

**REVIEW**

by

**Prof. Dr. Krasimira Ilieva Ikonomova, MD, PhD**  
**Head of Clinical Laboratory and Immunology**  
**National Multidisciplinary Transport Hospital – Sofia**

on

**Thesis for the academic and scientific degree “Doctor”**

of

**Dr. Vesselina Stoyanova Koleva – Topova**

**COMPARATIVE EVALUATION OF THE CLINICAL APPLICATION OF  
BIOMARKERS AND ALGORITHMS IN ONCOGYNECOLOGY –  
CA 125, HE 4, ROMA and CPH-I**

**Scientific supervisors:**

**Prof. Dr. Tanya Deneva, MD, PhD**  
**Assoc. Prof. Dr. Pavel Bochev, MD, PhD**

**Scientific specialty – “Clinical Laboratory”**  
**Field of higher education “Healthcare and Sports”**  
**Professional field 7.1. Medicine**

Among malignant diseases in women, endometrial cancer ranks third, and ovarian cancer ranks sixth. Nonspecific clinical symptoms in the early stages of these diseases, as well as the lack of effective screening strategies, lead to late diagnosis and limitation of therapeutic options.

CA125 is the first tumor marker for ovarian carcinoma. However, its specificity is limited due to its increase in benign conditions - endometriosis, pelvic inflammatory disease, pregnancy. This leads to false-positive results and makes it difficult to interpret it as an independent diagnostic tool.

HE4 (Human epididymis protein 4) is significantly increased in malignant processes in the ovary and uterine carcinoma. In benign gynecological diseases, HE4 is increased rare compared to CA125. The limitations in the clinical application of HE4 are related to its dependence on age and renal function. There is still no definitive opinion on the independent use of HE4 in the diagnosis and monitoring of patients with oncogynecological diseases.

The development of multimarker models is an approach to overcome the limitations of the independent use of biomarkers. The ROMA index includes the study of CA125, HE4 and menopausal status. The limitations of the index are related to the correct definition of menopausal status, especially in women in perimenopause, on hormone therapy or after hysterectomy. The Copenhagen Index (CPH-I) is a model that includes HE4, CA125 and the patient's age, without the use of menopausal status.

Data on the clinical effectiveness of multimarker models are more limited, especially outside the original populations for which the algorithm was developed and validated. This requires an analysis of CA125 and HE4 in the Bulgarian population. It is also necessary to

monitor the correlations between them when including them in algorithms such as ROMA and CHP-I.

According to the latest data from the National Cancer Registry, the number of newly diagnosed cancer patients has increased by 1.5 times compared to 1993. The demographic collapse in our country requires urgent measures to improve early diagnosis, monitoring and prognosis of oncogynecological diseases. The creation of algorithms for diagnostic behavior is a basis on which recommendations for screening programs can be developed. The approval of an accessible methodology for selecting patients with high risk of ovarian and endometrial carcinoma will allow their rapid referral to specialized centers for oncogynecological treatment.

This determines the topic of the proposed work for review as topical, contemporary and significant.

The PhD thesis is written on 228 standard pages. It is structured as follows: title page, list of abbreviations used and substantive text of the dissertation, grouped into chapters: introduction – 2 pages, literature review – 42 pages, aim and objectives – 2 pages, material and method – 15 pages, results – 121 pages, discussion - 15 pages, conclusions – 2 pages, contributions – 1 page. The dissertation is illustrated with 105 figures, 53 tables and 2 appendices. The bibliography includes 156 sources, with the majority of the articles being published in the last ten years.

## **Literature review**

The literature review includes eight sections, ending with a summary of the unresolved problems on the topic. First, an overview of the prevalence of ovarian and uterine carcinoma is made. Data on the nature of tumor markers are summarized - origin, presence in various biological samples, methods of determination. It is emphasized that despite the widespread use of molecular, genetic and proteomic analyses, indirect immunochemical methods are still the most commonly used and best methodologically characterized. Methodological difficulties in the determination of tumor markers include the lack of certified reference material, because of which method-dependent values are used. In practice, tumor markers are defined as diagnostic, prognostic, predictive and monitoring. There is still a lack of ideal tumor markers, which should be highly specific, highly sensitive, easily and reliably detectable. It is emphasized that tumor markers are not sufficient for diagnosis and should be evaluated in conjunction with other diagnostic methods and the clinical picture. A trend in the development of tumor diagnostics is the creation of panels of biomarkers that are examined simultaneously. Mathematical algorithms for predicting probabilities and risk stratification are also proposed.

