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Endometrial Cancer and the Glycoprotein Osteopontin: An Explorative Surgical Cohort Study on Potential Diagnostic Applications

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Abstract

Background/Objectives: One of the most prevalent gynecological diseases in developed nations is endometrial cancer (EC), whose incidence is steadily rising. Osteopontin (OPN) is a crucial extracellular matrix molecule linked to tumors. Our main goal was to investigate the potential relationship between OPN and EC, by comparing its levels in blood and endometrial tissue in 40 patients with malignant versus 40 with benign uterine disorders. Our secondary aim was to analyze OPN (in blood and endometrial tissue) and the levels of some related molecules (Cyclin-Dependent Kinase Inhibitor 3-CDKN3 and Integrin αvβ3, in endometrial tissue), also considering established serum tumor markers. **Methods:** Patients undergoing hysterectomy for EC (cases) or for benign diseases (controls) at the Department of Obstetrics and Gynecology of the AOU Maggiore della Carità, Novara (Italy), were recruited for this single-center prospective observational study. **Results:** The mean amount of OPN in blood and endometrium was significantly higher in cases than in controls ($p < 0.0001$). Integrin αvβ3 and CDKN3 were significantly higher in cases ($p < 0.0001$); no significant connections were found considering OPN, Integrin αvβ3, CDKN3 and weight, body mass index, age, thyroid-stimulating hormone (TSH), glycated hemoglobin, specific cancer staging/grading and molecular classification. **Conclusions:** These preliminary findings require validation in future prospective, independent cohorts. The levels of OPN, Integrin αvβ3, and CDKN3 were significantly elevated in cases compared to controls. No major confounding has been identified in preliminary evaluations, supporting their potential utility in a future diagnostic panel, though these findings require verification in larger cohorts.

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Keywords: endometrial cancer; Osteopontin; tumor markers; integrin αvβ3; *CDKN3*

1. Introduction

Endometrial cancer (EC) is one of the most common gynecological cancers in developed countries [1,2]. Environmental risk factors, such as chronic inflammation and oxidative stress, are closely linked to the oncogenic pathway [3,4]. Due to its ability to bridge inflammation and tumorigenesis, Osteopontin (OPN) is a key extracellular matrix molecule in oncology (Figure 1) [5,6]. It is a phosphorylated glycoprotein rich in aspartic acid, expressed by a single copy gene as a 34-kDa protein. The human gene contains seven exons, mapping to chromosome 4(4q13) [7]. It is a multifunctional protein related to cell viability and the secretion of interleukins, interferon-γ, Integrin αvβ3, and Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) [5,8]. OPN plays important roles in some pathological conditions: pre-eclampsia, cardiovascular diseases, endometriosis, and cancer [7,9]. Osteopontin has, in fact, been characterized in many tumors, e.g., those of the head, breast, liver, and colon [7,8]. Multiple functions in the oncological processes have been attributed to OPN, but its possible role has been poorly understood for EC [10,11]. OPN is expressed in the secretory epithelial cells of endometrium and in stromal uterine cells of the decidua [12]. In vitro studies across various EC cell lines showed that OPN could be consistently expressed; cells with knockdown/reduced expression exhibited lower migration and invasive capacity [13,14]. OPN also appears to be involved in apoptotic processes. The inhibition of OPN expression in endometrial lines can reduce cell migration and tumor angiogenesis [15]. There is evidence in the literature for the oncogenic action of OPN, for example, by increasing the expression of Forkhead box M1(FoxM1), or acting on the extracellular protein kinase signaling pathway [11,14]. The different expression of OPN could identify the biological diversity of cancers, but OPN as a possible tumor marker has not been deeply investigated [10,16]. As known in the literature, the combination of blood markers such as CA125, along with clinical/ultrasonographic parameters, can assist in risk stratification and the prediction of regional lymph node metastasis in endometrial carcinoma [17]. Preoperative screening is relevant to its diagnosis [18]. In FIGO stage 1 carcinomas, OPN in plasma evaluation was able to correctly identify the majority of cases, for which there had been no significant changes in known tumor markers [19]. The positivity of OPN was also found to be an independent prognostic factor for disease-free survival (DFS) ($p = 0.035$) [19]. From the pilot data evaluated so far, inhibition of OPN expression appears also associated with increased susceptibility to radiotherapy. At present, OPN has not yet been evaluated in the context of the most recent molecular categorization and considering some interconnected molecules such as Integrin αvβ3 and CDKN3 (Cyclin-Dependent Kinase Inhibitor 3). Integrin αvβ3 controls how stromal and epithelial cells interact with the extracellular matrix (ECM) in healthy endometrial tissue, affecting cell adhesion, motility, and shape [20]. Its expression shifts physiologically during the proliferative and secretory phases of endometrium, helping to prime the uterus for embryonic nidification [21,22]. The Integrin αvβ3 also experiences functional changes in the neoplastic endometrium that promote tumor growth and the cells' ability to invade [19].

