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DOMENIUL MEDICINĂ GENERALĂ

Correlation of myometrial invasion in endometrial cancer in patients with metabolic syndrome

DOCTORAL THESIS ABSTRACT

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1. GENERAL PART

Chapter 1. Endometrial cancer

The definition of uterine cancer includes two types of neoplasia: endometrial cancer (EC) and uterine sarcoma. EC originates in the endometrial lining and is more commonly diagnosed, while uterine sarcoma, a less common form, develops in the myometrium [1].

Globally, cancer of the uterine corpus is recognized as the 15th most common form of cancer, regardless of sex or age, with 420,369 new cases diagnosed in 2022. In terms of cancer mortality rate, it ranks 19th with 97,723 deaths recorded in the same year. Worldwide incidence among females puts uterine cancer in 6th place after breast, lung, colorectal, cervical and thyroid neoplasms. An analysis of the incidence of uterine cancer in developed (high-income) countries shows that it ranks fourth after breast, colorectal and lung cancers. The incidence rate in these countries is 15.84 per 100,000 women and the mortality rate is 2.45 per 100,000 women. In upper middle-income countries (including Romania) uterine cancer ranks 6th (incidence rate 8.5 per 100,000 women, mortality rate 1.6 per 100,000 women) [2].

Several well-established risk factors contribute to EC, which can be broadly categorized as follows: endocrine factors, metabolic factors and genetic factors. The prognostic factors in the etiology of EC are clinical factors (age, menopausal status, race), anatomopathologic factors (uterine factors - histologic subtype, histologic grade, myometrial invasion, lymphovascular space invasion, invasion of lower uterine segment and cervical stroma, and extrauterine factors - positive peritoneal cytology, adnexal involvement, serosal involvement, lymph node metastasis) [3-6].

Chapter 2. The role of metabolic syndrome in endometrial cancer

MetS is a group of cardio-metabolic risk factors that significantly increase the risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [7]. However, specific diagnostic criteria for MetS have shown substantial variation between different organizations over the past three decades. This heterogeneity in the definitions of MetS presents challenges for clinical practice, epidemiologic research, and public health initiatives.

Obesity contributes to endometrial carcinogenesis through a number of molecular and metabolic mechanisms. Adipose tissue goes beyond simply storing fat, acting as an active endocrine organ. Adipocytes synthesize a wide range of hormones, including leptin, adiponectin, and interleukin-6 (IL-6). These findings emphasize the contribution of adipose tissue

to the creation of a complex hormonal microenvironment that may modulate cancer risk [8-10]. Chronic inflammation, a complex process triggered by cell dysfunction, is a second potential mechanism involved in EC aetiology. German researchers elucidated the potential roles of RI, MetS and inflammation in increasing the risk of EC in postmenopausal women. The results of their study suggest a complex interplay between hormonal and metabolic factors, highlighting the potentially significant role of inflammation, beyond hormonal imbalances, in the development of EC [11].

Obesity induces adipose tissue dysfunction, characterized by chronic low-grade inflammation. This inflammation, unlike transient acute inflammation, manifests as a persistent change. It is characterized by infiltration of adipose tissue with lymphocytes and macrophages. Macrophages become polarized towards a proinflammatory M1 phenotype, further amplifying the inflammatory response [12-15]. Obesity is associated with impaired insulin signaling, partly due to inflammatory signals generated by dys-functional adipose tissue. Insulin resistance (IR) is a major consequence of this dysfunction, resulting from disrupted insulin signaling. Unlike other receptors in the tyrosine-kinase family, insulin and IGF receptors rely on scaffolding proteins to transmit intracellular signals.

Intracellularly, insulin activates insulin receptor substrate proteins (IRS) (IRS-1 through -6) by phosphorylating tyrosine. This crucial step is disrupted in most cases of IR. TNF-α, a proinflammatory cytokine, exacerbates this dys-function by promoting inhibitory serine phosphorylation of IRS-1. This modification, commonly observed in insulin-resistant cells and tissues, blocks intracellular transmission of the insulin message. Specific kinases, such as JNK and inhibitor of nuclear factor kappa-B kinase beta (IKKβ), play a crucial role in this disruptive process [16].

There is also a worrying global increase in EC, particularly in developed countries, a trend that parallels the increasing prevalence of DM, a condition demonstrably linked to the development of EC. A meta-analysis revealed a 72% higher risk for EC in women diagnosed with DM compared to the non-diabetic population [17]. While the precise mechanisms underlying this association remain under investigation, two potential pathways are postulated: hormonal dysregulation and chronic hyperglycemia. Studies support a positive association between hyperglycemia and the incidence of EC. A large Swedish study observed an increased risk of EC in diabetic women (HR 1.46) and those with abnormal glucose metabolism (HR 1.41) [18]. Similar findings emerged from a case-control study, in which fasting hyperglycemia correlated with an increased risk of EC (OR 1.36) [18]. Meta-analyses

have further strengthened this association, demonstrating a significantly increased risk of EC in people with DM (RR 1.89) [19].

