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USE OF SENSORS ARRAY ANALYSIS TO DETECT OVARIAN CANCER THROUGH BREATH, URINE AND BLOOD

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Alla mia famiglia, ai miei nonni,

a tutti coloro che ci sono stati, ci sono e ci saranno....

Daniela Luvero

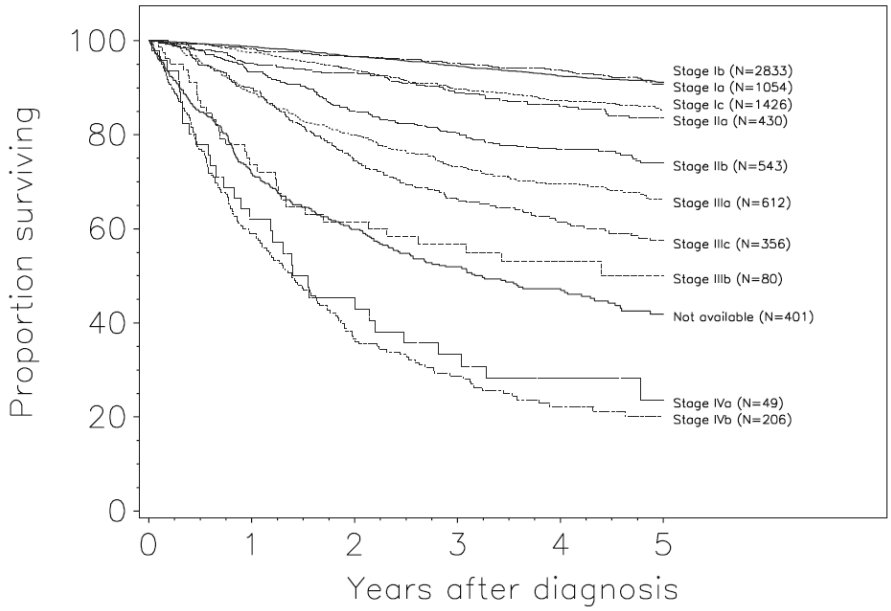
INTRODUCTION

1. BACKGROUND AND STATE OF THE ART

1.1 Epidemiology of ovarian cancer

According to the Global Cancer Observatory, ovarian cancer is the eighth most common cancer in women, with 313.959 new cases and 207.252 new deaths worldwide, in 2020. In Italy, 5370 new cases and 3285 of deaths were registered last year. Most of the cases, around 75-80%

Figure 1: Survival by FIGO stage



Epithelial ovarian cancer is predominantly a disease of perimenopausal and postmenopausal women with 80% to 90% of cases occurring after the age of 40. The median age at the time of diagnosis is 58.

Ovarian malignant germ cells tumors account for only 3% of all ovarian malignancies and they are the most common ovarian malignancies in young women with an average age at diagnosis is 20 years. The two main categories

of ovarian germ cell tumors include dysgerminomas and non-dysgerminomatous tumors. Dysgerminomas are composed of undifferentiated germ cells and account for 40% of germ cell malignancies. Non dysgerminomatous cancers are composed of abnormally differentiated germ cells. This category includes the immature teratoma, endodermal sinus tumor, embryonal tumor, polyembryoma, and choriocarcinoma. Signs and symptoms in these patients are consistent: abdominal pain associated with a palpable pelvic-abdominal mass is often reported.

Ovarian sex cord-stromal tumors account for 7% of all malignant ovarian neoplasms and develop from the gonadal non-germ cell component such as granulosa, Sertoli or Leydig cells. The clinical presentation of patients with ovarian sex cord-stromal tumor most often is associated with the excessive production of steroid hormones (menstrual irregularities, virilization); abdominal swelling and pain are also frequent. Most of these tumors are benign, and are generally localized unilaterally.

The majority of ovarian malignant germ cells and sex cord-stromal tumors are associated with a favorable prognosis and the last two decades have seen great improvements in their management. The results are an excellent example of the value of cooperation of different disciplines (surgery, radiotherapy, chemotherapy). Moreover, treatment of these pathological entities has to be individualized according to patient age, stage of tumor and degree of differentiation, as detailed in the following chapter.

Hereditary ovarian cancers generally occur about 10 years earlier.

The disease occurs sporadically in over 90% of the cases. An estimated 10% of all epithelial ovarian carcinomas, are familial. Nearly 75% of the cases of hereditary ovarian cancers is represented by the so-called breast and ovarian cancer syndrome, with germline mutations of the BRCA1 and BRCA2 genes. Women who carry a BRCA 1 or BRCA2 mutations have a 60% and 27% lifetime risk of developing ovarian cancer respectively.

Less frequent familial ovarian cancer is associated with hereditary nonpolyposis colon cancer or Lynch II cancer syndrome.

The most important known risk factor is a family history of ovarian cancer. A genetic counseling and discussion of various preventive strategies screening, oral contraceptives and prophylactic oophorectomy is recommended in the high-risk women. A history of infertility, low parity, and a long time from menarche to menopause are reported to be associated with increased lifetime risk of ovarian cancer. These observations have led to the concept of "incessant ovulation" as being a factor in the genesis of epithelial ovarian malignancies.
There is a significant protective association between oral contraceptive use and ovarian cancer. The protective effect appears to increase with the duration of the oral contraceptives use: a review of the literature demonstrated a 10-12% decrease in risk associated with use for one year and an approximate 50% decrease after five years of use.

1.2 Etiology

The etiology of EOC is complex and not clearly defined. The genome encodes proteins that control the function, growth, and division of cells. DNA damage and mechanisms to repair exist in order to decrease the likelihood of genetic mutation and cell transformation. In addition, the immune system is designed to recognize early changes in carcinogenesis and destroy cancerous cells to keep the balance in cell proliferation and cell death. Accumulation of disruptions in these homeostatic control mechanisms can lead to uncontrolled proliferation and cancer. The six hallmarks of cancer introduced by Hanahan et al. include sustaining proliferative signaling, evading growth suppressors, resisting cell death, unlimited replication capability, inducing angiogenesis, and activating invasion and metastasis. Cancer related inflammation is postulated to be the seventh hallmark, with smoldering inflammation in the tumor environment, that promotes genetic instability and accumulation of genetically altered cancer cells. The interplay between milieu and genes is a fundamental mechanism in cancer, with epigenetic events like DNA methylation and histone modification as

a link. Ovarian carcinogenesis, as in most cancers, involves multiple genetic alterations and molecular changes, with important key pathways related to chronic inflammation. The crosstalk and signaling interactions between cancer cells and their supporting stroma evolves during the tumor development.

Inflammation and epithelial ovarian cancer

Chronic inflammation underlies the progression of ovarian cancer. Pathways that link inflammation and cancer have been identified, an intrinsic pathway driven by genetic events (RAS, MYS, and TP53 mutations) and an extrinsic inflammatory driven pathway. The mitochondria, the organelle that supply energy to the cells, have an important role in coordinating life and death signaling in the convergence of these pathways, promoting inflammation by producing free radicals and activating transcriptions factors, such as nuclear factor kappa B (NFκB), signal transducer and activator of transcription 3 (STAT3) and hypoxia-inducible factor (HIF) to orchestrate the production of inflammatory mediators and generate cancer related inflammation. Inflammatory responses, with communication between stromal microenvironment and the epithelial compartment, play a role at all stages of cancer development, including initiation by generating genotoxic stress, promotion by inducing cellular proliferation, malignant conversion, and metastasis by enhancing angiogenesis and invasion. The immune system has a dual function which is called cancer immunoediting; both protecting the host against tumor development and as well as promoting the tumor to grow. The response from the body to cancer is not a unique mechanism, actually there are many parallels with inflammation and wound healing, "a wound that does not heal ". Use of anti-inflammatory drugs like aspirin has been related to reduction in the long-term risk of several cancers and the risk of distant metastasis, which further supports the relation between inflammation and cancer. Key features of cancer-related inflammation include leukocyte infiltration, prominent tumor associated macrophages (TAMs), vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX2), cytokines (small cell signaling molecules) such as tumor necrosis factor alpha (TNFα), transforming growth factor beta (TGFβ),

interleukin-1 (IL-1), IL-6 (CXCL6) and chemokines (chemotactic cytokines) like IL-8 (CXCL8), growth regulated alpha protein (GROα; CXCL1) and Monocyte chemoattractant protein-1 (MCP-1; CCL2). MCP-1 attract TAMs, which are the major players in the cancer related inflammation, and enhances angiogenesis and tissue remodeling. The interaction of the cytokines is strongly regulated with positive and negative feedback aiming to maintain balance in the immune control. Ability of malignant cells to interact with and influence their environment is critical for the development of cancer, and chronic inflammation coordinate a cancer supporting microenvironment.

Incessant ovulation and the gonadotropin theory, with exposure to follicle stimulating hormone (FSH) and luteinizing hormone (LH), have been considered to play a major role in ovarian cancer development. Ovulation is an inflammatory process, which involves repeated minor trauma of the ovarian surface and exposure to estrogen rich follicular fluid, cytokine release, influx of inflammatory cells to the ovarian stroma. Every month the epithelial cells are affected by increased oxidative stress, production of reactive oxygen and nitrogen species (ROS and RNS), cell damage, elevations of cytokines, proteases and prostaglandins, and the subsequent repair mechanisms with epithelial to mesenchymal transition (EMT) that place the cells at increased risk of developing mutations, and this repeated activity may initiate oncogenesis by causing DNA damage in adjacent cells. Even the fimbria of the fallopian tube is exposed to iron induced oxidative stress, by floating in the bloody peritoneal fluid, derived from retrograde menstruation. Many of the inflammatory cytokines and chemokines activated during ovulation have been found to exhibit overlap with that described in EOC. The TGFβ- family of multifunctional cytokines acts as tumor suppressors by inhibition of cell proliferation in normal tissue and in early tumorigenesis, but during oncogenesis switches its role to promote progression by interfering with EMT. IL-8 and anti-IL-8 antibody are present in serum from EOC patients, and increased secretion of VEGF, IL-6, GROα and IL-8 promoting cancer growth have been noted in the oncogenic RAS-signaling, present in one third of human cancers. IL-6 is a growth promoting and anti-

apoptotic factor, found with high plasma levels in advanced stage EOCs and is known to correlate with poor prognosis. The epidermal growth factor receptor (EGFR; HER1), a member of erythroblastic leukemia viral oncogene family (ERBB-family), (HER 1-4) of receptor tyrosine kinase, have a key role in the development of a normal follicle, and is also involved in activation of multiple signaling cascades, that cause growth and invasion of tumor cells, and has been related to poor outcome, among other factors via increased co-expression of IL-6 and plasminogen activator inhibitor-1(PAI-1).

Age is in general a risk factor for cancer, with changes in redox status and oxidative stress induced inflammatory reactions that lead to overall deregulation or acquired dysfunctional immunity, called immunosenescence. A typical feature of aging is a chronic, low-grade inflammatory status, an inflammatory aging with changes in the cytokine profile towards a pro-inflammatory condition, with increase of some chemokines; RANTES (CCL5), macrophage inflammatory protein 1 (MIP-1; CCL-3), IL-8, and MCP-1. GROα has been evaluated in this process and is supposed to be a cellular signal activated by extracellular oncogenic signals in aged epithelial cells, and may be a novel diagnostic marker for age-related pathology, including cancer. Endometriosis and pelvic inflammatory disease are related to both acute and chronic inflammation. Obesity is as well associated with a chronic state of low-grade inflammation. Increased concentrations of fatty acids, inflammatory cytokines and an influx of immune cells together with adipokines (cytokines secreted by adipocytes, which are regulators of metabolism and immunity, produced by the white adipose tissue contributes to the local inflammatory milieu in adipose tissue. The coagulation pathway is involved in cancer related inflammation, with IL-8 as the linking point. Coagulations factors promote not only formation of blood clots but also tumor cell proliferation, angiogenesis, invasion and metastasis. Fibrin formation can be inhibited by membrane associated endothelial protein C receptor (EPCR) via activated protein C (APC) in ascites and promote fluid expansion. In blood, soluble EPCR can cause a hyper-coagulation state associated with malignancy.

