Assigning site of origin in non-uterine high-grade serous cancers – Bridging the gap between research and clinical practice

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“Science is a process of disproving hypotheses”

Karl Popper, 'The Logic of Scientific Discovery' [Logic der Forschung, 1934].

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LIST OF ABBREVIATIONS

- ADJ: adjuvant chemotherapy
- BRCA1/BRCA2: Breast Cancer gene 1 and 2
- Ca125: cancer antigen 125; protein released in response to peritoneal irritation
- CT scan: computerized tomography scan
- EOC: epithelial ovarian cancer
- FIGO: International Federation of Gynecology and Obstetrics
- FTC: fallopian tube cancer
- HGSC: high-grade serous ovarian cancer
- HMGA2: High-mobility group AT-hook 2
- IHC: immunohistochemistry
- IRB: Institutional of Review Board
- MIB-1: E3 ubiquitin-protein ligase
- MUHC: McGill University Health Center
- NACT: chemotherapy given before surgery (neoadjuvant)
- N-EOC: non epithelial ovarian cancer
- NGS: Targeted Next Generation Sequencing
- No-NACT (ADJ): chemotherapy given after the surgery (no-neoadjuvant, adjuvant)
- non-HGSCs: non high-grade serous ovarian cancers
- OC: ovarian cancer
- OSE: ovarian surface epithelium
- PAX2: Paired Box Gene 2
- PPC: primary peritoneal cancer

- PTEN: Phosphatase and tensin homolog
- RSF-1: Remodeling and Spacing Factor 1
- SCOUTs: secretory cells outgrowths
- SEE-FIM protocol: Sectioning and Extensively Examining the Fimbriated end protocol
- STIC/TIC: serous tubal intraepithelial cancer/tubal intraepithelial cancer.
- STILs: serous tubal lesions in transition
- TH-BSO: total hysterectomy and bilateral salpingoophorectomy
- TILT: tubal intraepithelial lesions in transition
- TMI: tubal mucosa involvement
- TP53: tumor protein 53
- WHO: World Health Organization

ABSTRACT

Background and aims: In research studies it is now evident that the majority of high-grade serous ovarian cancers (HGSC) starts in the fallopian tube. However, this is not reflected in clinical practice. The criteria for assigning tubal origin used in research studies rely on identifying serous tubal intraepithelial carcinoma (STIC), or tubal mucosa involvement (TMI) in fallopian tubes examined *in toto* (examined in 2-mm section). The clinical criteria currently used recommended by the FIGO (International Federation of Gynecology and Obstetrics) and WHO (World Heath Organization) are based on the location of the dominant tumour mass. Recently, a consensus proposal based on research criteria has been put forward by a group of academic pathologists for adoption in clinical practice. However, there are concrete difficulties in applying research criteria to clinical practice. The reason for this is that routinely examining the fallopian tubes *in toto* is perceived as difficult to implement, STIC is difficult to standardize, and TMI has been challenged as a reliable criterion. This study aims to bridge the gap between research studies and clinical practice by evaluating what is currently done in clinical practice in comparison to the new recommendations relating to correct assignment of fallopian tube primary. This is done by examining all consecutive cases reported as ovarian, primary peritoneal, tubal cancer in a tertiary care gynecologic oncology center over a seven-year period. Therefore, the specific aim of this study is to evaluate which of the criteria proposed is already in use at our institution, and which is not and should be implemented. **Methods:** Retrospective analysis of all surgical pathology reports signed out as cancer of the ovary, peritoneum or fallopian tubes at a publically funded cancer centre relating to cytoreductive surgeries performed between January 2007 and December 2013. Surgical pathology reports were examined to identify pragmatic criteria for clinical adoption. **Results:** During the study period of 277 cases, 215 (125 HGSC and 90 non-HGSCs) had fallopian

tubes examined *in toto*, which represents 91% of the cases. The primary was assigned as ovary, peritoneum, fallopian tube, tubo-ovarian and uncertain in 48%, 17.6%, 19.2%, 8.8%, and 6.4% respectively of HGSC cases vs. 95.6%, 1.1%, 3.3%, 0%, and 0% respectively of non-HGSCs. (STIC) was seen only in 12.8% of HGSC and TMI in 56%. If TMI was used systematically as a criterion to assign tubal origin, the assigned primaries would be: 29.6% primary ovarian cancers, 11.2% primary peritoneal, 56 % tubal and 3.2% uncertain. We then compared the frequency of TMI in HGSC vs non-HGSCs and we found that only five cases of non-HGSCs had TMI. **Discussion:** These results suggest that all components of the proposed criteria, examination of the tubes *in toto*, identification of STIC and TMI, is already being done. However, it was not used to assign site of origin. Therefore, the proposed criteria appear to be implementable. **Conclusion:** Examination of the fallopian tubes *in toto* and meticulous reporting of TMI appear feasible in clinical practice and may help bridge the gap between research and clinical practice. Increasing the proportion of HGSC cases attributed to tubal primary and correctly assigning site of origin of HGSC is of clinical importance because it has implications for screening and early detection.

Key words: fallopian tube cancer, STIC, tubal mucosa, origin of HGSC

RESUME

Contexte et objectifs: Dans les études de recherche, il est maintenant évident que la majorité des cancers séreux de haut grade non-utérins (CSHG) démarre dans la trompe. Toutefois, cela ne se reflète pas dans la pratique clinique. Les critères d'attribution de l'origine aux trompes utilisés dans les études de recherche reposent sur l'identification du carcinome séreux intraépithéliale des trompes (CSIT), ou du cancer dans la muqueuse tubaire (CMT) dans des trompes examinées *in toto* (examine en sections de 2 mm). Cette identification se fait en utilisant le protocole de Sectionnement et Analyse Exhaustive des Trompes (SEE-FIM). Les lignes guides cliniques pour les pathologistes reposent quant à elles sur les protocoles de la FIGO et de l'OMS basés sur la localisation de la masse tumorale dominante. Récemment, une proposition de consensus sur la base de critères de recherche a été mise en avant par un groupe de médecins universitaires pour adoption dans la pratique clinique. Cependant, il y a des difficultés dans l'application des critères de recherche à la pratique clinique. L'examen de routine des trompes en utilisant le protocole de SEE-FIM est perçu comme difficile à mettre en œuvre, le CSIT est difficile à standardiser et le CMT a été contesté en tant que critère fiable. Cette étude vise à combler le fossé entre les études de recherche et la pratique clinique en évaluant ce qui se fait actuellement dans la pratique clinique en comparaison avec les nouvelles recommandations relatives à l'assignation de l'origine du CSHG. Cela a été fait en examinant tous les cas consécutifs signalés comme cancer des ovaires, du péritoine ou des trompes dans un centre de soins de gynécologie oncologique tertiaires sur une période de sept ans. L'objectif principal de cette étude est d'évaluer quelles sont les étapes et critères proposés pour l'assignation de la source primaire des cancers des ovaires, du péritoine et des trompes. Cela afin de proposer des critères pour traiter les cas présumés de CSHG dans la pratique clinique. **Méthodes:** Une analyse

