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Invited critical review

HE4 in the differential diagnosis of ovarian masses

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ABSTRACT

Ovarian masses, a common finding among pre- and post-menopausal women, can be benign or malignant. Ovarian cancer is the leading cause of death from gynecologic malignancy among women living in industrialized countries. According to the current guidelines, measurement of CA125 tumor marker remains the gold standard in the management of ovarian cancer. Recently, HE4 has been proposed as emerging biomarker in the differential diagnosis of adnexal masses and in the early diagnosis of ovarian cancer. Discrimination of benign and malignant ovarian tumors is very important for correct patient referral to institutions specialized in care and management of ovarian cancer. Tumor markers CA125 and HE4 are currently incorporated into the "Risk of Ovarian Malignancy Algorithm" (ROMA) with menopausal status for discerning malignant from benign pelvic masses. The availability of a good biomarker such as HE4, closely associated with the differential and early diagnosis of ovarian cancer, could reduce medical costs related to more expensive diagnostic procedures. Finally, it is important to note that HE4 identifies platinum non-responders thus enabling a switch to second line chemotherapy and improved survival.

Contents

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1. Introduction

Ovarian masses represent a common finding among both pre- and post-menopausal women. The reported prevalence varies widely depending upon the population studied, the criteria employed and the imaging equipment. Ovarian masses can be benign or malignant and include neoplasms, ovarian endometriomas and lesions of functional or inflammatory origin. Epithelial ovarian cancer is the most frequent malignant tumor accounting for 70% of malignant neoplasms [1]. The number of women diagnosed with a benign adnexal mass is much higher than the number of ovarian cancer cases. In pre-menopause, most ovarian masses are benign. The overall incidence of ovarian cancer in pre-menopause is approximately 1:1000 increasing to 3:1000 at the age of 50 [2]. Ovarian masses are also common in post-menopausal women, although the prevalence is lower than in pre-menopausal women. Ovarian carcinoma (OC) is the leading cause of death from gynecologic cancer for women living in industrialized countries [3]. At the present, the global annual incidence is approximately 204,499 cases per year with a mortality of 124,860 patients per year [4]. The expected number of new ovarian cancer cases in Europe in 2012 was 65,538 with 42,704 deaths [1]. If carcinoma is diagnosed at an early stage, it has an excellent prognosis since it can be treated before spreading to surrounding tissue [5].

Currently, as many as 70% of patients are diagnosed at International Federation of Gynecology and Obstetrics (FIGO) stage III and IV. This late diagnosis is generally related to the asymptomatic behavior of the disease in the early stages. Although certain epithelial ovarian cancer screening tests have been shown to decrease mortality rates, the possibility of efficient screening that may be used in normal practice remains elusive [6].

It is crucial to correctly characterize whether ovarian masses are benign or malignant.

Patients with diagnosed ovarian cancer should be treated in specialist units that provide the most comprehensive cancer care [7], whereas in cases of benign ovarian masses expectant or conservative surgical management should be performed in order to reduce morbidity and fertility preservation [8]. In fact, in premenopausal women preservation of fertility is an important issue. Although these women are rarely thought to have cancer, they in fact account for up to 20% of all ovarian malignancies [9]. Even in selected cases of early stage invasive disease or borderline ovarian tumors (BOT), conservative treatment is a therapeutic option in young women who want to preserve childbearing capacity [8,10,11].

Prediction models have been developed to assist clinicians to triage patients to appropriate treatment pathways; however, none has gained universal acceptance in routine daily practice. Most of these algorithms include the serum CA125 biomarker and this limits their utility in women of reproductive age [12]. Serum CA125 levels are frequently normal in BOT and early stage invasive ovarian cancer [13], and can show a false positive increase in numerous benign tumors or conditions that irritate the pelvic peritoneum (e.g. endometriosis, fibroids, pregnancy, infection and surgery) [14].

Recently, human epididymis protein 4 (HE4) has been proposed as emerging biomarker in the differential diagnosis of pelvic masses.

Currently a combination of physical examination and serum biomarker measurement are the pillar of medical diagnosis. Serum CA125, newly discovered HE4 and imaging have the highest positive predictive value (PPV): 33.8% in pre- and 74.0% in post-menopausal women [15].

The lack of early symptoms to guide timely image-driven investigations, severely limits the possibility of detecting the disease at its early stages. Availability of sensitive and specific ovarian cancer biomarkers is very much needed to reduce the mortality risk due to late diagnosis.