Dislipidemia, o afecțiune caracterizată prin profiluri anormale ale lipidelor serice, a apărut ca o preocupare semnificativă în contextul dezvoltării EC. Studii recente din China raportează o prevalență îngrijorător de ridicată a dislipidemiei care depășește 40% în rândul populației adulte. În special, dislipidemia transcende rolul său stabilit ca factor de risc pentru bolile cardiovasculare, demonstrând o asociere îngrijorătoare cu diverse tumori maligne, inclusiv cancerul de sân, ovarian și EC. În mod intrigant, dislipidemia pare să fie intim legată nu numai de inițierea, ci și de progresia EC, reprezentând potențial cea mai proeminentă alterare metabolică observată în această afecțiune malign [20].

2. SPECIAL PART (PERSONAL CONTRIBUTIONS)

Chapter 3. General objectives and hypotheses

3.1. General objectives of the doctoral thesis

The present work has centered its attention on the degree of myometrial invasion in patients diagnosed with EC with associated MetS. To comprehensively address this topic, the research will pursue the following scientific objectives:

1. To quantify the correlation between the presence of MetS and the degree of invasiveness in a well-defined cohort of patients diagnosed with EC. To define MetS in the study population, established diagnostic criteria such as the NCEP ATP III criteria will be used to define MetS. The degree of invasiveness will be determined based on established pathologic criteria used for FIGO staging of EC. This objective will involve the use of robust statistical methods to assess the existence and strength of a statistically significant association between MetS and invasiveness.
2. Building on the initial findings of the correlation analysis, this research can deepen to explore potential biological mechanisms that might link various components of MetS to aggressive EC behavior.
3. The ultimate goal may be to develop a risk stratification model that incorporates MetS status. This model could serve as a valuable tool to improve patient management within the healthcare system. By identifying women at higher risk of developing aggressive EC based on MetS status, this model could facilitate earlier

intervention and improve patient outcomes. The feasibility of achieving this goal will depend on the robustness of the observed correlation between MetS and depth of invasion. A strong correlation would provide a solid basis for the development of a clinically useful risk stratification model. Such a model could be particularly valuable in the context of the Romanian healthcare system, where both MetS and EC are growing public health concerns.

3.2. Research hypotheses

This doctoral thesis focuses on a central hypothesis that probes the potential association between MetS and EC aggressiveness. We hypothesize that patients diagnosed with EC who also present with MetS are more likely to have a higher degree of invasiveness compared to those without MetS. In rudimentary terms, this research aims to investigate whether women with MetS are more likely to have a more aggressive form of EC characterized by deeper myometrial infiltration.

Chapter 4. General research methodology

4.1. Study type

This study uses a retrospective, descriptive, analytic-observational (case-control) approach to investigate endometrial lesions.

4.2. Study population

The present study included a cohort of 670 patients divided into two distinct groups:

- Group 1 (n=192): patients diagnosed with EC, histopathologically (HP) confirmed.
- Group 2 (n=478): patients diagnosed with EH, HP confirmed.

Patient data were collected from hospitalization records obtained from the external and internal archives of Elias Hospital. Anonymized electronic health records served as data source. The data span a comprehensive eight-year period from January 1, 2015 to December 31, 2022. In order to conduct our study, relevant data was collected and centralized in a Microsoft Excel table. This computer tool served as a database, ensuring efficient organization and easy management of the information.

The common variables for the two groups of patients included in the present study are: age, ethnicity, level of education, marital status, number of abortions, parity and type of delivery, age and time (preoperative or postoperative) of menopause onset, tobacco use and

number of pack-years, personal history of COC use, metabolic factors (HTA, DM, TG, HDL-C, BMI), serologic factors (complete blood count, fibrinogen and CRP). I mention that in the absence of the abdominal circumference variable, we used the BMI variable. We also used the HP report cards, grouping them on this basis into the two groups (EC and EH).

Variables included strictly for the EC group: tumor stage and grade, cervical stromal involvement, parametrial involvement, adnexal involvement, LVSI, ganglion and organ metastsis. For a complete staging of these cases I mention that we also followed information from the anatomopathological and imaging spheres, namely magnetic resonance imaging and computed tomography).

Inclusion criteria for the study were:

- histopathologic diagnosis of EC or EH confirmed by endometrial biopsy or operative specimen.
- women older than 18 years of age.

Exclusion criteria were represented by:

- women younger than 18 years of age;
- patients whose ECs were not primary cancers;
- presence of other concomitant gynecologic malignancies or severe general medical conditions (severe cardiovascular disease, neoplastic disease, etc.).

Confidentiality and Ethics: This study was approved by the Institutional Review Board of SUU Elias, Bucharest, Romania (approval number: 7172/26.08.2022). To ensure participants' confidentiality, all data were anonymized before analysis.

Statistical Methods: This study used the Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA) and XLSTAT (version 2023.3.1.1416) for statistical analyses. Prior to analysis, data were assessed for normality using appropriate statistical tests. Normally distributed continuous variables were presented as mean ± standard deviation (SD). Categorical variables were summarized as frequency (n) and percentage (%).