rétrospective de tous les rapports de pathologie chirurgicale ayant été diagnostiqués comme cancer des ovaires, du péritoine ou des trompes relatives aux chirurgies cytoréductives réalisées entre le 1erJanvier 2007 et le 31 Décembre 2013 dans un centre dédié au cancer et financé publiquement. Nous avons examiné les rapports de pathologie chirurgicale pour identifier les critères pragmatiques pour adoption clinique. **Résultats:** Sur 277 cas, 215 (125 CSHG et 90 non-CSHGs) ont eu les trompes examinées *in toto*, ce qui représente 91 % des cas. La source primaire du cancer a été considérée comme étant les ovaires, le péritoine, les trompes, tuboovarienne ou non classifiable dans respectivement 48%, 17.6%, 19.2%, 8.8% et 6.4% des CSHG contre respectivement 95.6%, 1.1%, 3.3%, 0% et 0 % des non-CSHGs. CSIT n'a été observé que dans 12.8% des CSHG et CMT dans 56%. Si le CMT était utilisé comme critère pour attribuer l'origine tubaire, les proportions seraient : 29.6% cancer de l'ovaire, 11.2% cancer du péritoine, 56 % cancer des trompes et 3.2% non défini. On a aussi comparé la fréquence du CMT parmi les cas CSHG et non-CSHGs. Seuls cinq cas ont été trouvés. **Discussion:** Ces résultats suggèrent que toutes les critères proposés, examen des tubes *in toto*, identification des CSIT et CMT, sont déjà réalisées. Cependant, ils n'ont pas été utilisés pour assigner le site d'origine. Par conséquence, les critères proposés semblent être réalisables. **Conclusion:** L'examen intégral des trompes et les rapports minutieux de CMT semblent réalisables dans la pratique clinique et peuvent aider à combler le fossé entre la recherche et la pratique clinique. Le fait d'attribuer l'origine correcte du CSHG est très importante car cela à des conséquences sur le dépistage et le diagnostic précoce.

Mots-clés: cancer des trompes, CSIT, muqueuse tubaire, origine du cancer de l'ovaire de haut-grade séreux.

PREFACE AND CONTRIBUTION OF AUTHORS

Prior to starting this study, I have familiarized with the literature review and proposed a study design. I have received guidance and feedback of my supervisor, Dr. Lucy Gilbert. I have also received guidance and feedback from all the members of my thesis committee and particularly Dr. Olga Basso for the outline of the study. I have received a training session by the senior pathologist and member of my committee, Dr. Arseneau. The teaching consisted of an overview of how specimens are handled and description of the terms used and how to interpret the pathology reports. I have also received continuous guidance and support from all the members of my committee and particularly from Dr. Gilbert and Dr. Basso throughout every stage of my work. Dr. Lapointe, as the chair of the committee, guided me during the meetings and whenever I contacted him for feedback.

STATEMENT OF RESEARCH PROBLEM

Ovarian cancer (OC) is the most common cause of death from gynecologic malignancy and the fifth most common cause of cancer-related death in women in North America (1, 2). There are many subtypes of OC, but the high-grade serous cancer (HGSC) subtype is responsible for at least 70% of cases related to this disease (2). Ovarian cancer is also the most deadly gynecologic cancer (2). The high death rate associated with HGSC is due to the fact that this disease is often diagnosed at an advanced stage (3). It is now recognized that HGSC has eluded all attempts at early diagnosis because, in the majority of cases, it starts in the fallopian tube –not in the ovary- and disseminates widely into the peritoneal cavity early in the course of the disease involving the ovaries and the peritoneal cavity in the metastatic process (4, 5). Therefore, in scientific circles there is strong evidence that the majority of non-uterine HGSC of the ovary and the peritoneum are metastatic HGSC of the fallopian tube. However, in clinical practice, fallopian tubal cancer continues to be reported as a rare disease (6). In 2014, the FIGO (International Federation of Gynecology and Obstetrics) staging unified the staging system of ovarian, peritoneal and fallopian tube cancer. While this is accompanied by the mandate that primary site must be assigned as tubal, ovarian, peritoneal or undesignated, no guidance is offered for assigning primary site (7). The WHO (World Health Organization) classification of tumors of the female genital tract released in 2014 leaves the assignment of site of origin to the *"experience and professional judgement"* of the reporting pathologist and Tumor Board (8, 9). Incidence data derived from surgical pathology reports and tumor registries indicate that fallopian tube cancer is 32 times less frequent than ovarian cancer, and 18 times less frequent than primary peritoneal cancer (6). The reason why fallopian tube cancer is only rarely reported by gynecologic oncology pathologists as the primary source of the malignancy is that the current criteria for assigning the primary are based on the location of the

dominant tumor mass/masses (FIGO and WHO) (10, 11). In surgical specimens of HGSC cases, the fallopian tube is rarely the site of a dominant tumor mass, the bulky tumor masses are instead in the ovary and the omentum (12). Scientific/academic criteria for the assignment of origin of HGSC to the fallopian tube require: 1) the examination of the fallopian tube *in toto*: in order to do that, a variety of protocols have been described, which essentially served the same purpose. The most commonly used is Sectioning and Extensively Examining the Fimbriated end (SEE-FIM) protocol (13); 2) the identification of the precursor lesion for HGSC, known as serous tubal intraepithelial carcinoma (STIC – please refer to page 25 for further explication); 3) the identification of cancer in the tubal mucosa (TMI), which is considered resistant to metastasis. If tubal mucosa is involved by cancer (TMI), the cancer is believed to have originated in the fallopian tube (14). However, the fact that even in academic tertiary care gynecologic oncology units, which have credited the scientific concept that the fallopian tube is the site of origin of HGSC, continue to sign out far more cases of HGSC as ovarian or primary peritoneal cancer rather than tubal cancer, highlights the practical difficulty of adopting these criteria in the real world of surgical pathology reporting. The difficulties range from the additional technical workload involved in examining the fallopian tubes *in toto* for all cases of suspected ovarian/peritoneal/fallopian tube cancers, to the challenge of identification and reproducibility of STIC, even among experienced gynecologic oncology pathologists (15, 16). Gynecologic oncology pathologists have little difficulty identifying TMI, but this criterion, which was before considered as being pathognomonic of tubal primary, has been challenged recently by a study showing metastasis from other cancers into the fallopian tube (17). Given these practical difficulties, the gap between clinical and research reports with respect to recognizing the fallopian tube as the site of the primary in the majority HGSC continues.

This gap has profound clinical implications. Identifying the site of origin of a cancer is a fundamental tenet of cancer medicine and is essential for initiating effective strategies for the early diagnosis of HGSC. Recently, a consensus proposal based on research criteria has been put forward by a group of academic pathologists for adoption in clinical practice. However, there are concrete difficulties in applying research criteria to clinical practice. The reason for this is that routinely examining the fallopian tubes *in toto* is perceived as difficult to implement, STIC is difficult to standardize, and TMI has been challenged as a reliable criterion. This study aims to bridge the gap between research studies and clinical practice by evaluating what is currently done in clinical practice in comparison to the new recommendations relating to correct assignment of fallopian tube primary. This is done by examining all consecutive cases reported as ovarian, primary peritoneal, tubal cancer in a tertiary care gynecologic oncology center over a seven-year period with the specific aim of evaluating which of the criteria proposed is already in use at our institution, which is not, and should be implemented.

In this study, the term HGSC is used to refer only to high-grade serous cancers of the ovary, peritoneum and fallopian tube leaving out all other types of HGSC (e.g. endometrial).

INTRODUCTION

The ovary: normal female pelvic anatomy

The ovary is an intraperitoneal organ, located in the ovarian fossa, next to the uterus (Fig. 1). In females, two ovaries are present, one on the left and one on the right side. The ovary has endocrine as well as reproductive functions: it produces estrogen, progesterone, and testosterone. Ovarian pathology can be classified as endocrine, reproductive system and neoplasms. As illustrated in Fig. 2, the ovary is in close contact with the omentum. The omentum is a large fold of visceral peritoneum that hangs down from the stomach. It extends from the greater curvature of the stomach, passing in front of the small intestines and reflects on itself to ascend to the transverse colon before reaching to the posterior abdominal wall.