The objective of this review is to assess the overall diagnostic value of measuring HE4 for the differential diagnosis of ovarian masses and Epithelial Ovarian Cancer identification.

2. Genetic biomarkers

Tumors are caused by the accumulation of genetic injuries, but the genetic mutations and pathways involved in the early ovarian carcinogenesis are largely unknown. Since the close relationship between genetic alterations and ovarian carcinogenesis, during the last decade, the study of researchers has been focused on providing novel ovarian cancer biomarkers [5].

Gene mutations in BRCA1 and BRCA2 account for the majority of families with hereditary breast and ovarian cancer syndrome [16]. The risk of developing ovarian cancer in a woman with a BRCA1 mutation is 39–46%, while it is 12–27% in a woman with a BRCA2 mutation [17]. Despite substantial improvement in managing ovarian cancer risks owing to BRCA1/BRCA2 mutations, the guidelines recommend prophylactic bilateral salpingo-oophorectomy (PO) by age 40 years. Guiding recommendations about these interventions are difficult, as the relative risk for either BRCA1 or BRCA2 mutations have been proposed being different [18].

Previous studies showed that both BRCA proteins participate in multiple functions, such as DNA repair, transcriptional regulation of gene expression, and cell cycle [19].

Actually, advances in genomic technologies quicken the finding of other cancer susceptibility genes. To date, at least 16 genes, including, RAD51C, RAD51D, BRIP1, BARD1, CHEK2, MPE11A, NBN, PALB2, RAD50, MLH1, MSH2, MSH6, PMS2, and TP53 have been associated with hereditary ovarian cancer [6].

The gene-expression profiling can be a considerable biomarker for early detection of ovarian cancer, allowing various information including prognosis, prediction of chemotherapy response, mechanisms of chemoresistance, and finally the characterization of different histologic or genetic subtypes. The rapid advances in new generation sequencing (NGS) technology in terms of higher throughput and lower cost, together with the development of multiple genomic sequence enrichment methods, have contributed significantly to both the research and clinical applications of cancer genome sequencing.

It is now widely expected that the second generation sequencing will offer the in-depth characterization of the cancer cell genome and further advance the fields of pathogenesis of cancer and personalized oncology for patients.

3. Epithelial ovarian cancer

Epithelial Ovarian Cancer (EOC) is the most common type, which is further divided into endometrioid, clear cell, mucinous, low-grade serous ovarian carcinoma (LGSOC), and high-grade serous ovarian carcinoma (HGSOC). HGSOC is the most common and aggressive subtype of EOC, accounting for the majority of new cases [20]. HGSOC was long thought to arise from the ovarian surface epithelium (OSE) or inclusion cysts derived from them, but recent evidence has recognized the distal fimbrial epithelium of the fallopian tube as the font for at least a subset of HGSOC.

To establish experimental models for the study of the initiation of EOC, much effort has been dedicated to the genetic modification of cells from an OSE or fimbrial origin, either in tissue culture or *in vivo*. Attempts to model HGSOC have been particularly challenging and have yielded inconsistent results [21]. On the basis of a series of morphologic and molecular genetic studies, it has been proposed a dualistic model that groups various types of epithelial ovarian cancers into two broad categories, nominated type I and type II.

Type I tumors include low-grade serous, low-grade endometrioid, mucinous, and clear cell carcinomas. These neoplasms typically present as large cystic masses restricted to one ovary; have a relatively indolent course; and are associated with mutations in *KRAS*, *BRAF*, *PTEN*, *PIK3CA*, *CTNNB1*, *ARID1A*, and *PPP2R1A* that disturb signaling pathways. These molecular alterations result in morphologic changes, which are reflected by a stepwise progression from benign through varying degrees of atypia (borderline tumor), then to noninvasive and finally to invasive and metastatic carcinoma.

Type II tumors are collected of high-grade serous, high-grade endometrioid, undifferentiated carcinomas, and malignant mixed mesodermal tumors (carcinosarcomas). These tumors are aggressive and typically present at an advanced stage, which adds to their high mortality rate. Unlike type I tumors, which are relatively genetically stable, type II tumors demonstrate several chromosomal aberrations at diagnosis, but these remain relatively stable over the course of the disease [21].

4. Ovarian serum biomarkers

The role of tumor markers in diagnosing and monitoring ovarian cancer is well established [22]. The current benchmark biomarker for ovarian cancer detection CA125 was originally identified following the development of the OC 125 antibody for the coelomic epithelial antigen produced the mesothelial cells from the peritoneal, pleural, and pericardial cavities. It has been used to predict the presence of malignancy in women with a pelvic mass, monitor response to chemotherapy, and detect relapse after initial response to treatment [23,24].

