF-α and IL-1β, which stimulate the local release of SF levels in pancreatic cancer due to the action of this

inflammatory mediators[53]. Thus, SF could be increased in cancer patients as response to inflammatory cytokines.

The high difference between the types of cancer and the behavior of ferritin as a biomarker was evident, even though the results are highly heterogeneous. The heterogeneity between the studies may be due to several factors such as the tumor stage. In the Goswami *et al.,* study the meta-analysis showed contradictory results in relation to the rest of the studies, having higher SF values in cancer patients. It can be associated to the presence of a greater number of women with advanced stages of the disease. A recent systematic review evaluating SF levels and the risk of breast cancer turned out without association (RR 1.13; CI 0.78,1.62; $p>0.05$)[54], similar results are presented in our study, so SF was an ineffective biomarker for breast cancer.

Another reason for high heterogeneity can be the age and the sex proportionally different between groups in Alexandrakis *et al.* and Maxim & Veltri studies. Men have higher SF levels compared to women[15,22,39,40]. Meanwhile, older women have SF levels higher than younger one[22,55], and this may be related to menstrual blood loss[56].

In head and neck cancer two studies exhibited SF mean levels lower to 35 ng/ml. Although the authors do not describe control adults with iron deficiency, these values are in contrast to the rest of the studies. Therefore, the results of the SF levels in this cancer type must be interpreted with care. Hu *et al.* found that SF levels was not biomarker for head and neck cancer, however, it may be a potential biomarker to predict tumor progression[57].

Furthermore, in patients with colorectal or prostate cancer it was found that they had lower SF levels compared to the healthy group. This is not considered a definitive data since we only analysed one study per group. Despite this, the clinical studies in the literature show agreement with our results about to colorectal cancer[58]. Schneider *et al.,* adds that SF levels up to 20 ng/ml have a tendency to predict colorectal cancer (OR 10.66; CI 6.88,16.51;

$p<0.05$). Therefore, our results are similar to previous meta-analysis, where ferritin levels are significantly lower compared to the control group. (SMD -1.569; CI -2.718,-0.420; $p<0.01$)[59].

On the other hand, the SF was significantly associated with prostate cancer according to Wang *et al.,* where was suggested that it may be a non-invasive biomarker[60]. These results are contradictory to ours and other hematologic parameters are necessary to understand the behaviour of this hormone-dependent cancer.

Despite the fact that in hepatocellular, mesothelioma and ovarian cancers the ferritin levels was significantly higher, the results should be looked at carefully due to the low number of studies, constituting a limitation of our review. On the other hand, the high variability of SF behaviour between types of cancer was a limitation when interpreting the regression analysis to evaluate publication bias.

Conclusion

SF levels in patient with cancer were higher compared to healthy adults and showed changed according to disease progression. Therefore, it could be considered as a biomarker for pancreatic cancer, renal cell carcinoma, lung cancer, and head and neck cancer.

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Disclosure statement

The authors declare that they have no conflict of interest.

Data availability statement

Not applicable

Data deposition

Not applicable

Supplemental online material

Supplement 1: Search strategy

Supplement 2: The list of excluded articles and their causes

Supplement 3: Characteristics of the included studies

Supplement 4: Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages and the control group.

Authors' contributions

Conceptualization: [Wilmer Ramírez-Carmona, Beatriz Díaz-Fabregat]; Methodology: [Wilmer Ramírez-Carmona, Beatriz Díaz-Fabregat, Juliane Avansini Marsicano, Rosana Leal do Prado]; Formal analysis and investigation: [Wilmer Ramírez-Carmona, Beatriz Díaz-Fabregat]; Writing - Original Draft: [Wilmer Ramírez-Carmona, Beatriz Díaz-Fabregat, Andreia Yuri Yoshigae, Ariana Musa de Aquino]; Writing - Review & Editing: [Wellerson Rodrigo Scarano, Anthony César de Souza Castilho, Juliano Pelim Pessan, Leonardo de Oliveira Mendes]; Supervision: [Leonardo de Oliveira Mendes].

References

1. Moustarah F and Mohiuddin SS: Dietary Iron. *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2020.

2. Akiba S, Neriishi K, Blot WJ, Kabuto M, Stevens RG, Kato H, and Land CE: Serum ferritin and stomach cancer risk among a Japanese population. *Cancer* **67**, 1707-1712, 1991.

3. Sangkhae V and Nemeth E: Regulation of the Iron Homeostatic Hormone Hepcidin. *Adv Nutr* **8**, 126-136, 2017. doi:10.3945/an.116.013961.

4. MacKenzie EL, Iwasaki K, and Tsuji Y. Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxid Redox Signal* **10**, 997-1030, 2008. doi:10.1089/ars.2007.1893.

5. Paul BT, Manz DH, Torti FM, and Torti SV: Mitochondria and Iron: current questions. *Expert Rev Hematol* **10**, 65-79, 2017. doi:10.1080/17474086.2016.1268047.

6. Kalainayakan SP, FitzGerald KE, Konduri PC, Vidal C, and Zhang L: Essential roles of mitochondrial and heme function in lung cancer bioenergetics and tumorigenesis. *Cell Biosci* **8**, 56, 2018. doi:10.1186/s13578-018-0257-8.

7. Lui GY, Kovacevic Z, Richardson V, Merlot AM, Kalinowski DS, and Richardson DR: Targeting cancer by binding iron: Dissecting cellular signaling pathways. *Oncotarget* **6**, 18748-18779, 2015. doi:10.18632/oncotarget.4349.

8. Alkhateeb A, Zubritsky L, Kinsman B, Leitzel K, Campbell-Baird C, Ali SM, Connor J, and Lipton A: Elevation in multiple serum inflammatory biomarkers predicts survival of pancreatic cancer patients with inoperable disease. *J Gastrointest Cancer* **45**, 161-167, 2014. doi:10.1007/s12029-013-9564-9.

9. Gao Y, Wang J, Zhou Y, Sheng S, Qian SY, and Huo X: Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for Colorectal Cancer. *Sci Rep* **8**, 2732, 2018. doi:10.1038/s41598-018-21048-y

10. Brown RAM, Richardson KL, Kabir TD, Trinder D, Ganss R, and Leedman PJ: Altered Iron Metabolism and Impact in Cancer Biology, Metastasis, and Immunology. *Front Oncol* **10**, 476, 2020. doi:10.3389/fonc.2020.00476.

11. Moher D, Liberati A, Tetzlaff J, and Altman DG: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* **6**, e1000097, 2009. doi:10.1371/journal.pmed.1000097.

12. Stang A: Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* **25**, 603-605, 2010. doi: 10.1007/s10654-010-9491-z.

13. Islam MM, Iqbal U, Walther B, Atique S, Dubey NK, Nguyen PA, Poly TN, Masud JH, Li YJ, and Shabbir SA: Benzodiazepine Use and Risk of Dementia in the Elderly Population: A Systematic Review and Meta-Analysis. *Neuroepidemiology* **47**, 181-191, 2016. doi: 10.1159/000454881.

14. Sterne JA, Gavaghan D, and Egger M: Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol* **53**, 1119-1129, 2000. doi: 10.1016/s0895-4356(00)00242-0.

15. Goswami B, Rajappa M, Gupta N, Mahto M, Hadke NS, and Mishra TK: Breast cancer: interaction between oxidant-antioxidant dynamics and inflammation in Indian females. *Cancer Biomark* **6**, 95-103, 2010. doi: 10.3233/CBM-2009-0122.

16. Güner G, Kirkali G, Yenisey C, and Töre IR: Cytosol and serum ferritin in breast carcinoma. *Cancer Lett* **67**, 103-112, 1992. doi: 10.1016/0304-3835(92)90132-f.

17. Rajizadeh A, Mozaffari-Khosravi H, Zavar-Reza J, and Shiryazdi SM: Comparison of hematological parameters, iron levels, and oxidative stress in women with and without breast cancer: A case- control study. *Med J Islam Repub Iran* **31**, 114, 2017. doi: 10.14196/mjiri.31.114.

18. Gackowski D, Kruszewsk M, Banaszkiewicz Z, Jawien A, and Olinski R: Lymphocyte labile iron pool, plasma iron, transferrin saturation and ferritin levels in colon cancer patients. *Acta Biochim Pol* **49**, 269-272, 2002.

19. Gür T, Demir H, and Kotan MÇ: Tumor markers and biochemical parameters in colon cancer patients before and after chemotherapy. *Asian Pac J Cancer Prev* **12**, 3147-3150, 2011.

20. Cook MB, Kamangar F, Weinstein SJ, Albanes D, Virtamo J, Taylor PR, Abnet CC, Wood RJ, Petty G, Cross AJ, and Dawsey SM: Iron in relation to gastric cancer in the Alpha-tocopherol, Beta-carotene Cancer Prevention Study. *Cancer Epidemiol Biomarkers Prev* **21**, 2033-2042, 2012. doi: 10.1158/1055-9965.

21. Bhatavdekar JM, Vora HH, Goyal A, Shah NG, Karelia NH, and Trivedi SN: Significance of ferritin as a marker in head and neck malignancies. *Tumori* **73**, 59-63, 1987.

22. Ho S, Leung SF, Leung WT, Tsao SY, Kwan WH, Choi P, and Johnson PJ: Strong association between hyperferritinaemia and metastatic disease in nasopharyngeal carcinoma. *Eur J Cancer B Oral Oncol* **32,** 242-245, 1996. doi: 10.1016/0964-1955(95)00084-4.

23. Maxim PE and Veltri RW: Serum ferritin as a tumor marker in patients with squamous cell carcinoma of the head and neck. *Cancer* **57**, 305–311, 1986. doi: 10.1002/1097-0142(19860115)57:2<305::aid-cncr2820570219>3.0.co;2-d.

24. Richie JPJ, Kleinman W, Marina P, Abraham P, Wynder EL, and Muscat JE: Blood iron, glutathione, and micronutrient levels and the risk of oral cancer. *Nutr Cancer* **60**, 474–482, 2008. doi: 10.1080/01635580801956477.

25. Vinzenz K, Schönthal E, Zekert F, and Wunderer S: Diagnosis of head and neck carcinomas by means of immunological tumour markers (Beta-2-microglobulin, immunoglobulin E, ferritin, N-acetyl-neuraminic acid, phosphohexose-isomerase). *J Craniomaxillofac Surg* **15**, 270-277, 1987. doi: 10.1016/s1010-5182(87)80066-5.

26. Yuan C, Yang K, Tang H, and Chen D: Diagnostic values of serum tumor markers Cyfra21-1, SCCAg, ferritin, CEA, CA19-9, and AFP in oral/oropharyngeal squamous cell carcinoma. *Onco Targets Ther* **9**, 3381–3386, 2016. doi: 10.2147/OTT.S105672.

27. Giannoulis E, Arvanitakis C, Nikopoulos A, Doutsos I, and Tourkantonis A: Diagnostic value of serum ferritin in primary hepatocellular carcinoma. *Digestion* **30**, 236–241, 1984. doi: 10.1159/000199114.

28. Tatsuta M, Yamamura H, Iishi H, Kasugai H, and Okuda S: Value of serum alpha-fetoprotein and ferritin in the diagnosis of hepatocellular carcinoma. *Oncology* **43**, 306–310, 1986. doi: 10.1159/000226388.

29. Zhao Y, Wang M, Cui C, Zhang L, Liao F, Li H, and Wu X: Significance of combined tests of serum golgi glycoprotein 73 and other biomarkers in diagnosis of small primary hepatocellular carcinoma. *Cancer Biomark* **15,** 677-683, 2015. doi: 10.3233/CBM-150508.

30. Alexandrakis MG, Passam FH, Perisinakis K, Ganotakis E, Margantinis G, Kyriakou DS, and Bouros D: Serum proinflammatory cytokines and its relationship to clinical parameters in lung cancer patients with reactive thrombocytosis. *Respir Med* **96**, 553-558, 2002. doi: 10.1053/rmed.2002.1328.

31. Gulen ST, Karadag F, Karul AB, Kilicarslan N, Ceylan E, Kuman NK, and Cildag O: Adipokines and systemic inflammation in weight-losing lung cancer patients. *Lung* **190**, 327-332, 2012. doi: 10.1007/s00408-011-9364-6.

32. Shi HB, Li XD, Jiang JT, Zhao WQ, Ji M, and Wu CP: Serum ferritin is elevated in advanced non-small cell lung cancer patients and is associated with efficacy of platinum-based chemotherapy. *J Cancer Res Ther* **10**, 681-685, 2014. doi: 10.4103/0973-1482.139156.

33. Ji M, Li XD, Shi HB, Ning ZH, Zhao WQ, Wang Q, Zhu LN, Liu Y, and Wu CP: Clinical significance of serum ferritin in elderly patients with primary lung carcinoma. *Tumour Biol* **35**, 10195-10199, 2014. doi: 10.1007/s13277-014-2317-y.

34. Sukiennicki GM, Marciniak W, Muszyńska M, Baszuk P, Gupta S, Białkowska K, Jaworska-Bieniek K, Durda K, Lener M, Pietrzak S, Gromowski T, Prajzendanc K, Łukomska A, Waloszczyk P, Wójcik JZ, Scott R, Lubiński J, and Jakubowska A: Iron levels, genes involved in iron metabolism and antioxidative processes and lung cancer incidence. *PLoS One* **14**, e0208610, 2019. doi: 10.1371/journal.pone.0208610.

35. Wang X, Zhang Y, Sun L, Wang S, Nie J, Zhao W, and Zheng G: Evaluation of the clinical application of multiple tumor marker protein chip in the diagnostic of lung cancer. *J Clin Lab Anal* **32**, e22565, 2018. doi: 10.1002/jcla.22565.

36. Luger TA, Linkesch W, Knobler R, and Kokoschka EM: Serial determination of serum ferritin levels in patients with malignant melanoma. *Oncology* **40**, 263-267, 1983. doi: 10.1159/000225740.

37. Sezgi C, Taylan M, Sen HS, Evliyaoğlu O, Kaya H, Abakay O, Abakay A, Tanrıkulu AC, and Senyiğit A: Oxidative status and acute phase reactants in patients with environmental asbestos exposure and mesothelioma. *ScientificWorldJournal* **2014**, 902748, 2014. doi: 10.1155/2014/902748.

38. Lodh M, Goswami B, Gupta N, Patra SK, and Saxena A: Assessment of oxidative stress and inflammatory process in patients of multiple myeloma. *Indian J Clin Biochem* **27**, 410-413, 2012. doi: 10.1007/s12291-012-0222-y.

39. Macciò A, Madeddu C, Massa D, Mudu MC, Lusso MR, Gramignano G, Serpe R, Melis GB, and Mantovani G: Hemoglobin levels correlate with interleukin-6 levels in patients with advanced untreated epithelial ovarian cancer: role of inflammation in cancer-related anemia. *Blood* **106**, 362-367, 2005. doi: 10.1182/blood-2005-01-0160.

40. Pinto V, Marinaccio M, Garofalo S, Vittoria Larocca AM, Geusa S, Lanzilotti G, and Orsini G: Preoperative evaluation of ferritinemia in primary epithelial ovarian cancer. *Tumori* **83**, 927-929, 1997.

41. Nitti D, Fabris C, Del Favero G, Farini R, Grassi F, Farini A, Baccaglini U, Pedrazzoli S, Piccoli A, Lise M, and Naccarato R: Serum ferritin in pancreatic disease. An accurate test of malignancy? *Digestion* **25**, 258-262, 1982. doi: 10.1159/000198842.

42. Fabris C, Farini R, Del Favero G, Grassi F, Nitti D, Piccoli A, Brosolo P, and Naccarato R: Combined evaluation of serum ribonuclease and ferritin: any advantages in pancreatic cancer diagnosis? *Oncology* **41**, 393-395, 1984. doi: 10.1159/000225861.

43. Kuvibidila S, Gauthier T, Warrier RP, and Rayford W: Increased levels of serum transferrin receptor and serum transferrin receptor/log ferritin ratios in men with prostate cancer and the implications for body-iron stores. *J Lab Clin Med* **144**, 176-182, 2004. doi: 10.1016/j.lab.2004.03.017.

44. Essen A, Ozen H, Ayhan A, Ergen A, Tasar C, and Remzi F: Serum ferritin: a tumor marker for renal cell carcinoma. *J Urol* **145**, 1134-1137, 1991. doi: 10.1016/s0022-5347(17)38555-5.

45. Ozen H, Uygur C, Sahin A, Tekgül S, Ergen A, and Remzi D: Clinical significance of serum ferritin in patients with renal cell carcinoma. *Urology* **46**, 494-498, 1995. doi: 10.1016/S0090-4295(99)80261-1.

46. Singh KJ, Singh SK, Suri A, Vijjan V, Goswami AK, and Khullar M: Serum ferritin in renal cell carcinoma: effect of tumor size, volume grade, and stage. *Indian J Cancer* **42**, 197-200, 2005.

47. Stroud J: A mechanistic theory explaining hyperferritinaemia in haemophagocytic lymphohistiocytosis. *Med Hypotheses* **122**, 165-171, 2019. doi: 10.1016/j.mehy.2018.11.015.

48. Alkhateeb AA, Han B, and Connor JR: Ferritin stimulates breast cancer cells through an iron-independent mechanism and is localized within tumor-associated macrophages. *Breast Cancer Res Treat* **137**, 733-744, 2013. doi: 10.1007/s10549-012-2405-x.

49. Porporato PE, Filigheddu N, Pedro JMB, Kroemer G, and Galluzzi L: Mitochondrial

metabolism and cancer. *Cell Res* **28**, 265-280, 2018. doi: 10.1038/cr.2017.155.

50. Battaglia AM, Chirillo R, Aversa I, Sacco A, Costanzo F, and Biamonte F: Ferroptosis and Cancer: Mitochondria Meet the "Iron Maiden" Cell Death. *Cells* **9**, 1505, 2020. doi: 10.3390/cells9061505.

51. Torti SV, Manz DH, Paul BT, Blanchette-Farra N, and Torti FM: Iron and Cancer. *Annu Rev Nutr* **38**, 97-125, 2018. doi: 10.1146/annurev-nutr-082117-051732.

52. Mantovani A, Allavena P, Sica A, and Balkwill F: Cancer-related inflammation. *Nature* **454**, 436-444, 2008. doi: 10.1038/nature07205.

53. Alkhateeb A, Zubritsky L, Kinsman B, Leitzel K, Campbell-Baird C, Ali SM, Connor J, and Lipton A: Elevation in multiple serum inflammatory biomarkers predicts survival of pancreatic cancer patients with inoperable disease. *J Gastrointest Cancer* **45**, 161-167, 2014. doi: 10.1007/s12029-013-9564-9.

54. Chang VC, Cotterchio M, and Khoo E: Iron intake, body iron status, and risk of breast cancer: a systematic review and meta-analysis. *BMC Cancer* **19**, 543, 2019. doi: 10.1186/s12885-019-5642-0.

55. Ellidag HY, Eren E, Akdag M, Giray O, Kiraz K, and Yilmaz N: The relationship between serum ferritin levels and serum lipids and HDL function with respect to age and gender. *Ukr Biochem J* **88**, 76-86, 2016. doi: 10.15407/ubj88.06.076.

56. Yu Q, Zhou Y, Suturina L, Jaisamrarn U, Lu D, and Parke S: Efficacy and Safety of Estradiol Valerate/Dienogest for the Management of Heavy Menstrual Bleeding: A Multicenter, Double-Blind, Randomized, Placebo-Controlled, Phase III Clinical Trial. *J Womens Health (Larchmt)* **27**, 1225-1232, 2018. doi: 10.1089/jwh.2017.6522.

57. Hu Z, Wang L, Han Y, Li F, Zheng A, Xu Y, Wang F, Xiao B, Chen C, and Tao Z: Ferritin: A potential serum marker for lymph node metastasis in head and neck squamous cell carcinoma. *Oncol Lett* **17**, 314-322, 2019 doi: 10.3892/ol.2018.9642.