The fallopian tube: normal anatomy

The fallopian tube is an organ situated in the pelvis. It is not in direct contact with the ovary, but their fimbriated end fluctuates on the surface of the ovaries and helps the eggs to be brought into the uterus. It is macroscopically divided in five regions: interstitium, isthmus, ampulla, infundibulum, and fimbria (Fig. 3). Microscopically it is made of mucosa, muscolaris, and serosa. This histological structure applies to the whole extent of the fallopian tube except the fimbria that only has mucosa.

Clinical presentation of ovarian cancer

To date, there are no effective screening strategies that are recommended for the early diagnosis of ovarian cancer (OC). Usually the presentation of ovarian cancer is mild and is characterized by unspecific symptoms, such as bloating, nausea, vomiting, and heartburn, for which the patients seek doctor attention. In the most common scenario, patients go through many tests, most of which

do not focus on gynecologic organs. When the tests do not target the right organ, it is likely to give negative results. As a consequence of a non-conclusive work up, patients are often referred to the gynecologist as the last alternative. If ovarian cancer is suspected, the gynecologist may ask for an ultrasound and a blood test for a tumor marker, cancer antigen 125 (Ca125). These tests are diagnostic for ovarian cancer only at an advanced stage only.

Treatment of OC

Once OC is suspected/diagnosed, the conventional approach consists of surgery and chemotherapy. There are three goals of surgical intervention for patients with suspected ovarian malignancies: establishing the diagnosis, staging, primary cytoreductive or interval surgery/debulking (18). The chemotherapy precedes and/or follows the surgical treatment. This will be discussed in more detail in the next few paragraphs:

Primary cytoreductive surgery

If the gynecologic oncologist believes that complete resection of all the macroscopically evident disease is achievable through surgery, primary cytoreductive surgery is performed. This is usually performed by laparotomy using a midline incision of the abdomen. The surgery includes at least a Total Hysterectomy (TH), Bilateral Salphingo-Ophorectomy (BSO) and omentectomy, referred to as 'primary cytoreductive surgery' because it is offered as the upfront treatment (before chemotherapy). The surgery is said to be 'optimal' if no macroscopic evident disease is left at the end of the procedure. After the operation, the patient may or may not need chemotherapy, primarily depending on the type of OC. For HGSC the standard chemo-treatment is Carboplatin and Paclitaxel for six cycles.

Interval surgery/debulking

Alternatively, if the gynecologic oncologist considers complete resection of all the macroscopically evident disease not achievable through surgery due to the extensive spread of disease, a biopsy is done to establish the diagnosis. The biopsy is usually done under ultrasound or CT (computerized tomography) scan guidance. It is usually taken from the omentum. Once the diagnosis is confirmed, the patient can undergo chemotherapy (usually three cycles) for shrinking of the tumor mass before the surgery. Chemotherapy given before surgery is referred to as Neoadjuvant chemotherapy. Surgery that follows neoadjuvant chemotherapy is called interval surgery/debulking. The surgery is aimed to remove as much disease as is possible and usually includes at least a total hysterectomy, bilateral salpingoophorectomy and omentectomy. The surgery is said to be 'optimal' if no macroscopic evident disease is left at the end of the procedure. Postoperatively, the patient will usually receive three more cycles of chemotherapy, Carboplatin and Paclitaxel.

Fallopian tube cancer

For several years, fallopian tube cancer has been considered a different entity than ovarian cancer, with a distinct FIGO (International Federation of Gynecology and Obstetrics) staging system, management and follow up. In 2014, the FIGO staging has unified the staging system of ovarian, tubal and peritoneal cancer (7).

BACKGROUND AND LITERATURE REVIEW

Ovarian cancer is the fifth cause of cancer related deaths in women, and the first cause of gynecologic cancer deaths overall (1). Epidemiologic studies show that one in 75 women will develop this disease during their life time (1, 2). Ovarian cancer has a relatively low incidence but a high case fatality ratio (19). Although its incidence rates have decreased by 0.9% per year over the past decade (from 2003 to 2012), it still causes more deaths than any other gynecologic cancer (1). The majority of the patients are diagnosed at late stages, stage III being the most common at diagnosis (20). At stage III and IV, the five-year survival rate is only 10 to 30% (21). Ovarian cancer is not a single disease, but rather a group of diseases, each with different morphology and biological behavior (11, 22-26).

Pathology of ovarian cancer

The ovary is composed of surface epithelium, germ cells, and supporting sex cord stromal cells (23). Although primary ovarian malignancies can arise from any of these components, 90% are epithelial in origin (2). These epithelial tumors can be divided into five main types: serous carcinoma (68-71%); endometrioid carcinoma (9-11%); clear-cell carcinoma (12-13%); mucinous carcinoma (3%); transitional (1%), and mixed (6%) (27). HGSC account for at least 70% of all ovarian cancers (20, 28). Each of these tumor types are inherently different diseases, as indicated by differences in epidemiologic and genetic risk factors, precursor lesions, patterns of spread, molecular events during oncogenesis, response to chemotherapy, and prognosis (26, 29). Cancers that arise from the germ cells are much less common (dysgerminomas, yolk sac tumors, and immature teratomas) and account for 3% of ovarian cancers. The potentially malignant sex cord-stromal tumors account for only 1%–2%, and are mainly granulosa cell tumors (30, 31).

Origin of Ovarian Cancer

The Traditional View

Historically, it was thought that epithelial ovarian cancer originated from the ovarian surface epithelium. However, rather than resembling the surface epithelium of the ovary, which consist of flat cuboidal mesothelial cells, epithelial tumors resemble parts of the mullerian tract (i.e., fallopian tubes, uterus and endocervix). Benign counterparts of the cells of ovarian carcinomas are not found in the normal ovary. This discrepancy was explained by proposing that invaginations of the ovarian surface epithelium into the underlying stroma resulted in the formation of inclusion cysts, called cortical inclusion cyst (CIC) that would eventually undergo metaplasia and malignant transformation (32). Epithelial ovarian inclusion cysts are differentiated from serous cystadenoma by an arbitrary size limit of 1 cm (33). It was believed that the epithelial inclusion cysts through a process of mullerian neometaplasia would then resemble morphologically the epithelium of the fallopian tube, endometrium, or endocervix (32). Thus, serous tumors of the ovary resemble the fallopian tube, endometrioid tumors resemble the endometrium, and mucinous tumors resemble the endocervix. There are reports of early or *in situ* lesions involving the ovarian surface epithelium (OSE) (22, 34, 35) and of experimental models of transformation of OSE giving rise to tumors that resemble ovarian carcinoma in humans (36-38). However, the rarity of such reports suggest that other mechanisms may be more likely and common. Another theory proposed is that these tumors come from a 'secondary mullerian system' (39, 40). The secondary mullerian system consists of microscopic structures lined by mullerian epithelium that can be found at extraovarian sites (e.g. endosalpingiosis) or in the ovary. It may account for HGSC that does not start in the fallopian tube, i.e., primary peritoneal cancer (41). However, serous carcinomas only rarely are reported to occur in the hilum of the ovary (42). From early 2000, evidence has accumulated to

point to another and far more common site of origin of HGSC: the tubal fimbriae. The fallopian tube, rather than cortical inclusion cyst from ovarian surface epithelium or the secondary mullerian system, is now considered as the site of origin of many if not the majority of HGSC of the ovary (4, 12, 15, 43-48).