The mucin CA125 has a high sensitivity, but its clinical use in the management of EOC patients is limited because it is also frequently elevated in women with other malignancies and with benign gynecologic disorders as well as benign diseases associated with inflammatory conditions of the pleura, pericardium, and peritoneum [25,26]. However, according to the current guidelines measurement of serum CA125 antigen remains the gold standard in the follow-up EOC [26].

In the recent years, research has focused on new biomarkers such as mesothelin, inhibin, osteopontin and Ca72.4 even if the benefits of these markers are unclear, as, although sensitivity is increased, specificity is sub-optimal [27–30].

4.1. Mesothelin

The mesothelin gene encodes a 71-kDa precursor protein that undergoes physiological cleavage by a furin like protease to produce two main proteins, the first is the 31-kDa NH2-terminal megakaryocyte potentiation factor (MPF), which is secreted into the blood, the second COOH-terminal product is a 40-kDa fragment referred to as mesothelin, which is attached to the cell membrane and is overexpressed in several cancers, including mesothelioma, ovarian and pancreatic cancers, and some squamous cell carcinomas. In patients with ovarian carcinoma, Scholler et al. [31] have described a 42 to 44-kDa protein termed soluble mesothelin-related peptide (SMRP). These findings indicate that a high expression of mesothelin in both tissue and serum indicates a poor prognosis. The mechanism of release of mesothelin from the cell surface is not clear. Many studies showed that serum mesothelin levels are related to the FIGO surgical pathological staging and pathological grade in EOC patients. Patients with advanced stage and low differentiation tumors showed higher levels of SMRP most recent study reported the expression of mesothelin in ovarian tissue correlated to chemotherapy resistance and poor prognosis suggesting a role for mesothelin in diagnosis and disease staging [32].

4.2. Inhibin

Inhibins were initially isolated from gonadal fluids based on their relevant abilities to inhibit follicle stimulating hormone (FSH) secretion from the pituitary. Successively, these proteins were recognized as members of a family of growth factors, the transforming growth factor-beta (TGFb) superfamily, with multiple functions as confined regulators of gonadal biology. Inhibin A and Inhibin B act as antagonists and are structural homologues of activins, including the activin b-subunit and a unique a-subunit.

It has been validated that the alteration of the inhibin/activin pathway may contribute to the development of epithelial ovarian cancer due to the alteration of the crosstalk between granulosa and epithelial cells [33].

In a recent study, Walentowicz et al. propose the association of high levels of inhibin A with a poor prognosis and a low survival at 5 years [33].

4.3. Osteopontin

Osteopontin (OPN) is a secreted, integrin-binding phosphoprotein that has been associated with cancer and is overexpressed in different tumor types [34]. Physiologically, OPN is secreted by osteoblasts and the epithelial cells of multiple organs as well as by activated T lymphocytes, macrophages and leukocytes at the site of inflammation. Some authors showed that OPN-c, an OPN splicing variant, contributed to the increased proliferation, migration and invasion of ovarian cancer cells [35,36]. However, this glycoprotein is strongly associated with progressive tumor stage, poor patient prognosis and metastasis formation. Although several studies have focused on the role of OPN in ovarian cancer screening, the utility of OPN for differentiating between malignant and benign ovarian tumors has not been sufficiently elucidated.

4.4. Carbohydrate antigen 72-4

Carbohydrate antigen 72-4 (CA72.4) is another biomarker for EOC; the level of this 200–400 kDa glycoprotein rises in gastric, cholic, breast, and ovarian adenocarcinomas. It can be used alone or in association with CA125. The sensitivity of CA72.4 is lower than CA125 in detecting EOC, but the levels of this marker are not affected by pregnancy or the menstrual period. There is evidence in the literature that CA72.4 levels can be found slightly increased with endometriosis, benign ovarian tumors, or inflammatory conditions [37,38].

Some authors have demonstrated the role of the biomarker CA72.4 combined with CA125 as a predictive factor of epithelial ovarian cancer recurrence( ).

Moore RG et al. 2007 showed that the combination of more tumor markers including HE4, together with CA72.4 increased the sensitivity and specificity in the diagnosis of ovarian cancer in patients with pelvic masses [39].

5. Human epididymis protein 4 (HE4): a new biomarker

5.1. Gene and protein biology

Recently, human epididymis protein 4 (HE4) has been proposed as emerging biomarker in EOC differential diagnosis, it allows a differential diagnosis from pelvic mass, can detect the disease at early stage, and for monitoring the response to chemotherapy and to estimate the prognosis of ovarian cancer.

