

TO THE MEMBERS OF THE DEPARTMENT COUNCIL  
DEPARTMENT OF CLINICAL LABORATORY  
FACULTY OF MEDICINE, MEDICAL UNIVERSITY –  
PLOVDIV

**OPINION**

by Assoc. Prof. Ivanka Slaveykova Nenova-Chilova, MD, PhD  
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regarding a dissertation project for the award of the educational and scientific degree  
“Doctor”

Field of Higher Education 7. “Healthcare and Sports”  
Professional Field 7.1. Medicine  
Doctoral Programme “Clinical Laboratory”.

Author: Dr. Vesselina Stoyanova Koleva-Topova

Form of doctoral study: independent preparation

Department: Clinical Laboratory, Faculty of Medicine

Title: “Comparative evaluation of the clinical application of biomarkers and algorithms in  
oncogynecology – CA125, HE4, ROMA and CPH-I.”

Scientific supervisors:

Prof. Tanya Deneva, MD, PhD, Department of Clinical Laboratory, Faculty of Medicine,  
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Assoc. Prof. Pavel Bochev, MD, PhD, Clinic of Nuclear Medicine, Acibadem City Clinic  
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**Relevance of the Topic**

The incidence of ovarian carcinoma in developed countries shows a slight downward trend, in contrast to countries with socio-economic challenges. A characteristic feature of this malignant disease is its late diagnosis due to the absence of alarm symptoms. The clinical course remains unfavorable with respect to remission duration and overall survival (5-year survival rate of 51% for the period 2015–2021). There are groups at high (genetic) risk for developing this disease. For these reasons, surveillance programs are of significant importance, and for affected women, early diagnosis, prognostic assessment, including the use of non-invasive biomarkers, and the choice of optimal treatment are crucial.

The incidence of benign adnexal tumors is higher than that of malignant ones. The accuracy of assessment is important in order to determine whether urgent surgical intervention or conservative management with follow-up, including imaging studies, should be undertaken. For the differentiation of malignant from benign pelvic tumors, in addition to examination data, tissue- and tumor-specific biomarkers with high sensitivity are helpful.

### **Knowledge of the Problem**

The dissertation demonstrates good understanding of the epidemiology, risk factors, and pathogenesis of ovarian and endometrial carcinoma. The latest scientific achievements in this field are presented, and results from numerous clinical studies are cited.

As a clinical practice problem, the diagnosis of a pelvic mass suspected for ovarian carcinoma is discussed. Substantial information in this regard is provided by algorithms/models of indicators, in this case ROMA (Risk of Ovarian Malignancy Algorithm) and CPH-I (Copenhagen Index).

Risk prediction is an important step prior to the choice of treatment. Preoperative serum biomarker levels, and particularly their dynamics following treatment, have prognostic value regarding the likelihood of recurrence.

Serum tumor markers, in addition to imaging studies, provide information in the diagnosis of recurrence or progression of the malignant process.

These are the main directions in which the role of HE4 (human epididymis protein 4) is investigated in comparison with CA125 and the ROMA and CPH-I algorithms.

Unresolved scientific problems requiring future investigations are outlined. In this regard, the lack of unified reference ranges and data on biological variations in Bulgarian women for certain tumor markers (HE4) is emphasized. The significance of HE4 as a prognostic and monitoring marker for ovarian carcinoma is insufficiently studied. There are insufficient scientific data on the prognostic role of CPH-I in the Bulgarian population, including an accepted pathological cut-off value.

### **Methodology of the Study**

The study is defined as retrospective, with a prospective evaluation of progression-free interval and overall survival. The study was approved by the Scientific Ethics Committee at MU-Plovdiv and Acibadem City Clinic UMHAT "Tokuda", Sofia. The inclusion and exclusion criteria are well considered for maximal precise evaluation of the investigated indicators.

The reliability of the data is ensured by the large number of cases as well as contemporary statistical methods of analysis.

Statistical analysis was performed using parametric and non-parametric statistical methods, descriptive, frequency, correlation, regression, and graphical analysis. Statistical data processing was performed using the statistical package SPSS v.25.0.

### **Characteristics and Evaluation of the Dissertation and Contributions**

The dissertation project submitted for opinion comprises 228 standard pages, 105 figures, and 53 tables. The main text contains: title page, contents, list of abbreviations – 2 pages; introduction – 2 pages; literature review – 42 pages; aim and objectives – 2 pages; materials and methods – 15 pages; results – 121 pages; discussion – 15 pages; conclusions and contributions – 3 pages. Publications and participation in scientific forums – 1 page; bibliography – 14 pages. A total of 156 literature sources are cited, 50% of which are from the last 5 years. An appendix (2 pages) lists the figures and tables.

The literature review analyzes results from clinical studies outlining the main directions of the problem, structured as follows:

- History and chronology of the discovery of HE4 as a tumor marker and laboratory diagnostic methods for its determination.
- Diagnostic value of HE4 and CA125 for differentiation of malignant from benign ovarian lesions. The significance of HE4 is emphasized, having the highest sensitivity and high specificity as a standalone marker for early ovarian carcinoma.
- Preoperative prognosis based on tumor markers. Preoperative HE4 values correlate with clinical stage and tumor aggressiveness.
- Tumor markers for monitoring treated patients with ovarian carcinoma. Significant changes in serum HE4 values may indicate recurrence earlier than CA125, which is necessary when deciding on treatment modification.
- There are data that HE4 in endometrial carcinoma is an independent prognostic factor for survival.
- Advantages of multi-marker models and algorithms in the diagnosis of unspecified pelvic masses. The role of ROMA, OVA-1, and CPH-I in preliminary diagnosis of malignant pelvic tumor and referral to a specialized treatment center.