Sites of origin of non-uterine HGSC

Therefore, there are three possible primary sites of origin of non-uterine HGSC (10):

- the ovary, from surface epithelium that undergoes metaplasia and malignant transformation/neometaplasia or more likely cancer arising from areas of mullerian inclusions or endosalgingosis in the ovary – primary ovarian cancer
- the areas of mullerian inclusions or endosalgingosis in the peritoneum -primary peritoneal cancer.
- the fallopian tube mucosa – primary tubal cancer

Assigning the site of origin by gynecologic oncology pathologists

In clinical practice, when a pathologist receives the surgical specimens (tissues removed at surgery), he/she uses the FIGO (International Federation of Gynecology and Obstetrics) and WHO (World Health Organization) criteria for the assignment of HGSC primary site, which are based on the localization of the tumor mass (10, 11). Of note, the WHO classification of tumors of the female genital tract released in 2014 leaves the assignment of site of origin to the *"experience and professional judgement"* of the reporting pathologist and Tumor Board (8). For all practical purposes, cancers of the ovary, peritoneum and fallopian tube are reported together in clinical practice. This is because they are considered as being one disease, HGSC, and because there are

practical difficulties in assigning site of origin. The treatment of ovarian, peritoneal and fallopian tube cancers is the same; differentiating site of origin is important for preventive strategies.

Traditional criteria recommended by the FIGO and WHO criteria and followed by gynecologic oncology pathologist for assigning site of origin /primary are as follows:

a) **Primary ovarian cancer:** *"Tumors were classified as ovarian in origin if none of the above criteria […]"* (to assign peritoneal origin) *"[…] were fulfilled. In addition, tumors that were >5mm on the ovarian surface (unless the tumor was imbedded in a desmoplastic plaque characteristic of secondary ovarian involvement) […]"* (are) *"[…] classified as primary ovarian, even if no tumor was present in ovarian"* (10).

b) **Primary peritoneal cancer (reviewed in 2014):** prior to 2014 primary peritoneal cancer was defined by size criteria: *"1) Both ovaries must be either physiologically normal in size or enlarged by a benign process. 2) The involvement in the extra ovarian sites must be greater than the involvement on the surface of either ovary. 3) Microscopically, the ovarian component must be one of the following: a) nonexistent, b) confined to ovarian surface epithelium with no evidence of cortical invasion, c) involving ovarian surface epithelium and underlying cortical stroma but with any given tumor size less than 5 x 5 mm, d) tumor less than 5 x 5 mm within ovarian substance associated with or without surface disease. 4) The histological and cytological characteristics of the tumor must be predominantly of the serous type that is similar or identical to ovarian serous papillary adenocarcinoma, any grade"* (10). Since 2014 primary peritoneal cancer is defined as HGSC in the peritoneum when fallopian tubes and ovaries are not enlarged or are enlarged due to benign conditions (8).

c) **Primary fallopian tube cancer:** according to the traditional view, *"1) The tumor arises from the endosalpinx. 2) The histological pattern reproduces the epithelium of the tubal mucosa. 3) Transition from benign to malignant epithelium is found. 4) The ovaries are either normal or with tumor smaller than that of the tube"* (49, 50). According to the new recommendations (4, 9, 12, 14, 15, 43-48, 51) *"Primary site should be assigned as tubal in the presence of STIC or invasive mucosal carcinoma in the fallopian tube, or when part or all of the tube is inseparably incorporated within a tubo-ovarian mass. […]. In cases where HGSC occurs following previous removal of tubes and ovaries which were not fully sampled and uterine origin has been excluded, the primary site should be assigned as 'tubo-ovarian'." (9, 14).*

Consensus of opinion in scientific literature

Although the mesothelial origin of HGSC as believed in the past cannot be excluded (45), there is now compelling evidence that the majority of cases of primary ovarian cancers are actually cancers which originated from cells that are not native to the ovary. Clear cell and endometrioid adenocarcinomas of the ovary appear to start in areas of endometriosis, i.e. ectopic endometrium/mullerian tissue in the ovary (52). The origin of transitional cell tumors is not well established. With respect to serous tumors, particularly high-grade serous cancer (HGSC), as indicated above, the most common site of origin of HGSC appears to be the precursor epithelial lesions in the distal fimbriated end of the fallopian tube (4, 12, 15, 43-48, 51) called serous tubal intraepithelial carcinoma or STIC (– please refer to page 25 for further explication).

Recognizing the role of fallopian tube as the site of origin of HGSC: an overview

The fallopian tube was first suggested as a potential site of serous carcinoma development in 1896 by Doran (51). Over the years, and particularly since early 2000's, several studies have investigated the role of the fallopian tube in HGSC (4, 12, 15, 43-48, 51). Particularly, Piek et al. in 2001 and Medeiros et al. in 2006 found lesions within the tubal fimbria in risk-reducing salpingo-oophorectomy specimens. These lesions were defined as STIC and were found in women with known mutation in Breast Cancer gene 1 and/or Breast Cancer gene 2 (BRCA1/BRCA2 genes), who are at high risk to develop ovarian cancer. These women are offered prophylactic risk reducing surgery which consists of bilateral salpingo-oophorectomy because of their substantially higher risk of lifetime HGSC – about 45% in BRCA1 and 11% in BRCA2 (53). Although the surgery is done as a prophylactic measure, an occult or early in invasive cancer can be found in 2 to 17% of the cases (54-59). The importance of examining the fallopian tubes carefully and in its entirety – Sectioning and Extensively Examining the Fimbriated end (SEE-FIM) protocol, was first recognized in this population. As the practice of using the SEE-FIM protocol for handling the fallopian tubes in all risk-reducing surgeries became more common, the role of the fallopian tube in HGSC carcinogenesis became evident (6, 15, 43, 45, 60). The findings in the high risk population suggested the possibility that STIC represented the long sought precursor of non-uterine HGSC (46, 61, 62). An immunohistochemical study has supported the tubal phenotype of 80% of ovarian cortical inclusion cysts, showing that they expressed a mullerian marker, PAX8, rather than a mesothelial marker, calretinin (63). Over the years, considerable evidence has emerged pointing to the tubal origin of HGSC, both in women with BRCA mutation as well as in sporadic cases of HGSC (4, 12, 15, 43-48, 51).

Importance of examining the fallopian tubes *in toto*

One of the developments that facilitated the shift in assigning the primary of HGSC in research studies is the recognition and adoption in academic pathology units of detailed examination of the fallopian tube in its entirety, *in toto*. Thorough examination of the fallopian tube can be done effectively by several methods (Table 1): in 1996 the University of California San Francisco Gynecologic Oncology Program introduced a protocol for risk-reducing total hysterectomy and bilateral salpingoophorectomy (TH-BSO). This consisted in analyzing both ovaries, the fallopian tubes, peritoneal and random omental biopsies plus collection of peritoneal washings for cytology, serial sectioning of entire fallopian tubes and ovaries at 2-mm intervals and microscopic examination of all sections (58).