58. Schneider C, Bodmer M, Jick SS, and Meier CR: Colorectal cancer and markers of anemia. *Eur J Cancer Prev* **27**, 530-538, 2018. doi: 10.1097/CEJ.0000000000000397.

59. Feng Z, Chen JW, Feng JH, Shen F, Cai WS, Cao J, and Xu B: The association between serum ferritin with colorectal cancer. *Int J Clin Exp Med* **8**, 22293-22299, 2015.

60. Wang X, An P, Zeng J, Liu X, Wang B, Fang X, Wang F, Ren G, and Min J: Serum ferritin in combination with prostate-specific antigen improves predictive accuracy for prostate cancer. *Oncotarget* **8**, 17862-17872, 2017. doi: 10.18632/oncotarget.14977.

Figure captions

Figure 1: Flow-Diagram of the systematic review and meta-analysis.

Figure 2: Meta-analysis relating to serum ferritin levels (ng/ml) in cancer and control groups.

Figure 3: The funnel plot analysis for publication bias.

Flow-Diagram

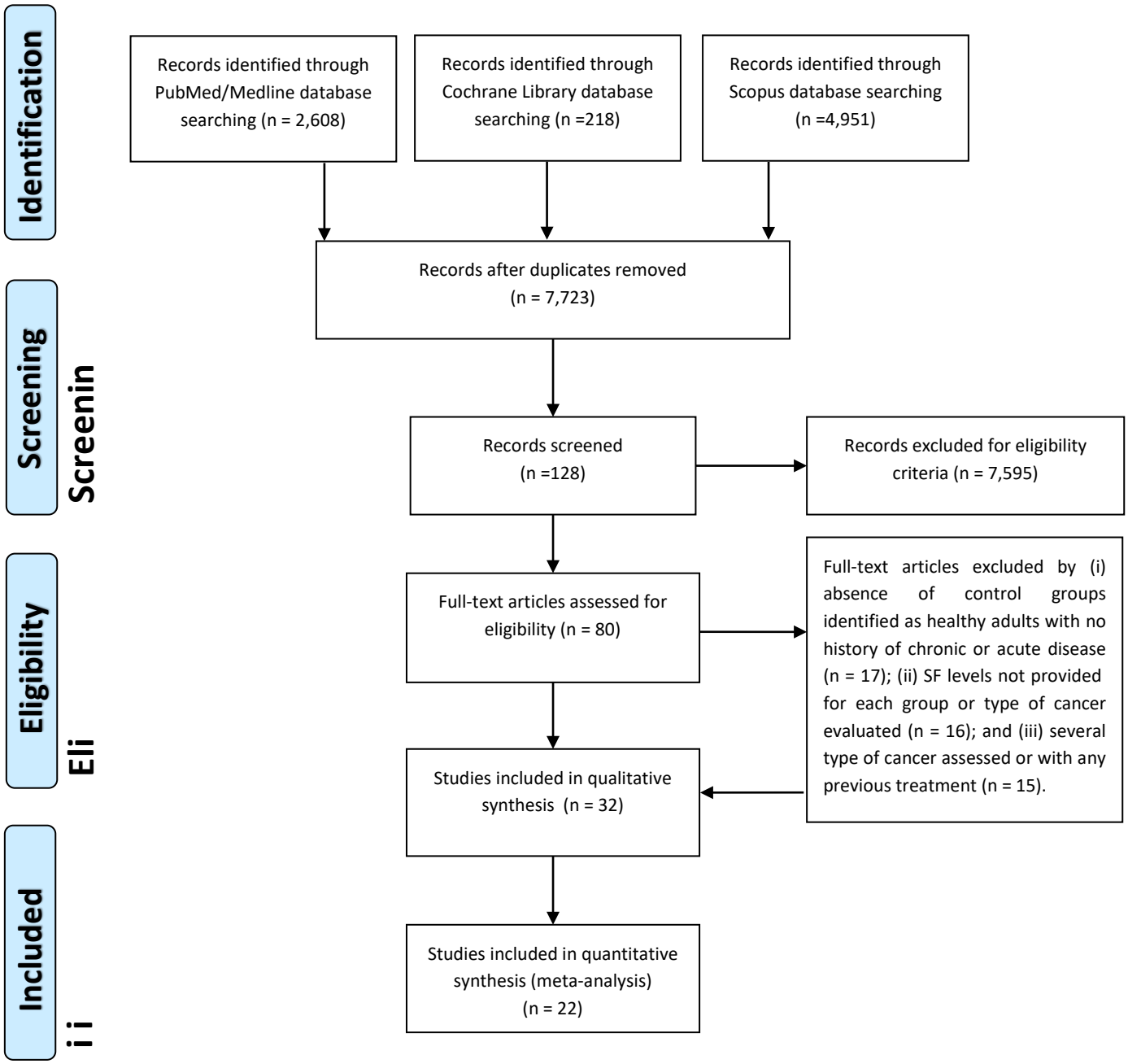

Figure 1: Flow-Diagram of the systematic review and meta-analysis.

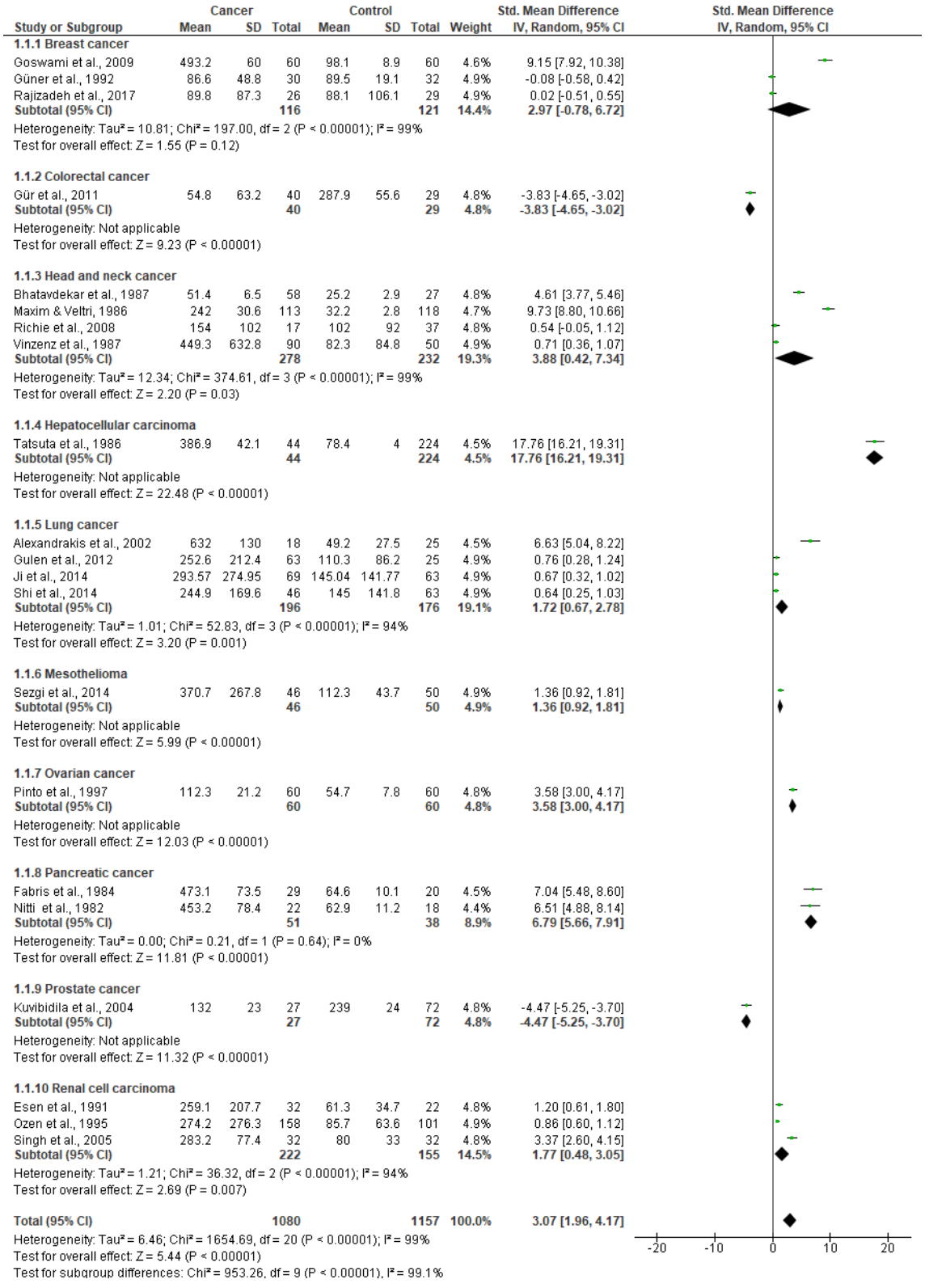

Figure 2: Meta-analysis relating to serum ferritin levels (ng/ml) in cancer and control groups. SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and higher global ferritin values in cancer patients compared to controls.

Table 1: Serum ferritin levels (ng/ml) in TNM stages and the control group.

All stages				
Stage I	4.60(-2.21,11.41)˚↑	-157.68(-172.84,-142.53)˚*↑	-1.95(-3.63,-0.27)*↑	-0.66(-1.61,0.28)˚↑
Stage II	5.60(-1.31,12.51)˚↑	-2.58(-4.58,-0.57)*↑	-1.43(-2.67,-0.20)*↑	
Stage III	4.89(2.72,7.06)˚*↑	-1.70(-3.12,-0.29)˚*↑		
Stage IV	8.40(6.99,9.82)*			
Compared with	**Control**	**Stage IV**	**Stage III**	**Stage II**
Head and neck cancer				
I	14.53(12.72,16.34)*Δ	-115.54(-132.58,-98.49)*	-0.85(-2.69,0.99)↑	0.12(-0.22,0.46)
II	13.49(11.80,15.18)*Δ	-1.57(-4.13,0.99)↑	-0.94(-2.51,0.62)↑	
III	6.66(5.77,7.54)*Δ	-0.16(-0.42,0.11)		
IV	9.56(8.41,10.71)*Δ			
Compared with	**Control**	**Stage IV**	**Stage III**	**Stage II**
Ovarian cancer				
I	-1.39(-2.33,-0.44)*Δ	-276.00(-357.73,-194.27)*Δ	-3.08(-4.22,-1.95)*Δ	-0.93(-2.17,0.30)Δ
II	0.34(-0.44,1.13)Δ	-3.14(-4.87,-1.41)*Δ	-2.40(-3.35,-1.45)*Δ	
III	3.56(2.92,4.20)*Δ	-4.72(-5.99,-3.45)*Δ		
IV	7.69(6.14,9.24)*Δ			
Compared with	**Control**	**Stage IV**	**Stage III**	**Stage II**
Renal cell carcinoma				
Stage I	0.83(0.02,1.63)*Δ	-324(-361.02,-288.58)*Δ	-3.43(-5.29, -1.58)*Δ	-3.21(-4.99, -1.43)*Δ
Stage II	3.08(1.76,4.40)*Δ	-4.44(-6.38,-2.50)*Δ	-1.57(-3.10,-0.05)*Δ	
Stage III	4.43(2.83,6.04)*Δ	-2.30(-3.64,-0.96)*Δ		
Stage IV	7.53(5.53,9.52)*Δ			
Compared with	**Control**	**Stage IV**	**Stage III**	**Stage II**

Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages and the control group. SMD (CI 95%), *p<0.05, ˚ significate differences between subgroups of cancer, ↑ considerable heterogeneity > 75%, and Δ heterogeneity not applicable.

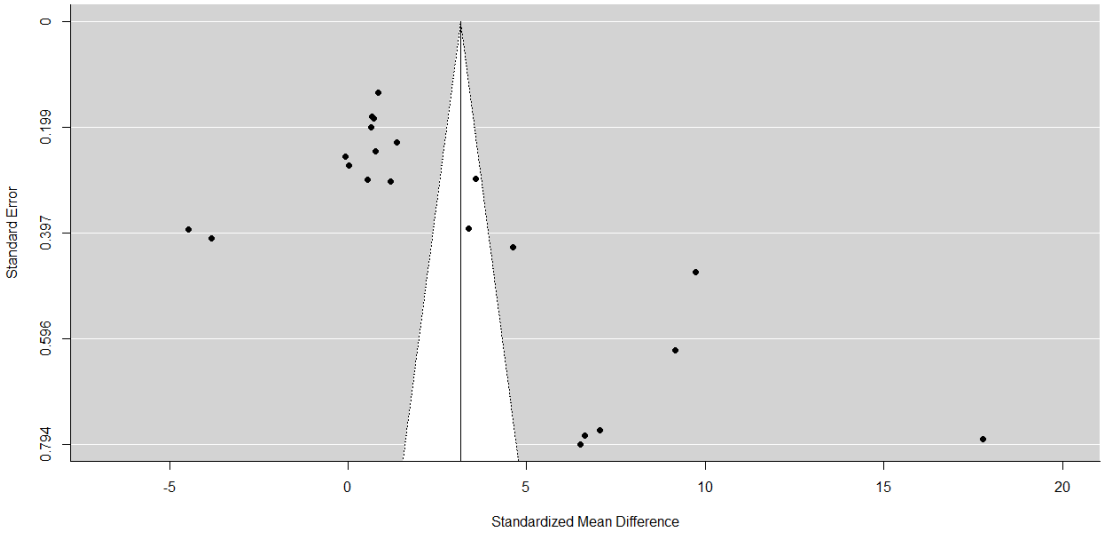

Figure 3: The funnel plot analysis for publication bias shows an asymmetry graphic. The Egger's test ($p < 0.0001$).

ARTIGO 2

DIMENSÃO FRACTAL APÓS O TRATAMENTO COMBINADO DO ACETATO DE ABIRATERONA E O NVP-BEZ235 SOBRE A REMODELAÇÃO TECIDUAL NA CARCINOGÊNESE PROSTÁTICA

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O trabalho está apresentado sob a forma de artigo, segundo as normas do periódico o qual foi submetido: Prostate International , Fator de impacto: 1,625 Qualis: A3.

Resumo

Introdução. O remodelado tecidual prostático é um evento característico após o uso de terapias anti-tumorais, porém são necessários métodos de alta acurácia para observá-los. A análise fractal surge como método quantitativo, diminuindo a subjetividade das analises histopatológicas. O presente estudo teve como objetivo validar a análise fractal após o tratamento da terapia combinada com acetato de abiraterona e o NVP-BEZ235, além de avaliar o efeito das drogas sobre a remodelado tecidual na carcinogênese prostática. **Métodos**. Quarenta ratos da linhagem Fischer 344 foram submetidos à indução tumoral e aleatoriamente divididos em três grupos experimentais: acetato de abiraterona, NVP-BEZ235, combinação de ambas as drogas e o grupo controle submetido somente ao processo de indução. Posteriormente à eutanásia, os lobos prostáticos ventral e dorsolateral foram removidos e preparados para realização da análise fractal, quantificação do volume de colágeno, altura epitelial e estereologia. **Resultados**. Na próstata ventral, o grupo submetido à administração das drogas combinadas possuiu maiores valores de dimensão fractal comparados aos demais grupos, além disso, nas análisis estereológicas a altura epitelial foi maior nesse grupo. Por outro lado, a associação das drogas apresentou menor altura do epitélio e melhor organização das fibras de colágeno comparado ao grupo controle. Na próstata dorsolateral, a administração de NVP-BEZ235 isoladamente apresentou maior altura epitelial comparado ao grupo das drogas combinadas, resultado semelhante foi encontrado no compartimento epitelial na análisis estereológica. Além disso, melhor organização das fibras de colágeno foi observado. **Conclusão**. A análise fractal é útil para avaliar as alterações morfológicas prostáticas em terapias anti-carcinogênicas, além disso, a combinação das drogas mostrou-se eficaz na reorganização do microambiente prostático em ambos os lobos.

Palavras-chave: Antineoplásicos; Colágeno; Fractal; Neoplasias Prostáticas.

1. Introdução

O câncer da próstata (CaP) é caracterizado por uma proliferação celular descontrolada, onde o receptor de andrógeno (AR) desempenha um papel fundamental na oncogênese[1]. Este tipo de tumor é frequentemente tratado com inibidores farmacológicos do AR[2] e, desta forma, a privação androgênica por castração cirúrgica ou química tem sido empregada como o principal tratamento para redução significativa da doença[3]. No entanto, esta terapia por si só não é considerada o tratamento ideal para erradicar o CaP, pois apesar da eficácia inicial da privação de andrógenos, a maioria dos doentes em estágio avançado eventualmente desenvolvem resistência a esta terapia e progridem para CaP resistente à castração[4]. Neste cenário, a combinação de antiandrógenos com terapias que interfiram em outras vias de sinalização envolvidas na progressão tumoral tem sido apontada como uma estratégia mais eficaz[5].

O antiandrógeno acetato de abiraterona, utilizado na terapia do CaP, inibe a enzima 17α-hidroxilase-$C_{17,20}$-liase CYP17 necessária para a síntese de hormônios esteróides[6] (Figura 1). Apesar da melhora na sobrevida após tratamento com este fármaco, a resistência à droga invariavelmente ocorre e contribui para a progressão da doença[3,7]. Varias drogas têm sido testadas com o intuito de inibir outras vias de sinalização como as vias de reparo de fosfatidilinositol-4,5-bisfosfato 3-quinase PI3K/AKT/mTOR[8] e assim conter o progresso do CaP[9].

NVP-BEZ235 é um derivado de imidazol [4,5-*c*]quinolina que inibe a atividade de todas as isoformas de PI3K e mTOR ligando-se e bloqueando seus sítios catalíticos[10]. Por ser um inibidor dual de PI3K e mTORC1/2, o NVP-BEZ235 tem atividade tumoral superior, uma vez que a ativação de AKT via mTORC2 é também inibida[11] (Figura 1). A combinação do acetato de abiraterona com o NVP-BEZ235 já foi testada obtendo resultados promisores

favorecendo a diminuição na resposta inflamatória do tumor e nas lesões hiperplásicas prostáticas[5].

Para o diagnóstico do câncer prostático é classicamente utilizada as análises histopatológicas, as quais estão sujeitas à subjetividade e experiência do profissional. Estudos quantitativos em pesquisas sobre carcinogênese, como o método fractal, têm papel cada vez mais importante, mostrando-se útil na análise de diversos componentes estruturais dos órgãos[12]. Tal ferramenta nada mais é do que a análise matemática de estruturas celulares irregulares, a fim de entender mais objetivamente como ocorrem os processos intra e extracelulares[13]. Esse tipo de análise possui grandes aplicações nas áreas de tecnologia e medicina, mostrando-se útil tanto para análises nos compartimentos prostáticos epitelial quanto estromal[14], sendo capaz de diferenciar lesões malignas de benignas, além de facilitar a classificação do câncer de próstata[15].

Assim, o presente estudo teve como objetivo validar a análise fractal após o tratamento da terapia combinada com acetato de abiraterona e o NVP-BEZ235, além de avaliar o efeito das drogas sobre a remodelado tecidual na carcinogênese prostática.

2. Material e Método

2.1. Desenho do estudo

Foram utilizados 40 ratos machos da linhagem Fischer 344 provenientes do CEMIB/UNICAMP com 30 dias que foram mantidos no biotério de pequenos mamíferos do Departamento de Morfologia do Instituto de Biociências de Botucatu (UNESP, *campus* de Botucatu) até completarem 12 semanas de idade parainício dos experimentos. O experimento foi aprovado pelo comitê de ética no uso de animais (CEUA) do Instituto de Biociências de Botucatu, UNESP (Protocolo. 559-CEUA).