CDKN3 is a dual-specificity phosphatase that regulates the cell cycle [23]. CDKN3 acts as a possible multifunctional biomarker and has a clinically significant role in EC [23]. Significant CDKN3 overexpression in EC could encourage unchecked cell division and the development of cancer [23]. As for Integrin αvβ3, its described increase in endometrial tumor tissues may raise the possibility that it could be used as a diagnostic marker to differentiate between normal and malignant endometrial tissue [23].

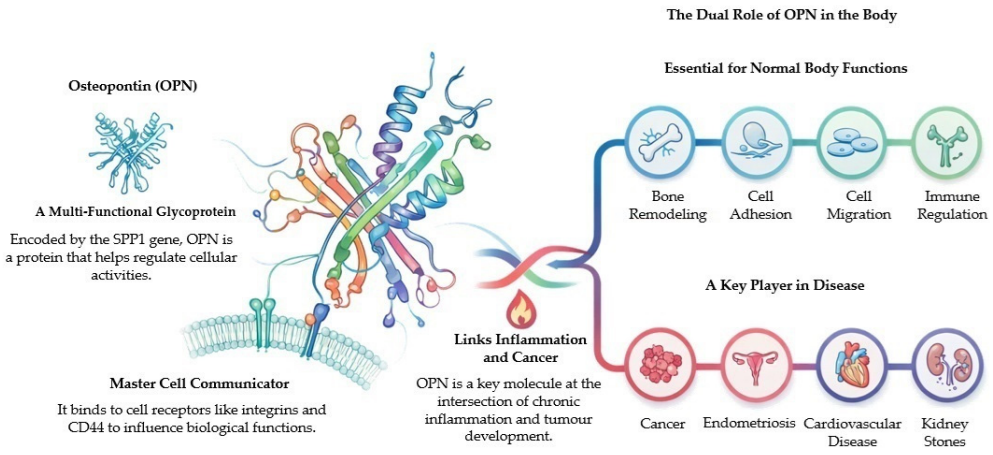


Figure 1. Osteopontin glycoprotein and its possible functions. CD44: Cluster of Differentiation 44; OPN: Osteopontin; SPP1: Secreted Phosphoprotein 1. Created through BioRender.com (accessed on 16 January 2026).

Several classifications of EC are based either on clinical and endocrine features (e.g., type 1 and type 2 tumors) or on histopathological characteristics (e.g., endometrioid versus serous histology). It is essential to distinguish between histologic grade (denoting tumor differentiation, i.e., low-grade [grades 1–2] vs. high-grade [grade 3]), and FIGO stage (denoting the anatomical spread of the disease). While traditional staging and grading guide clinical management [24], the modern diagnostic landscape incorporates the four established molecular subgroups defined by the Cancer Genome Atlas (TCGA) and updated in recent consensus guidelines [25,26]: TP53-mutant/p53-abnormal (p53abn), POLE-mutant (POLEmut), mismatch repair-deficient (MMRd), and no specific molecular profile (NSMP). These molecular classes exhibit distinct prognostic and therapeutic behaviors, superseding traditional criteria alone [26]. This further correlation could be crucial because each oncological variant presents different molecular abnormalities allowing other subclassifications.

Objectives

In this scenario of different suspected onco-etiopathogenetic factors, our primary objective was to study the values of OPN in the context of an EC exploratory surgical cohort, analyzing its levels in blood and endometrial tissue in patients affected by uterine malignant versus benign diseases. Our secondary aim was to evaluate the levels of some related molecules (CDKN3 and Integrin αvβ3, in endometrial tissue), in light also of the established blood tumor markers. These are pilot aims that have to be configured in prospective independent cohorts.

2. Materials and Methods

This is an exploratory surgical cohort single-center prospective observational study that recruited patients who underwent hysterectomy with/or without salpingoophorectomy/lymphadenectomy at the Maggiore della Carità Hospital, Novara, Italy. The research was planned in accordance with STROBE Guidelines for Observational Studies [27], approved by the local Ethical Committee with protocol number CE019/2023.