Another method to analyze the fallopian tube *in toto* was proposed by Crum et al. in 2006, the Sectioning and Extensively Examining the Fimbriated end (SEE-FIM) protocol (64). The protocol calls for 2 mm serial sections along the whole length of the fallopian tube and longitudinal sectioning of the fimbria. This has since been adopted by the majority of academic units as the standard protocol for handling and examining the fallopian tubes in risk reducing surgery for BRCA1/BRCA2 mutation carriers, and increasingly even in known or suspected cases of invasive HGSC. There are some variations to this protocol, such as applying the SEE-FIM but using deeper sections at 100 micron intervals (57), multiple level sections (65). Examining the fallopian tubes *in toto* using one of these methods, allows pathologists to identify a specific lesion, called STIC. This is thought to be the precursor of HGSC.

Using the SEE-FIM protocol, Medeiros et al. in 2006 examined 26 patients affected by BRCA1/BRCA2 mutation, who underwent prophylactic surgery. In women with BRCA1/BRCA2 mutations the prophylactic surgery consists of a total hysterectomy and bilateral

salpingoophorectomy to reduce the risk of developing breast and ovarian cancer (46). They found five tubal cancers, and no ovarian cancers. In this study, among the fallopian tube cancers, three out of five tumors were present in the fimbria, one in both fimbria and ampulla, and one was present in the ampulla of the fallopian tube (46). The following year, the same group published a study of 63 consecutives cases, of OC; in 41 of the cases (75%) the tubal mucosa (endosalpinx) was involved. Particularly, in 29 cases (71%) there was a STIC. among the 29 cases with STIC, 27 (93%) were found within the fimbria. They concluded that serous cancer rarely presents with a dominant tubal mass, that although the fimbria is the actual site of origin, the cancer grows preferentially at a remote site, because of the permissibility of the micro environment (4). According to Kindelberger et al, STIC arises in the fallopian tube, often in the fimbria, and subsequently drops or seeds on to the surface of the adjacent ovaries and the peritoneal cavity and develops into HGSC (4). The SEE-FIM protocol would allow the identification of STIC in both high risk and the general population.

Serous tubal intraepithelial carcinoma (STIC)

Serous tubal intraepithelial carcinoma (STIC) is defined as a proliferation of non-ciliated cells with cytological atypia and high-cytoplasmic ratio, exhibiting epithelial stratification, loss of polarity, mitotic activity, cellular exfoliation, and epithelial fractures. It is accepted as the precursor lesion in some cases of HGSC (4, 12, 15, 43-48). STIC is thought to arise in the fallopian tube in response to cancer stimuli, such as gene mutation (please see next section “TP 53 gene and p53 mutation”). As described above, there is growing evidence that STIC is more often found within the fimbria rather than the ampulla region of the fallopian tube (14, 47).

Early reports of STIC and its role in the fallopian tube origin of HGSC came from studies on women with a genetic predisposition to HGSC because of mutations in BRCA1/BRCA2 (43). It

would appear that STIC is evident in these fallopian tubes because they are diagnosed at early stages and their fallopian tubes are extensively analyzed as per the SEE FIM protocol (13). STIC is found to coexist with the majority of HGSC from women diagnosed with early stage HGSC (44, 66). Studies on molecular alterations in fallopian tubes from patients with BRCA1/BRCA2 mutation, suggest that both STIC and HGSC have a common histological origin (6, 12, 15, 45). However, BRCA1/BRCA2 germline mutation is responsible for only 10-15% of all OC (67, 68).

Other candidate precursor lesions besides STIC

Other lesions have been described and these resemble STIC but do not fulfill the criteria to be named as it. These are (69):

1. STILs: Serous Tubal Lesions in Transition
2. TILT: Tubal Intraepithelial Lesions in Transition
3. SCOUTs: Secretory Cells Outgrowths

   A SCOUT is a proliferation of secretory cells of the tubal epithelium with minimal cytological atypia, pseudostratification not altered and low MIB-1 index. It usually does not show p53 mutations. However, it shows alterations in PTEN and PAX2 (70, 71).
4. "p53 signature": a p53 positive SCOUTs

   A "p53 signature" is a SCOUT with p53 mutation and is defined as a linear, p53 immunopositive segment of tubal cells with at least 12 consecutive secretory cell nuclei (72).

Evidence supporting that "p53 signature" is the precursor of STIC

Studies have shown evidences that "p53 signature" is in fact the precursor of STIC. For instance, Lee et al. in 2007 and Carlson et al. in 2008 have shown that the kind of mutations of TP53 in "p53 signatures", STIC and synchronous pelvic HGSC are the same (12, 72). Lee et al. in 2007 have shown that there are peculiar DNA damages in these lesions (72). Medeiros et al. in 2006 and Carlson et al. in 2008 have shown that STIC most likely arises in the fimbria (46, 60), and the percentage of STIC is higher in patients with HGSC (71).

TP53 gene and p53 mutation

TP 53 is a tumor suppressor gene. Its main role, but not the only one, is to induce the apoptosis of the cell in the picture of the normal cell cycle. It is mutated in more than 50% of human cancers. P53 expression is evaluated with immunohistochemistry (IHC) in OC, and is found to be over-expressed in at least 97% HGS ovarian cancers (73, 74). Jarboe et al. in 2008 proposed that the p53 overexpression is actually the first step in the pathogenesis of the STIC (69). There were observations that the mutations that lead to over expression of the p53 are the same, both in the ovarian mass and in the STIC, among the cases of HGSC studied (4, 5, 14, 16, 17, 46, 47, 75). On the other hand the presence of the p53 mutation, that lead to its over-expression, is more common than the STIC in the general population (47).

The level of expression of protein p53 indicates whether the TP53 gene is normal (non-mutated, or wild type) or mutated. In normal tissues, protein p53 is not over-expressed in presence of a wild type TP53 gene. In abnormal tissues, such as in cancer, p53 is over-expressed in response to a mutation of the TP53. However, as the possible spectrum of TP53 mutations can vary, so can the IHC results of protein p53. Therefore, though the majority of TP53 mutations lead to an over-expression of protein p53, a minority of these lead to a non-expression of protein p53 (null

phenotype of p53 IHC) (73). The BRCA1/BRCA2 genes, like TP53 gene, codify for proteins (BRCA1 and BRCA2 respectively) that are responsible for repairing DNA damages. The BRCA1/BRCA2 can be inactivated either via gene mutation or hyper-methylation of BRCA 1 promoter (73). DNA hyper-methylation is a biochemical process in which a methyl group (-CH_3) is added to a cytosine or adenine DNA nucleotides. A promoter of a gene is the region in the DNA that initiates the transcription of that particular gene into a protein. When the promoter is hyper-methylated it cannot be transcribed into a protein anymore. As a result, the DNA damage cannot be repaired efficiently and the cancer results (73).

The Tubal Mucosa Involvement (TMI)

According to Kindelberg et al. 2007 *"The first is that the tubal mucosa appears resistant to direct mucosal implantation. The salpingeal mucosa is rarely the site for implants from endometrioid, mucinous or borderline serous neoplasms. Metastatic mucinous tumors likewise frequently involve the ovarian or tubal serosal surfaces, yet spare the mucosa"* (4). Thus, can tubal mucosal involvement (TMI) be a good indicator? Several other experts on tubal pathology and carcinogenesis too have indicated that the tubal mucosa is resistant to metastasis and therefore TMI could be a good marker. Singh et al. in 2014 have proposed a pragmatic algorithm for assigning primary site in HGSC (14). They suggest that in the presence of TMI, the fallopian tube should be assigned as the primary (14). A group of influential academic gynecologic oncologists and gynecologic pathologists led by Singh in January 2016 have released a *"Consensus statement on unifying practice worldwide"* (9). They introduce criteria for assignment of primary in HGSC (Tab 1) and they recommend a uniform approach in assigning primary site of origin in HGSC (76). The International Collaboration on Cancer Reporting has already included most of the criteria prosed by Singh et al. in their dataset (76).