The following section traces the clinical application of CA125 as a marker for screening and early detection of ovarian carcinoma. Its role in differentiating processes in the pelvis, in monitoring therapy and detecting disease recurrence is emphasized. Limitations in the clinical applicability of CA125 are associated with insufficient specificity (especially in young patients) and low sensitivity (below 50%) in stage I of the disease. To overcome the limitations, a risk of malignant disease index (RMI) is used, including menopausal status, pelvic ultrasound examination and serum CA125 concentration.

Human Epididymis Protein 4 is expressed in ovarian carcinoma cells and plays a key role in tumor cell adhesion and migration. It is actively released into body fluids and is rapidly eliminated through the circulation. HE4 is rarely elevated in benign diseases. In ovarian carcinoma stage I, the marker shows very high sensitivity - 77.8%. The level of HE4 in serum depends on the patient's age, and a higher body mass index (BMI) is associated with lower HE4 values. The preoperative level of HE4 is a marker of cancer aggressiveness and a predictor of

death. HE4 can predict ovarian cancer recurrence earlier than CA125. The combination of CA125 and HE4 provides more information than the two markers separately. The increase in HE4 and CA125 is not a reason to start treatment, but requires intensive search with more sensitive and specific methods such as PET-CT and MRI.

The significance of HE4 as a marker for endometrial carcinoma has been reviewed. Compared with the traditionally used tumor marker CA125, HE4 shows higher sensitivity. Its concentration correlates with histopathological markers of disease severity, survival and recurrence.

The Risk of Ovarian Carcinoma Algorithm (ROMA) predicts the probability of ovarian carcinoma in patients with a space-occupying process in the pelvis. A Predicted Probability (PP) is calculated, according to which patients are categorized as high or low risk. The use of ROMA creates the opportunity to refer high-risk women to highly specialized activities for more successful treatment. ROMA has been found to have better sensitivity and specificity than RMI.

The Copenhagen Index CPH-I is an algorithm whose main advantage is that it is independent of menopausal status and the relative subjectivity of ultrasound examination

In conclusion, unresolved problems discussed in the literature review are highlighted. They are related to the diagnostic sensitivity and specificity of CA 125, HE4 and their limitations depending on hormonal status, age and histological type of the tumor. There is a lack of information on reference values of the markers and multimarker models specific to the Bulgarian population. Their in-depth study is a prerequisite for their establishment as an easy and accessible methodology for clinical selection of high-risk patients. The introduction of the markers will help monitor the course of the disease and select appropriate therapy.

Overall, the literature review shows good information, critical analysis and logical unification of scientific facts. The conclusions from the literature review are logical and make a smooth transition to the next chapter of the dissertation.

## **Objective**

The objective of this dissertation is to compare the clinical application of the biomarkers CA125 and HE4 and the ROMA and CPH-I algorithms in patients with oncogynecological diseases.

## **Tasks**

The goal is formulated clearly and precisely. Five specific tasks are logically derived.

## **Material and method**

The the clinical contingent includes 1647 women, distributed in the following groups: healthy non-pregnant women - 246 (of which 124 premenopausal), healthy pregnant women - 52, patients without gynecological disease with varying stages of renal failure - 94; patients with benign ovarian cysts - 942 (of which 741 premenopausal); patients with epithelial ovarian carcinoma - 150; patients with uterine carcinoma - 74; patients with other carcinomas/metastases in the ovaries – 47; patients with other non-gynecological non-oncological diseases – 42. The data were selected from the laboratory information system of Acibadem City Clinic, Tokuda for the period 2011-2023. The study was approved by the Research Ethics Committee of the hospital. All participants signed a standard informed consent and completed a questionnaire regarding

menstrual status and past history of oncogynecological disease. The necessary clinical and demographic data of the patients were taken from the hospital information system. The methods for determining the tumor markers HE4 and CA125 are based on chemiluminescent microparticle immunoassay (CMIA) on an Architect/Abbott analyzer. The calculation methods of the ROMA and CPH-I algorithms are based on the values of CA125 and HE4 in combination with the menopausal status or age of the patient. The formulas for calculating the algorithms have been developed as a software program and are part of the Architect/Alinity Abbott analytical platform. The results of the algorithms are presented in %. The kinetic method of Jaffe was used to determine serum creatinine, after which the glomerular filtration rate in ml/min was automatically determined. The laboratory methods used are modern and reliable. They allow for obtaining reliable results, corresponding to generally accepted world standards for such studies. The results obtained are processed with modern statistical methods - statistical package SPSS v.25.0.

## Results

The results, a significant part of which have been published, are presented in 5 sections. They are logically systematized and well illustrated with appropriate tables and figures.

***In a group of 246 healthy women, reference ranges for HE4, CA125, ROMA and CPH-I were derived for the Bulgarian population.*** In the different age periods, the confidence intervals of the upper reference ranges overlap, which makes it unjustified to derive reference ranges for HE4, CA125, ROMA and CPH-I, dependent only on age. In postmenopausal women, a significant difference in the values of HE4, ROMA and CPH-I was found between women from 50 to 59 and over 59 years of age.