The aim is correctly and precisely formulated and corresponds to the topic of the dissertation.

The five objectives are in accordance with the stated aim.

The materials and methods are correctly and precisely selected to achieve the aim and objectives.

A total of 1,647 patients were included: healthy controls and patients with benign and malignant gynecological diseases, who signed informed consent. Oncological diseases were confirmed by histological examination, and non-epithelial ovarian tumors were excluded.

The laboratory methods for determination of tumor markers HE4 and CA125 are described precisely.

Modern statistical analysis methods were applied for data processing. A software program was created for calculation of the CPH-I algorithm; a software program provided by Abbott Architect was used for the ROMA index.

The material and applied methods allow formulation of precise and reliable conclusions and outline the contributions of the dissertation.

In the chapter “Results,” the presentation follows the stated objectives and is written in a clear and precise scientific style. The description of the results is supported by extensive graphical and tabular material.

1. Reference ranges for HE4, CA125, ROMA, and CPH-I were determined, differentiated according to menopausal status. A positive correlation with age was found for HE4, ROMA, and CPH-I, and a negative one for CA125. Four age groups were studied, and the conclusion is that application of reference ranges for these parameters solely according to age is not justified.

HE4 values increase with age (a plateau up to 50 years and rapid increase above 70 years), regardless of hormonal status.

Reference ranges for HE4 in pregnant women were determined, differentiated according to gestational age, without statistically significant difference compared to healthy premenopausal women.

A positive correlation was found between HE4 concentration and serum creatinine concentration.

2. Diagnostic significance of HE4, CA125, ROMA, and CPH-I for detection of ovarian carcinoma

Standalone HE4 application has excellent diagnostic efficiency for differentiation of ovarian carcinoma from benign tumors and unsatisfactory efficiency for differentiation of clinical stages and histological types of OC.

CA125 has very good diagnostic value for diagnosing OC among benign tumors.

Both ROMA and CPH-I, when used independently, have excellent diagnostic efficiency for differentiation of ovarian carcinoma from benign tumors.

3. Prognostic significance of preoperative HE4, CA125, ROMA, and CPH-I values for overall survival

All tumor markers show a negative correlation with overall survival but exert indirect effect. The key factors determining survival are age, histological variant, and clinical stage. Only CPH-I has a direct effect on overall survival. Independently, these tumor markers do not reach statistical significance in multifactorial analysis. HE4 has the highest prognostic value in ovarian and endometrial carcinoma.

4. Endometrial carcinoma and tumor markers

HE4, ROMA, and CPH-I show good diagnostic efficiency in differentiating endometrial carcinoma from benign uterine diseases; CA125, ROMA, and CPH-I differentiate well endometrial from ovarian carcinoma; CPH-I and ROMA have the best diagnostic efficiency for endometrial carcinoma.

## 5. Applicability of tumor markers for follow-up of treated ovarian carcinoma

HE4 and CPH-I have prognostic value regarding risk of recurrence, time to progression, and overall survival, while CA125 is a weaker marker and ROMA is borderline.

**In the discussion section**, well-reasoned conclusions are presented for each objective.

Objective 1: The proposed upper reference limits of HE4 (for premenopausal and postmenopausal women) have practical value. A correlation between patient age and serum creatinine level on one hand, and HE4 concentrations on the other, was established.

Objective 2: Conclusions are based on observations of 1,338 patients with benign and malignant gynecological tumors. The advantages of algorithms over standalone tumor markers for diagnosis of pelvic masses are emphasized. According to the author, CPH-I has the highest diagnostic value and it is an innovative marker. As a standalone marker, HE4 is applicable for differentiation of ovarian carcinoma from benign diseases.

Objective 3: Preoperative HE4 values and algorithms based on it show potential for preoperative prognostic assessment. Clinical factors are leading; biomarkers are complementary prognostic tools.

CPH-I has good prognostic value for overall survival in univariate and multivariate analyses, representing an original contribution of the study. Preoperative HE4 values may be useful in determining the type of surgical treatment particularly when fertility preservation is possible.

Objective 4: Endometrial carcinoma and tumor markers. CPH-I and ROMA demonstrate the best diagnostic efficiency, however data from other studies are lacking. CPH-I has an advantage over ROMA due to its independence from menopausal status. It is practically applicable for automatic integration in laboratory information systems.

Objective 5: Applicability of tumor markers for follow-up of treated ovarian carcinoma. HE4 is a particularly sensitive indicator for early recurrence of diagnosed and treated ovarian carcinoma (insufficiently observed patients), in contrast to routinely used CA125.

The conclusions correspond to the objectives section and reflect the obtained results.

The dissertation submitted for review concludes with the formulation of scientific contributions, contributions with scientific-applied character, and contributions with methodological significance – three of them with original character.

### **Evaluation of publications and personal contribution of the doctoral candidate**

Three publications and two congress participations are listed. Two of the publications are in journals indexed in Scopus and/or Web of Science, in which Dr. Koleva is first author.

## CONCLUSION

I consider that the dissertation meets all requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation, and the Regulations of MU – Plovdiv.

The dissertation demonstrates that the doctoral candidate Dr. Vesselina Stoyanova Koleva-Topova possesses in-depth theoretical knowledge and professional skills in the scientific specialty “Clinical Laboratory,” demonstrating qualities and skills for independent conduct of scientific research.

In view of the above, I confidently give my positive evaluation and propose to the members of the extended Department Council to proceed with the procedure for awarding the educational and scientific degree “Doctor” to Dr. Vesselina Stoyanova Koleva-Topova in the doctoral program in Clinical Laboratory.

Date: 16 Mar 2026

Signature: \_\_\_\_\_

Заличено на основание  
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