HE4 was initially identified in the epithelium of the distal epididymis. This protein was discovered to be a protease inhibitor involved in sperm maturation [40,41].

This protein has a WAP-type four-disulfide core (WFDC) domain and is encoded by the *WFDC2* gene. It is suggested that those genes evolved by repeated duplications. Genes at the WFDC locus are variably conserved across species and may play a role in natural immunity with both antimicrobial and anti-inflammatory activity. The HE4 gene extends over 8 kb DNA and contains five exons. Full-length HE4 is the result of splicing of exons 1, 2, 4, and 5. Exons 3 and 4 can exist in three forms, two of which can be spliced. Intracellular immunofluorescence studies revealed that HE4 is distributed in a region of the cytoplasm with a perinuclear pattern reminiscent of the endoplasmic reticulum and the Golgi [42].

Its role as a potential biomarker for ovarian cancer emerged after cDNA comparative hybridization experiments based on the observation of an increased primary expression of HE4 in some ovarian cancers, relative to normal tissues [43].

5.2. Analytical methods

5.2.1. Enzyme-linked immunosorbent assay (EIA)

HE4 can be measured by immunometric techniques: manual or semi-automated enzyme-linked immunosorbent assay (EIA), chemiluminescent microparticle immunoassay (CMIA) and electrochemiluminescence immunoassay (ECLIA) automated.

The first evaluation method to detect HE4 was solid phase EIA, non-competitive immunoassay based upon the direct "sandwich" technique using two monoclonal antibodies, 2H5 and 3D8, directed against two epitopes in the C-WFDC domain of HE4. This assay has been developed for the determination of HE4 in human serum. The HE4 based enzyme-linked immunosorbent assay recognizes full-length HE4 and splice variants V2 and V3, but not splice variants V1 and V4. This assay is characterized by an equivalent sensitivity, but a higher specificity compared to the CA125 between malignant and benign adnexal masses [44].

5.2.2. Chemiluminescent microparticle immunoassay (CMIA)

The ARCHITECT HE4 assay (Abbott Diagnostics Division, Chicago, USA) is a two-step chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of HE4 antigen in human serum. Differences between the commercial EIA method (Fujirebio Diagnostics) and the ARCHITECT HE4 assay (Abbott Diagnostics Division) are the technology for signal detection (colorimetric *vs.* chemiluminescent), the measurement ranges (15–900 pmol/L for EIA and 20–1500 pmol/L for CMIA with an automated 1:10 dilution protocol that extends the linear range up to 15,000 pmol/L).

5.2.3. Electrochemiluminescence immunoassay (ECLIA)

The Cobas Electrochemiluminescence utilizes a specificity chemiluminescence reaction on the electrode surface induced by the electrochemical reaction. It is actually the perfect combination of electrochemical reaction and chemiluminescence reaction. The measuring ranges for HE4 with ECLIA is 15.0–1500 pmol/L.

With the method EIA and ECLIA the suggested cut-off is 150 and 140 pmol/L respectively, while using the CMIA procedure the cut-off proposed is 70 pmol/L in premenopausal women and 140 pmol/L in postmenopausal women (Table 1).

However, it is important to consider that for HE4, as for many tumor markers, is not correct to express the result as positive or negative, since has not been validated a diagnostic cut-off.

Clinically important variations in HE4 serum concentration occur on the basis of age among healthy women, which underscores the need to address specific age subgroups. Further studies will be required determining whether trends of HE4 values provides even greater specificity, as is the case with CA 125.

5.3. Clinical relevance

Various factors aside from malignancy may influence serum HE4 levels and should be carefully considered in interpreting the results of HE4 [45,46].

HE4 levels in healthy subjects increased with age and smoking habitus, but its serum levels are not affected by the menstrual cycle, oral contraceptive use or endometriosis [47–51] (Table 2). Conversely, elevated HE4 levels have been observed in patients with chronic renal disease which may result from decreased elimination or increased production from the damaged renal tubules [52]. However it should clarify the relationship between HE4 levels and early stage renal failure. The elevated HE4 serum concentrations detected in patients with renal failure resolutely suggested that HE4 must be interpreted carefully in patients with renal failure. This is important, because acute renal failure may be frequently present in some patients during chemotherapy treatment.