Chapter 5. Descriptive analysis of the study groups

5.1. Introduction

Our study explores a key hypothesis related to the comparative clinical and demographic characteristics of EC and EH patient groups. We hypothesize that there is a correlation between the clinical and demographic characteristics of patients at increased risk of developing EC. This hypothesis aligns with established research suggesting that these factors con-

tribute to an increased hormonal load, potentially influencing endo-metasthelial cell growth and possibly leading to malignancy.

This study aims to characterize the endometrial lesions of women hospitalized in the Elias Hospital, Bucharest, Romania. By identifying areas for improvement in patient care, we hope to ultimately improve women's reproductive health outcomes.

Specific objectives:

- To identify the most susceptible age groups for specific endometrial pathologies.
- To characterize socio-demographic and economic factors associated with endometrial lesions.
- Investigate potential associations between endometrial pathology and personal medical history.
- Classify lesions based on clinical presentation and pathologic anatomy.
- Assess the distribution of malignant and benign endometrial lesions.
- Analyze the frequency of malignant endometrial lesions according to histologic stage and grade.

5.2. Material and method - see general research methodology

5.3. Results

Baseline characteristics: Patients diagnosed with EC had menarche at a significantly younger age (mean: 11.11±1.10 years) compared to their counterparts in the EH group (mean: 13.77±1.12 years). No significant disparities were observed between groups in terms of parity (mean 1.52 children in EC vs. 1.62 children in EH) or mean age at menopause (48.71 years in EC vs. 48.37 years in EH). However, a notable difference emerged when examining OC use. The prevalence of OC use was demonstrably higher in the EC group (37.5%) compared to the EH group (25.5%). EC patients had a significantly higher mean BMI (36.58 ± 5.598) compared to the EH group (29.945 ± 5.315).

Characteristics of the EC group:

Histologic distribution: HP examination of EC specimens revealed a pre-dominance of EEC, accounting for 85.43% of cases. This finding aligns with established data, highlighting CEE as the most common and generally favorable histological subtype in EC. Less common histologic subtypes have been identified, including mixed carcinomas (4.69%), serous carcinomas (4.17%), clear cell carcinomas (3.65%), mucinous carcinomas (2.08%)

and carcinosarcomas (1.56%). In particular, serous carcinomas and carcinosarcomas are usually associated with a more aggressive clinical course.

FIGO Staging: The FIGO staging system has been used to classify the stage of disease of patients with EC, providing valuable prognostic information regarding patient outcomes. It is important to note that the majority of patients (71.87%) had early stage disease (stage I). This finding suggests a potentially favorable prognosis, as early stage EC is generally associated with better treatment outcomes. A significant proportion of patients (18.22%) were diagnosed with stage III disease, indicating a more advanced stage requiring more aggressive treatment strategies. Stages 0, II and IV were seen in a smaller proportion of patients (2.60%, 6.25% and 1.04%, respectively).

Lymph node invasion: Assessment of lymph node involvement is crucial to guide surgical management and to tailor treatment plans for patients with EC. In the majority of cases (88.54%) no LNI was present.

5.4. Conclusion

This study identified a number of statistically significant differences between patients with EC and those with EH. These findings suggest that there are a number of factors that may increase the risk of developing one of these two conditions. Further studies are needed to confirm these findings and to elucidate the underlying mechanisms by which these risk factors may contribute to the development of EC and EH.

Chapter 6. The role of metabolic factors in endometrial cancer diagnosis

6.1. Introduction

The working hypothesis that initiated the present study started from the existence of a statistically significant association between lipid profile, metabolic factors and risk of developing EC, with significant implications for early diagnosis, prevention and treatment personalization.

In terms of specific objectives, we pursued three important aspects, namely:

1. Detailed assessment of the lipid profile by comparing total cholesterol, triglyceride (TG), HDL-C and LDL-C levels in patients with EC and EH; analyzing subgroups of patients with specific characteristics such as age, BMI, menopausal status to identify possible variations in the association between lipid profile and EC risk; Exploring the potential of triglyceride-triglyceride-glycemic index (TyG) and TG/HDL-C ratio as

additional biomarkers for EC risk, given their involvement in atherosclerosis and inflammation, factors associated with the neoplastic process.

2. Comprehensive assessment of metabolic factors by measuring insulin resistance (IR) by a recently established method, the TyG index, a surrogate biomarker for the HOMA-IR index; by assessing fasting blood glucose levels to identify possible abnormalities of carbohydrate metabolism.
3. To identify lipid and metabolic biomarkers for early diagnosis by assessing the diagnostic performance of individual biomarkers; by developing biomarker panels combining multiple lipid and metabolic markers to improve the accuracy of early diagnosis of EC.

6.2. Material and method

Study design

We conducted a single-center, retrospective, descriptive, analytic, retrospective study to assess the potential association between metabolic factors including hyperglycemia, lipid profile abnormalities and the presence of modifiable risk factors and the development of EC.

Study population

Two separate cohorts were created for this study. The first cohort comprised 192 women over the age of 18 years with a HP result of EC. The second cohort included 198 women over the age of 18 years diagnosed HP with EH in the same time period as the EC cohort.