Origin and pathogenesis

There is uncertainty surrounding the site of origin of EOC. Surface epithelium of the ovary (OSE), epithelial invaginations, inclusion cysts inside the ovaries, and dysplastic lesions from the fallopian tube and the uterus have been suggested to be the origin of EOC. Auersperg state that both ovarian epithelium and the oviduct originate in the embryonic pleuripotential mesothelial coelomic epithelium and are therefore able to produce similar tumors. Dysplasia of OSE differentiates into epithelia resembling Müllerian duct derivates, serous tumors will be like the fallopian tube epithelium, endometrioid tumors similar to endometrium in the uterus, and mucinous tumors like epithelium of endocervix. Homebox (HOX) genes are strongly expressed in ovarian cancer, and not in normal epithelium. These genes contain transcription factors that determine cellular identity, and play a key role in the embryonic development, were the HOXA9 becomes expressed in the fallopian tubes, HOXA10 in the developing uterus, HOXA11 in the lower uterine segment and cervix and HOXA13 in the upper vagina. It is thought that appropriate expression of these genes is an early step in neoplasia of the ovarian epithelium, as they induce aberrant epithelial differentiation.

During the past 10 years, more evidence has led to a paradigm shift in the process of etiology and pathogenesis in the framework of different origins that may develop after distinct pathways, from the ovary, tube, peritoneum and endometrium. Immunohistochemical, morphologic and molecular genetic analysis proposes that EOC is more like metastases. Serous fallopian tube carcinoma was found more often in women harboring BRCA1 and BRCA2 mutation than in sporadic cases. A suggested candidate precursor lesion for EOC, called serous tubal intra epithelial carcinoma (STIC), is present in the non-ciliated epithelium of the distal fimbria of the fallopian tube. STIC is then supposed to implant onto the ovarian and /or peritoneal surfaces, and after an occult period will develop into fast growing high-grade serous cancer. P53 signature, an early alteration in p53 function, is proposed to occur before STIC. Different gene alterations have been discovered in the oviduct, as secretory cell

outgrowths (SCOUT), increased in frequency as a function of older age and serous cancer. The p53 signature and its malignant counterpart STIC have proposed the link between the fallopian tube, peritoneal and ovarian serous carcinomas. Supporting this theory is that the pared box gene 8 (PAX8), a marker of Müllerian-type epithelium was found expressed in high-grade serous cancer, but not in OSE, whereas calretinin, a marker of mesothelioma and OSE was not detected in EOC or in the tube. Complexity of regulation on a genomic level with DNA repair mechanisms, as well as NOTCH pathways (an evolutionarily conserved pathway that regulates cell-fate determination during development and maintains adult tissue homeostasis) and the regulatory network of the transcription factor FOXM1 (forkhead box protein M1) - signaling are involved in the high-grade serous cancers.

The low-grade serous cancers are distinct tumors that might develop from more clearly defined lesions, such as cystadenom, adenofibroma and borderline tumors, in more indolent stepwise manner. These tumors evolve from OSE, invaginations and inclusion cyst inside the ovaries via alteration in the RAS-RAF signaling pathway, which is responsible for normal cell growth, differentiation and survival, due to mutation in KRAS (GTPases, molecular switches for a variety of cellular signaling events) and BRAF (a kinase cascade, that send a signal from the surface of the cell to the DNA in the nucleus).

The recent literature suggests that ovarian low-grade serous tumors and their non-invasive implants, ovarian epithelial inclusion glands and endosalpingiosis (fallopian tube-like epithelium is found outside of the fallopian tube) might arise from the fallopian tube rather than through Müllerian metaplasia of the OSE. Low-grade endometrioid and clear cell cancer may arise from endometriosis via retrograde menstruation, with multi-factorial etiology including genetic, hormonal and immunological factors. AT-rich interactive domain-containing protein 1 A (ARID1A), tumor- suppressor gene mutation frequently found in these lesions can be an early event in the transformation into cancer. Mutation of the catenin-interacting protein 1 (CTNNB1) the gene that encodes β-catenin (regulating cell-cell adhesion and gene transcription), and phosphatase and tensin homolog

(PTEN) a tumor suppressor gene (involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly) are found primarily in low-grade endometrioid, whereas phosphatidylinositol 3-kinase (PIKC3CA) oncogene mutations characterize clear cell cancer. Approximately 80% of all mucinous tumors are benign, and most of the remainder borderline. Mucinous tumors harbor high frequency of KRAS mutations; these tumors often show gastrointestinal differentiation and have also been related to the endocervix. Mucinous and transitional cells tumors (Brenner) were recently reported to develop from transitional epithelial cells located near the tubo-peritoneal junction. However, the majority of invasive mucinous tumors are metastases to the ovary, often from the gastrointestinal tract including colon, appendix or stomach, if appropriate examined only 3-4% are left as ovarian carcinomas.

Dualistic model - Type I and Type II

Kurman proposed a novel tumor origination and progression model, based on morphological and molecular genetics, dividing EOC into type I and type II tumors. This simplistic approach indicates that the two tumor types develop via two different pathways, slow-growing type I and rapid-growing highly aggressive type II tumors (Table 1).

Low-grade serous, low-grade endometrioid, all clear cell, mucinous, and transitional (Brenner) carcinomas are classified as type I, were each histological type has a distinct molecular profile. Type II tumors are the most common, and include high-grade serous, high-grade endometrioid, undifferentiated carcinoma and malignant mixed mesodermal tumors or carcinosarcomas .Low-grade type I carcinomas exhibit low-grade nuclei with infrequent mitotic figures. They evolve in a slow stepwise process from defined benign or borderline lesions to invasive cancer. These tumors harbor frequent somatic mutations, encoding mismatch repair proteins and signaling proteins governing cell proliferation, such as BRAF, KRAS, β-catenin, PTEN or ERBB2 (HER2) genes, but lack TP53 mutations.

Table 1. Pathogenesis of slow-growing Type I and aggressive Type II EOC.

EOC	%	Precursor lesion	Gene mutation	Genom	Tempo
Type I	**25**	Ovary; Cystadenoma → → → → Borderline → LGSC Tube; Endosalpingiosis → LGSC Uterus; Endometriosis →→ Clear Cell and LG-Endometrioid Cervix, G-I, Ovary, Tube; → → Borderline → Mucinous	KRAS, BRAF, ERBB2, PIKC3CA, PTEN, ARIDA1A β-catenin, PTEN, ARID1A KRAS	Stable	Slow Step-wise
Type II	**75**	Tubal fimbria/ovarium; STIC →HGSC ? → HG-Endometrioid	TP53, BRCA1-2 TP53	Chaotic	Fast

LGSC=low-grade serous carcinoma; HGSC = high-grade serous carcinoma; G-I=gastro-intestinal; STIC = serous tubal intraepithelial carcinoma

Type I tumors are in general larger, in earlier stages and in younger women when diagnosed compared to type II EOC, and consequently type I EOC have a better prognosis. Type II tumors are more aggressive and genetically highly instable with frequent mitotic high-grade nucleus, with increased expression of Ki-67 (a cellular marker of proliferation), and estrogen receptor (ER) is expressed in circa 75%. Majority of the tumors have TP53 mutation, and almost half of the cases have mutation or dysfunction of BRCA1/2 (10-20% have mutation of BRCA1/2 and 10-40% hypermetylation or dysfunction of BRCA1). These aggressive tumors account for 75% of all EOC, and are responsible for 90% of death in the disease.

1.3 Histological classification

The task forces of FIGO endorse the histologic typing of ovarian tumors as presented in the WHO publication no. 9, 1973, and recommend that all ovarian epithelial tumors be subdivided according to a simplified version of this (Table 2)

Table 2: Histological classification of ovarian tumors

HISTOLOGIC TYPE	ANALOGOUS CELL TYPE
Serous (75%)	Endosalpingeal
Mucinous (10%)	Endocervical
Endometrioid (10%)	Endometrial
Clear cell	Mullerian
Transitional cell (Brenner tumor)	Transitional
Squamous cell tumor	Squamous
Mixed epithelial	Mixed
Undifferentiated	Anaplastic
Unclassified	Mesothelioma, etc

The types of tumors classified are as follows: serous, mucinous, endometrioid, clear cell (mesonephroid), undifferentiated and unclassified.

1 Serous tumors

- Benign serous cystadenomas
- Of borderline malignancy: serous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)
- Serous cystadenocarcinomas

2 Mucinous tumors

- Benign mucinous cystadenomas
- Of borderline malignancy: mucinous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrativedestructive growth (carcinomas of low potential malignancy)
- Mucinous cystadenocarcinomas

2 • Endometrioid tumors

- Benign endometrioid cystadenomas
- Endometrioid tumors with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)
- Endometrioid adenocarcinomas

3 Clear cell tumors

- Benign clear cell tumors
- Clear cell tumors with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (low potential malignancy)
- Clear cell cystadenocarcinomas

4. Brenner

- Benign Brenner
- Borderline malignancy
- Malignant
- Transitional cell

5 Undifferentiated carcinomas: a malignant tumor of epithelial structure that is too poorly differentiated to be placed in any other group.

6 Mixed epithelial tumors: these tumors are composed of two or more of the five major cell types of common epithelial tumors (types should be specified).

7 Cases with intraperitoneal carcinoma in which the ovaries appear to be incidentally involved and not the primary origin should be labeled as extra-ovarian peritoneal carcinoma.

Histopathologic grade (G)

5. GX: Grade cannot be assessed
6. G1: Well differentiated
7. G2: Moderately differentiated
8. G3: Poorly or undifferentiated

1.4 Risk Factors

Multiple endogenous and exogenous risk factors have been shown to influence ovarian cancer development. Advancing age is one of the major risk factors and accumulated genetic damage is likely involved. Cellular senescence (CS) could have a role in aging and age-related diseases. Hereditary factors are involved in about 10-15% of cases, with history of earlier breast cancer, hereditary breast and ovarian cancer (HBOC), resulting from a BRCA1 or BRCA2 gene mutation and hereditary nonpolyposis colorectal cancer (HNPCC) gene, or TP53 mutation. Carriers of BCRA1or BRCA2 mutation have increased lifetime risk of ovarian cancer up to 50-60% respective 25%, and are estimated to cause 65-85% of all heredity cases. HNPCC or germline mismatch repair (MMR) gene mutations (MLH1, MLH2, MSH6) linked to Lynch syndrome accounts for 10-15% of heredity cases and have an increased lifetime risk of 8% for EOC, and highest for those with MLH2, MSH6 mutation. These women tend to be at younger age and with non-serous tumor presenting in an early stage. "Incessant ovulation theory", introduced by Fathalla 1971, more ovulations over a lifetime increases the risk of getting EOC by creating an unfavorable microenvironment. Poor reproductive history with long duration, low parity, early menarche, late menopause, and infertility, has been associated to increased risk of EOC. Endometriosis defined as endometrial implants outside the uterus, transported via retrograde menstruation, usually present on ovaries and peritoneum in the pelvis. Acute and chronic inflammation in combination with immune dysfunction is acting in endometriosis. Several characteristics are shared with invasive endometrioid and clear cell cancer; both harbor similar cytokines and genetic defects, and have a capacity to spread distantly.

The observation that the incidence of ovarian cancer increases after menopause, and the increase in gonadotropin levels at the same time generated the "Gonadotropin theory" 1975 by Stadel. Hormonal effect with increased estrogenic stimulation of the OSE as a result of excessive gonadotropin (FSH, LH) secretion, related to menopause, ovulation, or infertility

therapy and hormone replacement therapy (HRT) has been implicated as possible risk factor for EOC. Higher levels of androgens, which are increased in menopausal or obese women and seen among women with polycystic ovarian syndrome (PCOS), were associated with an increased risk of ovarian cancer, whereas progesterone had protective effect. In a populations study (n=29 000) in Sweden, obesity was related to significant excess risk for endometrial- (standard incidence ratio (SIR) 2.9), cervix- (1.4) and ovarian cancer (1.2). Pelvic inflammatory disease (PID) was coupled to increased risk in a study of 200 000 women in Taiwan. Local inflammation like asbestosis and talc exposure has also been related to EOC.