The most important uses of HE4 in EOC are: differential diagnosis, "Risk of Ovarian Malignancy Algorithm" (ROMA), and screening. The prognostic value of this biomarker is still controversial. Indeed, Jiang et al. [53] suggest that HE4 overexpression enhanced several malignant phenotypes in cell culture and in a mouse model, while Kong X et al. [54] conversely, found that HE4 plays a protective role in the progression of EOC by inhibiting cell proliferation, this effect is mediated by intracellular HE4, which may function by regulating the MAPK and PI3K/Akt signal transduction pathway in vitro.

6. Differential diagnosis of ovarian masses

In the past, when ultrasound was not routinely used, the presence of a palpable ovarian mass in a postmenopausal woman was considered an indication to surgery with bilateral salpingo-oophorectomy [55]. More recent studies suggest the possibility to perform a conservative management in these patients according to the ultrasonographic characteristics of the cyst, the CA125 value and the patient's preference. Identification of the subgroup of patients most likely to benefit from consultation with a gynecologic oncologist may be a clinical challenge. Differential diagnosis can be sometime difficult but it is mandatory particularly in the presence of benign conditions such as small ovarian endometrioma, that do not necessarily require surgical treatment [29].

Discriminating benign from malignant disease is important not only to ensure appropriate management by a gynecologic oncology surgeon in the setting of malignancy, but also to avoid unnecessary procedures, including surgery, and anxiety in women with asymptomatic, nonmalignant conditions. To date, no single prediction model or set of referral

Table 1
Laboratory test used to detection of HE4.

Manufacturer kit	Standard range pmol/L	Limit of detection pmol/L	Precision % CV	Cut-off pmol/L
Manual EIA Fujrebio®	15–900	15	≤15	<150
Automated CMIA Abbott®	20–1500	0.18	≤10	<70 premenopausal <140 postmenopausal
Automated ECLIA Roche®	15–1500	15	≤10	<140

Table 2
Comparison of CA125 *vs.* HE4 in benign gynecologic diseases.

Benign disease	CA125 Menopausal status		HE4 Menopausal status	
	Pre	Post	Pre	Post
Ovarian cyst	15%	13%	6%	13%
Germ cell tumors	19%	25%	2%	0%
Cystadenomas	20%	22%	20%	19%
Benign, non-specified	35%	18%	5%	14%
Endometriosis/endometrioma	72%	18%	3%	6%
Abscess/PID/hydrosalpinx	40%	33%	13%	13%
Menstruation	Elevated	–	Not elevated	–
First trimester pregnancy	Elevated	–	Not elevated	–
Infertility	Elevated	–	Not elevated	–

guidelines for the evaluation of an adnexal mass has received widespread acceptance.

A careful and documented assessment of the patient should be performed according to the current guidelines. Clinical assessment always includes family history, physical examination, imaging, and laboratory tests (including CA125 results, if available). A physical examination of the woman is essential and should include abdominal and vaginal examination. In the presence of acute pain the diagnosis of ovarian cyst torsion, rupture or hemorrhage should be considered.

According to the Dearking modified ACOG guidelines, suspicion criteria of an ovarian cancer are:

in premenopause:

a) Very elevated CA125 (>67 units/mL);
b) Ascites;
c) Evidence of abdominal or distant metastasis;

in postmenopause:

a) Elevated CA125 (>35 units/mL);
b) Nodular or fixed pelvic mass;
c) Ascites;
d) Evidence of abdominal or distant metastasis.

As already reported, CA125 is unreliable in differentiating benign from malignant ovarian masses in premenopausal women because of the increased rate of false positives. In these women CA125 levels can be high in benign gynecological diseases such as endometriosis, fibroids, pelvic infections, but also in physiological conditions such as pregnancy or different phases of the menstrual cycle [56,57]. HE4 level is not influenced by pregnancy or the menstrual cycle phase and it never increases in patients with endometriosis or other benign ovarian masses [58,59]. Therefore HE4 may be the best biomarker in premenopausal women. Imaging is used to detect and characterize adnexal masses and to stage ovarian cancer both before and after initial treatment, although the role for imaging in screening for ovarian cancer has not been established.

The risk of encountering an unexpected ovarian malignancy after modern preoperative screening is 0.9% to 13% [60].

Transvaginal ultrasonography (TVUS) is the first line of imaging to study an adnexal mass. TVUS evaluates the size, structure (cystic, solid or mixed), the vascularization with the use of power-color doppler and the relationship with the surrounding structures. Over the past several years scoring system have been proposed to provide more objective criteria in discrimination between benign and malignant masses with a sensitivity close to 100% and specificity vary between 84 and 92%.