A sample size of 40 cases vs. 40 controls was defined through statistical evaluation, assuming a percentage of subjects with EC equal to 30%, associated with a predictive model with AUC equal to 0.70 and a power of 90%, at a significance level alpha = 0.05. Statistical analysis is described in the Supplementary Materials. The tissues were obtained from women suffering from malignant versus benign uterine pathologies, during the routine surgical

procedures for their condition and upon signature of the informed consent, included in a prospective and consecutive manner following the surgery waiting list for the procedure. The inclusion criteria were a previous negative cancer history during the last ten years; positive histological diagnosis for women with EC; and the diagnosis of benign uterine pathologies for the controls. Women unable or unwilling to participate in the study, and/or affected by any type of cancer in the last 10 years, with final histological results different than the initial diagnosis or with only blood or endometrial samples, were excluded. Common clinical values (i.e., hemoglobin, glucose, etc.) were obtained from the preoperative routine blood examinations. Anamnestic data were retrieved from medical records and entered into the password-protected "Electronic Data Capture" (REDCap https://project-redcap.org) online computerized platform. Each patient was assigned a sequential identification number after the pseudo-anonymization procedure. On the day of the hysterectomy, two tubes of blood were taken from the patient. Surgical staging was carried out based on the latest guidelines and the new molecular classification [26]. Endometrial and blood samples were transported to the Laboratory of Human Physiology to be treated and stored. The techniques used were ELISA and Western blot (Supplementary Material), as described previously [28,29].

3. Results

Initially, 70 cases and 75 controls were enrolled. Finally, 40 cases and 40 controls were included in the study after excluding some patients who had oncological cancer within the preceding ten years, had final histological results that differed from the original diagnosis, or had only a blood or endometrial sample (Figure 2). All 40 malignant cases in our cohort are histologically confirmed endometrioid endometrial carcinomas (low-grade, predominantly FIGO grades 1 and 2); for the controls, they were primarily diagnosed with uterine leiomyoma or genital prolapse. All the cases were in menopause, twenty-two women had hypertension, seven had diabetes, and five smoked cigarettes. These cases have the following mean values (Tables 1 and S1A): age 68 years, BMI 30 kg/m^2, Hb 13.6 g/dL, preoperative CRP (C-Reactive Protein) 0.57 mg/dL, cholesterol 194 mg/dL, HDL 60 mg/dL, LDL 118 mg/dL, glycated hemoglobin 5.9%, TSH 1.9 mU/L, and CA125 20.5 U/mL.

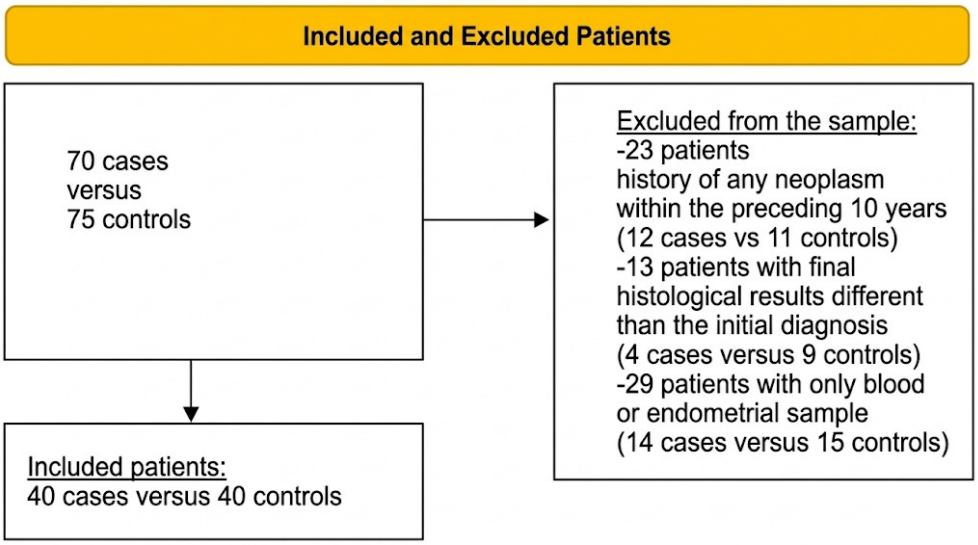


Figure 2. Sample flowchart with excluded and included patients (cases vs. controls).