Risk factors are found to have different effects in the different histological types of EOC. With longer use of oral contraceptives the risk decreased with 20% for each 5 years, and after 15 years the risk was halved, and the protective effect was on all types of EOC except for mucinous cancer. In a recent study (n=849 EOC/n=169 391 healthy women under mean 5.1 years), HRT was associated to increased risk of serous and endometrioid EOC (RR=1.3), but to decrease in risk for the mucinous type (RR=0.37). In the same study, obesity (BMI >30) was found to be related to increased risk in endometrioid EOC (RR=1.67), similar to endometrial cancer of the uterus, but decreased risk was found for serous, mucinous and clear cell cancer. Infertility itself is a risk factor for EOC, and it is still debated if fertility drugs will increase the risk for EOC or not. Physical activity, smoking dietary fat, and other life style factors may also affect the risk. Prevention of ovulation have been considered as protective against ovarian cancer; oral contraceptives, multiparty and long lactation periods, as well as obliteration of the tubes by tubal ligation, prophylactic oophorectomy and hysterectomy.

1.5 Diagnosis of ovarian cancer

Epithelial ovarian adenocarcinoma (EOC) is the most deadly of the gynecologic tumors. Although there have been advances in surgery and chemotherapy, the

survival rate for this disease remains low. The majority of cases diagnosed with EOC have already spread to the upper abdomen with omental cake and peritoneal metastasis. Noninvasive diagnostic procedures are lacking, therefore invasive surgery is needed followed by pathologic anatomic diagnosis (PAD) to confirm the definite diagnosis. The principle cause of the poor survival rate for the patients is diagnosis at a late stage, when a radical surgery is not possible or unsuccessful. If diagnosed in stage I, when the cancer is confined to the ovaries, the 5-year survival is over 90% after optimal surgery, this in contrast to less than 30% when the cancer had spread to the abdomen or outside the abdomen (stage III or IV). Unfortunately, less than 25% of cases are diagnosed early, and as a consequence the overall 5-year survival in EOC is less than 50%. Early diagnosis means possibility to cure with surgery and continuous life without sequel from expensive cytotoxic drugs and extensive operations. Correctly diagnosed EOC followed by treatment at the right level of care will improve the survival of women and decrease the number of unnecessary extensive operations in the benign cases, which leads to improved quality of life for all women with ovarian tumors.

Retrospective studies show that women with ovarian cancer present with non-specific symptoms including abdominal pain and bloating, changes in bowel habit, urinary and/or pelvic symptoms. Cachexia is uncommon and women with advanced disease often look surprisingly well. Most women with ovarian cancer are diagnosed when they already have advanced disease. On average, a GP will see only one new case every five years. Patients who present with non-specific gastrointestinal symptoms may be misdiagnosed as suffering from irritable bowel syndrome.

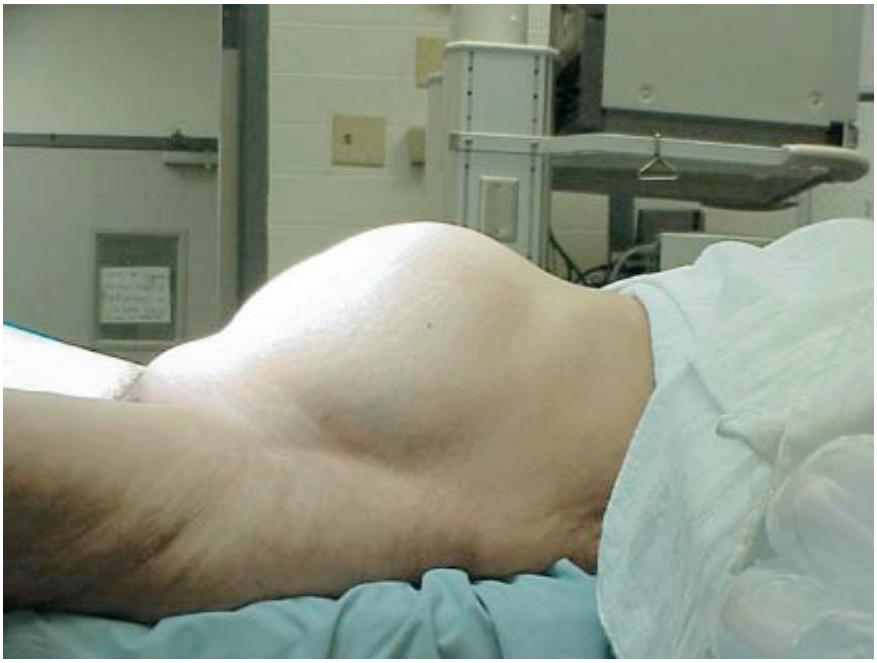
Ovarian cancer typically is portrayed as a "silent killer" without appreciable signs or symptoms until advanced disease is obvious clinically. A pelvic or pelvic-abdominal mass is palpable in most patients with ovarian cancer (Fig.2).

In general, malignant tumors tend to be solid, nodular, and fixed, but there are no pathognomonic findings that distinguish these growths from benign tumors

Therefore, at diagnosis 62% of the cases of epithelial ovarian cancers presents at advanced disease (FIGO stage III-IV) because most women with the disease do not have any symptoms for long time.

The most common presenting symptom is abdominal discomfort or pain with or without abdominal distention due to the presence of ascites or large abdominal masses.

Figure 2: patients with palpable pelvic-abdominal mass



The symptoms of nausea, dyspepsia, constipation, anorexia and intolerance to the food are common but, unfortunately, non-specific. These patients must undergo complete physical examination; greater attention should be directed toward the bimanual pelvic examination to detect adnexal masses. Ultrasonography is frequently used to aid in the evaluation of adnexal pelvic masses. The preoperative evaluation of patients with suspected ovarian carcinoma should include a serum Ca125 level.

Ca125 has proven to be the most useful, currently available, marker for epithelial ovarian cancer, primarily because of its utility in monitoring the results of therapy. The value of screening for ovarian cancer is uncertain. Even if a

significant reduction in mortality could be demonstrated with screening programs that use ultrasound and serum markers, this approach may not be practical because of the high cost associated with screening for a low incidence disease.

Approximately 80% of patients with advanced ovarian cancer have elevated concentrations of CA125 in the blood serum. However, no more than 50% of patients with clinically detectable stage I disease have elevated CA125 levels.

A large randomized controlled trial (RCT) of 78,216 women aged 55 to 74 in the United States demonstrated that testing for CA125 blood serum level combined with transvaginal ultrasound (TvUS) conferred no benefit in screening for ovarian cancer in the general population compared to usual care. In this study, ovarian cancer was diagnosed in 212 women (5.7 per 10,000 person-years) in the intervention group and 176 (4.7 per 10,000 person-years) in the usual care group (rate ratio 1.21, 95% confidence interval (CI) 0.99 to 1.48). There were 118 deaths from ovarian cancer in the intervention group (3.1 per 10,000 person years) and 100 deaths (2.6 per 10,000 person-years) in the usual care group (mortality risk ratio (RR) 1.18, 95% CI 0.82 to 1.71).

In the last decades, many other markers and diagnostic tools were used to improve the diagnosis of ovarian cancer with poor results.

There are two scoring systems for assessing malignancy risk, the Risk of Malignancy Index 1 (RMI 1) and the Risk of Malignancy Index 2 (RMI 2), each of which calculates scores using ultrasound features, menopausal status and preoperative CA125 level according to the equation: RMI score = ultrasound score x menopausal score x CA125 level in U/ml.

The original RMI 1 scoring system and the revised RMI 2 system are both outlined in Table 3.

The RMI 2 score gives greater weight to the ultrasound findings and menopausal status than the RMI 1 score.

Table 3: The risk of malignancy index (RMI) scoring system

Feature	RMI 1 Score	RMI 2 Score
Ultrasound features: • multilocular cyst • solid areas • bilateral lesions • ascites • intra-abdominal metastases	0 = none 1 = one abnormality 3 = two or more abnormalities	0 = none 1 = one abnormality 4 = two or more abnormalities
Premenopausal Postmenopausal	1 3	1 4
CA125	U/ml	U/ml
RMI score = ultrasound score x menopausal score x CA125 level in U/ml		

It has been suggested that RMI and other explicit scoring systems may be less sensitive than ultrasound morphological scores, but are more specific. Other morphological scoring systems are being developed that may supersede RMI 1, but these require more extensive external validation and may be more complicated to use in practice.

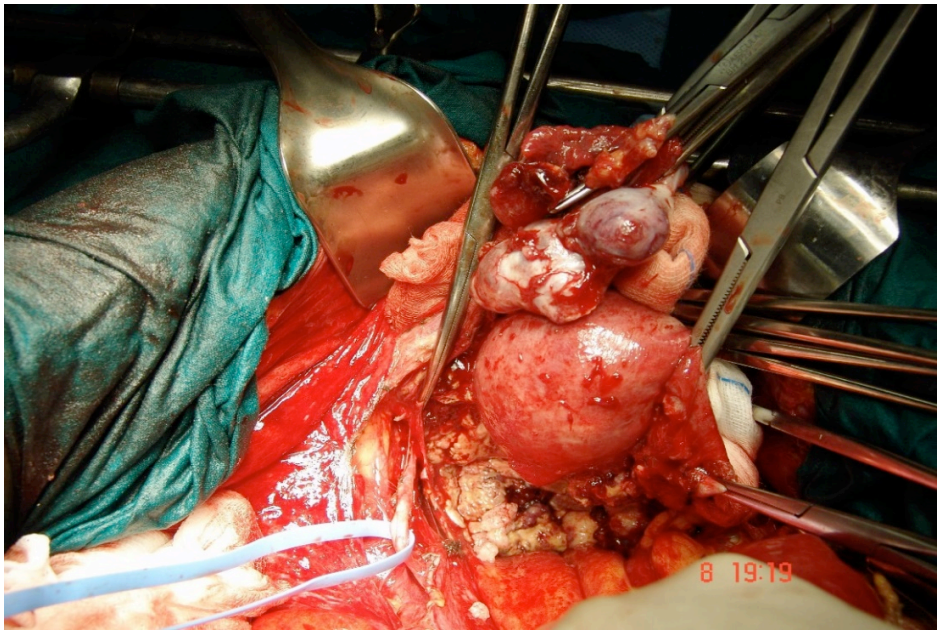
Risk of Malignancy Index 1 score with threshold of 200 should be used to predict the likelihood of ovarian cancer. Patients with an RMI 1 score greater than 200 should be referred to a gynecology oncology multidisciplinary team.

However at this moment screening for ovarian cancer in the general population should not be performed outwit the research setting.

1.6 Patterns of spread and surgical staging

Spread of ovarian cancer beyond the ovary occurs in three ways. First, the tumor can penetrate the ovarian capsule and directly invade contiguous organs such as the uterus, fallopian tube, bladder, rectum, or the pelvic peritoneum (Fig.3).

Figure 3: Ovarian cancer- surgical findings



Second, the tumor cells may spread via the lymphatics involving the pelvic and para-aortic lymph nodes. Lymph node metastases occur in as many as 20% of early stage cancers and the majority of advanced-stage cancers. Third, ovarian cancer cells will escape from areas where the tumor has penetrated the capsule of the ovary and escape into the peritoneal cavity. These free tumor cells are then spread throughout the abdomen by respiratory motion of the diaphragm, peristalsis of the intestine, and the changes in position of normal daily activities. This latter form of spread is the most devastating because it results in the dissemination of tumor cells throughout the peritoneal cavity.

Therefore, patients affected by epithelial ovarian cancer have metastases most commonly in the peritoneal cavity, and, occasionally also in extra-peritoneal locations. The risk for early peritoneal seeding depends on stage as well as biological factors not included in the current FIGO 1988 staging system.