In order to use a common descriptive language for ultrasound readings, and better compare the data a study IOTA (International Ovarian Tumor analysis, still ongoing), has compiled an accurate classification of ovarian masses based on the content of the mass, the surface, the walls, the septa, the presence of papillary vegetations and the vascularization. This classification distinguishes different types of adnexal lesions: unilocular, unilocular solid multilocular, multilocular solid, solid. Some masses cannot be classified due to poor visualization (large cone shadow due to calcification as in some dermoid cysts) [61]. The study IOTA 2010 proposes an index of risk of malignancy based on the sonographic findings, the state of menopause and the serum concentration of CA125 suggesting a score ultrasound (U) from 0 to 3 according to multilocularity, the solid component, the bilateral presences of masses and the presence of ascites and eventual metastasis [61]. According to the literature about 8% of the lesions remain indeterminate at adnexal sonographic investigation. In this group are included lesions such as the tumors with low degree of malignancy, mucinous forms, fibroids and struma ovarii. In these cases the use of biomarkers together with imaging could be an important tool to formulate the diagnosis. Indeed, the high value of HE4 in a complex mass is strongly indicative for malignant lesion [62]. These tumors have proven difficult to classify with transvaginal ultrasound, and remain a diagnostic challenge for which accurate second-stage tests would be performed.

Several authors report magnetic resonance imaging (MRI) as the best method to investigate pelvic female thanks to the multiparametric capabilities of the method. MRI is used in the evaluation of benign conditions such as endometriosis and in the staging of malignant lesions of the uterus [63,64].

A correct diagnosis of any adnexal mass is essential to define appropriate treatment pathways. Some studies suggest that MRI, compared with other imaging modalities, may play a role in the assessment of adnexal masses 'difficult to classify' Conventional MRI combined with dynamic contrast-enhanced and Diffusion Weighted Imaging (DWI) may play a critical role in this cohort of patients [65].

Multi-detector computed tomography (MDCT) is the procedure of choice for preoperative staging of ovarian cancer and to determine the resectability of tumors. Recent studies show that MDCT had a sensitivity of approximately 93% with a specificity ranging between 91 and 96% for lesions larger than one centimeter. These parameters dramatically fail for lesions less than 0.5 cm in diameter, with a sensitivity that, in these cases, is around 43% [66]. Sensitivity reaches 100%, for implants located at the level of the hepatic dome, in the evaluation of the degree of infiltration of the liver parenchyma [67].

PET–CT (positron emission tomography–computed tomography) is a reliable imaging technique for suspected recurrence, particularly in women with rising CA125 levels, in the presence of negative results of conventional imaging.

In the follow-up is very important to correlate the level of biomarkers with imaging.

It is interesting to note that HE4 combined with sophisticated imaging techniques is a good marker for the early diagnosis. In addition, HE4 serum levels combined with MDCT may improve the monitoring management of women affected by ovarian cancer [68]. However, PET-CT demonstrates high sensitivity (75–97%) and accuracy ranging (92–96%). Those parameters are higher than those reported for MDCT (SE 61–92%) [69].

The PET–CT demonstrates a higher level of accuracy in the detection of para-aortic lymph node involvement and peritoneal metastases, this is even greater than the accuracy for recurrence at the vaginal dome [70].

7. Risk of malignancy algorithm: ROMA

Subsequently the discovery of serum HE4, researches have focused on its role in differentiating between epithelial ovarian cancer and benign masses. HE4 was combined in two high-risk disease evaluate formulas, called "risk of malignancy algorithm" (ROMA) formulated for premenopausal and for postmenopausal women with adnexal

mass. Unlike other algorithms as ROCA or RMI, ROMA combines the diagnostic power of the CA125 and HE4 markers with menopausal status lacking the imaging evaluation. This algorithm has been approved by the FDA as a useful indicator for differentiating malignant from benign pelvic masses. Although this index has been enhanced as a diagnostic instrument, high ROMA scores have been also reported to be independently associated with a negative prognosis in some patients with ovarian cancer [71].

Many studies report that ROMA algorithm implement better in the premenopausal population than in the postmenopausal women. A predicted probability (PP) greater than 13.1% suggests a high risk in the premenopausal women, whereas PP value higher than 27.7% indicates a high risk in the postmenopausal women. Using this algorithm, 93.8% of epithelial ovarian cancers were correctly defined as high risk [70, 72]. ROMA sensitivity and specificity suggests its use for the triage of woman with an adnexal mass to gynecologic oncologist [73–82] (Table 3). Currently the role of ROMA is controversial, to elucidate the clinical relevance of this algorithm numerous international studies were designed.