A indução de um microambiente carcinogênico foi realizada em todos os animais com metodologia desenvolvida e cedida pelo Laboratório de Carcinogênese Urogenital e Imunoterapia e Instituto de Química/UNICAMP. A indução tumoral consistiu em: (i) pré-tratamento com Cipionato de Testosterona; (ii) administração intraprostática de N-metil-N-nitrosouréia; e (iii) tratamento semanal com Cipionato de Testosterona.

O pré-tratamento com Cipionato de Testosterona consistiu de 100mg/Kg da droga via subcutânea [Deposteron-Sigma pharma] por três dias consecutivos. Posteriormente, os animais foram anestesiados por Cloridrato de Xilazina 2% (5mg/kg i.m.; König, São Paulo, Brasil) e Cloridrato de Cetamina 10% (60mg/kg, i.m.; Fort Dodge, Iowa, EUA) e realizada incisão suprapúbica de 0,5 cm para inoculação do carcinógeno N-metil-N-nitrosouréia (MNU - Sigma, St. Louis, MO, EUA - 15 mg/Kg) dissolvido em citrato de sódio (1M pH 6,0) e veiculado com copolímero termosensível (Pluronic 127) na cápsula dos lobos ventral e dorsolateral prostáticos. Uma semana após a administração do carcinógeno, os animais receberam doses subcutâneas de 5mg/Kg de Cipionato de Testosterona (diluído em óleo de milho) duas vezes por semana durante 220 dias, o qual foi adaptado do protocolo original.

Após a indução tumoral os animais foram divididos aleatoriamente em concordância com as terapias propostas no estudo, com as drogas sendo administradas por gavagem. Grupo AA: Acetato de Abiraterona – receberam doses (14mg/dia, Cayman Chemical – em etanol 6% diluído em óleo de milho) e do veículo de diluição do BEZ (NMP:PEG - 1:9)[10,16]. Grupo BEZ: NVP-BEZ 235 – receberam doses (45mg/kg/dia, Cayman Chemical – diluído em NMP (1-Metil-2-pirrolidona – Sigma) e PEG300 (Polietilenoglicol 300) na proporção de 1:9 (NMP:PEG) [17–19], e do veículo de diluição do Acetato de Abiraterona (Etanol 6% em óleo de milho). Grupo AB: Acetato de Abiraterona + NVP-BEZ 235 – receberam doses (14mg/dia - 45mg/kg/dia, respectivamente). Grupo I: Controle-Indução da carcinogênese – receberam doses diárias dos veículos de diluição das drogas: etanol 6% diluído em óleo de milho

(Veiculo do Acetato de Abiraterona) e NMP (1-Metil-2-pirrolidona – Sigma) e PEG300 (Polietilenoglicol 300) na proporção de 1:9 (NMP:PEG) (Veiculo do BEZ)[10,16,20] (Figura 2). Todas as drogas foram administradas via gavagem por 10 dias.

2.2. Processamento das amostras

No dia pós-natal 234 os animais foram anestesiados[21,22] em câmara de CO_2 de acordo com a recomendação da Resolução Normativa nº 37 – 15 de fevereiro de 2018 do CONCEA – Conselho Nacional de Controle em Experimentação Animal, e eutanasiados por decapitação. Após o processo foram pesados e submetidos à laparotomia abdômino-pélvica para remoção e coleta da próstata ventral (PV) e dorsolateral (PDL) que foram pesadas em balança analítica (OhausTraveler; Ohaus Corporation, México, D.F.). Os fragmentos do segmento intermediário da PV e PDL foram rapidamente fixados por imersão em metacarn (6 metanol: 3 clorofórmio: 1 ácido acético) e mantidos em álcool 70%. Posteriormente, o material foi desidratado em soluções crescentes de etanol, clarificado em xilol e incluído em historesina (LeicaBiosystems Inc., Bufalo Grove, USA) ou paraplast (Oxford Labware, St. Louis, MO, USA).

2.3. Análise da estrutura prostática

Os fragmentos de próstata ventral e dorsolateral incluídos em paraplast foram seccionados com 4µm de espessura em micrótomo rotativo e submetidos às seguintes colorações:

1. Hematoxilina - Eosina (HE): análise da dimensão fractal e análises morfométrica-estereológicas.

2. Picrossírius: quantificação do volume relativo de colágeno e análise da dimensão fractal do colágeno.

As lâminas foram analisadas e os campos microscópicos digitalizados utilizando o sistema de análise de imagens (Image Pro-Plus) acoplado ao fotomicroscópio Leica.

2.3.1. Quantificação do volume relativo de colágeno

O volume relativo de colágeno foi quantificado nas secções histológicas coradas com picrossírius seguindo as instruções do software Image J (Instituto Nacional de Saúde, Estados Unidos – NIH), disponível gratuitamente na Internet (http://rsbweb.nih. gov/ij/). Foram analisadas secções histológicas de 5 animais/grupo, coradas com picrossírius, sendo fotografadas (10 campos histológicos/secção, aumento de 40x).

2.3.2. Dimensão Fractal

Para análise da dimensão fractal, 2 secções histológicas/animal (intervalo de 50 μm entre cada secção – 5 animais) coradas com HE e picrossirus foram fotografadas (10 campos histológicos/secção, aumento de 40x), binarizadas para leitura e a dimensão fractal estimada pelo método box-counting, por meio do software Image J. O software considera o box-counting em duas dimensões, permitindo a quantificação da distribuição de pixels nesse espaço, não considerando, portanto, a textura da imagem. A influência disso é que duas imagens com a mesma distribuição dos pixels, uma binarizada e outra em níveis de cinza, possuirão a mesma dimensão fractal. A análise das lâminas histológicas fractais foi baseada na relação entre a resolução e a escala avaliada, e o resultado quantitativamente expresso como a dimensão fractal do objeto que é DF= (Log Nr / log r^{-1}), sendo **Nr** a quantidade de elementos iguais necessários para preencher o objeto original e **r** a escala aplicada ao objeto. Com isso, a dimensão fractal calculada com o software Image J fica sempre entre 0 e 2, não distinguindo texturas diferentes.

2.3.4. Análise da Altura do Epitélio

A altura do epitélio do tecido prostático foi feita em secções histológicas coradas com HE seguindo as instruções do software Image J. Foram analisadas secções histológicas de 5 animais/grupo, coradas com HE, sendo fotografadas (10 campos histológicos/secção, aumento de 40x) e medidas 5 áreas distintas para cada foto (escala de 8.1025 pixels/μm).

2.3.5. Análise Estereológica

A análise estereológica foi realizada em secções histológicas coradas com HE. Foram analisadas secções histológicas de 5 animais/grupo, sendo fotografadas (10 campos histológicos/secção, aumento de 40x). A análise consistiu na utilização da grade de Weibel (1963), caracterizado como um sistema de linhas e pontos em um graticulado com 120 pontos e 60 linhas. Assim, obteve-se a proporção ocupada, nos diferentes grupos experimentais, para os seguintes compartimentos prostáticos: epitélio, estroma e lúmen.

2.4. Análise estatística

Os resultados foram avaliados utilizando o software estatístico Prism 5.0 (GraphPad), considerando um intervalo de confiança de um 95%. Foi utilizada análise paramétrica em todos os parâmetros avaliados, utilizando-se Anova seguido do post-teste de Tukey.

3. Resultados

Primeiramente, os achados demonstram que há um perfil lobo-específico na próstata como resposta aos tratamentos apresentados na dimensão fractal. Na próstata ventral, os animais submetidos à administração associada das drogas (Figura 3G e H) demonstraram maiores valores na dimensão fractal comparados com os grupos restantes (Figura 3I). Com relação à altura epitelial, o lobo ventral apresentou menores valores nos grupos submetidos às

administração dos fármacos de forma isolada ou em associação (Figura 3J). Além disso, a análise estereológica mostrou que o compartimento epitelial ocupou maior proporção nos grupo submetidos à associação das drogas quando comparado aos grupos I e BEZ, sem alteração nos compartimentos luminal e estromal (Figura 4A). O lobo ventral apresentou menor dimensão fractal nos grupos AA e AB quando comparados ao grupo I (Figura 5N), porém quando avaliamos o estroma prostático não apresentou alteração na área ocupada pelo colágeno (Figura 5M).

Na próstata dorsolateral não houve impacto dos tratamentos na dimensão fractal (Figura 6I), por outro lado, o grupo BEZ além de apresentar maior altura epitelial também apresentou aumento deste compartimento quando comparado aos demais grupos (Figura 4D). Com relação aos demais compartimentos, o grupo I apresentou aumento do estroma em relação aos demais grupos e aumento do lúmen quando comparado ao grupo AA (Figura 4E e F). Além disso, o estroma prostático não teve diferença na área ocupada pelo colágeno (Figura 7M), porém na organização dessas fibras foi observado que a associação das drogas apresentou redução na dimensão fractal quando comparados I e AA (Figura 7N).

4. Discussão

O presente estudo observou alteração na dimesão fractal em ambos os lobos prostáticos nos grupos submetidos ao uso isolado ou combinado das drogas quando comparados ao grupo induzido, evidenciando alteração na organização estrutural prostática tanto no epitélio quanto no estroma frente aos tratamentos. A análise fractal corroborou junto com as outras análises utilizadas, uma reorganização tecidual no microambiente prostático, demonstrando a validez do método fractal como ferramenta complementar nas análises histopatológicas, além de comprovar os efeitos das drogas, acetato de abiraterona e NVP-BEZ235, em melhorar os parâmetros morfológicos analisados.

O epitélio prostático, um epitélio colunar simples, é composto por uma camada de células secretoras luminais, caracterizado por baixos índices de proliferação e expressarem altos níveis de AR. Além deste tipo celular, o epitélio prostático possui também as células neuroendócrinas e as basais[23]. Estas últimas são consideradas as responsáveis pela aquisição de resistência à terapias de privação hormonal, o que leva ao surgimento de tumores mais agressivos. Isso ocorre devido à características únicas deste tipo celular, com independência à ação androgênica além de possuírem maior taxa de proliferação[24].

Com relação ao outro compartimento prostático, o estroma, é responsável por envolver o epitélio, sendo composto por células endoteliais, imunes, fibroblastos e miofibroblastos. As duas últimas são as principais células deste microambiente e que desempenham um papel crucial na remodelação da matriz extracelular pela produção de fibras colágenas[25].

No processo tumoral várias proteínas são acumuladas na matriz extracelular, grande parte derivada da alta atividade de fatores inflamatórios. Em muitos tipos de cânceres ocorre o acúmulo de colágeno tipo I e III, conjuntamente com a maior degradação do colágeno tipo IV[26]. A arquitetura do colágeno durante o processo do desenvolvimento do câncer apresenta-se alterada, como evidenciado em nosso estudo através da análise fractal. Egeblad *et al* (2010) relata que durante a oncogênese ocorre alta deposição e modificação pós-traducional onde a reestruturação do colágeno leva a diversas alterações, incluindo diferenciação celular, migração e sobrevivência de células cancerosas, culminando com a progressão do tumor[26].

Além da alteração na análise fractal, constatou-se que à exposição aos fármacos de maneira combinada promoveu redução da altura epitelial e alteração na organização do colágeno no tecido. A combinação das drogas tem demostrado seu efeito na redução da proliferação e aumento da morte celular em modelos de carcinogênese testada previamente[5]. Por outro lado, a droga NVP-BEZ235 isolada, além dos mecanismos já conhecidos, parece ter alguma influência sobre os AR, outros mecanismos continuam sendo desconhecidos na sua

totalidade, principalmente aqueles que relacionam a interação epitélio-estroma durante a oncogênese[5].

O medicamento NVP-BEZ235, além de atingir a via de sinalização androgênica, tem ação sobre a via PI3K/mTOR inibindo AKT, acarretando diminuição da proliferação celular, ao mesmo tempo que apresenta efeitos clínicos adversos, o que torna o fármaco pouco tolerado em pacientes com câncer prostático[5,27,28]. Observou-se que esta droga teve efeitos divergentes nos lóbos prostáticos em nosso estudo, provocando diminuição da altura epitelial no lobo ventral e aumento tanto da altura quanto do compartimento epitelial no lobo dorsolateral, o que pode estar associado às particularidades de cada lobo[29,30].

O acetato de abiraterona é considerada um dos principais fármacos no tratamento do câncer prostático, inibindo seletivamente a ação da enzima C17, 20-lyase e 17α-hydroxylase sobre o citocromo P450 (CYP) 17[31]. Dentro das drogas testadas, o acetato de abiraterona alcançou resultados mais expressivos no lobo ventral, porém sem alterações significativas quando administrada isoladamente sobre o lobo dorsolateral, sugerindo assim uma maior sensibilidade androgênica neste lobo.

Assim, podemos concluir que a análise fractal é útil para avaliar as alterações morfológicas prostáticas em terapias anti-carcinogênicas, sendo uma ferramenta complementar à outras análises histopatológicas. A combinação das drogas mostrou-se eficaz, levando à uma reorganização do microambiente prostático em ambos os lobos.

Declaração de conflito de interesse

Os autores declaram a não existência de conflitos de interesse.

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Referências

1. Chen X, Lu J, Xia L, Li G. Drug Resistance of Enzalutamide in CRPC. Curr Drug Targets. 2018;19(6):613–20.
2. Chen R, Dong X, Gleave M. Molecular model for neuroendocrine prostate cancer progression. BJU Int. 2018 Oct;122(4):560–70.
3. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol. 2019 May;20(5):686–700.
4. Francini E, Yip S, Ahmed S, Li H, Ardolino L, Evan CP, et al. Clinical Outcomes of First-line Abiraterone Acetate or Enzalutamide for Metastatic Castration-resistant Prostate Cancer After Androgen Deprivation Therapy + Docetaxel or ADT Alone for Metastatic Hormone-sensitive Prostate Cancer. Clin Genitourin Cancer. 2018 Apr;16(2):130–4.
5. Gonçalves BF, de Campos SGP, Fávaro WJ, Brandt JZ, Pinho CF, Justulin LA, et al. Combinatorial Effect of Abiraterone Acetate and NVP-BEZ235 on Prostate Tumor Progression in Rats. Horm Cancer. 2018 Jun;9(3):175–87.
6. Scott LJ. Abiraterone Acetate: A Review in Metastatic Castration-Resistant Prostrate Cancer. Drugs. 2017 Sep;77(14):1565–76.
7. de Bono JS, Chowdhury S, Feyerabend S, Elliott T, Grande E, Melhem-Bertrandt A, et al. Antitumour Activity and Safety of Enzalutamide in Patients with Metastatic Castration-resistant Prostate Cancer Previously Treated with Abiraterone Acetate Plus Prednisone for ≥24 weeks in Europe. Eur Urol. 2018 Jul;74(1):37–45.
8. Nevedomskaya E, Baumgart SJ, Haendler B. Recent advances in prostate cancer

treatment and drug discovery. Int J Mol Sci. 2018;19(5).

9. Yasumizu Y, Miyajima A, Kosaka T, Miyazaki Y, Kikuchi E, Oya M. Dual PI3K/mTOR inhibitor NVP-BEZ235 sensitizes docetaxel in castration resistant prostate cancer. J Urol [Internet]. 2014;191(1):227–34. Available from: http://dx.doi.org/10.1016/j.juro.2013.07.101
10. Maira S-M, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, et al. Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. Mol Cancer Ther. 2008 Jul;7(7):1851–63.
11. Sun Z, Li Q, Zhang S, Chen J, Huang L, Ren J, et al. NVP-BEZ235 overcomes gefitinib-acquired resistance by down-regulating PI3K/AKT/mTOR phosphorylation. Onco Targets Ther. 2015;8:269–77.
12. Luján E, Soto D, Rosito MS, Soba A, Guerra LN, Calvo JC, et al. Microenvironmental influence on microtumour infiltration patterns: 3D-mathematical modelling supported by in vitro studies. Integr Biol (Camb). 2018 May;10(5):325–34.
13. Bose P, Brockton NT, Guggisberg K, Nakoneshny SC, Kornaga E, Klimowicz AC, et al. Fractal analysis of nuclear histology integrates tumor and stromal features into a single prognostic factor of the oral cancer microenvironment. BMC Cancer [Internet]. 2015;15(1):409. Available from: https://doi.org/10.1186/s12885-015-1380-0
14. Frisch KE, Duenwald-Kuehl SE, Kobayashi H, Chamberlain CS, Lakes RS, Vanderby RJ. Quantification of collagen organization using fractal dimensions and Fourier transforms. Acta Histochem. 2012 Feb;114(2):140–4.
15. Stepan A, Simionescu C, Pirici D, Ciurea R, Margaritescu C. Fractal analysis and the diagnostic usefulness of silver staining nucleolar organizer regions in prostate adenocarcinoma. Anal Cell Pathol (Amst). 2015;2015:250265.

16. Ma BBY, Lui VWY, Hui CWC, Lau CPY, Wong CH, Hui EP, et al. Preclinical evaluation of the mTOR-PI3K inhibitor BEZ235 in nasopharyngeal cancer models. Cancer Lett. 2014 Feb;343(1):24–32.

17. Serra V, Markman B, Scaltriti M, Eichhorn PJA, Valero V, Guzman M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res. 2008 Oct;68(19):8022–30.

18. Marone R, Erhart D, Mertz AC, Bohnacker T, Schnell C, Cmiljanovic V, et al. Targeting melanoma with dual phosphoinositide 3-kinase/mammalian target of rapamycin inhibitors. Mol Cancer Res. 2009 Apr;7(4):601–13.

19. Fuereder T, Wanek T, Pflegerl P, Jaeger-Lansky A, Hoeflmayer D, Strommer S, et al. Gastric cancer growth control by BEZ235 in vivo does not correlate with PI3K/mTOR target inhibition but with [18F]FLT uptake. Clin cancer Res an Off J Am Assoc Cancer Res. 2011 Aug;17(16):5322–32.

20. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell. 2011 May;19(5):575–86.

21. Deckardt K, Weber I, Kaspers U, Hellwig J, Tennekes H, van Ravenzwaay B. The effects of inhalation anaesthetics on common clinical pathology parameters in laboratory rats. Food Chem Toxicol an Int J Publ Br Ind Biol Res Assoc. 2007 Sep;45(9):1709–18.

22. Nazian SJ. Serum concentrations of reproductive hormones after administration of various anesthetics to immature and young adult male rats. Proc Soc Exp Biol Med Soc Exp Biol Med (New York, NY). 1988 Apr;187(4):482–7.

23. Cunha GR. Role of mesenchymal-epithelial interactions in normal and abnormal development of the mammary gland and prostate. Cancer. 1994 Aug;74(3

Suppl):1030–44.

24. Bonkhoff H, Stein U, Remberger K. The proliferative function of basal cells in the normal and hyperplastic human prostate. Prostate. 1994;24(3):114–8.
25. Krušlin B, Ulamec M, Tomas D. Prostate cancer stroma: an important factor in cancer growth and progression. Bosn J basic Med Sci. 2015 May;15(2):1–8.
26. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. Curr Opin Cell Biol. 2010 Oct;22(5):697–706.
27. Wei XX, Hsieh AC, Kim W, Friedlander T, Lin AM, Louttit M, et al. A Phase I Study of Abiraterone Acetate Combined with BEZ235, a Dual PI3K/mTOR Inhibitor, in Metastatic Castration Resistant Prostate Cancer. Oncologist. 2017;22(5):503-e43.
28. Massard C, Chi KN, Castellano D, de Bono J, Gravis G, Dirix L, et al. Phase Ib dose-finding study of abiraterone acetate plus buparlisib (BKM120) or dactolisib (BEZ235) in patients with castration-resistant prostate cancer. Eur J Cancer [Internet]. 2017;76:36–44. Available from: http://dx.doi.org/10.1016/j.ejca.2017.01.024
29. Prins GS, Putz O. Molecular signaling pathways that regulate prostate gland development. Differentiation. 2008 Jul;76(6):641–59.
30. Lee CH, Akin-Olugbade O, Kirschenbaum A. Overview of prostate anatomy, histology, and pathology. Endocrinol Metab Clin North Am. 2011 Sep;40(3):565–75, viii–ix.
31. Thakur A, Roy A, Ghosh A, Chhabra M, Banerjee S. Abiraterone acetate in the treatment of prostate cancer. Biomed Pharmacother. 2018 May;101:211–8.