Collection of clinical and paraclinical data

Following meticulously standardized protocols, anonymized data were extracted from electronic medical records. The variables extracted covered a broad spectrum of patient information, categorized as follows:

- Demographic, clinical and histopathologic characteristics: see General research methodology.
- Biochemical markers:
    - Fasting blood glucose and HbA1c;
    - Complete lipid profile including TC, TG, HDL-C and LDL-C;
    - Triglyceride-to-glycemic index (TyG) and triglyceride-HDL-C ratio.

Blood samples were collected from all fasting patients within a preoperative window of 24-48 hours. In addition, established definitions and cut-off points for all biochemical

markers analyzed were rigorously applied to ensure optimal comparability of data within the study population.

The calculation of the TyG index used the established formula, defined as the natural logarithm (Ln) of [fasting triglyceride level (mg/dL) x fasting plasma glucose (mg/dL)] divided by 2, for clarity and consistency with the existing literature. TG/HDL-c ratio is simply the ratio of triglycerides (mg/dL) to high-density lipoprotein (mg/dL).

Statistical analysis

In the EC group, in particular, a receiver operating characteristics curve (ROC) analysis was performed to assess the diagnostic efficacy of the TyG index and TG/HDL-c ratio. ROC analysis assesses the ability of a biomarker to differentiate between two groups (in this case, different degrees of myometrial invasion). The analysis generates an ROC curve, and the area under the curve (AUC) serves as a quantitative measure of the discriminatory power of the biomarkers.

6.3. Results

This retrospective analysis investigated the baseline characteristics of a cohort of 390 patients. There was a statistically significant difference in mean age at diagnosis between the two groups (62.42 years ± 10.62 for EC vs 59.16 years ± 10.74 for EH, $p = 0.003$).This finding suggests a potential association between advancing age and an increased susceptibility to develop EC. Women with EC had an earlier menarche (11.11 years ± 1.10) compared to the EH group (13.74 years ± 1.20) ($p < 0.0001$).

Parity was significantly different between groups. The mean number of births was lower in the EC group (1.52) compared with the EH group (1.85), with a statistically significant difference. Similarly, the mean number of abortions showed a significant difference (2.05 in EC versus 2.87 in EH). The mean age at menopause did not show a statistically significant difference between the two groups (48.71 years in EC vs. 49.14 years in EH). A significant difference in mean BMI was observed between the EC and EH groups ($p < 0.0001$). Women with EC had a higher mean BMI (36.58 ± 5.598 vs. 32.16 ± 5.98).

While fasting blood glucose levels were marginally higher in the EC group compared with the EH group, the high standard deviation observed within the EC group suggests a wider range of individual values. HbA1c, a marker of long-term glycemic control, also showed a modest increasing trend in the EC group (5.90%) compared with EH (5.43%).

EC patients had slightly elevated total cholesterol (222.27 mg/dL) and LDL-C (141.68 mg/dL) compared to the EH group (215.15 mg/dL and 140.51 mg/dL, respectively).

Also, HDL-C levels were significantly higher in the EH group (50.90 mg/dL) compared to the EC group (45.47 mg/dL). In contrast, triglycerides were significantly elevated in the EC group (172.67 mg/dL) compared to the EH group (118.68 mg/dL).

The EC group showed a significantly higher mean TyG index compared to the control group (mean difference 0.48, 95% CI 0.39-0.57, $p < 0.0001$). Similarly, the TG/HDL-c ratio showed a statistically significant difference (mean difference 1.56, $p < 0.0001$) between groups (see Figure 6.1 for detailed data). 95% CI (1.27-1.86) emphasizes a potentially elevated mean TG/HDL-c ratio in the EC group compared to the EH group.

We further investigated the potential utility of these recently introduced markers (TyG index and TG/HDL-c ratio) in stratifying the depth of myometrial invasion in the EC cohort. ROC curve analysis was used to assess the ability of the TyG index to discriminate between tumors with less than 50% myometrial invasion and those with 50% or greater myometrial invasion. The resulting area under the curve (AUC) of 0.586 indicates a poor to moderate discriminatory ability. Similarly, ROC curve analysis of the TG/HDL-c ratio yielded an AUC of 0.603, suggesting a marginally improved discriminatory ability compared to the TyG index. However, this value still indicates a poor to moderate performance in differentiating between the two invasion depth categories.