Unfortunately, the studies are not consistent with each other, probably due to the different numbers of patients investigated, the different geographical origins, and finally the different analytical systems used. Actually this quick approach does not solve all the problems related to the differential diagnosis of pelvic masses. Potential limitations or benefits of ROMA in clinical management will be better investigated.

8. Screening

Despite recent advances in ovarian cancer cure, new methods of early detection remain of paramount importance, because they have the potential to clearly improve long-term survival. However, a number of novel insights into disease etiology, evolution and biomarker discovery suggest that a new era in screening is underway [83].

Longitudinal studies are ongoing in several countries to evaluate screening strategies using CA125 and/or transvaginal sonography and their impact on overall cancer detection and mortality. Other current serum markers that have been identified up to now have not proven adequate sensitivity or specificity for screening. Thus, new biomarkers that could improve the early detection for ovarian cancer are critically needed. In the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial, investigators evaluated some 35 different biomarkers in proximal prediagnostically collected serum samples from 118 women who subsequently developed ovarian cancer. In evaluating samples that were obtained before conventional diagnosis, the addition of 7 biomarkers to CA125 in a combined multimarker panel did not improve sensitivity over that obtained with CA125 alone at 98% specificity [57–84].

Despite this observation, it is encouraging that Anderson et al. [85] demonstrated a progressive increase in CA125 and HE4 between 1 and 3 years before diagnosis of ovarian cancer.

Currently, a phase I screening trial is underway to evaluate the validity of HE4 in a first/second-line multimodal screening strategy that combines CA125, HE4 and transvaginal at high risk for ovarian cancer [86].

9. Medical strategies

Standard initial treatment of epithelial ovarian cancer includes surgery to optimally cyto-reduce cancer within the peritoneal cavity before the initiation of cytotoxic chemotherapy. Although no randomized controlled trials have demonstrated the superiority of this management approach compared with beginning chemotherapy after histologic confirmation of the presence of a malignancy consistent with the diagnosis of ovarian cancer, extensive retrospective data have demonstrated that patients with the smallest volume of residual cancer within the peritoneal cavity before initiation of chemotherapy achieve the longest survival. Although the definitive value of surgery is unknown, in most patients it can be safely performed by qualified gynecologic oncologic surgeons. Under these circumstances, there seems to be little justification to deny a patient the potential, although not proven, benefits of surgery.

Considerable uncertainty within the gynecologic cancer community remains regarding the importance of achieving a particular degree of cyto-reduction. Traditionally, based on considerable retrospective data, a patient has been classified as having “optimal residual disease” if the maximum diameter of the largest residual tumor nodule present within the peritoneal cavity at the completion of surgery is <1 cm [87]. However, some surgical groups have challenged this concept by arguing that patients who have all microscopic cancer within the peritoneal cavity removed have a substantially superior survival compared with patients in whom any macroscopic cancer remains. In the opinion of these surgical groups, the goal of surgery should be to achieve this state even if it requires extensive radical surgery, including in the upper abdomen, liver, and chest. Further examination of the clinical relevance of the extent of surgery in achieving an optimal residual disease level is indicated [88].

Large numbers of phase III trials have been conducted during the past 30 years that have sequentially helped to define the current standard of care in the chemotherapeutic management of advanced ovarian cancer [89].

Carboplatin/paclitaxel has been widely accepted as the standard of care in treating primary EOC. Many studies have demonstrated similar progression-free survival (PFS) and overall survival (OS) between the standard of care and the various alternates regimens [90,91].

Approaches that have been examined and found to be no better than the current gold standard include: 1) doubling the dose intensity of the platinum drug, 2) high-dose systemic chemotherapy[92], 3) extending the duration of the paclitaxel infusion (from 24 to 96 h), and 4) adding one of several biologically active third drugs to the platinum/taxane doublet [91].

However, findings from a report from Japanese investigators have revealed that the delivery of paclitaxel on a weekly schedule improves both progression-free and overall survival compared with the standard every-3-week paclitaxel regimen in patients with advanced ovarian

Table 3
CA125 and HE4 performance in ROMA.