Challenges in bringing the research knowledge in clinical practice

As discussed earlier, accumulating evidence indicates that the fallopian tube is responsible for the majority of HGSC. However, tubal cancer is still considered a rare disease. One reason is the difficulty of identifying STIC in clinical practice. Despite various protocols for sectioning the fallopian tube extensively have been implemented (such as the SEE-FIM), about 40% of sporadic HGSC have no STIC, suggesting that these do not start in the fallopian tube (4, 77). When STIC is not present, it may appear logical to conclude that the cancer started in the ovary. However, it has been hypothesized that the STIC negative cancers may arise from normal tubal epithelium that that implant in the ovary and undergoes malignant transformation (25). A small minority of HGSC may arise from serous borderline tumors or low grade serous carcinomas (33). Even with these limitations, STIC in only 50-60% of all sporadic ovarian cancer (4, 12, 47). This may be because in the clinical setting the majority of HGSC is diagnosed at stage III. Therefore, it is likely that STIC may be obscured due to overgrowth by the invasive carcinoma (45). Therefore, the absence of STIC cannot exclude a tubal origin of HGSC. Another point of note is that although STIC was not thought to be a feature of other gynecologic cancers (4, 45, 72, 77, 78), some of the more recent studies have described lesions that are similar to STIC in association with endometrial serous adenocarcinoma (79, 80). Furthermore in 2015, McDaniel et al performed Targeted Next Generation Sequencing (NGS) on tumor blocks from four women, whom had uterine endometrioid carcinoma (81); their findings were surprising because they showed that at least some STICs may be metastatic lesions rather than represent precursor of HGSC (81). Moreover, findings of a study done by Seidman et al. on 388 tumor blocks in 2015 showed a possible association between STIC and endometrial hyperplasia and carcinoma (82). Therefore, it appears that the use of STIC in assigning tubal origin in HGSC is challenging. It would seem that, although

there is no doubt that STIC is the precursor lesion at least in some HGSC (5), there are inherent difficulties using this as a criterion to assign tubal origin (15, 16).

Recent studies showed that there is a significant inter-observer variability in the interpretation of these lesions (15, 16, 75). This variability may be reduced with the use of p53 and Ki-67 immunostains (16, 75). In fact, p53 mutations are more frequent than the STIC in the general population (47). This has been challenged by other studies that did not show higher presence of p53 mutation in fallopian tubes from patients with BRCA1/BRCA2 mutations (72, 83). However, this could be explained by the meticulousness with which the fallopian tubes are handled, the methodology used for sectioning the fallopian tubes and the number of tissue blocks examined (60, 70, 78). As ovarian cancer is diagnosed in late stages the extent of the disease is likely to obscure any precursor lesion (45). When precursor lesions are not evident, the over-expression of protein p53 at immunohistochemistry is the only remnant of a previously evident precancerous lesion. However, the p53 immunostains have an important problem: both null and over-expression may indicate a mutation (16, 75). Even though some authors still consider p53 immunostaining as a useful surrogate for TP53 mutation in the histological diagnosis of STIC (4, 14, 46), universal protocols for immunostaining technique are necessary to have uniform results. Moreover, similar p53 mutations as well as mutations in other markers, such as HMGA2, cyclin E, p16, RSF-1, fatty acid synthase, and PAX2 have been shown in patients with STIC and HGSC (5, 71). Of note, p53 signature can coexist with benign ovarian diseases (72).

It has been suggested that TMI is a good candidate to rule in tubal origin. In fact, it appears resistant to metastasis. However, a recent publication by Rabban cast doubt on this theory (17). In 100 cases

in which the fallopian tube harbored a metastasis from a non-gynecologic cancer, in 29, the tubal mucosa was involved.

HYPOTHESES

My hypotheses are the following:

1. Tubal mucosa involvement (TMI) is likely to be identified and reported in clinical practice by pathologists in fallopian tubes analyzed *in toto*. TMI is a valid criterion that could and should be used in clinical practice to correctly assign site of origin of HGSC.
2. TMI is a feature of HGSC and it is unlikely to be identified in non-HGSCs .

OBJECTIVES

There is a strong evidence in research studies that the majority of HGSC arise in the fallopian tube but pathology reports do not reflect this. Research studies use specific criteria –serous tubal intraepithelial carcinoma and tubal mucosa involvement (STIC and TMI) in fallopian tube thoroughly analyzed *in toto* (58). The objective of this study to identify ways of bridging the gap between research studies and clinical practice with respect to the assignment of the site of origin of high-grade serous ovarian cancer (HGSC). The reason why the criteria proposed in scientific studies have not yet been adopted in clinical practice is that there are practical difficulties in their routine application. Therefore, the specific aim of this study is to evaluate which of the criteria proposed is already in use at our institution, and which is not and should be implemented. The use of controls is intended for comparing the incidence of STIC and TMI in HGSC vs non high-grade serous ovarian cancers (non-HGSCs).

METHODS

Standard McGill University Health Center (MUHC) practice for processing upper genital tract specimens in cases of suspected or proven ovarian cancer

The standard practice for processing and reporting pathological specimens for suspected ovarian cancer at McGill University Health Center (MUHC) is to examine the fallopian tubes and ovaries *in toto,* using the protocol described by the University of California San Francisco Gynecologic Oncology Program (58). This consists of serial sectioning of the entire fallopian tubes and ovaries at 2-mm intervals and microscopic examination of all sections (58). The only difference between this protocol and the SEE-FIM is that the fallopian tubes are sectioned transversely rather than longitudinally, as in the SEE-FIM protocol. If an area of concern is encountered, it is evaluated by deeper serial sections. Henceforth, when the term *in toto* is used, it refers to the use of this protocol. This is the protocol used at MUHC.

The cases are routinely subjected to pathological examination on two occasions, first at the time of reporting of the surgical specimens, and then at multidisciplinary meeting or Tumor Board, when the gynecologic oncology pathologist presiding reviews the slides. The latter could be the same pathologist who signed out the case following the surgery, or by another pathologist. If there is a difference of opinion an addendum is added to the report after mutual agreement. No cases were reviewed or blocks re-cut for the purpose of this study. These results therefore reflect routine clinical practice in a publically funded, tertiary care gynecologic oncology center.

Study Population

This study is a retrospective analysis of consecutive cases of all eligible and evaluable cases that had primary cytoreductive surgery for a diagnosis of ovarian cancer (OC), primary peritoneal

cancer (PPC), fallopian tube cancer (FTC), at the McGill University Health Center (MUHC) between January 1st, 2007 and December 31st, 2013. In regards to the sample size of the cases and controls, no statistical calculations were used to define sample size. For both the case and the control group, all consecutive cases of ovarian/peritoneal/fallopian tube cancers operated within the given time frame were included.

Cases were potentially eligible for the study if (i) primary cytoreductive surgery have included resection of at least both adnexae and uterus (if present) and the omentum), ii) all pathology reports were signed out by a gynecologic oncology pathologists, and/or the case was reviewed by a gynecologic oncology pathologist for Tumor Board, iii) the fallopian tubes have been examined *in toto*.