It has been postulated that neoplasms originating in the ovary have two major routes of spread: the first being migration of exfoliated cells within the normal circulation of peritoneal fluid, reaching the domes of the diaphragm and

omentum through the para-colic gutters, followed by local stromal activation and then invasion; the other route is by lymphatic permeation. Six to eight lymphatic channels originate on the ovarian surface and drain by three main routes: along the infundibulopelvic ligament to the supracaval- and intercavoaortic nodes, along the broad ligament to the interiliac and upper gluteal nodes, and by the round ligament to the external iliac- and inguinal nodes. Lymphatic involvement, common in advanced stage patients can be explained in part by local invasive activity and local blood and lymph vessel angiogenesis, but may be considered a step earlier in metastatic activity, prior to parenchymal involvement. Further, there are no data indicating that the presence of lymph node disease is a marker for or a precursor of synchronous or late presenting parenchymal disease. There is evidence of a third way (although rare 1.9%) of hematogenous circulation of epithelial ovarian as has been shown in blood and bone marrow studies. Controversy exists as to the prognostic importance of these findings as at least one large study has not documented a worse outcome in case of the presence of ovarian cancer cells in bone marrow or blood.

Cancer staging is a description of the extent of the cancer. Cancer stages are defined by the growth of the primary tumor and its spread to other parts of the body. Clinical staging is based on tests done before surgery and pathologic staging on tests of tissue removed during the staging operation. An appropriate systematic surgical staging performed at the initial surgery is of great importance to find out how widespread the cancer is for planning further therapy. Microscopic examination, with assessment of specific histology type, grade and extent of disease is critical for predicting tumor behavior and for deciding the best therapeutic approach. EOC is staged I-IV according to FIGO (Table 4).

Table 4. Carcinoma of the ovary. FIGO staging

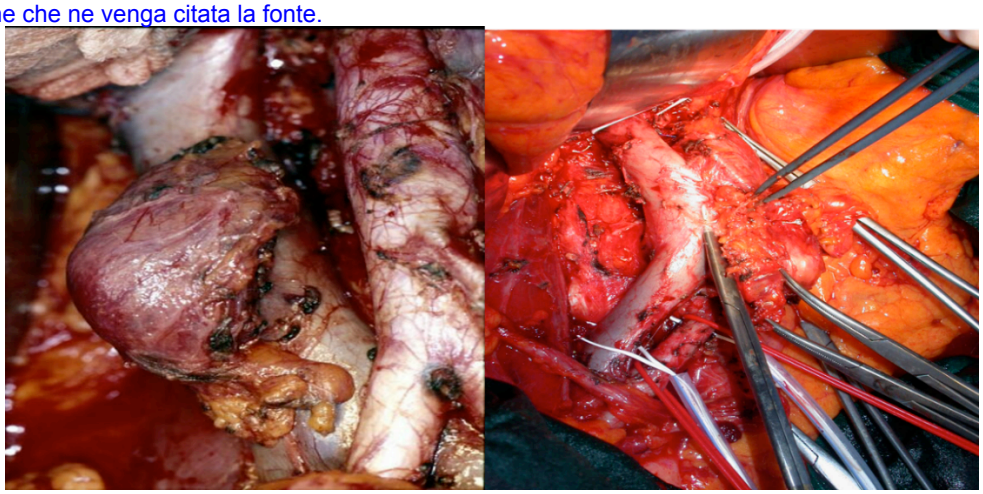
Stage	Definition
I	Tumor limited to the ovaries
IA	Tumor limited to one ovary; capsule intact, no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings
IB	Tumor limited to both ovaries; capsule intact; no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings
IC	Tumor limited to one or both ovaries with any of the following: capsule ruptured, tumor on ovarian surface, malignant cells in ascites or peritoneal washings.

II IIA IIB IIC	Tumor involves one or both ovaries with pelvic extension Extension and/or implants on uterus and/or tubes; no malignant cells in ascites or peritoneal washings Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings Pelvic extension with malignant cells in ascites or peritoneal washings.
III IIIA IIIB IIIC	Tumor involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph node metastasis. Microscopic peritoneal metastasis beyond pelvis Macroscopic peritoneal metastasis beyond pelvis 2 cm or less in greatest dimension Peritoneal metastasis beyond pelvis more than 2 cm in greatest dimension and/or regional lymph node metastasis
V	Distant metastasis (excluding peritoneal metastasis).

For all the patients, a comprehensive surgical staging should be performed (total hysterectomy, bilateral salpingo-oophorectomy, omentectomy, peritoneal biopsies, peritoneal washing cytology, lymph node evaluation in accordance with FIGO guidelines.

EOC confined to one or both ovaries is classified as stage I, spread to the uterus or other nearby organs in pelvis stage II, spread to the lymph nodes or abdominal lining is stage III (Figure 4), and spread to distant organs such as the lung or liver is classified as stage IV EOC. Liver capsule metastasis is stage III, metastasis of liver parenchyma stage IV (Figure 5), and pleural effusion must have positive cytology to be classified as stage

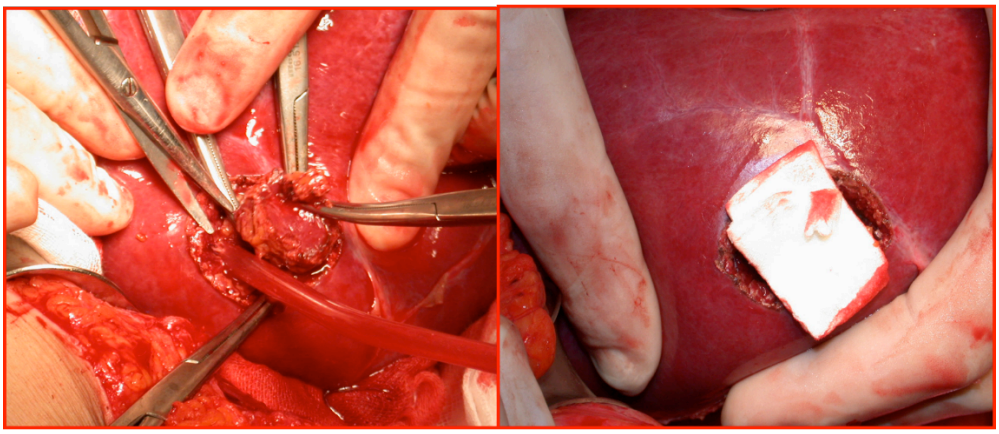
Figure 4: Lymph node metastasis



Ovarian cancer spreads by direct contact with other tissues in the pelvis, by exfoliated tumor cells transported through the fluid in the abdominal cavity and pleura, by invading lymph channels to spread through lymph nodes, and more seldom through the blood vessels to give metastases in other organs.

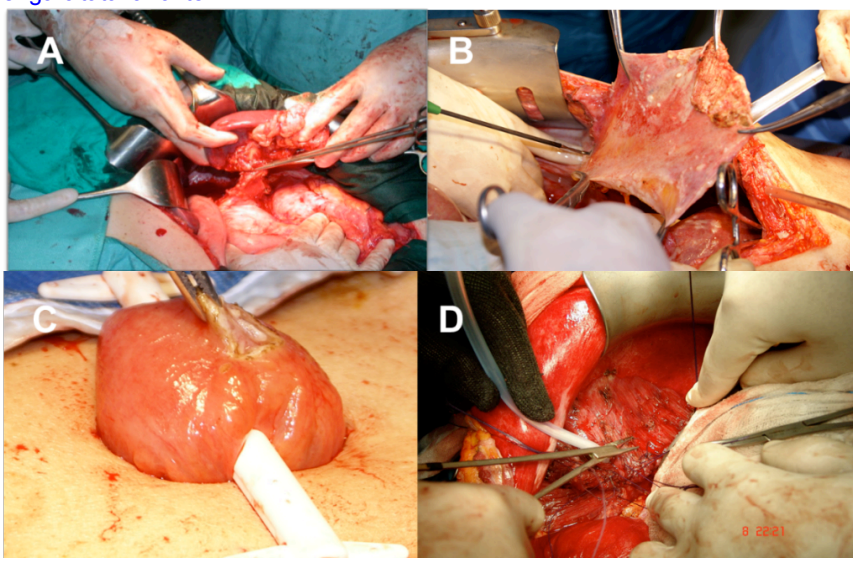
The biological behavior of EOC is unique in its early dissemination of detached cancer cells that are transported physically by peritoneal fluid.

Figure 5: Liver metastasis



The tumor implants invade the mesothelial cell layers lining the abdominal cavity, and at the surface of bowel, liver and other organs in the abdomen (Figure 6), but interestingly rarely invade deep into the peritoneum.

Figure 6: Tumor implants



A= Spleen; B= Pelvic Peritoneum; C=Larege Bowel; D= Diaphragm

Problems of "understaging", especially in apparent early stage diseases, are well-documented. In an often-cited report, Young et al showed that staging was often carelessly performed. They performed prospective systematic restaging of 100 patients referred as stage I to II b patients within 4 weeks IV (Figure 7).

Only 25% of the patients were found to have an initial surgical incision large enough for complete examination of the abdomen. Of the 68 patients restaged by laparotomy, 61 were referred by their physician as free of residual cancer, but at the time of restaging laparotomy 22 of these patients were upstaged. Out of a total of 100 patients, 31 were upstaged and 23 of these had stage III disease.

The most common sites of occult cancer are within peritoneal fluid or washings, the pelvic peritoneum or omentum, or in the subdiaphragmatic areas or nodes. McGowan et al, reported that only 54% of 291 patients with ovarian cancer received proper staging procedures.

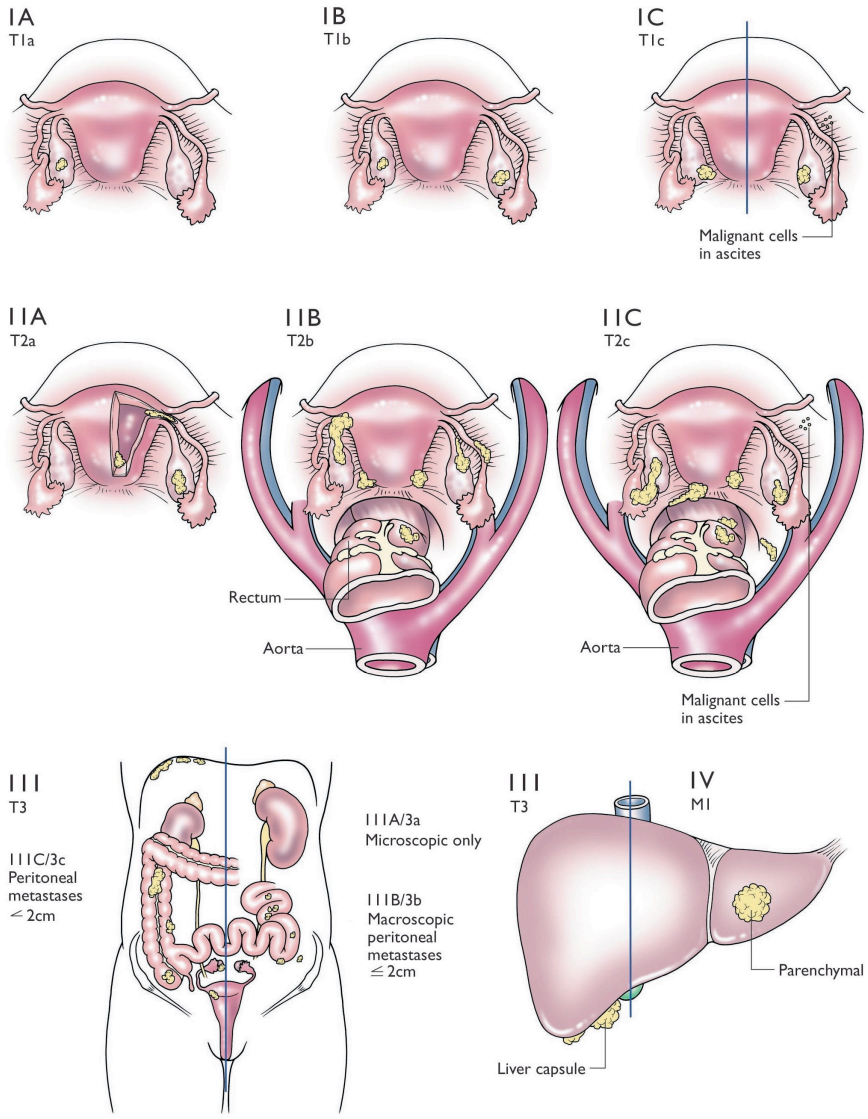
The completeness of staging varied depending on the type of specialist performing the procedure: gynecologic oncologists 97%, obstetricians-gynecologists 53% and general surgeons 35%. Trimbos et al showed in a multicenter study from Holland that proper staging was done only in 53% of the patients and in another study Muñoz et al found that only 15% of women with

presumptive stage I and II ovarian cancer received recommended staging and treatment.