Figure list

Figure 1

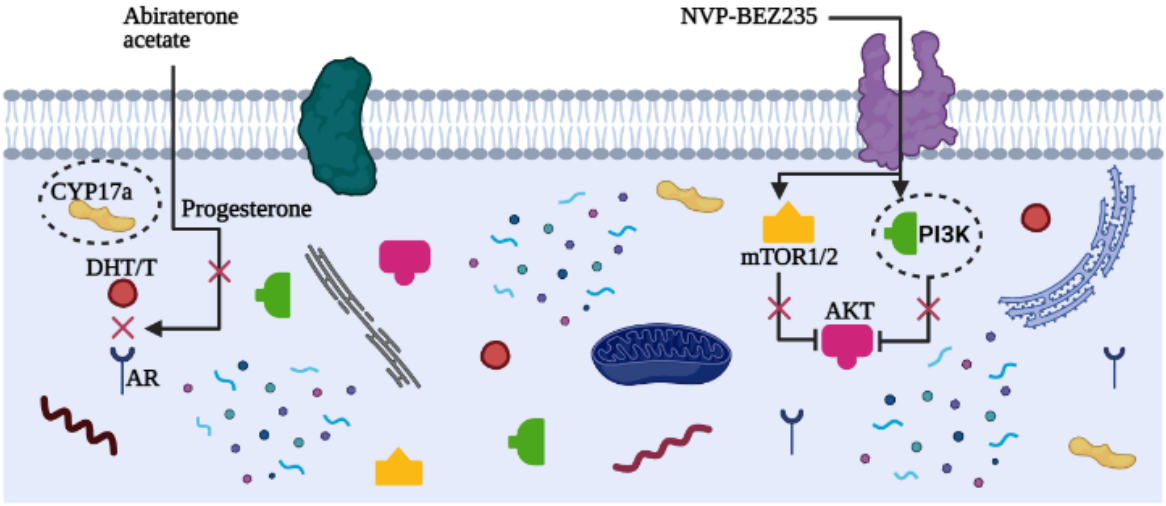


Figure 1. Signaling pathways of the drugs tested

Figure 2

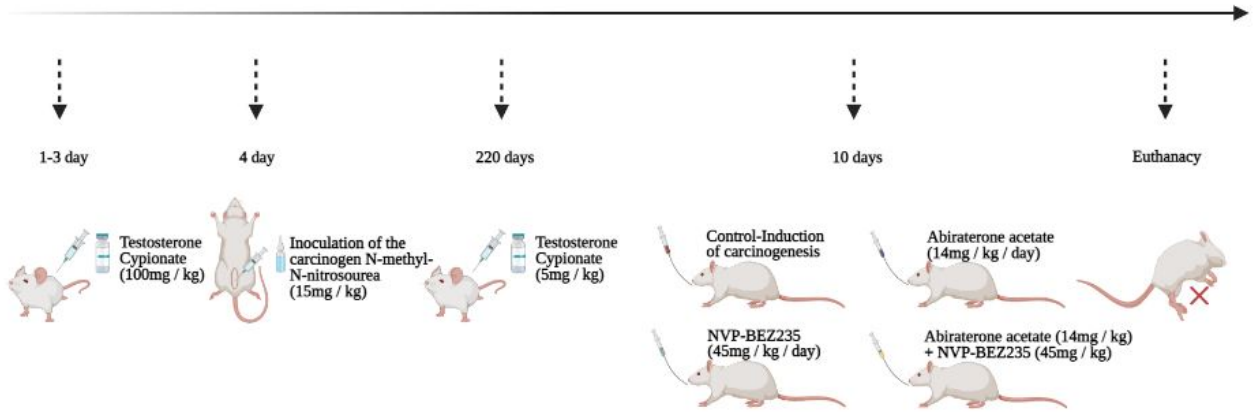
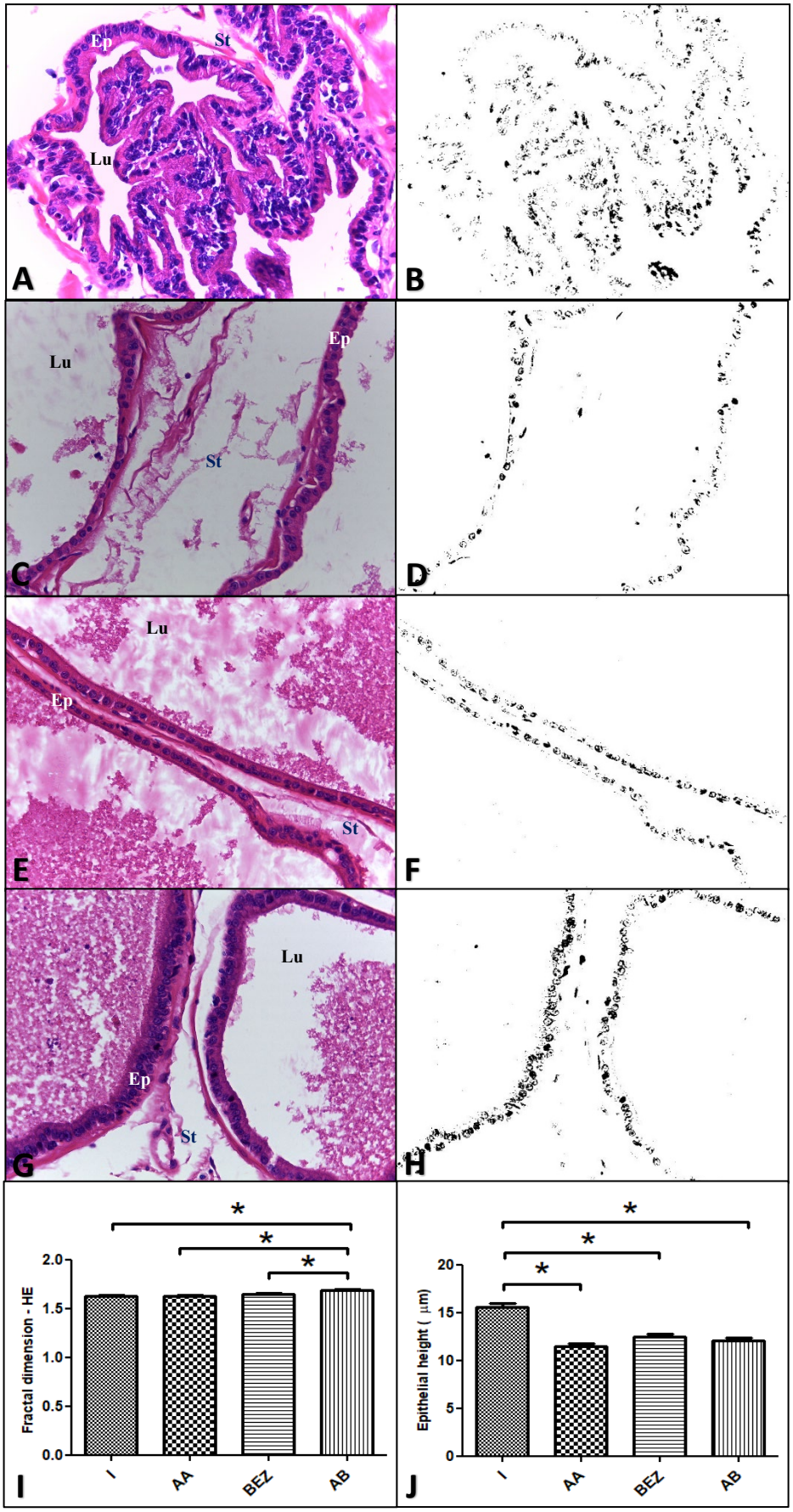


Figure 2. Experimental design representing the induction of carcinogenesis, drugs and procedures performed.

Figure 3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AA
BEZ
AB
Epithelial height (µm)
20
15
10
5
0
J
AA
BEZ
AB

Figure 4

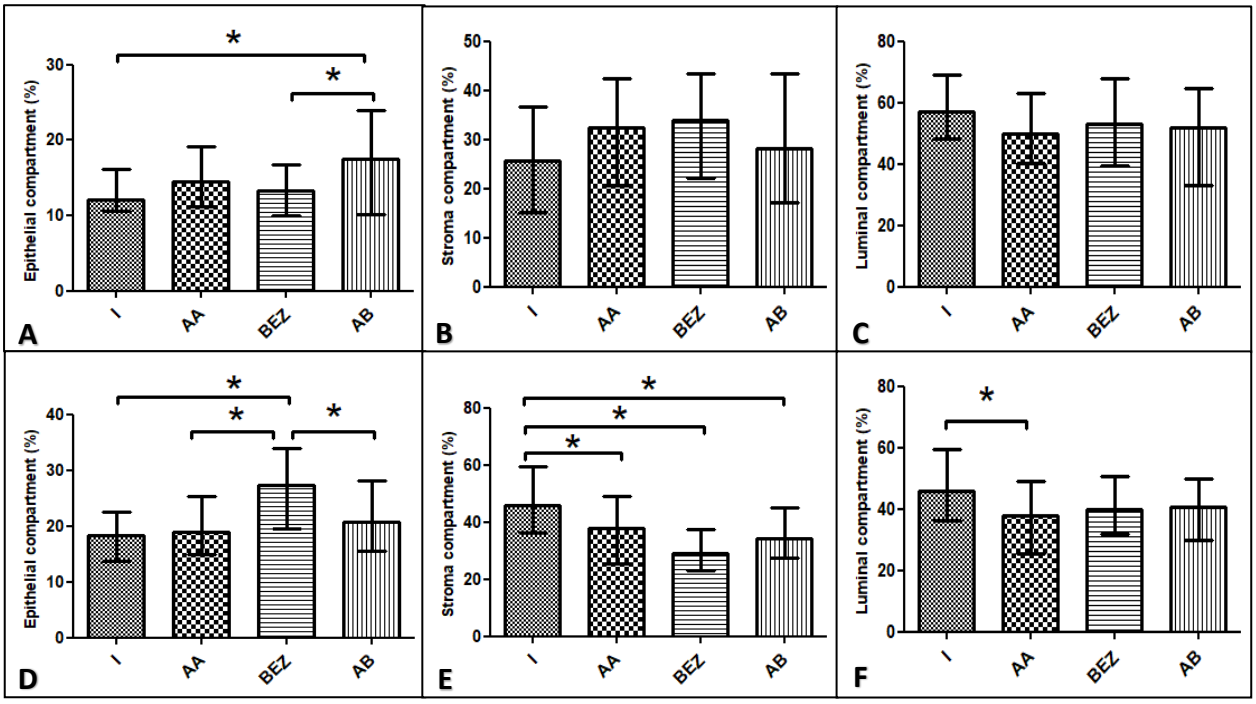

Epithelial compartment (%)
Stroma compartment (%)
Luminal compartment (%)
I
AA
BEZ
AB
A
B
C
D
E
F
*

Figure 5

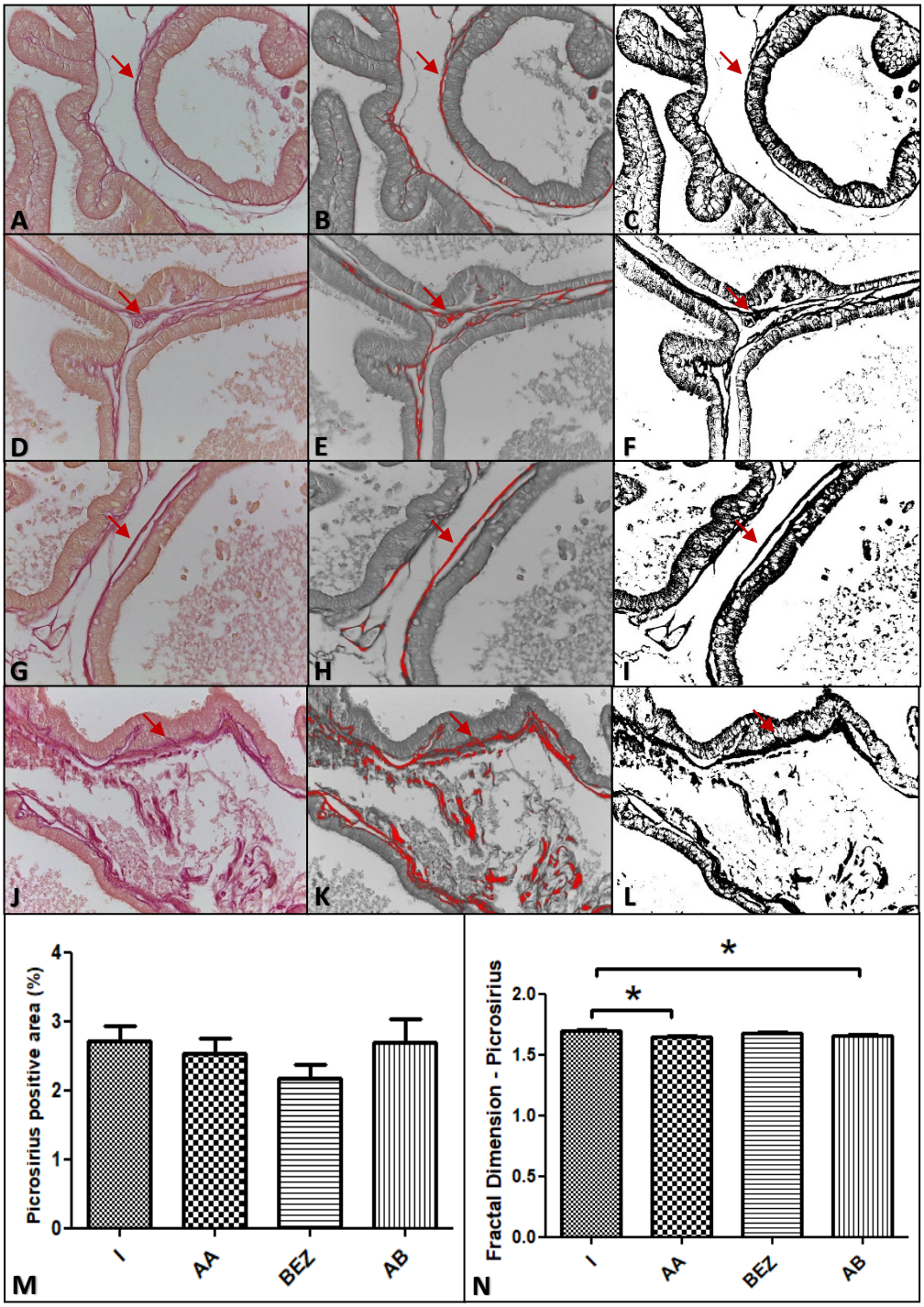

A
B
C
D
E
F
G
H
I
J
K
L
Picrosirius positive area (%)
0
1
2
3
4
I
AA
BEZ
AB
M
Fractal Dimension - Picrosirius
0.0
0.5
1.0
1.5
2.0
*
*
I
AA
BEZ
AB
N

Figure 6

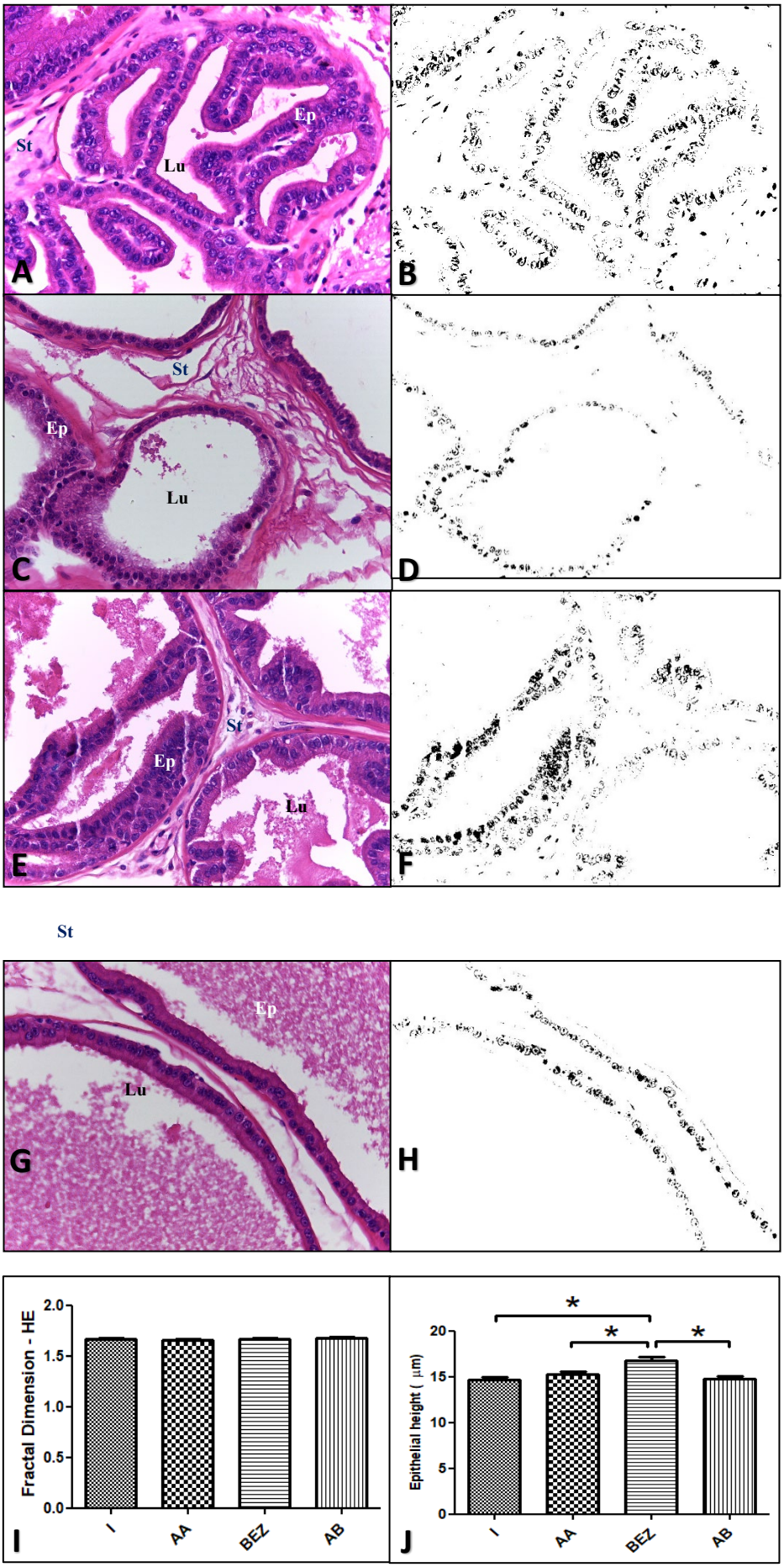
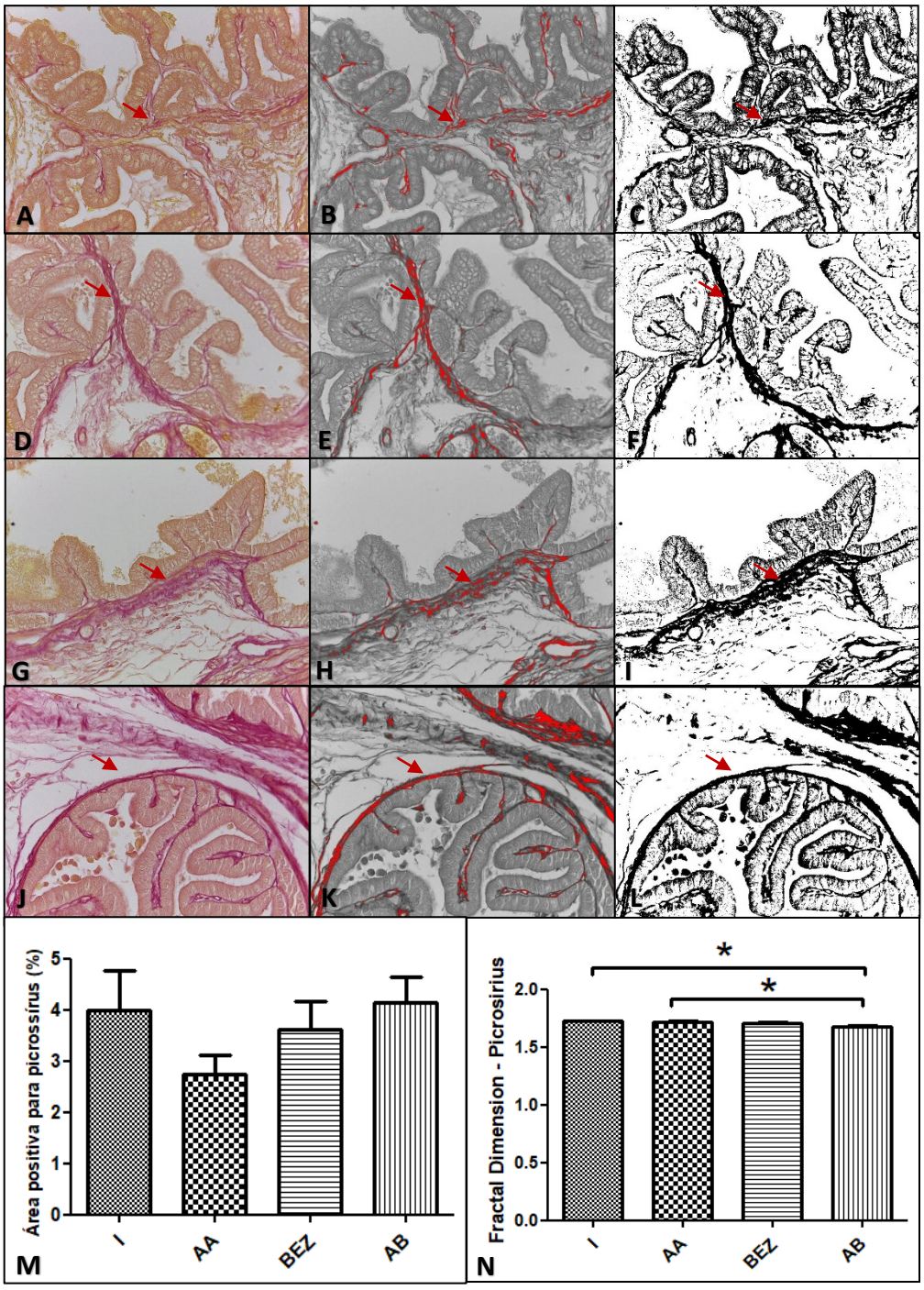