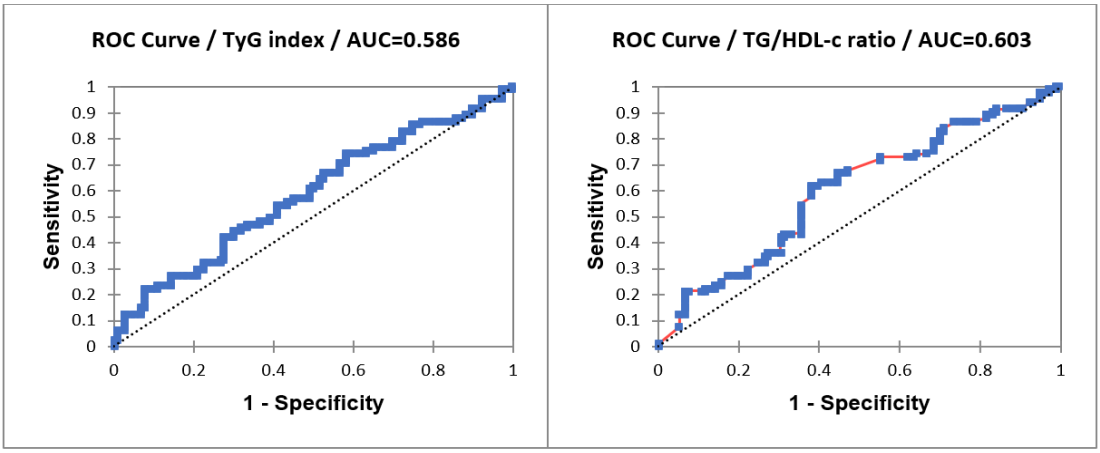

Figure 6.1. ROC curve analysis for TyG index and TG/HDL-C ratio in the EC group and association with myometrial invasion

6.5. Conclusion

This study contributes to the growing body of evidence that explores the complex interplay between metabolic health and gynecologic pathologies. Our analysis revealed distinct metabolic profiles between patients diagnosed with EC and EH. The EC group tended towards a less favorable metabolic profile, characterized by statistically significant increases

in serum TG and HbA1c levels, together with a higher mean BMI. In addition, the EC group showed a notable decrease in HDL-C concentrations.

These observations suggest a potential association between metabolic dysregulation and lipid abnormalities with the development of EC. While the TyG index and TG/HDL-c ratio did not demonstrate robust discriminatory power for assessing the invasiveness of myometrial-trial lesions in this study, exploration of alternative metabolic markers remains an important avenue for future research efforts.

Chapter 7. Role of serologic factors in the diagnosis of endometrial cancer

7.1 Introduction

EC results from a complex interplay between genetic predisposition, hormonal fluctuations and environmental exposures. This complicated 'dance' between various factors shapes the course of the disease, making diagnosis and treatment planning a multifaceted challenge.

The clinical outcome of patients with EC depends critically on established risk factors. The FIGO staging system, which incorporates factors such as depth of myometrial invasion and lymph node involvement, plays a crucial role in determining prognosis. In addition, other factors, such as histologic grade, completeness of tumor resection and serum CA-125 levels, also influence patient outcome [21].

In recent years, the potential utility of serologic parameters as biomarkers for EC has been increasingly emphasized. These readily available blood tests offer a non-invasive approach to potentially improve several aspects of EC management. This heightened interest stems from the ability of serologic parameters to provide valuable information about an individual's overall health status. Furthermore, these markers may potentially reflect underlying pathologic processes associated with the development of EC [22-24].

A causal link between inflammation and cancer was first made in 1863 by Virchow, who observed the presence of leukocytes in neoplastic tissues [25]. Beyond traditional markers, such as granulocytes and platelets, recent research has explored the utility of new inflammatory biomarkers in the diagnosis and prognosis of EC. These emerging markers, such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and monocyte/lymphocyte ratio (MLR), hold promise for improving our understanding of EC and possibly refining clinical management strategies.

The NLR has emerged as a potential independent prognostic factor in various malignancies, including EC. Studies suggest a correlation between NLR levels and patient outco-

mes, warranting further investigation into the underlying biological mechanisms. Similarly, PLR has demonstrated a potential association with clinicopathological factors such as tumor stage, grade and overall survival (OS) in patients with EC [26]. This finding emphasizes the potential of PLR as a prognostic marker for EC and further research is needed to elucidate the specific role of platelets in EC progression. Furthermore, preoperative MLR has shown promise as a predictor of disease recurrence in patients with stage I endometrioid EC [27].

The objective of this study was to compare several blood markers [red blood cell count, hemoglobin, hematocrit, hematocyte distribution width (RDW), platelet count, mean platelet volume (MPV), PDW, leukocyte count, granulocyte count, lymphocyte count, monocyte count, NLR (absolute neutrophil count to absolute lymphocyte count), PLR (absolute platelet count to absolute lymphocyte count), MRL (absolute monocyte count to absolute lymphocyte count), CRP, fibrinogen] in patients with benign and malignant endometrial pathology.

7.2. Material and method

The first group included 192 women over 18 years of age who were diagnosed with EC, and the second group of patients included 478 women over 18 years of age who were diagnosed with EH.

We extracted the following hematologic and biochemical markers:

- Complete bBlood count: It includes hematocyte count, hemo-globin level, hematocrit, RDW, leukocyte count, granulocyte count, lymphocyte count, monocyte count, platelet count, MPV and PDW;
- NLR, PLR, MLR;
- Immunology: PCR, fibrinogen.

7.3. Results

Patient population: Our study recruited a total of 670 women, who were further divided into two distinct groups based on their diagnoses:

- EC group: This group comprised 192 patients with a mean age of 62.42 ± 10.61 years.
- EH group: This group included 478 patients with a mean age of 53.12 ± 10.76 years.