Twenty-one controls were in menopause, nineteen were hypertensive, two were diabetic, and five were smokers (cigarettes). The mean values for controls were as follows: age of 58 years, BMI of 26.2 kg/m^2, Hb of 13.5 g/dL, preoperative CRP of 0.67 mg/dL, cholesterol

of 180.5 mg/dL, HDL of 59.2 mg/dL, LDL of 102.7 mg/dL, glycated hemoglobin of 5.4%, and TSH of 1.3 mU/L. Regarding the case cohort, the mean BMI was 30 kg/m^2. Specifically, 25% of patients were classified as overweight (BMI 25–29.9 kg/m^2), 55% presented with class 1–2 obesity (BMI 30–39.9 kg/m^2), and 20% presented with severe class 3 obesity (BMI ≥ 40 kg/m^2), demonstrating a right-skewed distribution towards higher adiposity.

Table 1. Laboratory data obtained from routine clinical evaluations. Hb (Hemoglobin), CRP (C-reactive protein), LDL (low-density lipoprotein) and HDL (high-density lipoprotein), IGF-1 (Insulin-like Growth Factor 1), TSH (Thyroid-Stimulating Hormone), and FT (Free Thyroxine), as mean ± SD in cases vs. controls.

Variable (Unit)	Cases (Mean ± SD)	Controls (Mean ± SD)	*p*-Value
Hb preoperative (g/dL)	13.7 ± 1.0	13.5 ± 1.5	>0.05
CRP preoperative (mg/dL)	0.6 ± 0.6	0.7 ± 0.6	0.237
CRP postoperative (mg/dL)	1.8 ± 1.1	1.6 ± 1.3	0.133
Total cholesterol (mg/dL)	194.4 ± 47.1	180.5 ± 31.0	>0.05
LDL (mg/dL)	118.2 ± 40.2	102.7 ± 25.5	>0.05
HDL (mg/dL)	60.1 ± 14.0	59.2 ± 19.0	>0.05
Triglycerides (mg/dL)	102.7 ± 41.2	86.8 ± 33.1	0.103
Insulin (µIU/mL)	13.6 ± 11.0	15.4 ± 13.0	0.512
Somatomedin/IGF-1 (ng/mL)	98.7 ± 34.4	130.8 ± 38.2	0.003
Glycated hemoglobin (%)	5.9 ± 1.0	5.4 ± 0.5	0.006
Glycemia (mg/dL)	103.7 ± 25.2	96.4 ± 16.0	0.486
TSH (mU/L)	1.9 ± 1.0	1.3 ± 0.7	0.003
FT4 (ng/dL)	1.3 ± 0.2	1.3 ± 0.3	0.468

Conversely, in the control group, 47.5% were overweight, and only 2.5% had grade 3 obesity, with a mean BMI of 26.2 kg/m^2.

Conditions were the most prevalent, followed by mental illnesses and endocrine problems, especially those that affected the thyroid. Information about menopause, exposure to tobacco smoke, usage of contraceptives within the previous ten years, comorbidities such diabetes, hypertension, a family history of cancer, and Pap-test results were gathered through the medical history.

Most cases' tumors were in initial stages: T1a (52.5%), T1b (35%); N0 (67.5%), Nx (20%), and N1 (12.5%) (Figure S1A,B, Table S1B). Tumor markers were detected in blood samples in the diagnostic stage (when performed) and then compared to OPN levels; CA125 was employed for diagnostic assistance in all cases (Figures S2 and S3, Table S2).

Our results (cases versus controls) were plasma OPN 12.8 ± 4.2 vs. 9.2 ± 1.6 ng/mL; endometrial OPN (as specific antibody/β-actin ratio) 17.9 ± 4.9 vs. 0.4 ± 0.2; integrin αvβ3 in endometrium (as specific antibody/β-actin ratio) 8.9 ± 2.7 vs. 0.2 + 0.8; and CDKN3 in endometrium (as specific antibody/β-actin ratio) 8.6 ± 2.7 vs. 0.2 ± 0.1 (Table 2, Figures 3, S4 and S5). The mean blood OPN values were substantially higher in cases than in controls ($p < 0.0001$) (Figure S4). Cases had considerably greater expression of endometrial OPN, Integrin αvβ3, and CDKN3, too ($p < 0.0001$) (Table 2, Figures 3 and S5). Based on our exploratory data, a plasma OPN threshold between 9 and 12 ng/mL showed an intermediate diagnostic performance for EC; values below 9 ng/mL suggested a lower probability of malignancy, whereas values exceeding 12 ng/mL were positively associated with an oncological diagnosis. Area under the ROC curve was 0.83 (95% CI 0.74 to 0.92). At a threshold of 9 ng/mL, sensitivity was 90% (36/40; 95% CI 76.3–97.2) and specificity

42.5% (17/40; 95% CI 27.0–59.1). At a threshold of 12 ng/mL, sensitivity was 52.5% (21/40; 95% CI 36.1–68.5) and specificity 97.5% (39/40; 95% CI 86.8–99.9).