The most frequently omitted step in the staging procedures in these studies, was the sampling of the retroperitoneal lymph nodes. Their conclusion was that general gynecologists should have better oncologic surgery education or patients should be referred to a center for gynecological cancer. Staging should be done through a vertical midline incision to allow palpation and biopsy of all peritoneal surfaces. It does not seem to be necessary to sample the subdiaphragmatic area routinely. In patients with stage II disease and peritoneal extension total excision of the pelvic peritoneum was recommended.

The emphasis on surgical staging has increased the interest about retroperitoneal nodal involvement associated with epithelial ovarian cancer. Data from the literature show that when cancer is apparently confined to the ovaries, positive nodes can be found in 4-25% of the cases, and if only data from systematic pelvic and paraaortic lymphadenectomy are considered, the node positivity rate ranges between 13 and 25% with a total percentage of 16%.

Figure 7: Carcinoma of the ovary. Staging ovarian cancer: primary tumor and metastases (FIGO and TNM)



When systematic lymphadenectomy (median number of nodes removed >20) was performed the median number of positive nodes was 2 (range 1-46) and in more than two-thirds of the cases metastases occurred in both the pelvic and paraaortic regions. This means that a considerable number of patients with

apparent stage I and II disease would be upstaged to stage IIIc as a result of lymphadenectomy. However, the 5-year survival (60%) for stage IIIc with only retroperitoneal spread is clearly higher than for stage IIIc with intraabdominal dissemination, which varies between 20%-30%.

As to advanced stage ovarian cancer, the incidence of lymph node metastases reported in literature varies between 3 and 40% for pelvic nodes and 2-49% for aortic nodes in different series. Some studies have shown that the incidence of nodal disease is highest in grade III or (33%) serous (27%) and clear cell (14.5%)tumors, while the chance of nodal disease in grade 1 and mucinous tumors is extremely small.

However only few reports have specifically addressed the prognostic significance of lymph node metastases in ovarian cancer. A therapeutic benefit of lymphadenectomy was suggested by some reports where lymphadenectomy was integral part of the surgical treatment for ovarian cancer. In particular, Burghardt et al. retrospectively analyzed the 5-year actuarial survival rate of patients affected by stage III ovarian cancer, optimally debulked, with or without pelvic lymphadenectomy: the observed survival rate was 53% and 13% respectively. Similar results were later reported by other studies. These data suggests that lymphadenectomy may improve survival of patients with advanced ovarian cancer optimally cytoreduced, with the limitations of non randomized studies. To confirm these observations an international randomized study comparing systematic lymphadenectomy versus lymphadenectomy of bulky nodes only in patients affected by advanced ovarian cancer is presently ongoing.

Concerning ovarian germ-cell and sex cord-stromal malignancies a proper surgical staging is important for both diagnosis and therapy. However germ cell tumors are generally much more chemo sensitive than are epithelial tumors: this may permit a more conservative surgery in well selected cases of patients.

1.7 Treatment

Surgery is the cornerstone of the treatment of ovarian cancer. Women affected by early stage of disease in which there is the desire to preserve fertility may be treated with a conservative approach; in case of aggressive histotype such as clear cell, mixed or indifferentiated carcinomas, the question of conservative surgery should not be considered. However some authors have suggested the possibility of a conservative approach in patients with unfavorable prognostic factors). In advanced ovarian cancer (FIGO stage IIC, III-IV), cytoreductive surgery is often a technical challenge. It is well known, since Griffith's report, that the survival (progression free and overall) outcome of these patients is directly related to the amount of residual disease left after primary cytoreductive surgery (Figure 8).

Many studies have subsequently confirmed these data. It is nowadays well accepted that the optimal residual disease left after primary debulking should be no macroscopic or minimal residual disease, whenever possible, before the start of first line chemotherapy.

The goal in the surgical management of advanced disease should be to remove all macroscopic disease. It is frequently not possible to do this with standard radical surgery The goal instead is to remove all tumor deposits measuring more than 1 cm in diameter (optimal cytoreduction).

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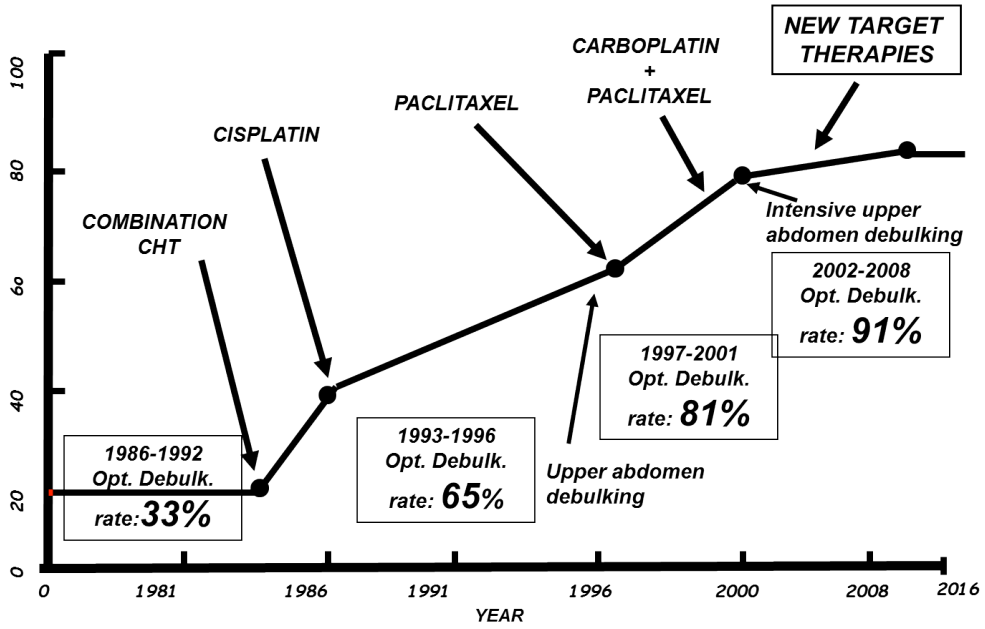
In the last decade clinicians have been paid special attention to the "interval debulking surgery" (IDS), defined as a surgical procedure with debulking intent performed midway a complete chemotherapy treatment. IDS should be considered a good opportunity in patients with suboptimal primary surgery, even if it cannot replace primary debulking which remains the gold standard in the management of advanced epithelial ovarian cancer patients. An EORTC trial comparing neoadjuvant chemotherapy-IDS-adjuvant chemotherapy versus primary cytoreductive surgery plus adjuvant chemotherapy is presently ongoing

to define the role of IDS in the management of advanced epithelial ovarian cancer (EORTC# 55971)

Figure 8. Progress in ovarian cancer management



Concerning ovarian germ cell and sex cord-stromal neoplasms the combination of surgery and chemotherapy makes excellent the outcome of these patients: in most of the cases the preservation of ovarian function and fertility, when desired, is feasible through a conservative surgical procedure (cystectomy or unilateral adnexectomy). Anyway, surgery should always include a staging procedure with sampling of pelvic and para-aortic lymph nodes, omentectomy, washings, and exploration of all peritoneal surfaces. The combination of bleomycin, etoposide and cisplatin is the standard regimen recommended for most high risk germ cell and sex-cord stromal tumors.

As surgery chemotherapy is the other cornerstone of the treatment of ovarian cancer. Among solid tumors, ovarian cancer is considered a highly chemo-sensitive malignancy. The following chapters will light up about the indications,

the timing and the different drugs used for an optimal medical management of this disease.

Frontline treatment with a platinum-paclitaxel combination is the internationally accepted standard of care in chemo-naïve advanced or recurrent ovarian cancer. At relapse, platinum compounds remain the mainstay of treatment. In platinum-sensitive disease i.e. where the treatment-free interval (TFI) is more than 6 months the response rates can be greater than 50%, but it is only 10–20% for platinum resistant disease (TFI <6 months) and less for platinum-refractory disease where the disease progresses on treatment. The latter are therefore usually treated with other non-platinum agents, such as liposomal doxorubicin, gemcitabine, topotecan, etoposide and hormonal therapies.

A challenge for improved treatment strategies for ovarian cancer is to develop new agents that are not only active against ovarian cancer but are also approvable by regulatory agencies such as the FDA and the European Medicines Agency (EMA).

Bevacizumab is the most studied antiangiogenic agent in ovarian cancer; in trials in the upfront as well as recurrent platinum-sensitive and platinum-resistant settings, addition of bevacizumab has resulted in PFS improvement, but no OS improvement. Currently, other antiangiogenic agents, such as nintedanib [vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor (TKI)] and trebananib (peptide-Fc fusion protein that inhibits binding of angiopoietin 1 and 2 to the Tie2 receptor), are being tested in the upfront and recurrent settings. Results of a phase III placebo-controlled study that tested carboplatin/paclitaxel $\pm$ nintedanib showed improved median PFS for the nintedanib arm versus placebo [17.3 vs. 16.6 months; hazard ratio, 0.84; 95% confidence interval (CI), 0.72–0.98; P = 0.0239]; data for OS are not mature. Many other trials in the upfront setting are currently awaiting mature results and are testing questions regarding dose and length of duration of antiangiogenics.

Studies of antiangiogenic agents in recurrent ovarian cancer have not demonstrated OS benefits until just recently. In ICON6, combining the oral VEGFR TKI cediranib with platinum-based chemotherapy in platinum-sensitive

recurrent ovarian cancer followed by cediranib maintenance improved OS in a preliminary analysis. PFS improved from 9.4 months with chemotherapy alone to 12.6 months for the cediranib/chemotherapy arm, while OS increased from 17.6 to 20.3 months with the cediranib/chemotherapy combination (hazard ratio, 0.70; P = 0.0419;). These results represent the first time a biologic therapy combined with standard-of-care chemotherapy has resulted in an OS benefit in ovarian cancer.

1.8 Need of screening and new diagnostic tolls

The overall incidence of ovarian cancer has remained constant for the last three decades. The overall 5-year survival for ovarian cancer in the United States has improved since 1974, from 37% to 44%, which is statistically significant.

However, despite enormous progress in treatment, not the same progress in diagnosis effort, there is no proof that routine screening with serum markers, sonography, or pelvic examinations decreases mortality.

Therfore, the majority of human ovarian carcinomas are diagnosed at stage III or IV, and 70% of these patients will die of disease within 5 years.

Ovarian cancer meets the World Health Organization's criteria for a disease that would benefit from screening. However, because current screening modalities have not been shown to reduce the morbidity or mortality of this disease, the National Institutes of Health (NIH) Consensus Panel on Ovarian Cancer currently recommends screening only for women at elevated-risk of disease due to a family history. Thus at this time most diagnoses of ovarian cancer start with evaluation of women's spontaneous complaints of suspicious symptoms or as a result of tests such as ultrasounds conducted for other reasons. In fact, although both ultrasound and tumour markers can preclinically detect a significant proportion of ovarian cancers, currently, there is still no accepted screening programme for ovarian cancer. Thus, it is essential to develop inexpensive and simple methods for early diagnosis.

Finding a screening test for ovarian cancer is challenging because ovarian cancer is not a common disease. High risk women can be identified who are more likely to benefit from intensive screening than average risk women, but only 10% of ovarian cancer occurs in these women. Multi-modal screening of women at high-risk for ovarian cancer using CA125 and transvaginal sonography (TVS) is recommended for those at highest risk, and is being studied in large efficacy trials in average-risk post-menopausal women. When used as a firstline screen, TVS may be sensitive but produces a relatively high rate of false positive results and a potentially unacceptable number of surgeries per cancer found. The use of CA125 as a first-line screen to select women for imaging by TVS as a second-line screen is a promising approach, but it has been reported that CA125 is elevated above reference levels in only 50% of clinically detectable early stage patients. Efforts are underway to improve the performance of CA125, and to identify additional biomarkers for ovarian cancer. The use of novel markers in a screening strategy is also being explored (NIH/NCI Grant P50 CA083636). These strategies use imaging prior to surgery to confirm the existence of a mass, and thus may be limited by the sensitivity of imaging.