Evaluation of hematologic indices, including erythrocyte count, hemoglobin concentration, hematocrit, and RDW, showed no significant differences between the EC and EH groups. Similarly, differential analysis of leukocytes, including granulocyte and monocyte

counts, showed no statistically significant variations between the groups. These findings suggest that standard hemacyte and leukocyte baseline parameters may not be discriminating factors in differentiating between EC and EH.

Interestingly, a key disparity emerged when lymphocyte and platelet counts were analyzed. The EC group had a significantly lower mean lymphocyte count compared to the EH group. This observation could indicate a potential suppression of the immune system in cancer patients, warranting further investigation into the underlying immunologic mechanisms associated with the development of EC. In contrast, the EC group had a significantly higher mean platelet count compared to the EH group.

Mean platelet volume (MPV) was also significantly elevated in the EC group compared to the EH group. However, there was no significant difference in platelet distribution width (PDW), a measure of platelet size variability, between groups. This suggests a potential role for increased platelet activation, rather than altered platelet production, in the EC context. In addition, CRP levels and fibrinogen levels were both significantly higher in the EC group compared to the EH group. Furthermore, the analysis revealed significantly elevated levels of NLR, PLR and MLR ratios in the EC group. These ratios, derived from readily available blood cell counts, provide information about the inflammatory state of the body. The collective increase in these inflammatory markers in the EC group strongly suggests a state of low-grade systemic inflammation, potentially associated with the pathogenesis or progression of EC.

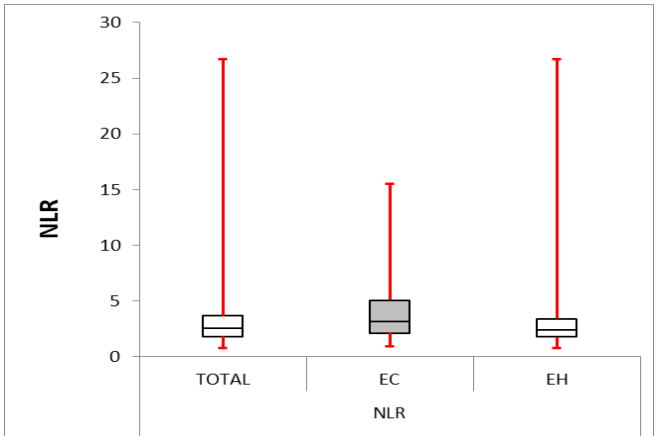

Figure 7.1. Neutrophil-to-lymphocyte ratio: a box plot comparing the NLR between the EC and EH groups is shown. The median NLR was significantly higher in the EC group (4.191) compared to the EH group (2.396, p-value = 0.004). The interquartile range (IQR) for the EC group (2.128-5.063) was higher than that of the EH group (1.786-3.421), suggesting a higher variability of the NLR within the EC group

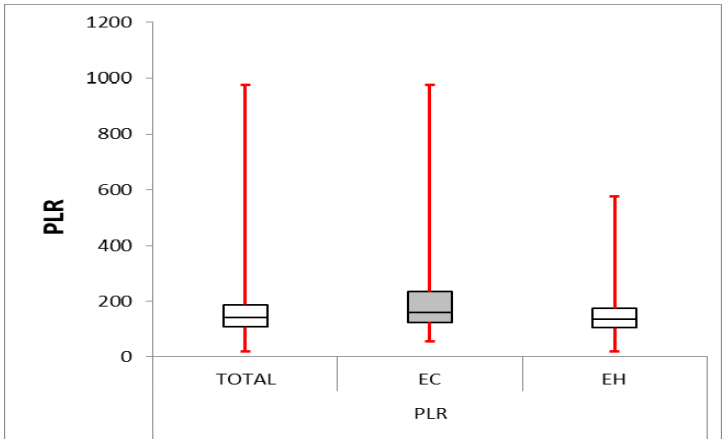

Figure 7.2. The platelet-to-lymphocyte ratio: shows a box plot comparing the PLR between the EC and EH groups. The median PLR was significantly higher in the EC group (161.204) compared to the other group (136.878, p-value < 0.0001). The IQR for the EC group (123.986-234.329) was more significant than for the EH group (105.089-174.808), suggesting a higher variability of PLR within the EC group.

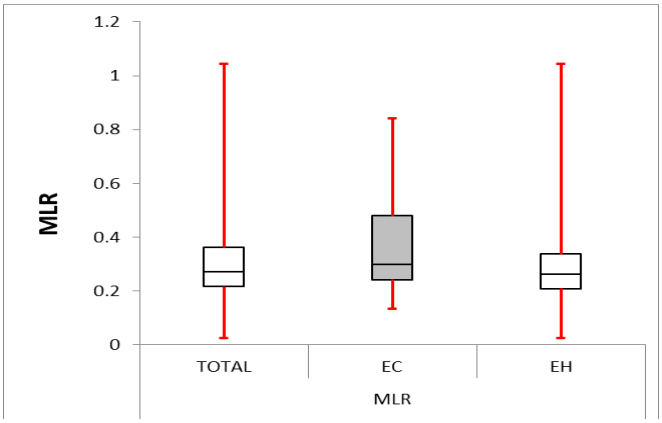

Figura 7.3. Monocyte-lymphocyte ratio: shows a box plot comparing the MLR between the CE and HE groups. The median MLR was significantly higher in the CE group (0.298) compared to the HE group (0.262, p-value < 0.0001). The IQR for the CE group (0.243-0.480) was higher than for the control group (0.208-0.337), suggesting a higher variability of MLR within the CE group

Figure 7.4 summarizes the diagnostic performance of the different markers in differentiating between two groups (EC group is noted as positive group and EH group is noted as negative group). CRP is observed to be the strongest predictor, with an area under the curve (AUC) of 0.961 (p-value < 0.000). Lymphocytes appear to be the weakest predictor, with an AUC of 0.377.