Author	Study size	CA125 analytical method	HE4 analytical method	Specificity	Sensibility	NPV
Moore RG [73]	472	CMIA	EIA	75%	93%	90%
Karlsen MA [74]	1218	CMIA	CMIA	76%	94%	–
Van Gorp T [75]	432	EIA	EIA	77%	85%	83%
Jacob F [76]	160	EIA	EIA	86%	79%	–
Lenhard M [77]	535	CMIA	CMIA	95%	77%	–
Molina R [78]	527	CMIA	CMIA	88%	90%	96%
Montagnana M [79]	153	EIA	EIA	81%	74%	–
Ruggeri G [80]	259	CMIA	EIA	75%	96%	–
Ortiz-Munoz B [81]	279	ECLIA	ECLIA	91%	93%	98%
Anton C [82]	120	ECLIA	EIA	76%	74%	–

cancer [93]. Important confirmatory data for these provocative results are provided by 2 phase III studies in breast cancer that have revealed the superiority of weekly paclitaxel (compared with the every-3-week schedule) [94]. Preclinical data have suggested that paclitaxel may have clinically relevant antiangiogenic effects, and it is possible that the weekly drug delivery schedule may optimize this biological effect. Weekly cisplatin, however, was not found to impart any benefit when compared with its standard regimen.

Three multicenter National Cancer Institute cooperative group trials have reported superior progression-free and overall survival rates associated with intraperitoneal administration of cisplatin compared with intravenous delivery of the agent in the primary treatment of small-volume residual advanced ovarian cancer, a finding confirmed in a meta-analysis of randomized trial data [95]. Although the definition of "small volume" differed among the studies, the most recent trials defined eligible patients as those whose largest residual cancerous mass was <1 cm in maximum diameter after surgical cyto-reduction.

According to the current guidelines measurement of serum CA125 antigen remains the gold standard in the follow-up EOC. HE4 has proposed as emerged and promising biomarkers capable of following the remission from disease as monitoring response to therapy. The suggestion that HE4 is a good indicator for the remission from the disease was recently reported by follow-up studies, in which it was shown that the values of HE4 correlated with the clinical response to treatment or remission from the disease, as documented by CT imaging [96]. Interestingly, in patients with recurrence of the increased expression of HE4 preceded by up to 5–8 months the CA125. In addition, other studies suggest that the evaluation of serum HE4 changes could improve the assessment of response to chemotherapy in patients affected by EOC. Interestingly, it has been demonstrated that high HE4 levels at third chemotherapy cycle are significantly associated with the platinum-based chemotherapy response, therefore HE4 is able to identify the platinum non responding patients with the possibility to switch to second line chemotherapy and with the opportunity to improve the survival[97]. This means that HE4 is not only a good indicator for the remission from illness but, being able to anticipate its expression, compared to that of CA125, is an ideal EOC marker for therapeutic strategies against relapse [98].

10. Conclusions

Ovarian cancer is an issue of great importance to public health. Early diagnosis is very important for a better chance of survival. Up to now the only clinically serum marker accepted for the diagnosis and follow-up of ovarian carcinomas is the CA125, which presents, as known, diagnostic limitations. Recently, the measurement of HE4 has proposed for the differential diagnosis of ovarian cancer in women with pelvic mass. This review supports the diagnostic role of serum HE4 alone or in combination with CA125. It may be a valuable approach for distinguishing patients with ovarian endometrioma or other benign adnexal masses from those with ovarian malignancy. Differentiating between benign and malignant diseases is very important for correct referral of patients to institutions specialized in the care and management of EOC. Moreover, HE4 compared to CA125, is potentially a better marker for the early diagnosis and could be an important initial indicator of the recurrence of the disease and promising prognostic factor of EOC. In addition, preoperative levels of HE4 were intensely linked to EOC prognostic factors because significantly enhance with age, FIGO stage, grade and residual tumor. Additionally, HE4 serum levels, reflected the course of the disease during and after chemotherapy. It is relevant that the congruence of HE4 outline with the disease progression, also suggest that the determination of the changes in serum concentrations of HE4 can help to evaluate the response to treatment and early relapse in patients with EOC and that HE4 is a good indicator for the remission from the disease with the clinical response to treatment as documented by CT imaging.

Finally, for a correct evaluation of the HE4 performance, it is essential to consider the influence of age, menstrual status, smoking habits and renal function. As occurs for many other tumor markers, a decrease in renal function leads to a significant increase of HE4. This is not an irrelevant question even considering that patients in follow-up may have renal failure due to chemotherapy. The availability of a good tumor marker for the differential diagnosis, early detection, treatment and recurrence of EOC, could reduce medical costs related to more expensive diagnostic procedures and it may have a reassuring effect on the patient.

Conflict of interest statement

None.

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