One of the most promising new serum biomarkers is human epididymis protein 4 (HE4).

Hundreds of possible markers have been identified, yet no test currently available approaches sufficient levels of accuracy (American College of Obstetricians and Gynecologists, 2002).

HE4 (gene name WFDC2) is a glycoprotein that is highly expressed by ovarian carcinomas and. Its highest normal tissue expression is in trachea and salivary gland. It has been proposed as a potential biomarker for ovarian cancer as it is expressed by 32% of ovarian cancers without CA125 expression, and, in combination with CA125, serum HE4 has been shown to improve prediction of malignancy in ovarian masses. HE4 was recently approved by the Food and Drug Administration (FDA) for use in the U.S. to monitor ovarian cancer patients for disease recurrence.

In literature, there are many article about the use of HE4 in ovarian cancer diagnosis. In our submitted work we have identified 49 articles that investigated the diagnostic role of HE4, on a total of 12631 women, with a total of 4549 ovarian cancer patients. Considering all studies, HE4 had a pooled sensitivity of 78.6 (95% CI, 0.72%-0.83%) and specificity of 86.3% (95% CI, 84.5%-87.6%) for the detection of borderline or malignant ovarian tumors.

In premenopausal women, HE4 had a pooled sensitivity of 71% (95% CI, 0.65%-0.77%) and specificity of 88.0% (95% CI, 86.0%-90.0%) for the detection of borderline or malignant ovarian tumors.

In postmenopausal women, the pooled sensitivity was 77.0% (95% CI, 72.0%-81.0%), and specificity was 91.0% (95% CI, 89.0%-94.0%), respectively.

In early ovarian cancer, the pooled sensitivity was 64.0% (95% CI, 61.0%-70.0%), and specificity was 87.0% (95% CI, 85.0%-89.0%). In late ovarian cancer, the pooled sensitivity was 89.0% (95% CI, 82.0%-92.0%), and specificity was 86.0% (95% CI, 84.0%-88.0%), respectively.

Our analysis confirms that HE4 has a diagnostic sensitivity similar to that of CA125. Moreover we showed a sensitivity of 64 % (0.61- 0.70) in early diagnosis of EOC, confirming to be higher than CA125 (45.9%), as reported in literature for this specific setting of patients. Although the evidence of diagnostic effectiveness in detecting early-stage tumours in post-menopausal women is of pivotal relevance, there are currently not enough studies for estimating HE4 performance in this clinical scenario. In particular, the focus on menopausal status is of relevance since guidelines assign the highest baseline risk index to post-menopausal women. The possibility that HE4 may differently perform according to the menopausal status is not marginal since higher HE4 concentrations are physiologically detectable in post- menopausal women and this may require the definition of specific clinical thresholds for this condition.

1.9 Why Electronic Nose?

Use of the olfactory sense (of smell) as an indicator of disease probably originated with Hippocrates around 400 BC. Observations that unusual human odors or aromas provided some indication of human ailments caused early medical practitioners to recognize that the presence of human diseases changed the odor of bodily excretions that could be used to diagnose certain common diseases.

Medical doctors have utilized the sense of smell to facilitate determinations of the physical state and general health of their patients for centuries. The application of smell as useful sensory clues used by physicians to identify the causes of human ailments resulted in the development of qualitatively descriptive odors (or aromas) and specialized terms used to describe and identify odors associated with specific human diseases and physiological disorders. Some descriptive aromas found to be associated with some common human diseases are presented in Table 3.

The use of olfactory information provided valuable additional information for physicians in assessing patient conditions and formulating accurate diagnoses before modern analytical equipment and chemical-detection devices became available for this purpose. Notice that in some cases the same term, such as "aminelike" for bacterial vaginosis and bladder infections, occasionally was used to describe common odors associated with completely different diseases. This occurred because different diseases can result in the production of very similar compounds even though the mechanism of disease is quite different. In other cases such as for use of the term "fishy" for hypermethioninemia and uremia, both of these diseases cause the buildup of similar or identical compounds in the blood due to similar physiological processes that are often referred to as in-born genetic or metabolic diseases resulting from the absence of certain enzymes or the failure of certain organs. Many other metabolic diseases caused by genetic enzyme deficiencies are associated with various distinctive odors due to the accumulation of undecomposed metabolites in the body.

Some descriptive aromas, such as maple syrup and pox stench, are so diagnostic that the aroma was named after the specific disease referred to by

name. Other diagnostic terms for descriptive aromas include fetor hepaticus, diabetic breath, and uremic breath which have been included in common medical vocabulary and continue to be used to some extent even in contemporary vernacula

Table 3. Descriptive aromas previously used for diagnosing human diseases

Disease / Disorder	Body source	Descriptive aroma	References
Anaerobic infection	Skin, sweat	Rotten apples	Pavlou & Turner, 2000
Bacterial vaginosis	Vaginal fluid	Amine-like	Pavlou & Turner, 2000
Bladder infection	Urine	Amine-like	Pavlou & Turner, 2000
Congestive heart failure	Heart	Dimethyl sulfide	Smith, 1982
Fetor hepaticus	Breath	Newly-mown clover	Hayden, 1980
Gout	Skin	Gouty odor	Liddell, 1976
Hyperaminoaciduria	Infant skin	Dried malt or hops	Liddell, 1976
Hypermethioninemia	Infant breath	Sweet, fruity, fishy	Liddell, 1976; Hayden, 1980
Isovaleric acidemia	Skin, breath	Sweaty, cheesy	Hayden, 1980; Pavlou & Turner, 2000
Ketoacidosis	Breath	Acetone-like	Hayden, 1980
Liver failure	Breath	Musty fish, feculent	Hayden, 1980; Smith, 1982
Maple syrup disease	Sweat, urine	Maple syrup	Liddell, 1976; Pavlou & Turner, 2000
Pseudomonas infection	Skin, sweat	Grape	Pavlou & Turner, 2000
Scrofula	Body	Stale beer	Liddell, 1976
Smallpox	Skin	Pox stench	Liddell, 1976
Trimethylaminuria	Skin, urine	Fishy	Pavlou & Turner, 2000
Typhoid	Skin	Freshly-baked bread	Liddell, 1976; Hayden, 1980
Uremia	Breath	Fishy, ammonia	Hayden, 1980
Yellow fever	Skin	Butcher's shop	Liddell, 1976; Hayden, 1980

Once modern analytical instrumentation became available in the twentieth century, the actual volatile compounds responsible for these characteristic smells began to be identified. Probably the first such identification was done by Linus Pauling, the noted chemist who was able to freeze out and identify some of the volatiles in urine using cold traps, followed by gas chromatography (Pauling et al., 1971). Many other discoveries of volatile organic compounds VOCs associated with specific human smells related to particular diseases

followed in subsequent years leading up to the identification of diagnostic bioindicators of disease.

VOCs are emitted as gases from certain solids or liquids. This is a very broad set of chemicals. VOCs include a variety of chemicals, some of which may have short and long-term adverse health effects. Concentrations of many VOCs are consistently higher indoors (up to ten times higher) than outdoors. VOCs are emitted by a wide array of products numbering in the thousands. Volatile Organic Compounds (VOCs) are a health hazard resulting in eye, nose, and throat irritation, headaches, loss of coordination, nausea, damage to liver, kidney, and central nervous system. Some organics can cause cancer in animals; some are suspected or known to cause cancer in humans. Key signs or symptoms associated with exposure to VOCs include conjunctival irritation, nose and throat discomfort, headache, allergic skin reaction, dyspnea, declines in serum cholinesterase levels, nausea, emesis, epistaxis, fatigue, and dizziness.

These compounds are highly correlated with the presence of specific diseases in the body as discussed in the following section.

The results of intense chemical analyses from numerous research studies have been the identification of many volatile biomarkers of disease and their associated chemical structures. The identification of unique molecular markers (volatile metabolites) associated with particular diseases has become an extremely effective and powerful tool for the early detection of diseased tissues and infectious agents in the human body. For example, the analysis of patients' breath odors has had a long history of application for the detection of various human diseases, not only respiratory diseases. Even though the human breath contains hundreds of volatile organic compounds at low concentrations, relatively few (less than fifty) of these are detected in the majority of healthy humans under normal physiological conditions. However, a much smaller number of aberrant VOCs are often found only in patients when disease is present somewhere in their bodies. Thus, the association of specific volatile metabolites, released within the expired human breath of patients, not only

provides indicators of particular diseases, but also reflect the overall physiological state as an indication of general health and a useful index of disease. These volatile markers of disease often are released several hours to several days before outwardly-noticeable physical symptoms of illness appear and thus provide early indicators of disease or physiological disorders. New molecular markers that are indicators of specific diseases, both infectious and noninfectious, are being increasingly revealed by new scientific research. Some examples of these volatile molecular biomarkers (or bioindicators) of disease and physiological disorders, reported hitherto by various researchers, are summarized in Table 4.

Table 4. Molecular biomarker VOCs of specific human diseases and disorders

Disease / Disorder	Volatile chemical biomarkers	References
Allograft rejection	Carbonyl sulfide	Studer et al., 2001
Breast cancer	C4-C20 alkanes	Phillips et al., 2003b
Cholera	p-menth-1-en-8-ol, dimethyl disulphide	Garner et al., 2009
Chronic hepatitis	Methyl-mercaptan, dimethyl sulfide	Kaji et al., 1978
Cirrhosis	Dimethyl sulfide, mercaptans	Chen et al., 1970
Cystic fibrosis	Leukotriene B4, interleukin-6, carbonyl sulfide, alkanes	Carpagnano et al., 2003; Phillips et al., 2004
Diabetes	Acetone, ethanol, methyl nitrate	Rooth & Ostenson, 1966; Crofford et al., 1997; Ping et al. 1997; Novak et al., 2007
Halitosis	Methanethiol, Hydrogen sulfide, methyl mercaptan, dimethyl sulfide	Kaizu, 1976; Van den Velde et al., 2009
Hepatic encephalopathy	3-methylbutanal	Goldberg, 1981
Histidinemia	2-imidazolepyruvic acid, 2-imidazolelactic acid, 2-imidazoleacetic acid	Bondy & Rosenberg, 1980
Liver cancer	Hexanal, 1-octen-3-ol, octane	Xue et al., 2008
Lung cancer	Alkanes, ketones, specific aromatic hydrocarbons (benzene derivatives)	Manolis, 1983; Gordon et al., 1985; Preti et al., 1988; Phillips et al., 1999b, 2003a
Maple syrup disease	2-oxoisocaproic acid	Bondy & Rosenberg, 1980
Necrotizing enterocolitis	2-Ethyl-1-hexanol	De Lacy Costello et al., 2008
Oxidative stress	8-isoprostane	Montuschi et al., 1999
Periodontal disease	Pyridine, picolines	Kostelc et al., 1981
Phenylketonuria	Phenylpyruvic acid, phenyllactic acid, phenylacetic acid	Bondy & Rosenberg, 1980
Schizophrenia	Pentane, carbon disulfide	Smith & Sines, 1960; Smith et al., 1969; Phillips et al., 1993
Tyrosinemia	p-hydroxyphenylpyruvic acid	Bondy & Rosenberg, 1980
Trimethylaminuria	Trimethylamine	Pavlou & Turner, 2000
Uremia	Dimethylamine, trimethylamine	Simenhoff et al., 1977

Analysis of expired human breath is considered particularly valuable because it can be monitored noninvasively (without causing physical damage to patients), yet provide information about the chemical and physiological state of the entire body. The reason that information about the physical health of the entire body is possible by the analysis of expired breath is because most volatile metabolites of infectious agents of disease, or those produced from abnormal tissues, are eventually eliminated from the body through the lungs, often soon after being formed within diseased tissues.

Alternatively, other less volatile abnormal metabolites are eliminated through the urine which may be similarly analyzed using aroma-sensing instruments such as electronic noses. Cao and Duan summarized some of the advantages and disadvantages of breath analysis for clinical practice and diagnosis. They found breath tests were noninvasive, easily repeated, and caused less discomfort and embarrassment to patients than blood and urine tests.