St
Ep
Lu
A
B
St
Ep
Lu
C
D
St
Ep
Lu
E
F
St
Ep
Lu
G
H
Fractal Dimension - HE
2.0
1.5
1.0
0.5
0.0
I
AA
BEZ
AB
I
Epithelial height (µm)
20
15
10
5
0
*
*
*
J
I
AA
BEZ
AB

Figure 7


A
B
C
D
E
F
G
H
I
J
K
L
Área positiva para picrossirius (%)
0
1
2
3
4
5
I
AA
BEZ
AB
M
Fractal Dimension - Picrosirius
0.0
0.5
1.0
1.5
2.0
*
*
I
AA
BEZ
AB
N

ANEXOS

ANEXO A

SUBMISSION RULES OF SCIENTIFIC REPORTS: NUTRITION AND CANCER

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ANEXO B

PREFERRED REPORTING ITEMS FOR SYSTEMATIC REVIEWS AND META-ANALYSES PROTOCOL

TITLE		
Title	1	Identify the report as a systematic review, meta-analysis, or both.
ABSTRACT		
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of what is already known.
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).
METHODS		
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.
RESULTS		
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).
DISCUSSION		
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.
FUNDING		
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.

ANEXO C

SUPPLEMENTAL MATERIAL

Supplement 1: Search strategy		
Database	**Search strategy**	**Results**
Cochrane Library	#1 MeSH descriptor: [Adult] explode all trees #2 MeSH descriptor: [Patients] explode all trees #3 (Adults OR Patient):ti,ab,kw #4 #1 OR #2 OR #3 #5 MeSH descriptor: [Neoplasms] explode all trees #6 (Neoplasia OR Neoplasias OR Neoplasm OR Tumors OR Tumor OR Cancer OR Cancers OR Malignancy OR Malignancies OR Malignant Neoplasms OR Malignant Neoplasm OR Neoplasm, Malignant OR Neoplasms, Malignant OR Benign Neoplasms OR Neoplasms, Benign OR Benign Neoplasm OR Neoplasm, Benign):ti,ab,kw #7 #5 OR #6 #8 MeSH descriptor: [Ferritins] explode all trees #9 (Ferritin OR Isoferritin OR Basic Isoferritin OR Isoferritin, Basic):ti,ab,kw #10 #8 OR #9 #11 #4 AND #7 AND #10	218 articles
PubMed/Medline	(((((((Adult[MeSH Terms]) OR (Adult[Title/Abstract])) OR (Patients[MeSH Terms])) OR (Patients[Title/Abstract])) OR (Adults[Title/Abstract])) OR (Patient[Title/Abstract])) AND ((((((((((((((((((Neoplasms[MeSH Terms]) OR (Neoplasms[Title/Abstract])) OR (Neoplasia[Title/Abstract])) OR (Neoplasias[Title/Abstract])) OR (Neoplasm[Title/Abstract])) OR (Tumors[Title/Abstract])) OR (Tumor[Title/Abstract])) OR (Cancer[Title/Abstract])) OR (Cancers[Title/Abstract])) OR (Malignancy[Title/Abstract])) OR (Malignancies[Title/Abstract])) OR (Malignant Neoplasms[Title/Abstract])) OR (Malignant Neoplasm[Title/Abstract])) OR (Neoplasm, Malignant[Title/Abstract])) OR (Neoplasms, Malignant[Title/Abstract])) OR (Benign Neoplasms[Title/Abstract])) OR (Neoplasms, Benign[Title/Abstract])) OR (Benign Neoplasm[Title/Abstract])) OR (Neoplasm, Benign[Title/Abstract]))) AND ((((((Ferritins[MeSH Terms]) OR (Ferritins[Title/Abstract])) OR (Ferritin[Title/Abstract])) OR (Isoferritin[Title/Abstract])) OR (Basic Isoferritin[Title/Abstract])) OR (Isoferritin, Basic[Title/Abstract]))	2,608 articles
Scopus	TITLE-ABS-KEY (Adult OR Patients OR Adults OR Patient) AND TITLE-ABS-KEY (Neoplasms OR Neoplasia OR Neoplasias OR Neoplasm OR Tumors OR Tumor OR Cancer OR Cancers OR Malignancy OR Malignancies OR “Malignant Neoplasms” OR “Malignant Neoplasm” OR “Neoplasm, Malignant” OR “Neoplasms, Malignant” OR “Benign Neoplasms” OR “Neoplasms, Benign” OR “Benign Neoplasm” OR “Neoplasm, Benign”) AND TITLE-ABS-KEY (Ferritins OR Ferritin OR Isoferritin OR “Basic Isoferritin” OR “Isoferritin, Basic”)	4,951 articles

Supplement 2: The list of excluded articles and their causes

Absence of control groups identified as healthy adults (with no history of chronic or acute disease) (n=17)

Carpagnano GE, Lacedonia D, Palladino GP, Koutelou A, Martinelli D, Orlando S, Foschino-Barbaro MP. Could exhaled ferritin and SOD be used as markers for lung cancer and prognosis prediction purposes? Eur J Clin Invest. 2012 May;42(5):478-86. doi: 10.1111/j.1365-2362.2011.02603.x. Epub 2011 Sep 28. PMID: 21955247.

Fracchia A, Ubbiali A, El Bitar O, Pacetti M, Sommariva E, Arreghini M, Longhini E, Bonalumi GP. A comparative study on ferritin concentration in serum and bilateral bronchoalveolar lavage fluid of patients with peripheral lung cancer versus control subjects. Oncology. 1999 Apr;56(3):181-8. doi: 10.1159/000011962. PMID: 10202271.

Gail MH, Muenz L, McIntire KR, Radovich B, Braunstein G, Brown PR, Deftos L, Dnistrian A, Dunsmore M, Elashoff R, et al. Multiple markers for lung cancer diagnosis: validation of models for localized lung cancer. J Natl Cancer Inst. 1988 Mar 16;80(2):97-101. doi: 10.1093/jnci/80.2.97. PMID: 3343691.

Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Akhmedkhanov A, Riboli E. Iron intake, body iron stores and colorectal cancer risk in women: a nested case-control study. Int J Cancer. 1999 Mar 1;80(5):693-8. doi: 10.1002/(sici)1097-0215(19990301)80:5<693::aid-ijc11>3.0.co;2-g. PMID: 10048969.

Knekt P, Marniemi J, Teppo L, Heliövaara M, Aromaa A. Is low selenium status a risk factor for lung cancer? Am J Epidemiol. 1998 Nov 15;148(10):975-82. doi: 10.1093/oxfordjournals.aje.a009574. PMID: 9829869.

Mahadavan L, Loktionov A, Daniels IR, Shore A, Cotter D, Llewelyn AH, Hamilton W. Exfoliated colonocyte DNA levels and clinical features in the diagnosis of colorectal cancer: a cohort study in patients referred for investigation. Colorectal Dis. 2012 Mar;14(3):306-13. doi: 10.1111/j.1463-1318.2011.02615.x. PMID: 21689307.

Mannello F, Tonti GA, Medda V, Simone P, Darbre PD. Analysis of aluminium content and iron homeostasis in nipple aspirate fluids from healthy women and breast cancer-affected patients. J Appl Toxicol. 2011 Apr;31(3):262-9. doi: 10.1002/jat.1641. Epub 2011 Feb 21. PMID: 21337589.

Milman N, Pedersen LM. The serum ferritin concentration is a significant prognostic indicator of survival in primary lung cancer. Oncol Rep. 2002 Jan-Feb;9(1):193-8. PMID: 11748482.

Moore AB, Shannon J, Chen C, Lampe JW, Ray RM, Lewis SK, Lin M, Stalsberg H, Thomas DB. Dietary and stored iron as predictors of breast cancer risk: A nested case-control study in Shanghai. Int J Cancer. 2009 Sep 1;125(5):1110-7. doi: 10.1002/ijc.24404. PMID: 19444907; PMCID: PMC2798105.

Pyo JH, Hong SN, Min BH, Lee JH, Chang DK, Rhee PL, Kim JJ, Choi SK, Jung SH, Son HJ, Kim YH. Evaluation of the risk factors associated with rectal neuroendocrine tumors: a big data analytic study from a health screening center. J Gastroenterol. 2016 Dec;51(12):1112-1121. doi: 10.1007/s00535-016-1198-9. Epub 2016 Mar 30. PMID: 27025841.

Robertson JF, Pearson D, Price MR, Selby C, Pearson J, Blamey RW, Howell A. Prospective assessment of the role of five tumour markers in breast cancer. Cancer Immunol Immunother. 1991;33(6):403-10. doi: 10.1007/BF01741602. PMID: 1878893.

Schneider C, Bodmer M, Jick SS, Meier CR. Colorectal cancer and markers of anemia. Eur J Cancer Prev. 2018 Nov;27(6):530-538. doi: 10.1097/CEJ.0000000000000397. PMID: 28692587.

Scholefield JH, Robinson MH, Bostock K, Brown NS. Serum ferritin. Screening test for colorectal cancer? Dis Colon Rectum. 1998 Aug;41(8):1029-31; discussion 1031-2. doi: 10.1007/BF02237395. PMID: 9715161.

Wild N, Andres H, Rollinger W, Krause F, Dilba P, Tacke M, Karl J. A combination of serum markers for the early detection of colorectal cancer. Clin Cancer Res. 2010 Dec 15;16(24):6111-21. doi: 10.1158/1078-0432.CCR-10-0119. Epub 2010 Aug 26. PMID: 20798228.

Wilhelmsen M, Christensen IJ, Rasmussen L, Jørgensen LN, Madsen MR, Vilandt J, Hillig T, Klaerke M, Nielsen KT, Laurberg S, Brünner N, Gawel S, Yang X, Davis G, Heijboer A, Martens F, Nielsen HJ. Detection of colorectal neoplasia: Combination of eight blood-based, cancer-associated protein biomarkers. Int J Cancer. 2017 Mar 15;140(6):1436-1446. doi: 10.1002/ijc.30558. PMID: 27935033.

Zhao J, Guo N, Zhang L, Wang L. Serum CA125 in combination with ferritin improves diagnostic accuracy for epithelial ovarian cancer. Br J Biomed Sci. 2018 Apr;75(2):66-70. doi: 10.1080/09674845.2017.1394051. Epub 2018 Feb 16. PMID: 29452533.

Zhao Z, Li C, Hu M, Li J, Liu R. Plasma ferritin levels, HFE polymorphisms, and risk of pancreatic cancer among Chinese Han population. Tumour Biol. 2014 Aug;35(8):7629-33. doi: 10.1007/s13277-014-1978-x. Epub 2014 May 6. PMID: 24798971.

Serum ferritin levels not provided for each group or type of cancer evaluated (n=16)

Abd Elmonem E, Tharwa el-S, Farag MA, Fawzy A, El Shinnawy SF, Suliman S. Hepcidin mRNA level as a parameter of disease progression in chronic hepatitis C and hepatocellular carcinoma. J Egypt Natl Canc Inst. 2009 Dec;21(4):333-42. PMID: 21415870.

Alemán MR, Santolaria F, Batista N, de La Vega M, González-Reimers E, Milena A, Llanos M, Gómez-Sirvent JL. Leptin role in advanced lung cancer. A mediator of the acute phase response or a marker of the status of nutrition? Cytokine. 2002 Jul 7;19(1):21-6. doi: 10.1006/cyto.2002.1051. PMID: 12200109.

Baharvand M, Manifar S, Akkafan R, Mortazavi H, Sabour S. Serum levels of ferritin, copper, and zinc in patients with oral cancer. Biomed J. 2014 Sep-Oct;37(5):331-6. doi: 10.4103/2319-4170.132888. PMID: 25179706.

Demir H, Akkus ZA, Cebi A, Cakir T, Izmirli M. Catalase, carbonic anhydrase and other biochemical parameters in esophageal cancers in Turkey. Asian Pac J Cancer Prev. 2010;11(4):1029-32. PMID: 21133619.

Döngel İ, Akbaş A, Benli İ, Bayram M. Comparison of serum biochemical markers in patients with mesothelioma and pleural plaques versus healthy individuals exposed to environmental asbestos. Turk Gogus Kalp Damar Cerrahisi Derg. 2019 Jun 28;27(3):374-380. doi: 10.5606/tgkdc.dergisi.2019.17557. PMID: 32082887; PMCID: PMC7021410.

Freng A, Daae LN, Engeland A, Norum KR, Sander J, Solvoll K, Tretli S. Malignant epithelial tumours in the upper digestive tract: a dietary and socio-medical case-control and survival study. Eur J Clin Nutr. 1998 Apr;52(4):271-8. doi: 10.1038/sj.ejcn.1600548. PMID: 9578339.

Havemann K, Gropp C, Scheuer A, Scherfe T, Gramse M. ACTH-like activity in immune complexes of patients with oat-cell carcinoma of the lung. Br J Cancer. 1979 Jan;39(1):43-50. doi: 10.1038/bjc.1979.6. PMID: 215184; PMCID: PMC2009814.

Khanna V, Karjodkar F, Robbins S, Behl M, Arya S, Tripathi A. Estimation of serum ferritin level in potentially malignant disorders, oral squamous cell carcinoma, and treated cases of oral squamous cell carcinoma. J Cancer Res Ther. 2017 Jul-Sep;13(3):550-555. doi: 10.4103/0973-1482.181182. PMID: 28862225.

Kishida T, Sato J, Fujimori S, Minami S, Yamakado S, Tamagawa Y, Taguchi F, Yoshida Y, Kobayashi M. Clinical significance of serum iron and ferritin in patients with colorectal cancer. J Gastroenterol. 1994 Feb;29(1):19-23. doi: 10.1007/BF01229068. PMID: 8199692.

Lian M, Zhang C, Zhang D, Chen P, Yang H, Yang Y, Chen S, Hong G. The association of five preoperative serum tumor markers and pathological features in patients with breast cancer. J Clin Lab Anal. 2019 Jun;33(5):e22875. doi: 10.1002/jcla.22875. Epub 2019 Mar 6. PMID: 30843272; PMCID: PMC6595372.

Matsha T, Brink L, van Rensburg S, Hon D, Lombard C, Erasmus R. Traditional home-brewed beer consumption and iron status in patients with esophageal cancer and healthy control subjects from Transkei, South Africa. Nutr Cancer. 2006;56(1):67-73. doi: 10.1207/s15327914nc5601_9. PMID: 17176219.

Mevio E, Benazzo M, Galioto P, Spriano P, Pizzala R. Use of serum markers in the diagnosis and management of laryngeal cancer. Clin Otolaryngol Allied Sci. 1991 Feb;16(1):90-2. doi: 10.1111/j.1365-2273.1991.tb01950.x. PMID: 2032368.

Orlandi R, De Bortoli M, Ciniselli CM, Vaghi E, Caccia D, Garrisi V, Pizzamiglio S, Veneroni S, Bonini C, Agresti R, Daidone MG, Morelli D, Camaschella C, Verderio P, Bongarzone I. Hepcidin and ferritin blood level as noninvasive tools for predicting breast cancer. Ann Oncol. 2014 Feb;25(2):352-7. doi: 10.1093/annonc/mdt490. Epub 2013 Dec 3. PMID: 24306042.

Panis C, Victorino VJ, Herrera AC, Freitas LF, De Rossi T, Campos FC, Simão AN, Barbosa DS, Pinge-Filho P, Cecchini R, Cecchini AL. Differential oxidative status and immune characterization of the early and advanced stages of human breast cancer. Breast Cancer Res Treat. 2012 Jun;133(3):881-8. doi: 10.1007/s10549-011-1851-1. Epub 2011 Nov 3. PMID: 22048816.

Tsionou C, Minaretzis D, Papageorgiou I, Nakopoulou L, Michalas S, Aravantinos D. Expression of carcinoembryonic antigen and ferritin in normal, hyperplastic, and neoplastic endometrium. Gynecol Oncol. 1991 Jun;41(3):193-8. doi: 10.1016/0090-8258(91)90307-q. PMID: 1869094.

Werner S, Krause F, Rolny V, Strobl M, Morgenstern D, Datz C, Chen H, Brenner H. Evaluation of a 5-Marker Blood Test for Colorectal Cancer Early Detection in a Colorectal Cancer Screening Setting. Clin Cancer Res. 2016 Apr 1;22(7):1725-33. doi: 10.1158/1078-0432.CCR-15-1268. Epub 2015 Nov 11. PMID: 26561557.

Several type of cancer assessed or with any previous treatment (n=15)

Bolarin DM. Serum ferritin in Nigerian patients with Burkitt's lymphoma and other malignant diseases. Acta Trop. 1983 Mar;40(1):71-7. PMID: 6134456.

Bolayirli M, Turna H, Orhanoğlu T, Ozaras R, Ilhan M, Ozgüroğlu M. C-reactive protein as an acute phase protein in cancer patients. Med Oncol. 2007;24(3):338-44. doi: 10.1007/s12032-007-0012-1. PMID: 17873311.