Inclusion criteria were as follows:

1. Patients with a first diagnosis of ovarian cancer received at the MUHC from January 1st, 2007 and through December 31st, 2013 (the time frame was arbitrary chosen);
2. Patients undergoing surgery for complete resection of the disease (cytoreductive surgery or interval surgery/debulking) at the MUHC. As described in the background, by cytoreductive surgery we refer to patients undergoing surgery for ovarian cancer before receiving chemotherapy treatment, whereas by interval surgery/debulking we refer to patients undergoing surgery after receiving chemotherapy.
3. Patients' fallopian tubes were analyzed *in toto*. The fallopian tube is thoroughly sectioned even when it looks macroscopically normal. When a tube is examined *in toto,* the entire fallopian tube is serially sectioned at 2 mm intervals and each section is analyzed microscopically.

4. Patients were categorized as having a primary ovarian, peritoneal, tubal or uncertain origin of mullerian HGSC.

Exclusion criteria were as follows:

1. Patients had been diagnosed with ovarian cancer outside the time frame (before January 1st, 2007 or after December 31st, 2013).
2. Fallopian tubes were not processed *in toto*.
3. Patients were diagnosed with benign or borderline ovarian tumors.

This study received approval from the McGill University Health Center – Institutional Review Board (MUHC-IRB). Cancer Registry and Tumor Board lists were obtained to identify all patients who were treated at MUHC for ovarian/peritoneal/fallopian tube cancer. After screening of these lists, 277 cases were identified. The pathological report of each patient was meticulously reviewed in the clinical record system and clinical charts to ensure that they fulfilled the eligibility criteria. Out of 277 patients, 215 (77.6%) patients fulfilled the above criteria. Of note, patients were further categorized into two groups: 125 patients diagnosed with high-grade serous (HGSC) cancers of the ovary, the peritoneum, and the fallopian tube; 90 patients diagnosed with all the other histological sub-types, which will be referred to as non high-grade serous cancers (non-HGSCs). The reason for including non-HGSC was because although TMI is considered a feature of HGSC (4), this has been recently challenged (17). However, he newly proposed research criteria established by Singh et al. 2016 are based on the presence of TMI (9). Therefore, I wanted to investigate the proportion of TMI found in the HGSC group as compared to the proportion found in non-HGSCs group to see whether there was a difference between the two.

From each pathology report, the following variables were abstracted

- age at diagnosis,

- stage at diagnosis (stage I, II, III, IV),
- attributed origin (ovarian, peritoneal, tubal, uncertain –unique primary cannot be assigned),
- presence of serous tubal intraepithelial carcinoma (STIC),
- presence of tubal mucosa involvement (TMI) after analysis under the microscope.
- maximal tumor dimension in the abdomen and its localization (ovary, fallopian tubes, omentum, and bowel).

No slide was reviewed, and members of the Tumor Board were not involved in data collection.

Statistical analysis

Characteristic of the study sample were tabulated as a function of HGSC vs. non-HGSCs (Table 3 and Table 5) and the frequencies of the characteristics of the women in the two groups were calculated.

Chi-squared test was used to compare the presence of STIC and TMI in HGSC vs non-HGSCs. The proportion of cases with tubal origin according to the FIGO criteria and the newly proposed research criteria based on Singh et al. 2016 (9) (referred to as “research criteria” from now on) are reported with exact binomial 95% confidence intervals.

RESULTS

During the study period, a total of 277 patients had cytoreductive surgery for a diagnosis of ovarian/primary peritoneal/fallopian tube cancer. Of these, 62 patients were excluded for the following reasons: four were diagnosed before 2007, nine had ovarian involvement from an extra ovarian primary, 23 did not have the adnexae removed during surgery or had had prior surgery at an outside institution, in 17 cases the fallopian tubes were not analyzed *in toto*, in 8 cases the pathology was not signed out or reviewed by a site specialized gynecologic pathologist. There was one case who previously had a prophylactic hysterectomy and bilateral salpingoophorectomy. Of note, 9% (17 plus 8 of 277) cases were not analyzed *in toto*. As a result, 215 cases were included for analysis (Figure 4).

The study population consisted of 215 consecutive cases of invasive ovarian cancer (after exclusion of borderline cases); of these, 125 were instances of non-uterine high-grade serous cancer (HGSC of the ovary, peritoneum, or fallopian tube according to the traditional FIGO and WHO criteria, reported in Table 2) and 90 were non-HGSCs. Cases were classified as HGSC of the ovary/peritoneum/fallopian tube/uncertain based on the FIGO and WHO criteria for assigning origin of HGSC. Eight of them had a small proportion of another component (high-grade endometrioid, sarcomatous). The 90 non-HGSCs included 82 epithelial and eight non-epithelial cancers of the ovary/fallopian tube (Table 3). For the purpose of this study, HGSC represent cases and non-HGSCs represent controls.

Assignment of site of origin

Of the 125 HGSC cases, 48% (60 of 125) were signed out by the gynecologic oncology pathologist as ovarian primary, 17.6% (22 of 125) as peritoneal, 28% (35 of 125) as tubal/tubo-ovarian and 6.4% (8 of 125) as uncertain (Table 4).

Differences in patient and tumor characteristics between HGSC and non-HGSCs

The median age in the HGSC and non-HGSC groups was 61 (range 34-87, interquartile range 14) and 53 years (range 18-88, interquartile range 14), respectively. The stage distribution differed in the two groups. Of the HGSC patients, 87.2% (109 of 125) were diagnosed in advanced stages (III & IV) vs. 18.9% (17 of 90) in the non-HGSC group (Table 5).

Consistent with the more advanced stage in HGSC cases, 41.6% (52 of 125) received neoadjuvant chemotherapy (NACT) (median three cycles, range 2-4), prior to their surgery compared to 2.2% (2 of 90) of the non-HGSC cases (Table 6). Data were stratified for chemotherapy status, i.e. patients who received NACT and patients who did not receive NACT (No-NACT), because NACT may have an impact on ability to detect STIC and TMI (Table 6).

Patients in the study are expected to be representative of the population of women with ovarian cancer given that all consecutive cases that met the inclusion criteria decided *a priori* were included without any further selection.

Dominant tumor mass

As evident in Table 6, the dominant tumor was in the ovary in 58.9% of HGSC cases who had not received NACT (No-NACT), with a median tumor volume of 222.8 cm^3. The omentum was the site of the largest tumor in 27.4% of patients who had not received NACT and in 46.2% of patients who had; the median volume of omental tumor was 293.9 cm^3 in women who had not received chemotherapy and 32.5cm^3 in those who had had NACT. In comparison, in only in 4% the fallopian tubes were the site of the dominant tumor mass; the median volume of fallopian tubal tumors was 3.6cm^3 and 3.3cm^3 respectively in patients who had not had NACT, and those who had. Median sizes of the ovaries appeared to be affected by chemotherapy. Particularly, it appeared to be smaller in patients who received NACT. For instance, NO-NACT patients in the HGSC

group had a median size of the largest tumor of 347.9cm³ vs 32.5cm³ in patients who received NACT. Findings were similar in the non-HGSCs group, where No-NACT patients had a median size of the largest tumor of 431cm³ vs 30 cm³ in patients who received NACT. However, the number of patients receiving NACT in the non-HGSCs group is very small, two of 90. The location of the largest tumor mass (the dominant mass) also appeared to be affected by chemotherapy. In fact, this was present within the ovary in 58.9% (43 of 73) of the No-NACT HGSC patients vs 36.5% (19 of 52) in the NACT subgroup. Furthermore, the size of the ovaries appeared to be affected by chemotherapy. For instance, the median size of the largest ovary in No-NACT HGSC patients was 222.8cm³ compared to 8.2cm³ in the HGSC patients who received NACT. Interestingly, the size of the fallopian tubes did not appear to be affected by chemotherapy. In fact, the median size of the largest fallopian tube in the HGSC group was almost the same in the No-NACT and NACT subgroups (3.6 vs 3.3cm³ respectively) (Table 6).