Breath samples closely reflected arterial concentrations and provided much less complicated mixtures than serum or urine analyses and more direct information on respiratory function than by other means. They listed limitations of breath testing for clinical practice to include the lack of standardization of analytical methods, the high water content of breath samples affecting detection, relatively expensive costs compared to simple chemical tests (but much less time-consuming for results), and the lack of well-established links between breath VOCs and certain kinds of diseases.

Biomarkers in chronic obstructive pulmonary disease (COPD) also may be useful in aiding diagnosis, monitoring exacerbations, evaluating effects of drugs, and defining specific phenotypes of disease. Frey & Suki found risk assessments, disease progression, and control of asthma and COPD required multidimensional fluctuation analysis of the dynamics of lung-function parameters that needed to be quantified and monitoring via precise biomarkers of these diseases using instruments capable of direct, electronic monitoring of these biomarkers.

The importance of the use of biomarkers for the detection of disease has become so prominent that Bentham Science, a leading international publisher of high quality scientific journals, decided to launch a new journal called Recent Patents on Biomarkers in January 2011 to publish reviews and research articles written by experts on recent patents and research relating to biomarkers in basic and applied, medical, environmental, and pharmaceutical research, and including patent biomarker applications, clinical development, and molecular diagnostics.

1.10 Electronic-Nose in Healthcare and Biomedicine

The discovery and recognition of particular volatile organic compounds (VOCs), released from various diseased human body parts or fluids derived from these tissues, have been found to be associated with specific human diseases through the use of specialized modern analytical instruments. These instruments have included such analytical machines as gas chromatographs working in tandem with mass spectrometers (GC-MS) and other such technical instruments used in analytical chemistry.

Electronic-noses are ideal instruments for biomedical uses because of their versatility, low cost, rapid output of results, capabilities of continuous operation (for physiological monitoring purposes), and the wide range of VOCs and other cellular chemical constituents that may be analyzed. The potential for miniaturization of electronic-nose devices also is great due to their micro circuitry and micro sensor components.

A variety of different types of e-noses, based on different working principles, have been used for biomedical tasks including conductive polymers (CP), metal-oxide semiconductor (MOS), quartz crystal microbalance (QCM), and surface acoustic waves (SAW) among others. Each e-nose technology has different advantages, disadvantages, and limitations that largely determine what types of medical applications that individual e-nose sensor types are best suited for in practical clinical settings.

The development and utilization of many new electronic-nose (e-nose) applications in the healthcare and biomedical fields have continued to rapidly accelerate over the past 20 years.

Innovative e-nose technologies are providing unique solutions to a diversity of complex problems in biomedicine that are now coming to fruition. A wide range of electronic-nose instrument types, based on different operating principles and mechanisms, has facilitated the creation of different types and categories of medical applications that take advantage of the unique strengths and

advantages of specific sensor types and sensor arrays of different individual instruments. Electronic-nose applications have been developed for a wide range of healthcare sectors including diagnostics, immunology, pathology, patient recovery, pharmacology, physical therapy, physiology, preventative medicine, remote healthcare, and wound and graft healing. E-nose biomedical applications range from biochemical testing, blood compatibility, disease diagnoses, drug purity, monitoring metabolic levels, organ dysfunction, and telemedicine.

E-noses in general have the advantages of providing patient laboratory results much faster than standard cultures or wet chemistry tests and the capability of providing early detections of diseases before symptoms appear. These characteristics have been compelling reasons for the development of e-nose systems for clinical medicine. Some recent uses of electronic noses in hospitals and universities around the world are presented in Table 5.

The potential applications of electronic-nose devices in the healthcare and biomedical industries will continue to expand with greater research and in-hospital testing as new ways of using these chemical-detection machines are discovered, and the breadth of capabilities widened, particularly in the area of coordinated uses in combination with other medical devices. The combined uses of e-noses with other electronic medical instruments will facilitate the development and availability of improved real-time information of patient conditions, leading to even more effective decisions and treatments by physicians in hospitals and clinics. The future potential of combining the capabilities of e-nose devices with other types of detection technologies are examined here in light of new technological discoveries in chemical sensor-detection that are currently emerging.

Table 5. Electronic-nose uses in hospitals and universities around the world

Country	Hospital, University or Research Facility	E-nose utilized	Application	References
USA	University of Pennsylvania	Experimental model	Distinguish cerebrospinal fluid	Thaler et al., 2000
USA	Merck Research Laboratories	Fox 4000	Flavor analysis for drug formulation	Zhu et al., 2004
United Kingdom	Birmingham Heartlands Hospital	Cyranose 320	Identify *Staphalococcus*	Dutta et al., 2005
Germany	University of Applied Sciences	DE 101	Detect renal dysfunction	Voss et al., 2005
USA	Cleveland Clinic	unspecified	Diagnose lung cancer	Erzurum et al., 2005
Belgium	University of Antwerp	PEN 2	Clinical diagnoses of bacteria	Moens et al., 2006
United Kingdom	South Manchester University Hospital	experimental model	Burn and wound infection types	Persaud, 2006
USA	University of Pennsylvania	unspecified	Diagnosis of diseases via breath	Anthes, 2008
USA	California Institute of Technology	JPL ENose	Detect & differentiate brain cancers	Kateb et al., 2009
Australia	Prince Charles Hospital	unspecified	Detect chronic lung disease	Dent, 2010
Netherlands	Amsterdam Academic Medical Center	Cyranose 320	Discriminate inflammation airway diseases	Lazar et al., 2010
Italy	Catholic University	experimental model	Asthma detection	Montuschi, 2010
Tanzania	National Institute of Medical Research	Bloodhound EN	Diagnosis of Tuberculosis	Kolk et al., 2010
United Kingdom	Gloucestershire Royal Hospital	NST 3320	Diagnosis of ventilator-associated pneumonia	Humphreys et al., 2011

2. RATIONALE

Detection of different malignancies using canine scent or electronic nose has been reported in peer-reviewed journals, indicating that this may represent a new diagnostic tool for malignancies. Hundreds of volatile organic compounds (VOCs) are emitted from the human body, and the components of VOCs usually reflect the metabolic condition of an individual. Body odors are the result of the combination of hundreds of emitted odorous that are originally secreted from various cells inside the body via metabolic pathways. The major sources of VOCs include breath, sweat, skin, urine, faeces and vaginal secretions. Basing on these premises, in 1989, Williams and co-workers presented the first hypothesis that dogs may be able to detect malignant tumors by scent. Dogs can be used as cancer detectors because they have an extraordinary sense of smell, with odour detection thresholds as low as parts per trillion.

Pickel was the first to use tumor tissue from melanoma in the training of such dogs.

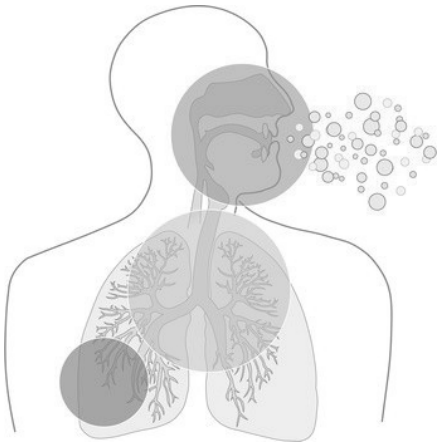
During the past two decades, an increasing number of authors have described cancer detection by dogs trained on various biological materials such as urine, breath, and stool.

Obviously, lung cancers are the best tumors to use for this experiment, because breath samples, that are directly emitted from cancer cells, can be immediately analyzed (Figure 1).

In the last years, some studies on ovarian cancer were published as well: here, a trained dog was used to discriminate between cancer and normal tissue.

Horvat in 2010 clearly demonstrated that human ovarian carcinoma tissues can be characterized by a specific odour, detectable by a trained dog. The same study showed that a dog can be trained to distinguish between different histopathological types and grades of ovarian carcinomas, including borderline tumours, as well as different healthy control samples. Double-blind tests showed 100% sensitivity and 97.5% specificity.

Figure 1. VOC



Their study strongly suggests that the characteristic odour emitted by ovarian cancer samples is also present in blood (plasma) taken from patients with the disease. This observation suggests that the specific cancer odour in the blood/plasma may be used for screening, diagnosis, and differential diagnosis of different malignant diseases

These authors recently demonstrated that human ovarian carcinoma tissues can be characterized by a specific, detectable odor. Especially, dogs can be trained to distinguish between different histopathological types and grades of ovarian carcinomas, including borderline tumors, as well as different healthy control samples. In addition to trained dogs, researchers also used electronic noses to detect cancer-related volatile organic compounds in the headspace above malignant tissues.

These detection methods, however, had relatively low sensitivity and were not shown to be tumor-specific as the only comparisons made were versus healthy material. In their current form, electronic devices probably lack the sensitivity to distinguish a specific cancer from other cancers, which is a crucial requirement for practical use.

To our knowledge, Horvat et al published one of the first study that used the electronic nose to test the detection of different volatile organic compound signals emitted by ovarian carcinoma and normal tissues. The electronic nose classified 84.4% of cancerous tissues correctly, while 86.8% of the control samples were classified accurately. However, in this study, the electronic nose analysis was done directly on cancer tissues and not on blood, urine or breath samples, so these data are not reproducible in clinical practice.

The specific odor of carcinomas is thus an important characteristic that is likely to play a crucial role in future early cancer diagnosis and in tumors follow up.

3. EXPERIMENTAL PART

INTRODUCTION

According to the Global Cancer Observatory, ovarian cancer is the eighth most common cancer in women, with 313.959 new cases and 207.252 new deaths worldwide, in 2020 (1). In Italy, 5370 new cases and 3285 of deaths were registered last year (1). Most of the cases, around 75-80% (2), are diagnosed in advanced stages, thus making the ovarian cancer a particularly lethal disease, named the "big silent killer". One of the main reasons is the lack of valid screening programs. Papanicolau test is extremely helpful in cervical cancer, ultrasounds in endometrial cancer, while in ovarian cancer nothing is really effective in detecting early disease to date. In clinical practice, clinical examination, transvaginal ultrasound (TVU), and serum biomarkers (the Carbohydrate Antigen 125 (CA 125) and the Human Epididymis Protein 4 (HE4)) dosage are used to investigate the presence of ovarian cancer OC, with a sensitivity of 88% and 95% respectively and a specificity of 84% for US and 76% for biomarkers; but these data are not enough and several early stages are missed (3). The aim of TVU is to observe both ovaries and calculate their volume. Any variation in morphology, or increase in volume, need to be better explored. Unfortunately, ultrasounds are extremely operator dependent, while CA125 may be elevated in OC, but also in endometriosis or other benign diseases, thus showing a low specificity (17). HE4 reaches a specificity of 86% (17) and it is usually dosed with CA125. All these methods have several limitations, as a consequence the great challenge now is to find an effective screening test for OC, and make a strong prevention. Many scientists have recently focused their attention on volatile organic compounds (VOC). They are gaseous molecules easily collected from breath, because they pass from the bloodstream into the lungs, but also from blood and urine. They might provide interesting information on several diseases, such renal dysfunctions, asthma,

but also solid tumors (4-8). In our university, Campus Biomedico of Rome, a brand new ongoing research is using the electronic nose to detect lung cancers. The original idea was born in 2008: several studies by Horvath et all demonstrated that human OC has a specific odour, that can be detected by a trained dog. In addition, the same dog was able to distinguish among several histopathological types and grades, as well as healthy control samples, with a sensitivity of 100% both in tissue and in blood tests; a specificity of 95% and 98% in tissue and in blood tests respectively. Unfortunately, dogs are not for clinical practice (9-10). Based on this discovery, Horvath et all used an electronic nose that can accomplish the same task, with a sensitivity of 84.4% and a specificity of 86.8% in tissue tests (11). This electronic nose was a combination of four gas sensors, operating at different temperatures, made of metal oxide to better differentiate between various gases. The nose produces some signals, which are reduced to numbers and analyzed through the Weka algorithm package. (11). In 2016 a similar device, called the electronic tongue, was introduced by Pascual et all to detect patients with prostate cancer. The electronic tongue recognizes an electrochemical fingerprint in urine samples with a sensitivity of 91% and a specificity of 73% (12). The e-tongue was an array of seven metal wire electrodes housed inside a steel cylinder. A Large Amplitude Pulse Voltammetry (LAPV) waveform was applied to each electrode and the resulting currents vs time profile for each electrode was measured. (12). Considering these studies, for the first time in literature, we investigated the potential use of sensors array analysis (the electronic nose and electronic tongue) to detect ovarian cancer not just from from breath and urine, but also from blood and plasma samples, with a special attention on their sensitivity and specificity. To increase the sensibility, we used both the electronic nose (made of 32 sensors) and the e tongue (made of 1500 sensors divided in three blocks of 500). As secondary endpoint, we correlated for the first time the fingerprints obtained from e-nose and e-tongue with CA125 and HE4.