Cross AJ, Sinha R, Wood RJ, Xue X, Huang WY, Yeager M, Hayes RB, Gunter MJ. Iron homeostasis and distal colorectal adenoma risk in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Prev Res (Phila). 2011 Sep;4(9):1465-75. doi: 10.1158/1940-6207.CAPR-11-0103. Epub 2011 Jun 17. PMID: 21685236; PMCID: PMC3168068.

Jaafari- Ashkavandi Z, Khademi B, Malekzadeh M, Shahmoradi Z. Serum Levels of Zinc, Copper and Ferritin in Patients with Salivary Gland Tumors. Asian Pac J Cancer Prev. 2019 Feb 26;20(2):545-548. doi: 10.31557/APJCP.2019.20.2.545. PMID: 30803218; PMCID: PMC6897009.

Jakobsen E, Engeset A, Sandstad B, Aas M. Serum ferritin and bone marrow haemosiderin in patients with malignancies and in healthy controls. Scand J Haematol. 1982 Mar;28(3):264-71. doi: 10.1111/j.1600-0609.1982.tb00525.x. PMID: 7089485.

Kenar G, Köksoy EB, Ürün Y, Utkan G. Prevalence, etiology and risk factors of anemia in patients with newly diagnosed cancer. Support Care Cancer. 2020 Nov;28(11):5235-5242. doi: 10.1007/s00520-020-05336-w. Epub 2020 Feb 21. PMID: 32086566.

Kuvibidila SR, Gauthier T, Rayford W. Serum ferritin levels and transferrin saturation in men with prostate cancer. J Natl Med Assoc. 2004 May;96(5):641-9. PMID: 15160979; PMCID: PMC2640669.

Li X, Asmitananda T, Gao L, Gai D, Song Z, Zhang Y, Ren H, Yang T, Chen T, Chen M. Biomarkers in the lung cancer diagnosis: a clinical perspective. Neoplasma. 2012;59(5):500-7. doi: 10.4149/neo_2012_064. PMID: 22668014.

Morita R, Yoshii M, Nakajima K, Kohsaka T, Miki M, Torizuka K. Clinical evaluation of serum ferritin to iron ratio in malignant diseases. Eur J Nucl Med. 1981;6(7):331-6. doi: 10.1007/BF00262528. PMID: 7250141.

Quintana Pacheco DA, Sookthai D, Graf ME, Schübel R, Johnson T, Katzke VA, Kaaks R, Kühn T. Iron status in relation to cancer risk and mortality: Findings from a population-based prospective study. Int J Cancer. 2018 Aug 1;143(3):561-569. doi: 10.1002/ijc.31384. Epub 2018 Apr 1. PMID: 29574909.

Saji S, Yokoyama Y, Niwa H, Takao H, Kida H, Kawata R, Tanemura H, Sakata K. Clinical studies on serum immunosuppressive acidic protein (IAP) and ferritin in gastric cancer patients: with special reference to preoperative value and influence of surgical stress. J Surg Oncol. 1986 Dec;33(4):215-22. doi: 10.1002/jso.2930330402. PMID: 3784555.

Stevens RG, Beasley RP, Blumberg BS. Iron-binding proteins and risk of cancer in Taiwan. J Natl Cancer Inst. 1986 Apr;76(4):605-10. doi: 10.1093/jnci/76.4.605. PMID: 3007843.

Tanaka M, Kato K. The measurement of ferritin in the leukemic blasts with a "sandwich" type enzyme immunoassay method. Cancer. 1983 Jan 1;51(1):61-4. doi: 10.1002/1097-0142(19830101)51:1<61::aid-cncr2820510115>3.0.co;2-8. PMID: 6336973.

Worwood M, Summers M, Miller F, Jacobs A, Whittaker JA. Ferritin in blood cells from normal subjects and patients with leukaemia. Br J Haematol. 1974 Sep;28(1):27-35. PMID: 4528565.

Zhang XZ, Su AL, Hu MQ, Zhang XQ, Xu YL. Elevated serum ferritin levels in patients with hematologic malignancies. Asian Pac J Cancer Prev. 2014;15(15):6099-101. doi: 10.7314/apjcp.2014.15.15.6099. PMID: 25124580.

Supplement 3: Characteristics of the included studies									
Study Identification (Newcastle Ottawa Scale)	**Country**	**Study Design**	**Participants**	**Exposure (n)/ Control (n) [age; sex; and race]**	**Selection**	**Results**	**Limitations**	**Conflict of interests**	**Funding**
Goswami *et al.*, 2009 (7)	India	Case-control study	120	Breast cancer (n=60) [female 60; mean age 49.2 years; race was not reported]/ Control (n=60) [female 60; mean age 47.7 years; race was not reported]	Case: patients with newly diagnosed, histopathologically confirmed breast carcinoma were enrolled in the stud. Single blood samples were taken from patients, before start of any therapy. Control: healthy controls, without history or laboratory evidence of malignancy and any infections during the past six months. None of the patients or controls had diabetes mellitus, liver disease, history of any infections during the past six months or rheumatoid arthritis.	SF levels were significantly higher in the cases compared to the controls ($p<0.01$). * ferritin levels were estimated by enzyme-linked immunosorbent assay (ELISA) using commercially available kits from Diaclone Research, France.	The small study population.	Not reported	Not reported
Güner *et al*., 1992 (9)	Turkey	Case-control study	62	Breast cancer (n=30) [female 30; race was not reported] / Control (n=32) [female 32; race was not reported] * They ranged in age from 30 to 65 years (mean 48.0 years)	Case: the breast cancer group consisted in lesions histopathologically diagnosed as 'carcinoma of the breast', of whom 6 had stage I, 13, stage II and 11, stage III pathology, according to the TNM classification system. The pathological classification of tumours was done according to the criteria of the World Health Organization. Inclusion in this investigation required the following conditions: (1) untreated primary breast carcinoma, (2) no anemia (Hb ≥ 12 g/dl); (3) no evidence of liver disease; (4) no iron	SF levels no significant difference has been determined between of the groups studied ($p > 0.05$). * SF levels were measured by an enzyme immunoassay method (BioMerieux).	Not reported	Not reported	Not reported

					overload or hemotransfusion in the last 6 months. Control: the control group consisted of the nursing, medical and laboratory staff, diagnosed to be 'healthy' from routine laboratory check-up.				
Rajizadeh *et al.*, 2017 (8)	Iran	Case-control study	55	Breast cancer (n=26) [female 26; mean age 45.9 years; race was not reported]/ Control (n=29) [female 29; mean age 42.8 years; race was not reported]	Case: inclusion criteria for the case group were as follow: females aged 25 to 65 years, a positive breast tissue biopsy, receiving no interventional treatment, no history of cysts or other cancers, no history of hormone replacement therapy, and no history of cardiovascular or hepatic disease, diabetes, or thalassemia. Control: inclusion criteria for the control group were as same as the case group, but without incidence of breast cancer, as confirmed by mammography. Individuals in both groups were frequency-matched by age and the presence or absence of menopause.	SF levels were not statistically different between the groups (p=0.007). * SF was measured by direct method using a kit (Acculite Clia Microwells Ferritin Test System, California, USA), for which the normal range for females was 10-126 ng/mL (by age).	As the selected cases were new cases, the sample size of this study was small, which was a limitation of this study.	None	This work This study was funded by Shahid Sadoughi University of Medical Sciences, Yazd, IR Iran (500 €).
Gackowski *et al.*, 2002 (6)	Poland	Case-control study	96	Colorectal cancer (n=45) [male 26, female 19; mean age 65.0 years; race was not reported]/ Control (n=51) [male 21, female 30; mean age 60.0 years; race was not reported]	Case: all the patients had histologically proven adenocarcinomas. Control: healthy controls. *non-smoker subjects	SF levels were not statistically different between the groups (p= 0.583). *SF levels were analysed using Vidas and Olympus Auto- Analyzers during routine laboratory tests.	Not reported	Not reported	This work was financed by grant from the State Committee for Scientific Research and the Maria Sklodowska-Curie

									Polish-American Joint Fund II.
Gür *et al.*, 2011 (9)	Turkey	Case-control study	69	Colorectal cancer (n=40) [sex, age and race were not reported]/ Control (n=29) [sex, age and race were not reported]	Case: patients diagnosed with colon cancer. Control: healthy volunteers.	SF levels were higher in control group than case group ($p<0.05$). * All serum markers obtained from serum samples and some biochemical parameters were determined by Modular equipment P800 and Roche/Hitachi apparatus in the Biochemical and Hormone Laboratory.	Not reported	Not reported	Not reported
Cook *et al.*, 2012 (8)	USA	Case-control study	682	Gastric cancer (n=341) [male 341; mean age 58.0 years; race was not reported]/ Control (n=341) [male 341; mean age 58.0 years; race was not reported]	Case: were subjects of the ATBC Study cohort who were diagnosed with gastric cancer through April 30, 2006, a follow-up of up to 21 years. Gastric cancer was defined according to the International Classification of Diseases, 9th Revision. Control: were matched to cases in a 1:1 ratio using the variables age at randomization (+/− 1 year) and date of blood draw (+/− 30 days). Each of these variables were highly correlated between cases and matched controls. Controls were required to be alive and cancer-free up until the date of cancer diagnosis of their matched case. * All participants were smokers.	SF levels were higher in control group than case group ($p=0.008$). * SF was quantitated using an immunoradiometric assay (Count-Acount Ferritin IRMA: Diagnostic Products Los Angeles).	They included sex-specific analyses only (male cohort) which avoided combination of the sexes which could result in type I or type II errors given the complexity of iron homeostasis in females. Lack of a female cohort as a comparison for the male results; and a population which includes only smokers,	Not reported	Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

							although we did adjust for duration and rate of exposure, where applicable.		
Bhatavdekar *et al.*, 1987 (8)	India	Case-control study	85	Head and neck cancer (n=58) [sex; age and race was not reported]/ Control (n=27) [sex; age and race was not reported]	Case: head and neck cancer patients with histologically proven cancer who attended the Gujarat Cancer and Research Institute entered the study. Control: age -matched blood donors (smokers and non-smokers) were selected as controls.	The mean ferritin level was significantly higher in patients ($p < 0.001$) than in normal subjects. * Ferritin was determined by an RIA kit (Diagnostics Products Co., Los Angeles, CA, U.S.A.).	Not reported	Not reported	Not reported
Ho *et al.*, 1996 (7)	China	Case-control study	463	Nasopharyngeal carcinoma (n=279) [sex; age and race was not reported]/ Control (n=184) [male 94, female 90; mean age for male 44.6 years, mean age 37.7 for female < 50 years old, and mean age 59.4 for female ≥ 50 years old; race was not reported]	Case: untreated patients with histologically proven NPC of stage I-IV (Ho's stage classification). Control: serum samples from volunteers with no apparent illness.	SF level in patients with localised disease (Ho's stage I-IV) had levels which were not significantly different from age, sex matched normal subjects and there was no relationship between mean serum ferritin levels and stage. * SF levels were measured using the (Hybritech TANDEM-R FER immunoradiometric assay kits).	Not reported	Not reported	Not reported
Maxim & Veltri, 1986 (8)	USA	Case-control study	231	Head and neck cancer (n=113) [male 88, female 25; mean age 56.7 years; race was not reported]/ Control (n=118) [male 55, female 63; mean age 25.1 years; race was not reported]	Case: sera were obtained from patients presenting at the West Virginia University Otolaryngology Clinic with a differential diagnosis of primary squamous cell carcinoma of the head and neck. The patients were staged according to the recommendations of the	The mean ferritin level was significantly higher in patients ($p < 0.001$) than in normal subjects. *A double antibody (sandwich) solid-phase enzyme immunoassay was used to quantitate serum	The potential for using this protein for early detection is limited because of the number of patients who have advanced	Not reported	Supported in part by funds from the West Virginia University Foundati

					American Joint Committee for Cancer Staging and scheduled for treatment by surgery, x-ray, or both. Sera were obtained at monthly intervals from these patients during their follow-up. Control: control sera were also obtained from normal healthy volunteers and laboratory personnel during the same intervals.	ferritin.	tumors with low serum levels of ferritin.		on-Otolaryngic Cancer Fund, Charleston Area Medical Center Foundation, and the Greater Kanawha Valley Foundation.
Richie *et al.*, 2008 (9)	USA	Case-control study	54	Oral cancer (n=17) [sex; age and race was not reported]/ Control (n=37) [sex; age and race was not reported]	Case: eligible cases were men and women with newly diagnosed histopathologically confirmed primary cancer of the oral cavity, excluding carcinomas of the lip, salivary gland, and nasopharynx. Control: control subjects were frequency matched to cases by age (within 10 years), sex, and month of interview. * All cases and controls were interviewed using a structured questionnaire that contained questions on smoking history, alcohol consumption and occupation. Never-Smokers subjects were included for the systematic review.	The mean ferritin level was significantly higher in patients than in normal subjects (p= 0.04). * SF was determined by a two-site immunoradiometric assay.	The concentrations of the serum nutrients reflect recent food intake and not long-term dietary patterns. Diurnal variation in blood biomarker levels, as have been observed for serum iron and transferrin saturation levels may impact these results, although fasting blood samples were obtained to help control	Not reported	This work was supported in part by NIH grants DE09514 and CA68384.

							for circadian fluctuations. Biomarkers levels in cases were measured post-diagnostically and may reflect recent changes in dietary habits.		
Vinzenz *et al.*, 1987 (7)	Austria	Case-control study	140	Head and neck cancer (n=90) [sex; age and race was not reported]/ Control (n=50) [sex; age and race was not reported]	Case: Patients with tumours of the head and neck region have been investigated, (according to the UICC tumour stage classification, 1978). All serum investigations were carried out sub- sequent to the diagnosis, yet before therapy. Control: healthy approximately age-matched controls.	SF levels in cancer patients were significantly increased for than healthy controls ($p < 0.0005$). Between the patients in the various subgroups, no stage-dependence was detected. * SF levels were tested by means of a competitive enzyme ynaec-assay based upon the "sandwich principle" (Enzygnost-[32-Microglobulin, Enzygnost-IgE, Behring Co.; Ferrizyme, Abbot Diagnostics, DN)	Not reported	Not reported	Not reported
Yuan *et al.*, 2016 (9)	China	Case-control study	199	Oral/oropharyngeal squamous carcinoma (OSCC/OPSCC) (n=169) [male 116, female 53; mean age 53.9 years; and race was not reported]/ Control (n=30) [male 19, female 11; mean age 51.8 years; race was not reported]	Case: newly diagnosed patients with OSCC/OPSCC in the Department of Oral and Maxillofacial Surgery in the First Affiliated Hospital of Chongqing Medical University. All patients were confirmed by pathological examination. The TNM classification and clinical stage were determined based on the 7th edition of the Union for International Cancer Control standards.	SF level were significantly higher than healthy control group ($p < 0.05$). However, the levels of serum ferritin showed no significant difference between patients with early OSCC/OPSCC and healthy control group ($p > 0.05$). SF in the middle-late stage of patients with OSCC/OPSCC (stage III + IV) were significantly higher than patients with the	Not reported	None	Not reported

					Control: healthy group received the routine physical examination, they had no systemic disease.	early OSCC/OPSCC and the healthy control group ($p < 0.05$). * The serum was separated and measured automatically using electrochemiluminescence immunoassay by Roche Elecsys 2010 analyzer (Roche, Basel, Switzerland).			
Giannoulis *et al.*, 1984 (6)	Greece	Case-control study	41	Hepatocellular carcinoma (n=19) [male 15, female 4; median age 61 years; and race was not reported]/ Control (n=22) [male 11, female 11; median age 48 years; and race was not reported]	Case: the diagnosis was based on percutaneous or operative biopsy and classified by histological criteria. Patients were excluded from the study if they had recent bleeding, iron-deficiency anemia or primary hemochromatosis determined by normal serum iron and TIBC. Control: normal controls were followed.	SF level were significantly higher in the patient group than control ($p < 0.0005$). * SF was measured by a 2-site immunoradiometric assay (Fer-Iron®, Ramco Lab., Houston, Tex., USA).	Not reported	Not reported	Not reported
Tatsuta *et al.*, 1986 (7)	Japan	Case-control study	268	Hepatocellular carcinoma (HCC) (n=44) [male 40, female 4; mean age 60.1 years; and race was not reported]/ Control (n=224) [sex, age and race was not reported]	Case: the histological and/or cytological diagnosis was verified in all patients with HCC at laparotomy, at autopsy, by laparoscopic examination or by aspiration biopsy. Control: the healthy subjects were patients with no evidence of symptons who underwent mass survey examination for cancer of the stomach, the pancreas and the liver, but who were found to be normal on laboratory investigation, and ultrasonographic and roentogenographic investigations.	SF level were significantly higher in patients with malignant hepatic disease than in healthy subjects ($p < 0.05$). * SF was assayed an enzyme 82 ynaecologi technique with an assay from Abbott Laboratories (North Chicago).	Not reported	Not reported	Not reported

Zhao *et al.*, 2015 (8)	China	Case-control study	90	Hepatocellular carcinoma (n=50) [male 36, female 14; median age 56 years; and race was not reported]/ Control (n=40) [male 22, female 18; median age 54 years; and race was not reported]	Case: they were all conformed to the diagnosis standard of criteria for diagnosis and treatment of common malignant tumors issued by the National Cancer Prevention and Control Office and the Chinese Anti Cancer Association. Control: healthy controls were collected after biochemical and immunological tests, to exclude hepatitis, cirrhosis, liver and gallbladder cysts, benign and malignant tumors and other diseases.	SF level were not statistically different between the groups (p = 0.155). * SF were quantified by electrochemiluminescence immunoassays.	The volumes of clinical data collected in this article were limited; more definite conclusions still needed more clinical data.	Not reported	Not reported
Alexandrakis *et al.*, 2002 (7)	Greece	Case-control study	43	Lung cancer (n=18) [male 10, female 8; median age 62 years; race was not reported]/ Control (n=25) [male 14, female 11; median age 39 years; race was not reported]	Case: the diagnosis of malignancy disease was based on clinical examination, imaging techniques, cytological and histological features. Control: healthy blood donors.	Serum ferritin (SF) levels were not statistically different between the groups (p>0.05). * SF levels were assessed by an immunoradiometric assay kit (Amersham Int, U.K.).	Not reported	Not reported	Not reported
Gulen *et al.*, 2012 (7)	Turkey	Case-control study	88	Lung cancer (n=63) [male 63; mean age 65.6 years; race was not reported]/ Control (n=25) [male 25; mean age 63.5 years; race was not reported]	Case: all patients were histopathologically confirmed to have lung cancer. None of the patients had undergone surgical resection or had received chemotherapy or radiotherapy at the time of sampling. Control: male volunteers, participants at the Chest Diseases Outpatients Clinic, in the same age range were admitted as the control group. *Both patients and controls with comorbidities that affect	SF levels were higher in lung cancer than control group (p=0.009). * Test for SF levels evaluation was not reported.	Not reported	None	The study was funded by the Adnan Menderes University Research Foundation.