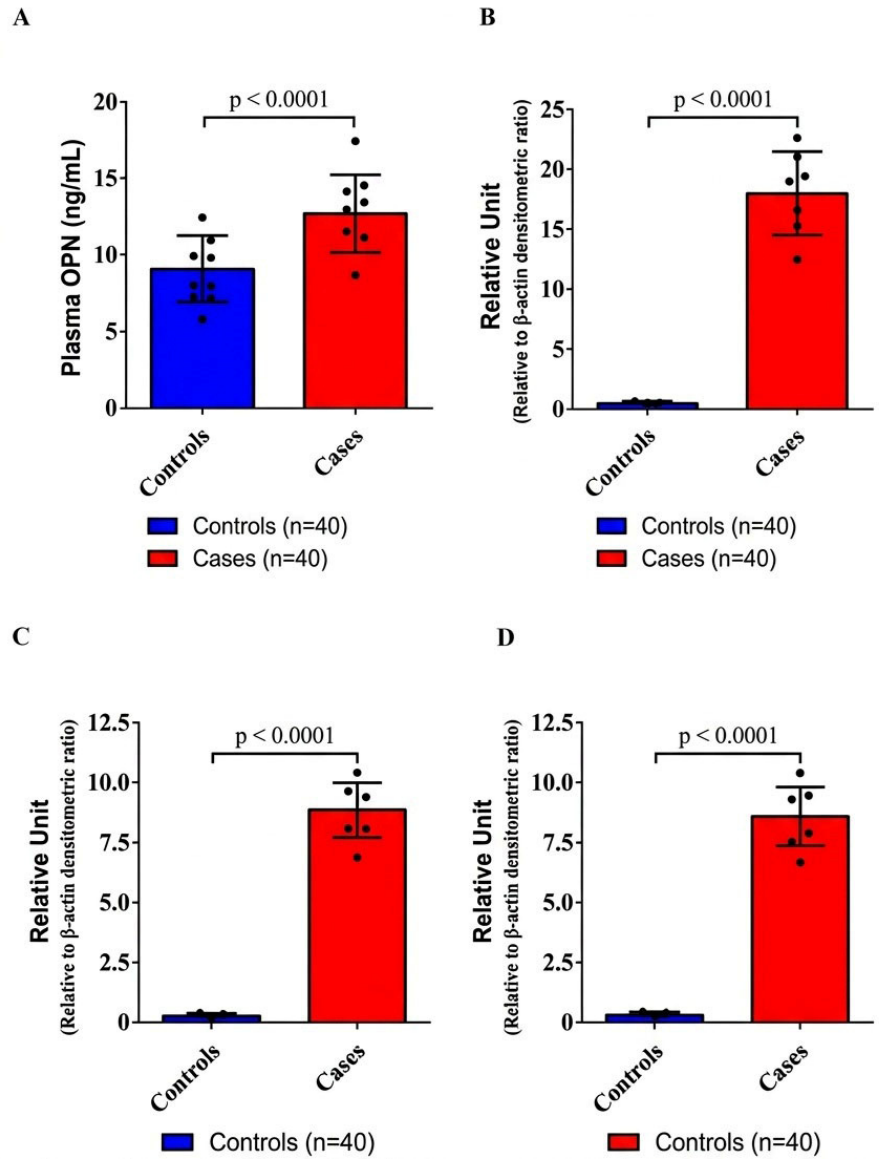


Figure 3. Plasma OPN (**A**), endometrial OPN (**B**), endometrial Integrin αvβ3 (**C**), endometrial CDKN3 (**D**). Individual data points and summary stats across cohorts (n = 40 cases vs. n = 40 controls). (**A**) Plasma OPN (ng/mL) evaluated via ELISA (duplicate average per patient). (**B**–**D**) Endometrial OPN, Integrin αvβ3, and CDKN3 relative protein expression evaluated via Western blotting, normalized against β-actin (triplicate average per patient). Significance evaluated via two-tailed Student's *t*-test ($p < 0.0001$ for all panels). Data are presented as group means ± standard deviation (SD). Individual data points overlaid on the bars represent representative biological replicates and assay runs (or jittered composite sampling subsets where high point density results in visual overlap). Due to parallel triplicate Western blot analyses and standardized densitometric normalization across pooled experimental runs, certain individual data values superimpose or cluster closely together. The full statistical evaluation, however, encompasses the complete cohort of independent biological samples per group.