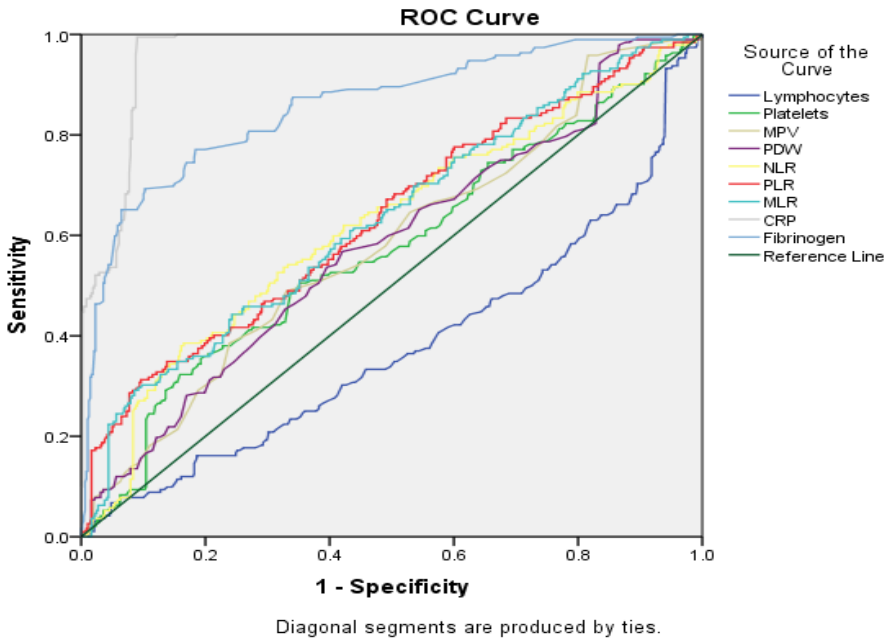

Figure 7.4. ROC curve for statistically significant markers

7.4. Conclusion

This study reveals significant differences in readily available serologic biomarkers between patients diagnosed with EC and those with EH. These readily available biomarkers, which include lymphocyte and platelet counts, MPV, NLR, PLR, MLR, CRP, and fibrinogen levels, suggest potential changes in both the immune system and hemostatic processes (processes involved in blood clotting) in patients with EC.

The accessibility of these biomarkers through routine blood testing suggests a distinct advantage. Unlike some diagnostic tools, they are affordable for health systems globally, a crucial factor given the prevalence of EC worldwide. While individual markers, such as NLR and PLR, may have a moderate ability to distinguish EC on their own, their true potential lies in the development of a panel of multiple biomarkers. Integrating this panel with established clinical data, such as patient history and imaging findings, holds great promise for improving the accuracy of EC diagnosis.

8. Conclusions and personal contributions

This doctoral work, entitled "Correlation of myometrial invasion in endometrial cancer in patients with metabolic syndrome", aims to highlight the involvement of metabolic syndrome, especially the role of high BMI, in the etiology of the most prevalent gynecologic neoplasia. The risk factors associated with MetS can be identified as early as childhood, and

prolonged exposure to these risk factors favors the development of endometrial abnormalities. The objectives initially set were successfully achieved. The results of the research carried out under this thesis, published in peer-reviewed journals with a cumulative impact factor of 8.8, constitute a significant contribution to the knowledge base of the international academic community on the association between EC and MetS.

This in-depth study investigates the relationship between metabolic status and gynecologic pathologies, focusing on the differentiation of EC from EH. The results highlight distinct metabolic and lipid profiles in patients with EC, providing valuable information for early diagnostic and risk stratification. We also identify promising serologic biomarkers that may facilitate non-invasive differentiation of EC.