***In a group of 52 healthy pregnant women, reference ranges for HE4 were derived.*** No statistically significant difference in the values of HE4 was found between pregnant women and non-pregnant premenopausal women.

***In a group of 94 women (40 with elevated creatinine) and 54 with creatinine within the reference range, the influence of impaired renal function on HE4 values was monitored.*** There was a positive correlation between HE4 concentration and creatinine concentration.

***In a group of 942 women (741 premenopausal) with benign ovarian formations and 150 women with proven ovarian carcinoma, the diagnostic value of HE4, CA125, ROMA and CPH-I for the detection of ovarian carcinoma was determined.*** First, the diagnostic value of HE4, CA125, ROMA and CPH-I for the differentiation of benign ovarian formations from healthy controls was determined. The analysis showed that the study of HE4, CA125, ROMA and CPH-I has poor diagnostic efficiency for the detection of patients with benign ovarian formations, compared to the group of healthy individuals. In the next stage, the diagnostic value of HE4, CA125, ROMA and CPH-I was determined for distinguishing patients with ovarian carcinoma from patients with benign ovarian formations and from healthy controls. The data show that ROMA and CPH-I have the best diagnostic reliability in distinguishing ovarian carcinoma from benign ovarian formations.

***In a group of 43 patients with benign non-gynecological diseases and in a group of 47 patients with oncological non-gynecological diseases, a non-specific increase in HE4 was found.*** These potential interferences should be taken into account when interpreting the results.

***In a group of 150 women with ovarian carcinoma, the prognostic value of HE4, CA125, ROMA and CPH-I in terms of overall survival was studied.*** The patients were followed for a period of 144 months. The overall mortality for the period was 71%. The median overall survival for all patients was 42 months. The one-year and three-year survival rates were 70% and

55%, respectively. In patients with a fatal outcome, the median survival was significantly shorter, at 17 months. The analysis showed that the age of the patients, the histological subtype (high-grade serous carcinoma) and the stage of the disease (stage IV disease) were key determinants of the prognosis in ovarian cancer. HE4 showed the highest prognostic value in terms of overall survival.

*In a group of 160 patients - 86 patients with ovarian carcinoma, 44 patients with endometrial carcinoma, 30 patients with other oncological localizations (breast carcinoma, colorectal carcinoma, primary peritoneal carcinoma and lung carcinoma) the prognostic value of CA125 and HE4 was studied.* In all three groups, HE4 had the highest prognostic value in terms of overall survival.

*In a group of 110 patients with proven ovarian carcinoma, the prognostic value of HE4, CA125, ROMA and CPH-I was assessed* in terms of recurrence rate, time to progression and overall survival, as well as the applicability and role of the markers in monitoring patients with ovarian carcinoma, including after first and subsequent recurrence. Patients were divided into three groups – 36 patients with no response (cancer-related death), 46 patients with a “progression” response (additionally proven by positive PET/CT with high tumor marker and negative CT), 28 patients with a “remission” response (imaging and biochemical). The minimum follow-up period was 33 months, and the maximum was 144 months.

HE4 and CPH-I best differentiate the groups in terms of prognosis, CA125 is a weaker marker, and ROMA is borderline. HE4 and CPH-I can be used for risk stratification, monitoring, and discussing the intensity of follow-up. CA125 remains useful for monitoring response, but not as a stand-alone prognostic marker.

*In a group of 74 women with proven uterine carcinoma, the clinical applicability of CPH-I, ROMA and the independent use of tumor markers HE4 and CA125 in patients with endometrial carcinoma was studied. ROMA and CPH-I have the best diagnostic efficiency in the diagnosis of uterine carcinoma.* The diagnostic efficiency of ROMA and CPH-I in differentiating uterine carcinoma from benign diseases is 85%/85%, respectively. The diagnostic efficiency of ROMA and CPH-I in differentiating uterine carcinoma from ovarian carcinoma is 79%/77%, respectively.

The obtained results cover the set goals and objectives of the work. They show the ability of the candidate to build a scientific hypothesis and critically analyze the data obtained.