Furthermore, an antibody-to-beta actin densitometric ratio greater than one for OPN, CDKN3, and Integrin αvβ3 in endometrial tissue could be indicative of a malignant diagnosis. When comparing cases and controls, significant differences were observed in the studied molecules. In preliminary analyses examining potential relationships between

OPN, Integrin αvβ3, CDKN3 and clinical parameters (such as weight, BMI, age, TSH, and glycated hemoglobin), most correlations did not reach statistical significance, suggesting that these factors may not act as major direct confounders, though residual confounding cannot be entirely ruled out. Only a weak correlation ($p = 0.04$) exists between elevated OPN in menopausal patients and controls. Although a correlation was observed between plasma OPN and CEA ($p = 0.0091$), and between Integrin αvβ3 and CA125 ($p = 0.0439$), it is prudent to evaluate these data further, given the sample size and the fact that CEA was tested in only a minority of patients. There are no significant associations with molecular classification, cancer stage, or grading.

Table 2. Plasma OPN and endometrial OPN (ng/mL), Integrin αvβ3, and CDKN3 expression (antibody/B-actin ratio). Values are presented as mean ± standard deviation (SD) in cases vs. controls. Sample size n = 40 independent subjects per group. Plasma OPN measurements represent the average number of technical duplicates per individual. Endometrial Western blot target protein densitometric signals were normalized to β-actin and derived from averaged technical triplicates per subject prior to statistical analysis. Differences evaluated via two-tailed unpaired Student's *t*-test.

Marker/Parameter	Assay Method	Cases (n = 40) Mean ± SD	Controls (n = 40) Mean ± SD	Mean Diff. (95% CI)	Exact *p*-Value
Plasma OPN (ng/mL)	ELISA (duplicates)	12.8 ± 4.2	9.2 ± 1.6	+3.6 (2.2 to 5.0)	<0.0001
Endometrial OPN (Relative Unit)	Western blot (triplicates)	18.0 ± 5.0	0.4 ± 0.2	+17.6 (16.0 to 19.2)	<0.0001
Integrin αvβ3 (Relative Unit)	Western blot (triplicates)	8.9 ± 2.7	0.2 ± 0.1	+8.7 (7.8 to 9.6)	<0.0001
CDKN3 (Relative Unit)	Western blot (triplicates)	8.6 ± 2.7	0.3 ± 0.1	+8.3 (7.4 to 9.2)	<0.0001

4. Discussion

OPN was identified as an integrin-binding ligand N-linked glycoprotein in osteoblasts in 1986 [10]. In oncology, OPN and other tumor markers have been examined [10]; however, there is currently little research on EC. It is one of the most significant gynecological neoplasms in Western nations, with increased incidence. This is probably due to the spread of several risk factors, particularly obesity, that also lower the age at which the disease first manifests [2,30,31]. In the population under study, there is an association between EC and obesity. Only 25% of the cases were overweight (BMI 25–29.9 kg/m^2), whereas 55% had a BMI between 30 and 39.9 kg/m^2 (grade 1 and 2 obesity) and 20% had a BMI ≥ 40 kg/m^2 (grade 3 obesity), according to the BMI ($p = 0.0122$).

OPN acts as an independent diagnostic biomarker in a variety of oncological situations, indicating a possible pertinent and transversal biological involvement between various neoplasia subtypes [8].

Although there is currently conflicting evidence in the literature, OPN has been demonstrated to have several pleiotropic effects in EC [10]. The growing understanding of the biological differences between the various EC subtypes has led to the development of increasingly sophisticated classifications, culminating in the TCGA molecular classification proposed by the Cancer Genome Atlas Research Network; it has allowed the identification of four molecular subgroups with different clinical behavior, prognostic value and potential therapeutic response, overcoming the limits of traditional classifications based exclusively on histological criteria [26]. Our population's predominance of more favorable TCGA subtypes and of T1 and T2 tumors indicates that most patients received an early diagnosis [1–4]. In our exploratory cohort, categorization was mainly based on standard clinicopathological parameters and established diagnostic workflows; genomic TCGA classification or surrogate

immunohistochemical panels do not correlate significantly with the data from our small preliminary cohort, highlighting the need for future studies to integrate these molecular frameworks with novel biomarkers such as OPN, CDKN3, and Integrin αvβ3.