					weight maintenance (diabetes, thyroid dysfunction, alcoholism, malabsorption, renal, and hepatic diseases) or that lead to systemic inflammation (infection, heart failure, collagen vascular diseases) and those who did not have precise information on his body weight status in the preceding 6 months were excluded from the study. The smoking history was similar in both groups (p = 0.079).				
Ji *et al.*, 2014 (7)	China	Cross-sectional study	132	Lung cancer (n=69) [male 52, female 17; mean age 70.6 years; race was not reported]/ Control (n=63) [male 33, female 30; mean age 72.6 years; race was not reported]	Case: the diagnoses were based on conventional clinical, radiographic, and histopathologic criteria, 37 patients were smokers. Control: normal elderly subjects who were also enrolled as negative controls. * All patients and controls received relative examinations which excluded primary ferritin metabolism disorders.	SF level of patients with cancer was significantly higher than that of healthy controls (p=0.000). * Reagents for electrochemiluminescence were purchased from Roche Co., Ltd (Germany). Electrochemiluminescence was performed with Roche COBAS e601 fully automatic serum immune analyzer purchased from Roche Co., Ltd (Germany).	Not reported	None	This work was funded by grants from 2007' The Twentieth Science and Technology Plan Supporting Projects of Changzhou provided by Yan Liu and The Jiangsu Health International Exchange Supporti

									ng Program provided by Xiao-Dong Li.
Shi *et al.*, 2014 (8)	China	Case-control study	109	Lung cancer (n=46) [male 32, female 14; mean age 59.9 years; race was not reported]/ Control (n=63) [male 33, female 30; mean age 50.6 years; race was not reported]	Case: the diagnoses were based on conventional clinical, radiographic and histopathologic or cytological criteria, and 25 patients were smokers. Patients who underwent symptomatic brain metastases and previous chemotherapy were excluded. Control: healthy subjects. * None of subjects enrolled in this study was treated with iron or suffered from other cancers, inflammatory and other known disorders affecting ferritin metabolism.	The expression levels of SF in patients with advanced lung cancer were significantly higher than that in healthy subjects (p=0.001). * The expression levels of SF in blood specimens from patients and healthy subjects were assayed using electrochemiluminescence method, and the reagent kit was purchased from Roche Co., Ltd. (Germany). According to the manufacturer's instructions, the normal range of the SF was identified at 15-200 ng/mL for men and 12-150 ng/mL for women.	Not reported	None	This work was funded by grants from the Major Bidding Project Changzhou Health Bureau (ZD200810) provided by Dr. Mei Ji.
Sukiennicki *et al.*, 2019 (7)	Poland	Case-control study	400	Lung cancer (n=200) [male 151, female 49; mean age 67.9 years; race was not reported]/ Control (n=200) [male 151, female 49; mean age 67.6 years; race was not reported]	Case: consisted of 200 consecutively collected patients with lung cancer diagnosed at the Clinical Thoracic Surgery Department in Szczecin and the IHCC between August 2009 and September 2015 by previous cohort study. Patients were not eligible if they were diagnosed with any other previous malignancy, and had to be untreated before blood sampling prior to enrolment in the study. The serum samples for determination of iron levels	The expression levels of SF in patients with lung cancer were significantly higher than that in control group (p=0.007). * The ferritin was determined by electrochemiluminescence immunoassay (ECLIA) using Cobas C Analyzer (Roche).	The data obtained in our study are insufficient to establish a causal relationship between iron and lung cancer risk, so we are not able to determine if detected higher body	None	This work was supported by National Science Centre, grant no. DEC-2013/11/N/NZ4/02250.

					and iron metabolism parameters in cases were collected at the time of lung cancer diagnosis but before therapy. 187 patients were smokers or had a history of smoking. Control: were selected from a population-based study of the approximately 2 million inhabitants of the West Pomerania region in Poland, designed to identify familial aggregations of cancer syndromes. For each diagnosed lung cancer case a single cancer-free control was selected. Control participants meeting the matching criteria were identified by review of the records of the population based study and invited for interview and a blood donation. Individuals using dietary supplements of iron were excluded. 184 patients were smokers or had a history of smoking.		iron is a marker of lung cancer diagnosis or a contributing etiologic factor.		
Wang *et al.*, 2018 (6)	China	Case-control study	347	Lung cancer (n=224) [male 151, female 73; mean age 64.0 years; race was not reported]/ Control (n=123) [male 78, female 45; mean age 60.1 years; race was not reported]	Case: the diagnosis of each patient was confirmed by cytological diagnosis and/or immunehistochemistry test. Control: who attended in physical examination in outpatient during the same period, except for those with a family history of lung cancer.	SF levels were significantly higher in patients with lung cancer than those with apparently healthy people ($p < 0.05$). * SF was quantified by a diagnostic kit (Huzhou HealthDigit corp.).	Not reported	Not reported	Not reported

Luger *et al.*, 1983 (6)	Austria	Case-control study	171	Malignant melanoma (n=91) [not reported] Stage I 35, Stage II 34, and Stage III 22 / Control (n=80) [not reported]	Case: the diagnosis of malignancy was confirmed by histology. All patient had been previously untreated or had received no treatment for 6 months. Patient with liver disease, on iron medication or recipients of blood transfusion were excluded. Control: healthy individuals of similar age with normal range of SF.	SF levels were higher in Stage III group than control group ($p<0.01$). * SF was assessed by a two-site IRMA technique according to the method of Halliday et al., 1975, as has been adapted in the commercially available kit by Behringwerke (Hoechst, Frankfurt,FRG).	Not reported	Not reported	Not reported
Sezgi *et al.,* 2014 (8)	Turkey	Cross-sectional study	96	Mesothelioma (n=46) [male 23, female 23; mean age 58.9 years; race was not reported] /Control (n=50) [male 24, female 26; mean age 58.3 years; race was not reported]	Case: the patients with confirmed mesothelioma diagnosis histopathologically were included. Control: healthy people with a mean of similar age and gender and who living in an area which not detected asbestos in soil analysis and without any disease. * The patients with chronic kidney failure, chronic heart failure, liver failure, and chronic obstructive pulmonary disease and those who have got active infection were excluded the study. The patients with malignancies other than mesothelioma were excluded from the study.	SF levels were significantly higher in patients group than control group ($p < 0.001$). * SF levels were determined by immunonephelometric method with an autoanalyser (Image 800 Beckman Coulter, Fullerton, CA, USA).	Not reported	None	None
Lodh *et al.*, 2012 (6)	India	Case-control study	40	Multiple myeloma (n=20) [male 11, female 9; mean age 52.1 years; race was not reported] / Control (n=20) [male 11, female 9; mean age 53.3 years; race was not reported]	Case: clinically diagnosed multiple myeloma. Control: healthy age and sex matched controls. The controls were chosen from hospital staff and healthy volunteers.	SF levels were higher in case group than control group ($p=0.014$). * SF levels were estimated by enzyme-linked immunosorbent assay (ELISA) using	Not reported	Not reported	Not reported

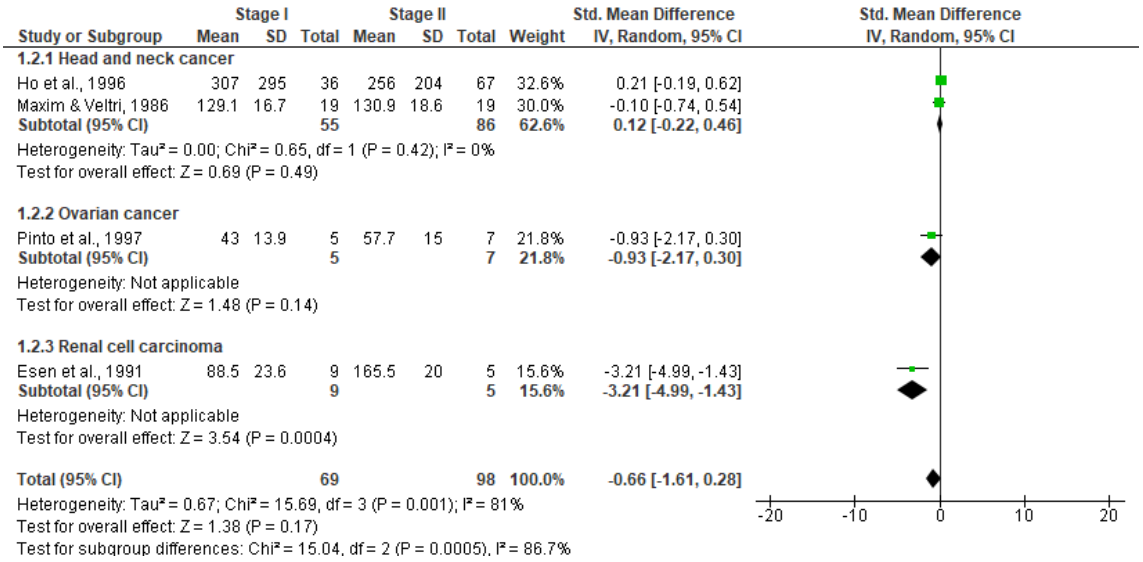
					*Subjects with a history of chronic inflammatory disorders, surgery or trauma in the past 3 months, anemia, renal or hepatic disorders were excluded from the study population.	commercially available. Kits from Diaclone Research, France.			
Macciò *et al.*, 2005 (8)	Italy	Case-control study	186	Ovarian cancer (n=91) [female 91; mean age 62.1 years; race was not reported]/ Control (n=95) [female 95; mean age 61.0 years; race was not reported]	Case: the diagnosis of ovarian cancer was histologically confirmed in all patients. The stage of ovarian cancer was carried out in accordance to the International Federation of Gynecology and Obstetrics classification system. Control: healthy women matched for age, weight, and height. * At the time of study, no patient had received surgery, radiotherapy, chemotherapy, or other medical interventions. No subject included in the study had evidence of infections, gastrointestinal disease, vaginal bleeding, or hemolysis.	SF levels in patients with stages III to IV epithelial ovarian cancer had a significantly higher serum ferritin levels in comparison to healthy individuals and patients at stages I to II ($p < 0.05$). * Did not report the evaluated method.	Not reported	Not reported	The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked ‘‘advertisement’’in accordance with 18 U.S.C. section 1734.
Pinto *et al.*, 1997 (8)	Italy	Case-control study	120	Ovarian cancer (n=60) [female 60; mean age 54.0 years; race was not reported] /Control (n=60) [female 60; mean age 50.0 years; race was not reported]	Case: ovarian carcinoma underwent surgery at the First Department of Obstetrics and Gynecology, University Medical School of Bari, Italy. Serum ferritin values were obtained preoperatively. To exclude the possible influence	SF levels were statistically significant on ovarian cancer group ($p = 0.005$). The serum ferritin levels increased markedly with advancing disease stage. * SF levels was evaluated	Not reported	Not reported	Not reported

					of iron deficit or accumulation on ferritin levels, the iron level in serum was measured at the same time. Preoperative ferritin levels were correlated with stage, histology and grading of ovarian cancer. Patients affected by other neoplasms, either synchronous or previous, or showing defective liver functioning, or having undergone neo-adjuvant chemotherapy were excluded from this study. Control: healthy, non-pregnant, age-matched controls, tested in the context of a gynecological screening program.	using the Immulite method.			
Fabris *et al.*, 1984 (7)	Italy	Case-control study	49	Pancreatic cancer (n=29) [male 18, female 11; 41 to 70 years old; race was not reported]/ Control (n=20) [male 14, female 6; 23 to 39 years old; race was not reported]	Case: the diagnoses were established on the clinical picture and on the results of relevant radiologic and/or histologic examinations. Control: healthy members of the medical staff.	SF levels were higher in pancreatic cancer group than control group ($p<0.01$). * SF determination was carried out using an immunoradiometric procedure (Fcr-Iron kit. Ramco Laboratories. Houston, Tex.).	Not reported	Not reported	Not reported
Nitti *et al.,* 1982 (8)	Italy	Case-control study	40	Pancreatic cancer (n=22) [male 12, female 10; 41 to 70 years old; race was not reported]/ Control (n=18) [male 13, female 5; 23 to 39 years old; race was not reported]	Case: the diagnosis was established in all the cases at laparotomy and by means of surgical biopsies. Control: healthy members of the medical staff with no previous gastrointestinal diseases.	SF levels were higher in pancreatic cancer group than control group ($p<0.01$). * SF was assessed by immunoradiometric procedure (Fer-Iron kit. Rarnco Laboratories. Houston. Tex.. USA), according to the instruction included in the kit.	Not reported	Not reported	Not reported

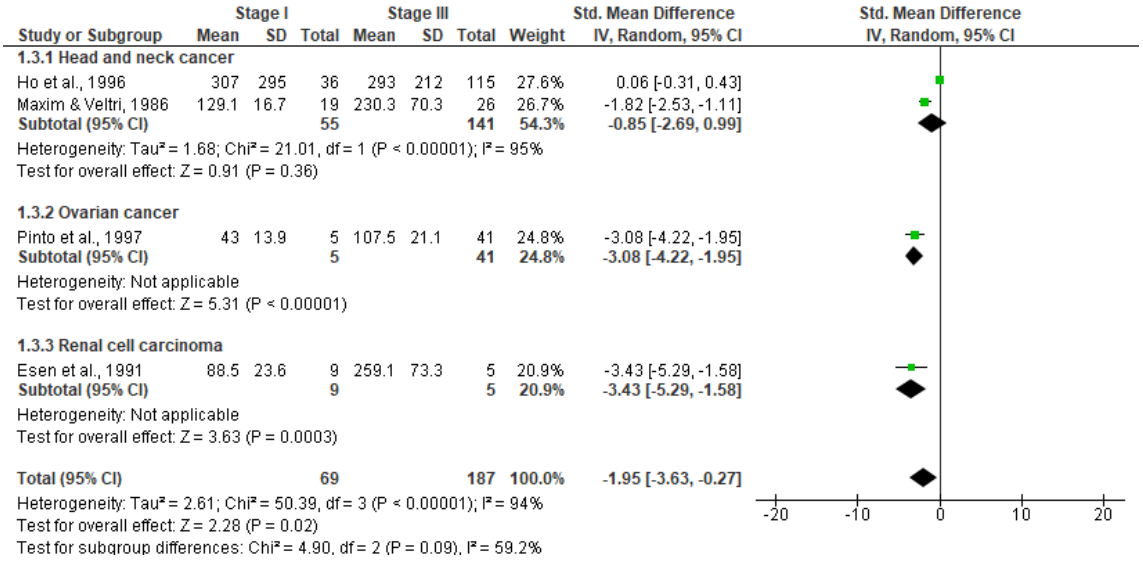
Kuvibidila *et al.*, 2004 (7)	USA	Case-control study	99	Prostate cancer (n=27) [male 27; mean age 59.2 years; African-American 25, and white 2]/ Control (n=72) [male 72; mean age 55.3 years; African-American 45, and white 27]	Case: all of the men participated in an ongoing prostate-cancer screening and early-detection program at the Louisiana State University Health Sciences Center in New Orleans. Prostate biopsy was offered to participants whose PSA values were greater than 2.5 ng/mL as a means of obtaining a definitive histopathologic diagnosis (newly diagnosed and untreated prostate cancer) Control: after prostate cancer was confirmed in the 27 men, frozen serum samples for controls were chosen from a pool of approximately 6000 on the basis of the following criteria: (i) a PSA level below the threshold (2.5 ng/mL) for the recommendation of prostate biopsy (n=66), (ii) a PSA level greater than 2.5 ng/mL but biopsy results negative for prostate cancer (n=6), and (iii) lack of clinical symptoms of any illness or complaints at the time of study entry.	SF levels were higher in control group than case group (p=0. 0117). * They measured serum ferritin in previously frozen serum samples using enzyme-immunoassay kits purchased from RAMCO (Houston, Texas), carefully following the instructions provided with the kits. Test samples, standards, and controls were studied in duplicate.	Not reported	None	Supported by general research funds from the Departments of Pediatrics and Urology.
Esen *et al.,* 1991 (7)	Turkey	Cross-sectional study	54	Renal cell carcinoma (n=32) [male 23, female 9; mean age 54.7 years; and race was not reported]/ Control (n=22) [mean age 52.6 years; sex and race was not reported]	Case: patients with histologically confirmed renal cell carcinoma were included. Patients with elevated liver function tests were excluded. Control: healthy matched blood donors screened by internal medicine department served as controls.	SF level in patients with renal cell carcinoma was significantly higher than that of control values ($p < 0.01$). * SF was measured using the ferritin radioimmunoassay kit (Omnia catalog IDS AJIFI).	Not reported	Not reported	Not reported

Ozen *et al.*, 1995 (9)	Turkey	Case-control study	259	Renal cell carcinoma (n=158) [male 161, female 61; median age 54 years; and race was not reported]/ Control (n=101) [sex, age and race was not reported]	Case: patients with histologically confirmed renal cell carcinoma were included. Patient were excluded from the study due to the lack of preoperative ferritin. Fasting serum ferritin were obtained from all patients in addition to complete preoperative radiologic and bio- chemical evaluation, including computed tomography of the abdomen and chest. Pathologic staging revealed 63 cases with Stage I disease, 22 with Stage II, 29 with Stage III, and 44 with Stage IV. Control: healthy matched blood donors in whom all biochemical evaluations were completely normal served as control subjects.	SF level in patients with renal cell carcinoma was significantly higher than that of control values ($p < 0.01$). * SF was measured using the ferritin radioimmunoassay kit (Omnia Ferritin Kit Code: AA-51Fl).	Not reported	Not reported	Not reported
Singh *et al.,* 2005 (8)	India	Case-control study	64	Renal cell carcinoma (n=32) [male 24, female 8; mean age 52.8 years; and race was not reported]/ Control (n=32) [sex, age and race was not reported]	Case: any patient with radiological evidence of RCC. Patients with liver disease, history of blood transfusion in last 6 months or anemia were excluded from the study. All patients were evaluated by detail history and physical examination, hemogram, liver function tests, renal function tests, chest X ray, and computerised tomography abdomen. Control: the controls comprised of age and sex-matched healthy volunteers.	SF level in patients with renal cell carcinoma was significantly higher than that of control values ($p < 0.001$). * Ferritin levels in serum were estimated using enzyme immunoassay method (Omega Diagnostic Ltd., Pathozyme-Ferritin Kit, FL, USA).	Not reported	Not reported	Not reported

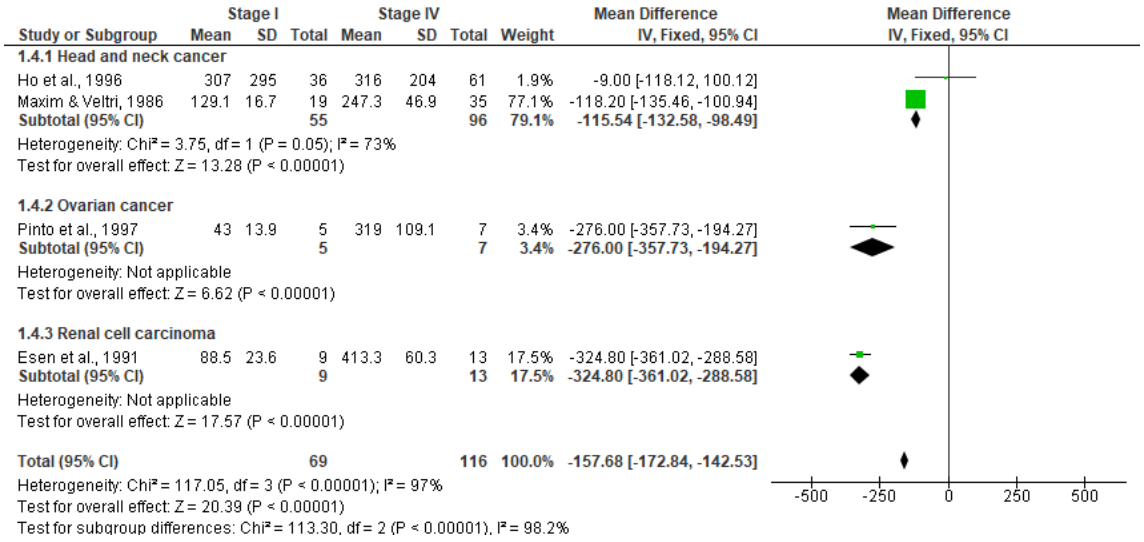
Supplement 4: Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages and the control group.