Thus, the conclusions of the thesis are as follows:

1. We assessed the demographic, epidemiologic characteristics of the two investigated groups and found the following:
    a. The EC group had an older mean age at diagnosis (62.42 ± 10.61 years vs 53.12 ± 10.79 years).
    b. Menarche showed lower mean age in the EC group (11.11 ± 1.1 years vs 13.77 ± 1.12 years).
    c. Parity (11.11 ± 1.1 vs. 13.77 ± 1.12 years) and number of abortions (2.05 ± 2.6 years vs. 2.44 ± 2.96 years) were more underrepresented in the EC group.
    d. Age of onset of menopause was insignificantly older in the EC group (48.71 ± 5.13 years vs 48.37 ± 4.89 years).
    e. Patients in the EH group were more likely to have never used OCs (37.5% used OCs of those with EC vs 11.62% of those with EH).
    f. Patients in the EC group were more likely not to have smoked ni- cigarettes (68.2% non-smokers with EC vs 30.3% non-smokers with EH).
    g. We also observed significantly higher percentage of metrorrhagia/ menorrhagia in both groups compared to no symptoms (89.6% of EC and EH patients).
    h. Women diagnosed with EC transitioned to menopause preoperatively in a significantly higher percentage (85.2% vs 48.6%).
2. We evaluated the metabolic profile among patients with EC and observed the following:

a. Patients with EC have significantly higher serum TG levels (172.67 ± 58.30 vs 118.68 ± 38.40).
b. HDL-C concentrations are significantly lower in patients with EC (a mean decrease of -5.42 mg/dL).
c. The newly assessed biomarkers, TyG index and TG/HDL-c ratio, pre-showed significant differences between the two groups (9.175 ± 0.48 vs 8.693 ± 0.39, respectively 4.01 ± 1.79 vs 2.44 ± 1.04).
d. TyG index and TG/HDL-c ratio were interrogated to obtain the degree of impact on myometrial invasion. These were associated with moderate discriminatory power-re discriminatory power (AUC = 0.586 and AUC = 0.603, respectively).
e. EC patients have significantly higher serum HbA1c levels (a mean value 0.47% higher) compared to the EH group.
f. Mean BMI is significantly higher in EC patients (a mean difference of 4.43 kg/m^2).

3. We evaluated the associations and utility of serologic biomarkers in the EC group, emphasizing the following:
    a. We observed significant differences in readily available serologic biomarkers (absolute lymphocyte and platelet counts, MPV, NLR, PLR, MLR, PCR and fibrinogen levels) between patients with EC and those with EH.
    b. Thus, lymphocyte counts are significantly lower in patients with EC (1.86 ± 0.72 vs 2.12 ± 0.79), while platelets and MPV are significantly increased (304.4 ± 84.24 vs 284.42 ± 78.65, respectively 10.82 ± 1.02 vs 10.53 ± 0.98).
    c. NLR is significantly increased in patients with EC (3.52 ± 1.97 vs 2.96 ± 2.35).
    d. PLR shows a significant increase in patients with EC (189.90 ± 102.25 vs 147.68 ± 63.66).
    e. MLR shows a significant increase in patients with EC (0.36 ± 0.16 vs 0.29 ± 0.13).
    f. CRP and fibrinogen levels are also significantly higher in patients with EC (31.91 ± 38.78 vs 1.13 ± 4.87, respectively 429.08 ± 86.78 vs 315.01 ± 65.57).

Personal contributions:

1. The present study involved a detailed comparative analysis of two groups of patients: patients diagnosed with EC and patients diagnosed with EH. The main objective of this analysis was to identify significant differences between the two pathologies, given the potential for a pro-gressive progression from EH to EC.
2. We analyzed in depth the metabolic and lipid profiles in patients with EC and EH. This allowed the identification of significant associations between metabolic de-regulation and lipid abnormalities, contributing significantly to elucidate the risk factors involved in the etiology of EC. The results obtained provide valuable insight into the mechanisms underlying the development of EC and may contribute to the optimization of prevention and screening strategies.
3. We investigated serologic biomarkers and highlighted their significant potential as promising tools for the early diagnosis of EC. Further identification and validation of these biomarkers may lead to significant improvements in the diagnostic accuracy and efficiency of clinical management strategies for patients with EC.
4. We have formulated future research directions with the aim of validating the findings and developing effective personalized diagnostic and treatment strategies for EC. Successful implementation of these directions will contribute significantly to improving patient prognosis and optimizing therapeutic outcomes in the context of EC.

Future research directions

1. Validation of the study with larger cohorts to confirm the observed associations between metabolic markers, serologic biomarkers and EC risk.
2. Assessing the role of dietary factors and daily activities in the prognosis of EC using prospectively collected data may offset potential bias.
3. Elucidation of the specific biological mechanisms underlying metabolic changes and serologic biomarkers in patients with EC, providing a deeper understanding of disease pathogenesis.
4. Development of a multiple biomarker panel that integrates identified serologic biomarkers with established clinical data to significantly improve the accuracy of EC diagnosis.

5. Implement and evaluate the efficacy of specific interventions aimed at modifying metabolic risk factors in patients with EC - including weight loss, dyslipidemia control and glycemic control - to determine their impact on disease prognosis.
6. Standardization of diagnostic criteria for MetS, a metabolic condition characterized by abdominal obesity, hypertriglyceridemia, low HDL-C, low HDL-C, arterial hypertension and RI, which has been associated with an increased risk of EC.

This research paper provides crucial insight into the complex interplay between metabolic status, serologic biomarkers and risk of developing EC. Addressing the proposed future research directions will facilitate a deeper understanding of this disease, paving the way towards effective strategies for early diagnosis, risk stratification and personalized intervention. Implementation of these strategies will significantly contribute to improving the prognosis and quality of life of EC patients

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