OPN seems to play a particularly significant function in the regulation of the tumor immune microenvironment, as interacting with the ECM [10]. By influencing the activities of T lymphocytes, macrophages, and Natural Killer cells in the endometrial tumor microenvironment and encouraging a persistent inflammatory state that promotes tumor growth and treatment resistance, OPN could aid in immune evasion [10]. Lastly, OPN also seems to be implicated in EC radio-resistance mechanisms [11]. Higher histological grades and more advanced disease stages are correlated with OPN overexpression, indicating a direct function of OPN in the development of endometrial neoplasms [19]. OPN's biological impact is further increased by its direct action at the intracellular level, where it influences the control of gene transcription and post-translational modifications. Numerous oncogenic factors, hormones, cytokines, and intracellular pathways—such as CDKNs and integrins—may regulate its expression [10,19]. Future research must go deeper into molecular impacts, such as those involving CDKN3 and Integrin αvβ3.

The tumor microenvironment, which consists of tumor-associated macrophages, fibroblasts, and myofibroblasts that can alter the immune response and encourage proliferation and invasiveness, has a significant impact on neoplastic progression [10]. OPN could be an interconnected molecule of interest, with pleiotropic functions involved in the processes of cell adhesion, migration and survival, as well as in the modulation of the immune response [11].

The current study is a component of an effort to explore the function of OPN and its functionally associated molecules, including CDKN3 and Integrin αvβ3, in the specific setting of EC. Although preliminary correlation analyses did not identify statistically significant confounding relationships between the investigated biomarkers (OPN, CDKN3, Integrin αvβ3) and major clinical parameters, the inherent demographic imbalances—specifically regarding age and menopausal status between the postmenopausal malignant cohort and the younger control group—must be carefully acknowledged as potential sources of residual confounding.

The purpose of integrating the clinical–demographic and histopathological data from our exploratory surgical cohort study was to shed light on the biological and clinical significance of these biomarkers, improving future diagnostic knowledge and providing new clinical avenues. OPN was considerably higher in our cases' blood and endometrial tumor tissue than in controls ($p < 0.001$). Furthermore, Western blot was used to analyze the expression of CDKN3 and Integrin αvβ3 in endometrium. CDKN3 is a dual-specificity phosphatase that regulates the cell cycle. Through its interaction with CDK2 and modulation of the p53-p21 axis, it plays a critical role in controlling the G1/S transition and mitosis [20]. According to published research, CDKN3 is overexpressed in cancerous tissue as opposed to healthy tissue, and this overexpression is linked to unchecked cell division and the advancement of cancer [20]. Our findings are consistent with those previously published [20,23]. As a dual-specificity phosphatase that interacts with cyclin-dependent kinases (such as CDK2), CDKN3 functionally modulates cell cycle progression and is closely intertwined with checkpoint signaling pathways, including the p53-p21 regulatory axis.

CDKN3 expression was statistically higher in the tumoral tissue of the cases than in the healthy tissue of controls ($p < 0.001$), with values of 8.6 ± 2.7 and 0.3 ± 0.1, respectively (Table 2). Simultaneously, Integrin αvβ3 expression was assessed in endometrium in our study (Figure 3). The αvβ3 integrin is a heterodimer composed of the αv and β3 subunits. It regulates various physiological and pathological processes, including cell adhesion and migration, angiogenesis, signal transmission via kinases, inflammation, and

oncogenesis [10,19,22]. Its levels were substantially higher in the tumor tissue of the cases than in the healthy tissue of the controls ($p < 0.001$).

Our preliminary findings could open new, more customizable diagnostic scenarios on the clinical history of patients affected by EC.

Given its capacity to stimulate angiogenesis, invasiveness, and immunosuppression, as well as, due to its correlation with CDKNs and Integrin αvβ3, OPN may be a promising biomarker [5]. High levels of OPN in plasma EC patients and tumor tissue have been linked in the literature to early diagnosis, increased biological aggressiveness, and a poor prognosis; specifically, OPN has shown better diagnostic capacities than CA125 in early FIGO stages [19]. The combination of CA19-9, CA125, leptin, thymidine kinase, CEA, CA15-3, and HE4 has been shown to have high sensitivity and specificity for detecting EC in terms of biomarkers [17]. According to published research, plasma OPN examination properly identified 62.1% of FIGO stage 1 carcinomas for which onco-markers like CA125 had not significantly changed. Combining blood markers like CA125 with additional novel markers like OPN and considering that biomarkers can differ between these selected groups could lead to an earlier EC diagnosis in future. OPN could be a crucial mediator of the processes connecting obesity, hormonal structure, chronic inflammation, and the tumor microenvironment in EC rather than a straightforward biomarker for diagnosis based on histological staging [10]. The possible association with OPN is intriguing: given the current great interest in multiplex marker panels, more research is needed to fully develop OPN's diagnostic potential. Preoperative screening may customize the diagnosis for each patient: the clinical parameters of interest related to OPN include the detection of cancer.