## **Discussion**

When evaluating the reference ranges for HE4 specific to the Bulgarian population, lower values were found than those declared in the manufacturers' leaflets. This is attributed to the difference in the cohorts of women studied and the use of different analytical platforms. It was found that the Roche-Cobas 6000 devices measured higher concentrations than the Architect-Abbott analytical platform. It is emphasized that the concentration of HE4 depends more on age than on menopausal status and there is no need to introduce age-specific reference ranges. HE4 is not affected by pregnancy in contrast to the well-known physiological increase in CA125 during pregnancy. The dependence of HE4 on impaired renal function requires careful interpretation of the results. In the Bulgarian population, lower reference ranges for ROMA and CPH-I are reported, as well as a difference in values between premenopausal and postmenopausal women. These data support the need for local validation of the algorithms and adaptation of cut-off values to increase specificity. The present study confirms that the use of serum markers alone is not suitable for population screening, due to significant overlap of values in healthy women and

patients with benign cystic formations. The highest diagnostic efficiency is achieved by the multimarker algorithms ROMA and CPH-I, which are superior to the independent determination of the markers. The main clinical application of HE4, CA125, ROMA and CPH-I remains the preoperative triage of patients with formations in the small pelvis and the differentiation of benign from malignant processes. The obtained results are fully comparable with the data from large meta-analyses. They contribute with original information about the diagnostic value of CPH-I, a marker that is less represented in the literature. None of the markers and algorithms have sufficient accuracy to reliably differentiate between histological variants of ovarian carcinoma or to stage the disease. This defines them as risk stratification tools, but not as substitutes for histological and imaging diagnostics. In ovarian carcinoma, the prognostic value of HE4, CA125, ROMA and CPH-I indicates that the markers have a limited but clinically significant role. HE4 has the highest prognostic value in terms of overall survival, while CA125 does not demonstrate a stable prognostic power. It has been established that HE4, CA125 and ROMA and CPH-I have also been found to have some importance in endometrial carcinoma. The data show that HE4 has a significantly higher diagnostic value compared to CA125. HE4 and the algorithms based on it show a good correlation with the stage of the disease. A progressive increase in HE4 has been reported in advanced stages. The markers and algorithms have the potential for preoperative prognostic assessment. It has practical significance in treatment planning and in choosing a therapeutic strategy. In endometrial carcinoma, the effectiveness of ROMA and CPH-I is highly dependent on the choice of cut-off values. Optimization of cut-off values by ROC analysis improves diagnostic efficiency. This supports the need for population- and nosology-specific adaptation of the algorithms. The present study contributes with a systematic and critical assessment of the diagnostic and prognostic role of HE4, CA125, ROMA and CPH-I. The development emphasizes the need for population-specific validation, careful choice of cut-off values and integration of biomarkers into a comprehensive clinical assessment.

### **Contributions**

Nine contributions are highlighted - 3 of an original scientific-theoretical nature and 6 of an applied nature. For the first time in Bulgaria, specific reference limits for HE4, CA125, ROMA and CPH-I have been developed. For the first time, a systematic assessment of the clinical significance of CPH-I in the diagnosis of uterine carcinoma has been conducted. The prognostic significance of CPH-I in patients with ovarian and endometrial carcinoma has been analyzed.

The significance of HE4, ROMA and CPH-I for the preoperative triage of patients with tumor formations in the small pelvis has been confirmed. The biological relationships of HE4 related to age, renal function and pregnancy have been confirmed for the Bulgarian population. It has been established that HE4 is not elevated in endometriosis and is superior to CA125 in specificity in the evaluation of premenopausal patients. The diagnostic superiority of HE4 over CA125 in endometrial carcinoma has been confirmed. The present study proves the comparable significance of CPH-I to ROMA in the evaluation of tumor masses in the small pelvis for early detection of the risk of oncogynecological disease. The study defines threshold values for CPH-I and suggests its inclusion in routine practice as a substitute for ROMA. Its main advantages are associated with independence from hormonal status and risk of incorrect definition of menopause. The indicator more reliably reflects the effect of age on HE4 values.

I share the contributions of the work, which have both a scientific-theoretical and a scientific-applied nature. They are of high national and international scientific and practical value

## **Publications**

There are 3 publications related to the dissertation in refereed medical journals. Two reports from scientific forums have also been presented.

## **Abstract**

The abstract meets the requirements. It reflects in a synthesized form the most important moments of the dissertation in all its sections.

## **Conclusion**

In conclusion, based on the material presented to me, I believe that Dr. Vesselina Koleva has acquired theoretical and methodological knowledge and professional skills, having completed the planned tasks accurately and systematically. I believe that the dissertation work in terms of volume, content and relevance meets the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria and I recommend to the esteemed Scientific Jury to award Dr. Vesselina Stoyanova Koleva - Topova educational and scientific degree "Doctor" in Professional Field 7.1 Medicine, Scientific Specialty "Clinical Laboratory".

27. 02. 2026  
Sofia

Заличено на основание  
Чл.5 §1, б."В" Регламент (ЕС)2016/679

  
Prof.. Krasimira Ilieva Ikonomova, MD, PhD