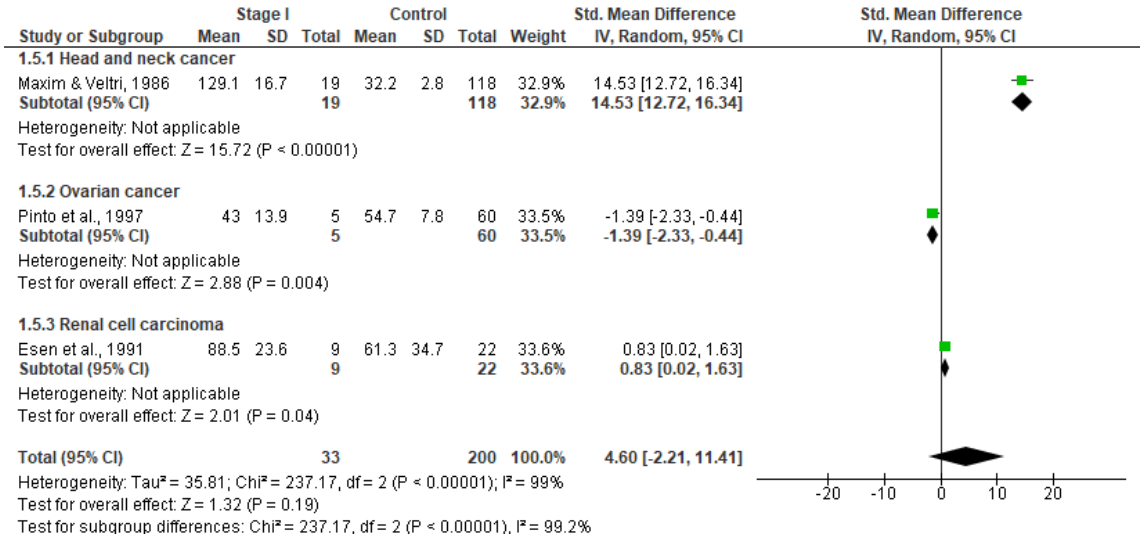
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage I and Stage II). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and not differences ferritin values in cancer patients and controls.



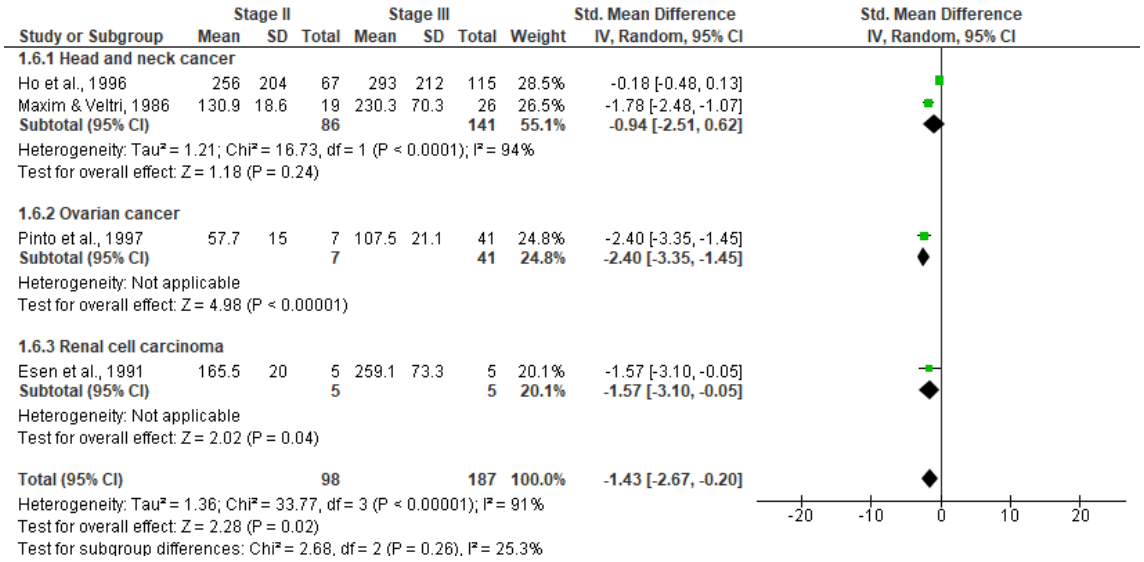
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage I and Stage III). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows higher global ferritin values in Stage III compared to Stage I.



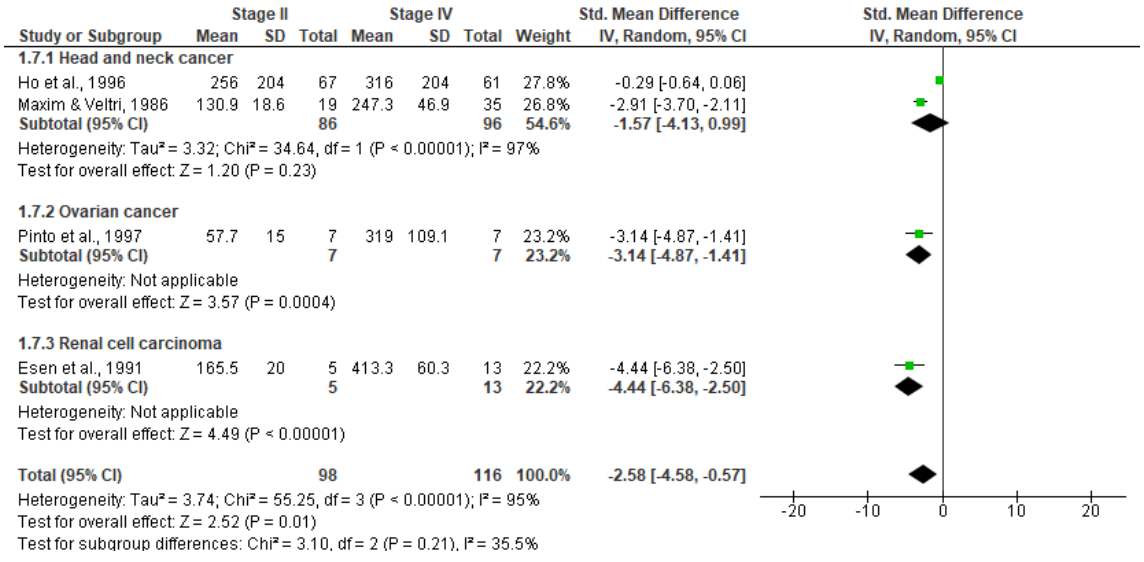
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage I and Stage IV). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and higher global ferritin values in Stage IV compared to Stage I.



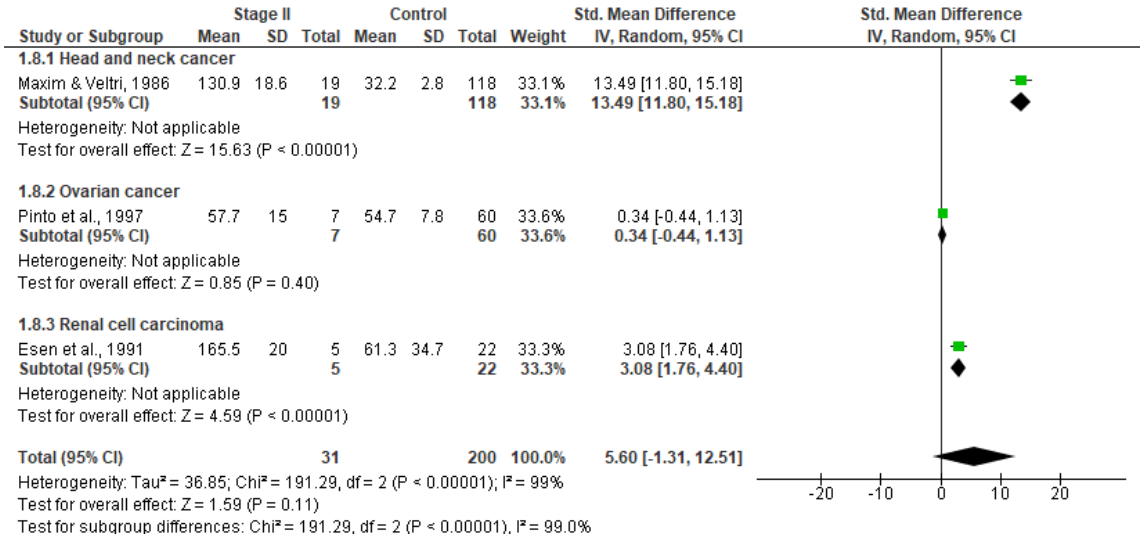
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage I and Control). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and not differences ferritin values in Stage I and controls.



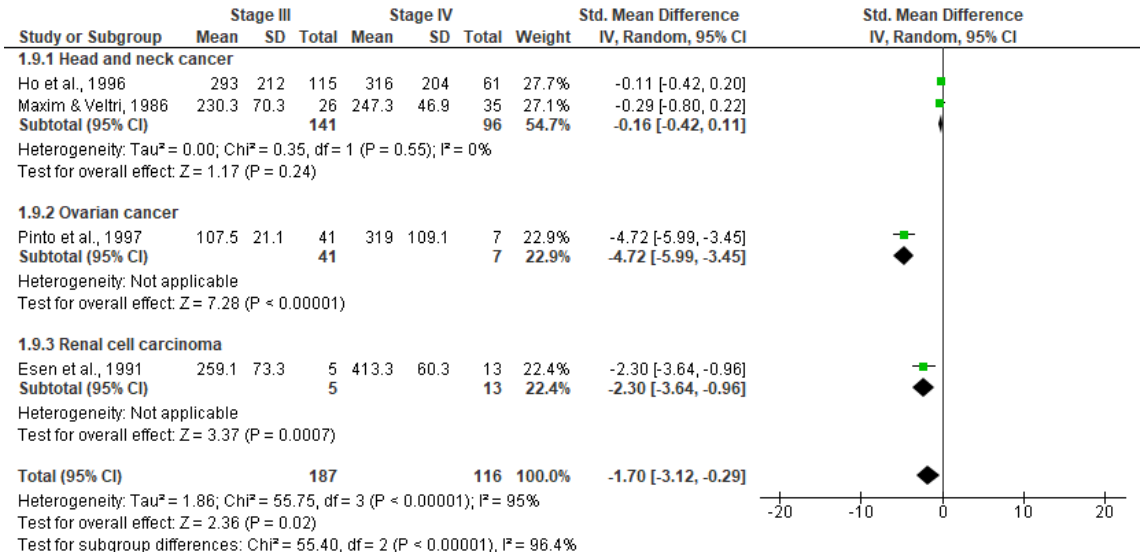
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage II and Stage III). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows higher global ferritin values in Stage III compared to Stage II.



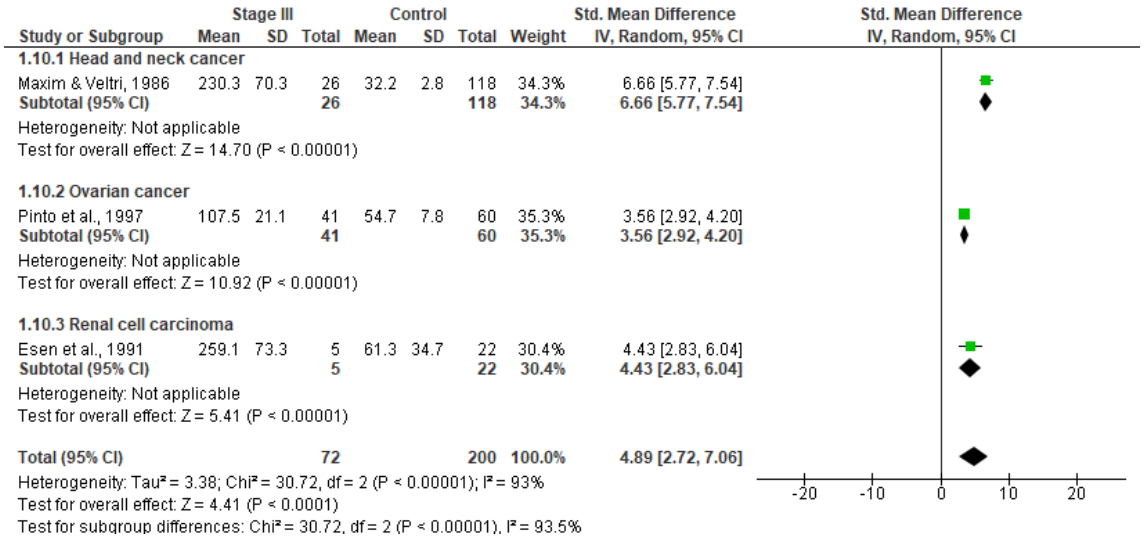
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage II and Stage IV). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows higher global ferritin values in Stage IV compared to Stage II.



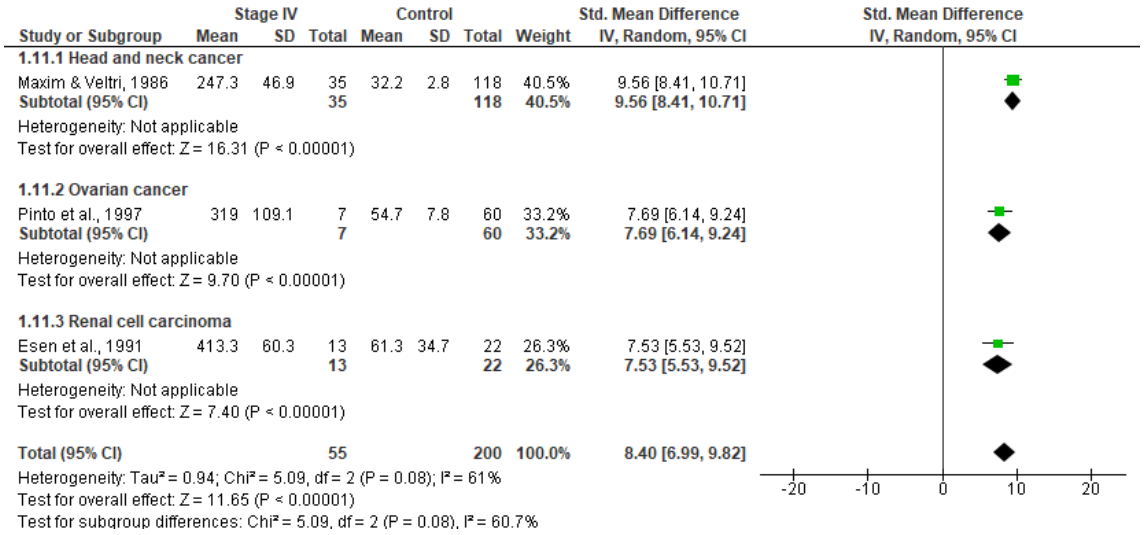
Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage II and Control). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and not differences ferritin values in Stage II and controls.



Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage III and Stage IV). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and higher global ferritin values in Stage IV compared to Stage III.



Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage III and Control). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows high variability among subgroups, and higher global ferritin values in Stage III compared to control.



Meta-analysis relating to serum ferritin levels (ng/ml) in TNM stages (Stage VI and Control). SD: standard deviation, IV: inverse variance, and CI: confidence interval. The graph shows higher global ferritin values in Stage IV compared to control.

ANEXO D

SUBMISSION RULES OF SCIENTIFIC REPORTS: PROSTATE INTERNATIONAL

Prostate International (Prostate Int, PI), the official English-language journal of Asian Pacific Prostate Society (APPS)and Korean Prostate Society (KPS)is an international peer-reviewed academic journal dedicated to basic and clinical studies on prostate cancer, benign prostatic hyperplasia, prostatitis, and other prostatic diseases. It is published four times per year, March 30, June 30, September 30, and December 30. Its formal abbreviation is Prostate Int. Original articles and topical reviews on various prostate-related conditions and problems are published in Prostate International, covering the state-of-the-art contents. Analysis articles, Technical reports, and invited/commissioned meeting reports are also published in Prostate International. Prostate International represents the only academic journal devoted to various prostatic diseases in Asian Pacific region. The incidence, characteristics, and management of various diseases may vary according to region and race. Prostate International brings solid coverage of prostatic diseases in Asian Pacific men. Prostate International also serves as a medium for cooperation amongst urologists and specialists from around the world focusing on various aforementioned prostatic conditions. All or part of Prostate International is indexed/tracked/covered by DOI/Crossref, Google Scholar, ScienceCentral,ScienceDirect,PubMed and Scopus.

Types of paper *1. Review article*Review articles shall be limited to an invited review article, which, by the Editorial Board, was selected as a significant theme from areas relevant to prostate disease field and whose authors were selected and referred on the basis of articles published in this or other journals. The submitted manuscript should be decided to be published via reviewing of the Editorial Board. The length of the manuscript should not exceed 3,500 words except for the cover, tables, figures, and references. The works in the references should not exceed 100.

2.Original article The manuscript for original articles should be organized in the following order: 1) title page, 2) abstract and keywords, 3) introduction, 4) materials (or subjects) and methods, 5) results, 6) discussion, 7) conflict of interest, 8) acknowledgments (if necessary), 9) references, 10) tables, 11) figures and photos, and 12) legends. The manuscript should be provided in MS Word file (doc, docx), double spaced on 212×297 mm (A4 size) with 2.5 cm margins at the top, bottom, and left margin. The length of the manuscript should not exceed 3,000 words except for the cover, tables, figures, and references. No more than 35 references can be cited. All manuscript pages are to be numbered consecutively, beginning with the abstract as page 1. Neither the authors' names nor their affiliations should appear on the manuscript pages. The use of acronyms and abbreviations is discouraged and should be kept to a minimum. When used, they are to be defined where first used, followed by the acronym or abbreviation in parentheses. Abbreviations are not allowed in the title. The names and locations (city, state, and nation) of manufacturers of equipment and nongeneric drugs should be given. When quoting from other sources, give a reference number in bracket after the author's name or at the end of the quotation.

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Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified (see relevant section below).

Articles covering the use of human samples in research and human experiments must be approved by the relevant review committee (see relevant section below).

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•You may use automatic page numbering, but do NOT use other kinds of automatic formatting such as footnotes.

•Put text, references, tables, and legends in one file, with each table on a new page.

Article Structure The text for Original Articles, for example, should include the following sections: Introduction, Materials and Methods, Results, and Discussion. The Introduction should be as concise as possible, without subheadings. The Methods section should be sufficiently detailed. Subheadings may be used to organize the Results and Discussion. Each section should begin on a new page. **Introduction**The Introduction should provide a brief background to the subject of the paper, explain the importance of the study, and state a precise study question or purpose. **Material and methods**The Methods section should describe the study design and methods (including the study setting and dates, patient samples or animal specimens used, with inclusion and exclusion criteria, the laboratory methods followed, or data sources and how these were selected for the study, the essential features of any interventions, the main outcome measures), and state the statistical procedures employed in the research.

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The Results section should comprise the study results presented in a logical sequence, supplemented with tables and/or figures. Take care that the text does not repeat data that are presented in the tables and/or figures.

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The Discussion section should be used to emphasize the new and important aspects of the study, placing the results in context with published literature, the implications of the findings, and the conclusions that follow from the study results.

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An abstract is required for the following article categories: Review Article, Original ArticleAbstracts should be no more than 300 words in length.Abstracts for Original Articles should be structured, with the section headings: Background, Methods, Results, Conclusion. Abstracts for Review Articles are unstructured in one single paragraph.

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1. Kim WT, Kim WJ. MicroRNAs in prostate cancer. Prostate Int 2013;1:3-9.

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2.Oguro, M, Imahiro, S, Saito, S, Nakashizuka, T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. http://dx.doi.org/10.17632/xwj98nb39r.1.

Book:

Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, editors. Campbell-Walsh urology. 9th ed. Philadelphia: Saunders; 2007.

Book chapter:

Klein Ea, Platz EA, Thompson IM, Epidemiology, etiology, and prevention of prostate cancer. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, editors. Campbell-Walsh urology. 9th ed. Philadelphia: Saunders; 2007. p. 2854-73.

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