To more clearly characterize the true clinical potential of OPN as an integrated biomarker and as a potential therapeutic target, further prospective studies with isoform-specific analysis and integration with the molecular categorization and immunological profiles will be required.

Strengths and Limitations

Several limitations of the present study must be acknowledged. First, the exploratory and single-center design introduces potential case–control spectrum bias and lacks external validation, restricting the immediate generalizability of our findings. Second, patient selection based on the availability of complete surgical specimens from hysterectomy introduces selection bias, excluding cases managed conservatively or with partial samples. Third, an inherent age and menopausal status imbalance exists between the older, exclusively postmenopausal malignant cohort and the younger, partially premenopausal control group; although bivariate analyses found no significant confounding effect with age, this variable requires cautious appraisal. Fourth, our cohort is characterized by small subtype strata, consisting predominantly of early-stage (T1/T2) type 1 endometrioid carcinomas, thus precluding definitive conclusions regarding high-grade or non-endometrioid variants. Finally, because OPN is deeply intertwined with systemic and chronic inflammation, overlapping inflammatory pathways in benign gynecologic pathologies (such as leiomyomas or prolapse) may modulate baseline levels, emphasizing that OPN acts as a non-specific indicator of endometrial tissue alterations rather than a standalone cancer diagnostic.

The present study possesses several notable strengths. The prospective and consecutive recruitment of patients based on surgical waiting lists minimizes selection bias and ensures a representative institutional cohort. Strict methodological criteria were applied, including the rigorous exclusion of patients with a prior oncological history within the preceding decade and the histological confirmation of type 1 endometrial carcinoma cases, thereby yielding a well-characterized phenotype. Obtaining endometrial samples immediately following the opening of the uterine cavity preserved tissue integrity for molecular analyses, avoiding contamination or degradation artifacts. Finally, this study represents one of the most recent

and comprehensive investigations exploring the concurrent diagnostic value of systemic and tissue-specific biomarkers (OPN, Integrin αvβ3, and CDKN3) in this clinical setting.

5. Conclusions

The values of OPN, Integrin αvβ3, and CDKN3 were significantly increased in cases compared to controls, showing no major apparent influence from standard demographic and clinical factors in our preliminary models, supporting their potential utility in future endometrial cancer assessment. Obviously, the possible diagnostic role of these molecules should be confirmed in future research. These preliminary results suggest that OPN may represent a biological intersection node between inflammation, endocrine alterations, and endometrial cancer, despite the absence of a direct association with tumor staging or grading, underlining the need for further studies to clarify its functional role. From a diagnostic perspective, the significant elevations observed for OPN, CDKN3, and Integrin αvβ3 may represent promising indicators of endometrial pathological activity.

In conclusion, plasma and endometrial OPN, Integrin αvβ3, and CDKN3 levels are significantly elevated in endometrial cancer cases compared to benign controls. These findings highlight preliminary, exploratory biomarker associations reflecting potential pathological alterations in the endometrial microenvironment. Given the exploratory design and baseline cohort differences, these results must be interpreted as hypothesis-generating and require rigorous validation in future prospective, independent cohorts before any definitive clinical or diagnostic utility can be established.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jcm15228843/s1. Statistical analysis, laboratory details: Figure S1. (A) Molecular staging of cases, considering: P53 level (null, mutant, wild type), POLE mutant, MLH1, PMS2, MSH6, MSH2, ER, PG, Ki67. (B) Grading of cases sample (G1, G2, G3); Figure S2. Graphic correlations about oncomarkers in controls vs cases; Figure S3. Graphic representation of plasma OPN and oncomarkers values in cases; Figure S4. Plasma OPN in cases and controls (ng/mL); Figure S5. Graphic correlations between plasma OPN (ng/mL), endometrial OPN, Integrin αvβ3, CDKN3 expression (as specific antibody/β-actin ratio) in cases vs controls; Table S1. (A) Demographic data and graphic comparison between groups. (B) Grading analysis of cases; Table S2. Oncomarkers and OPN (endometrium, blood), CDKN3, Integrin αvβ3 relations.

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