MATERIALS AND METHODS

We enrolled patients between 2017 and 2019 affected by an ovarian mass and suitable for a pelvic surgery and referred to University Campus Bio Medico of Rome. Women underwent surgery according to the inclusion and exclusion criteria, in a double blind analysis (See flow chart in Figure 1). In the first part of the study (training analyses) population was divided into two main groups according to pathology report obtained by surgery: (1) controls: women with adnexal masses with evidence of benign disease at histological evaluation (2) OC group: women with malignant adnexal masses (Figure 1).
The inclusion criteria were:

- patient with one or multiple ovarian masses
- age between 18 and 80 years.
- a good performance status (ECOG <2)
- no concomitant neoplasia
- no previous surgery or chemotherapy
- written informed consent.

The exclusion criteria were:

- the presence of a concomitant neoplasia
- non-ovarian disease at surgery
- the presence of an uncontrolled systemic disease.

We started with a preliminary analysis on the first women. As soon as they were admitted to the hospital, we collected breath by a pneumopipe. Nurses collected urine and venous samples. We immediately spin the blood in order to get plasma out (1500 laps in 15 minutes)in the centrifuge. We analyzed in the following hour all the samples: urine, plasma and blood. Responses obtained by sensors (32 sensors for E-nose and a total of 1,500 for E-tongue) were

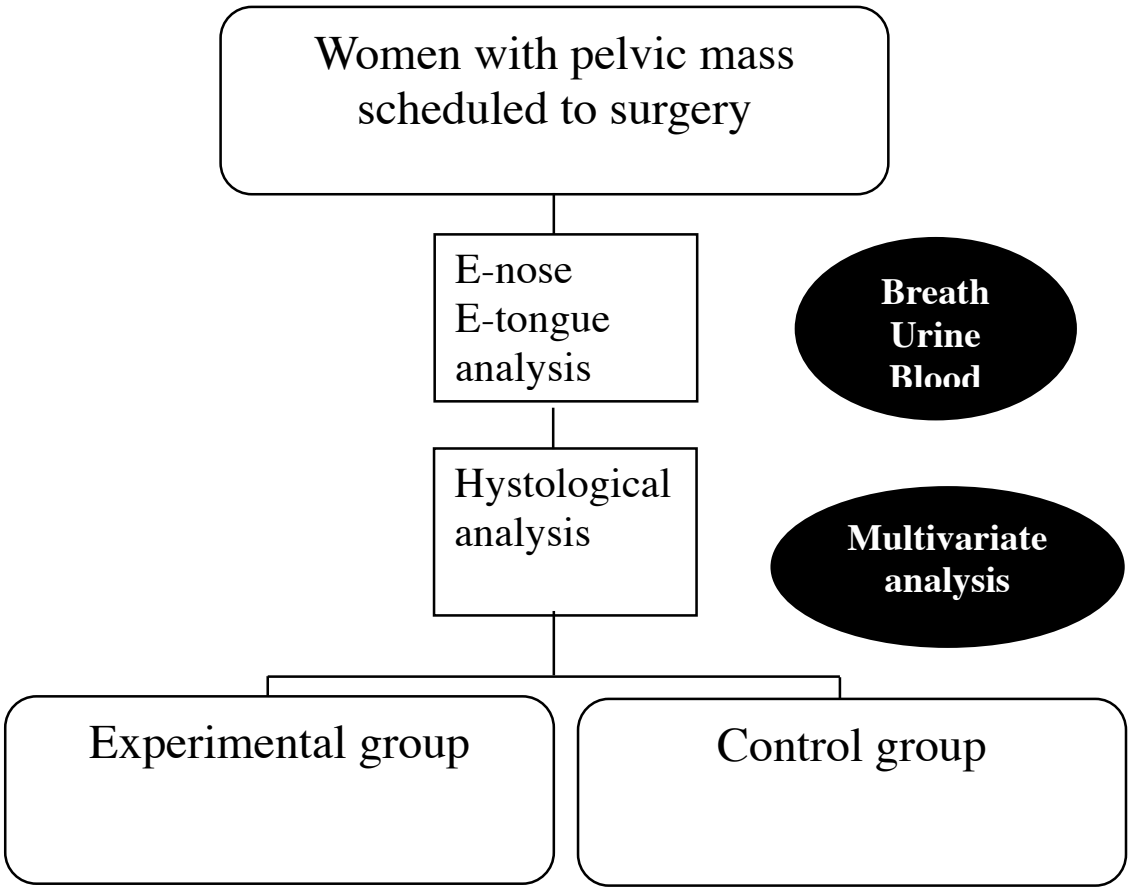
analyzed by a computer. Pneumopipes with breath samples were frozen and sent to our bioengineers. Through the leave-one-out analysis, the cross-validation method and the generation of confusion matrix we obtained the sensitivity and specificity of each sample (Figure 2).

As secondary endpoints, we proposed to identify a possible correlation between ca125 and HE4 and some VOCs emitted by patients with ovarian cancer through breath, blood and urine. These VOCs might match with specific fingerprints in sensor array in order to differentiate ovarian tumors from benign pathologies in an early onset.

We also applied the statistical model of PLS-DA (supervised method or Partial Least Squared Discriminant Analysis) for tenax (breath), urine and blood in order to find a possible fingerprint for serum tumor markers.

Finally, we compared our results with other diagnostic methods already used to discriminate ovarian cancer from benign masses.

Figure 1. Flow chart of study



RESULTS

We enrolled 196 patients, referred to University Campus Bio Medico of Rome between 2017 and 2019, affected by an ovarian mass and suitable for a pelvic surgery.

According to our criteria, 87 patients were not eligible for our study: 15 were elder than 80 years old, 3 did not have a good performance status (ECOG <2), 22 did not sign the written informed consent, 21 were younger than 18 years old, 26 were excluded because of inadequate blood sample, non adequate renal function, non adequate liver function, synchronous cancer, pneumonia and anesthesiology contraindication at surgery. Therefore, 109 patients remained, but 49 were excluded because they did not have ovarian diseases at surgery. As a consequence, only 60 suitable women were taken into

consideration, 30 controls and 30 cancers. Patients' characteristics are listed in table 1. The most diffused histotype for malignant mass was the serous carcinoma in 37 patients (71%), then endometrioid in 6 cases (11%). The stage III and high grade were the most represented. Among the benignant masses most present were serous and endometriosis cysts (29% both) (Tables 2 and 3) The e- tongue resulted to have a sensitivity of 96% in detecting OC in blood and urine samples and a specificity of 27%, which is very low. The e-nose had a sensitivity of 83% in detecting OC in breath samples and a specificity of 73%. Taken together, the e-nose and the e-tongue, reach a sensitivity of 88% and a specificity of 86%, with a VPP of 86% and a VPN of 84%, with a reduction of false positive cases (3/12, 25%).

Regarding the second endpoints, we proposed to identify a possible correlation between ca125 and HE4 and some VOCs emitted by patients with ovarian cancer through breath, blood and urine. After analysis we were not able to identify a specific fingerprint recognized by the sensors due to a too large error in the PLS model (see table 4).

Table 1 Patients' characteristic

TUMOR GROUP (52)		CONTROL GROUP (41)	p value
AGE, MEAN (SD)	60 (24-80)	59 (23-76)	0,22
MASS SIZE IN CM (MAIN DIAMETER)	7 (3-10)	6,5 (2,8-10,5)	0,3
BMI, MEAN ± SD	24	24,9	0,49
MENOPAUSE (%)	27	22	**0,01**
CA125, MEAN (SD)	313 (5-2130)	28,3 (3-179)	**<0,001**
HE4 , MEAN (SD)	906 (33-7287)	92 (37-144)	**<0,001**
SMOKING PATIENTS, N (%)	15	26	0,44
SURGERY, N (%)	100	100	0,1

Table 2 Histotypes malignant masses

HISTOTHYPE n. (%)	
ENDOMETRIOID	6 (11%)
SEROUS	37 (71%)
CLEAR CELL	2 (4%)
GERMINAL LINE OR SEXUAL CORD	4 (8%)
OTHER	3 (6%)
STAGE	
I	15 (29%)
II	9 (17%)
III	28 (54%)
RECURRENCE	17 (33%)
NAIVE	35 (67%)
GRADING	
G1	5 (10%)
G2	4 (8%)
G3	43 (82%)

Table 3 Histotypes benignant masses

HISTOTYPE BENIGN MASSES N (%)	
FIBROID	6 (14%)
ENDOMETRIOMA	12 (29%)
MUCINOUS	2(4%)
CYSTADENOFIBROID	6(14%)
ENDOMETRIOID ADENOFIBROID	1(2%)
SEROUS CYST	12(29%)
MATURE CYSTIC TERATOMA	2(4%)
PARATUBARIC CYST	2(4%)

Table 4 PLS model

	RMSE-C	RMSEC-V
Breath	1116	1127
Blood and plasma	1102	1131
Urine	N/A	N/A

RMSEC-C: Error in calibration
RMSEC-V: Error in validation
N/A: not applicable because of a too large error

CONCLUSIONS

The obtained sensitivity and specificity were compared to those of CA125 and HE4, serum markers currently used to identify ovarian cancer, that, taken together, in the ROMA score, have a specificity of 76,4% and a sensitivity of 95%. Unfortunately, we demonstrated that those biomarkers do not provide a specific fingerprint to be red by the electronic nose. Therefore, they can just be used together with the e- nose and e-tongue to increase the specificity. We compared our results with ultrasounds too, finding out a similar sensitivity (85% vs 88% US) and specificity (87% vs 84% US). While if we compare our results with imaging, computed tomography scan (CT) and positron emission tomography (PET), we get a much better sensitivity (85% vs 78% CT/PET) and specificity (87% vs 68%% CT/PET). According to data, our device is able to reach a similar sensitivity and specificity of biomarkers, ROMA SCORE, and ultrasounds, which is much higher in comparison to CT and PET. Raspagliesi et al agree in saying that the e-nose can achieve the same or even higher specificity values than CA125 and ultrasounds. It is clear that nothing could unfortunately reproduce the sensitivity (100%) and specificity (97,5%) of a trained dog.

In conclusion, this is the first time in literature that we analyze the efficacy of sensors array analysis in bood, urine and breath samples. Our preliminary results suggested the potential role of sensor array analysis for the detection of

OC in a selected group of patients, without overlooking comorbidities of patients, which can alterate the sensitivity as we have seen.

In the future, the next step will be to investigate in a larger population if altered VOCs might be predictive for higher risk of developing OC in comparison to normal VOCs, allowing to identify patients at risk, regardless the presence of ovarian masses (screening test), hopefully reducing the mortality rate.

In addition, our data suggest that the combination of the e-nose and the e-tongue, CA125 and HE4 could, in the future, be part of a diagnostic algorithm for a non- invasive screening of ovarian cancer patients. In addition, ideally, we think about a possible use of this algorithm in combination with the BRCA1 and 2 genetic testing, in order to identify patients at risk of OC, thus as first prevention to reduce the incidence of this cancer.

Unfortunately, as we know, the cost are not so contained for sensor array analysis, but If we think to all diagnoses that we can prevent we would have reached the greatest saving for our NHS. In fact, as reported by Eugenio Di Brino et al. in 2020, the cost of ovarian cancer therapy for National Healthcare System in Italy is around €90,000.00 per case, and an American study showed that the only use of genetic testing could be reduce the risk of OC by 40%, thus a correct use of this algorithm could be realistically cost-effectiveness.

In conclusion, this research is a preliminary but promising study, further study, of course, are needed to confirm our results.

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