Serous tubal intraepithelial carcinoma (STIC)

STIC was found in 12.8% (16 of 125) of the HGSC group and was more likely to be observed in patients who had upfront surgery compared with those who had received NACT (15% vs 9.6%). Of the HGSC diagnosed at early stages (I and II), 12.5% had STIC (2 of 16); of the HGSC diagnosed at advanced stages (III and IV), 12.8% had STIC (14 of 109). There were no STIC in the non-HGSCs group. However, the numbers were too small to make meaningful inferences (Table 7).

Tubal mucosal involvement (TMI) and reassignment of site of origin

Of the HGSC cases, 56% (70 of 125) had cancer in the tubal mucosa. The likelihood of finding TMI was higher in patients who had had upfront surgery 64.4% (47 of 73) compared with patients

who had had neoadjuvant chemotherapy 44.2% (23 of 52). Of the HGSC diagnosed at early stages (I and II), 75% had TMI (12 of 16); of the HGSC diagnosed at advanced stages (III and IV), 53% had TMI (58 of 109). This gave a p-value of .11 with the Fisher exact test, suggesting that there is no evidence that TMI is less identifiable at early stages. TMI was relatively infrequent in non–HGSCs occurring in only five cases (Tab 7). Therefore, only 5.6% of non-HGSCs had at least one between TMI and STIC vs 56% among HGSC (p-value <.0001 based on Chi-squared test).

After applying the research criteria, a total of 28% was reassigned to tubal from ovary, peritoneal, and uncertain (details are provided in Table 8). Thus, the research criteria based on the presence of TMI or STIC doubled the proportion of HGSC assigned to tubal origin, from 28% (95% C.I. 20.3, 36.7) to 56% (95% C. I. 46.8, 64.9). The two 95% C.I. indicate that the two estimates of tubal are significantly different from each other.

DISCUSSION

The results show that even in a tertiary care academic center, where pathologists are fully cognizant of the literature supporting tubal origin for the vast majority of non-uterine HGSC, only 28 % of such cases were reported as tubal cancer with 48% the cases being signed out as ovarian cancer. Nevertheless, the proportion reported as tubal primary is higher than that reported in recent large series (less than 5%) (84). This suggests that the reporting pathologists did not assign site of origin strictly according to the dominant tumor mass criteria recommended by FIGO and WHO (8, 10, 77, 85) (Table 2). If the volume criteria alone had been used to assign site of origin in this series, the ovary was the dominant tumor in 58.4% of cases not treated with prior chemotherapy. A close contender for the designation of based on dominant mass is the omentum (peritoneum). It contained the dominant mass in 27.4%-46.2% of the cases depending of preoperative chemotherapy use. In contrast, the fallopian tubes were unimpressive; in only in 4 % of cases was the tube the site of the dominant tumor mass. It is therefore not surprising that for some time, fallopian tubes remained unnoticed, with the culpability for the origin of the cancer falling predominantly on the ovary, and failing that on the peritoneum. After the Gynecologic Oncology Group defined the criteria for primary peritoneal cancer (PPC) in 1993 (10), the assignment of the peritoneum as the primary site of origin increased from seven to 23% in several institutions (86). This then has downstream effect on tumor registries, which rely on pathology reports, and then on national statistics. In an analysis of time-trends in the incidence of ovarian, peritoneal, and fallopian tube carcinomas (number of cases was 122,478) reported between 1995-2004 in the SEER, Goodman et al. in 2009 noted a 243% increase in the incidence of PPC and a 14% decrease in ovarian carcinoma (6).

The increase in reporting of PPC by surgical pathologists after publication of the GOG criteria suggests that easily adoptable criteria to standardize assignment of site primary could result in a gradual increase in pathological reporting of that primary over time. Why has this not happened with respect to fallopian tube cancers? The answer lies in difficulties (real and perceived) of adopting the criteria used in research studies as well as that proposed by Singh et al. in 2016 in routine clinical practice (9). Accurately assigning tubal primary is dependent on handling and examining the fallopian tubes in all cases of high-grade serous ovarian cancer (HGSC) using the Sectioning and Extensively Examining the Fimbriated end (SEE-FIM) protocol. Furthermore, it involves identification of serous tubal intraepithelial carcinoma (STIC) and tubal mucosa involvement (TMI). Adopting these measures has become the standard practice for dealing with pathologic specimens of risk reducing surgery, but these surgical specimens account for a small proportion of the workload of a surgical pathologist. In contrast the voluminous samples for analyses from the substantially more cases of cytoreductive surgery for HGSC are unmatched by any other cancer site, because unlike other sites when surgery in contraindicated in the context of widely metastatic disease, surgery is the gold standard treatment even in advanced stages. With bulky tumor masses, 10-70 times the size of the fallopian tube coming from the ovaries, omentum and other peritoneal surfaces, it is not surprising that the tubes have been traditionally ignored. Requiring the fallopian tubes to be processed using the SEE-FIM protocol, and examined for STIC and TMI substantially adds to the workload of the pathologist as well as the laboratory's technical staff if it has to be done in all cases of HGSC.

Therefore, my finding that in seven years only in 9% cases the tubes were not examined *in toto*, were surprising and encouraging. It suggests that if a protocol is adopted, it tends to be followed and becomes routine clinical practice, notwithstanding the increase in workload.

The two findings in the fallopian tube which has served in research reports to assign tubal primary are the presence of STIC and TMI. Singh et al. in 2014 and in 2016 have proposed a pragmatic algorithm for assigning primary site in HGSC (9, 14). They suggest that in the presence of either of these criteria, the fallopian tube should be assigned as the primary (9, 14).

From the description in the pathology reports, it appears that the pathologists did report on the presence of STIC and TMI in the tubes. However, it appears that although their findings were noted and described in the pathology reports, they were not used to assign the tumor as tubal primary HGSC.

STIC was noted only in a small proportion of the patients - 12.8% as opposed to 19% to 60% of HGSCs in research reports (4, 43, 46). This could partly be explained by the fact that 41.6% of patients received neoadjuvant chemotherapy (NACT). Even those who did not receive NACT had a huge burden of disease, which is known to overgrow and destroy fine detail such as STIC. Indeed in their series of 53 cases, examined prospectively for the specific purpose of evaluating their proposed criteria, Singh et al. found STIC only in 9.4% of cases (14, 87).

Another problem is the reproducibility of identifying STIC, which is low, with inter-observer and intra-observer agreement reported as fair (15, 16). Incorporation of morphologic and immunohistochemistry markers for p53 and Ki-67 immune-staining improved its performance (16, 75). However, this adds to the workload and may not be worthwhile for the limited gains it confers. Overall, it appears that unlike the case with prophylactic risk reducing surgery, in the context of clinical practice, STIC is unlikely to be a consistently useful criterion.

With respect to TMI, it was noted to be present in 64.4% of HGSC cases who had not received chemotherapy and 44.2% or those who had. The latter has been proposed as a criterion for assigning fallopian tube primary because the tubal mucosa is rarely involved in other histological

subtypes of ovarian cancer (4). This this has led to the belief that the tubal mucosa is relatively resistant to metastasis (4, 14, 47). In a study on twelve consecutive cases of serous carcinoma of the ovary, seven were found to have unilateral mucosal involvement of the fallopian tube that was ipsilateral to the dominant ovary mass. The unilateral fallopian tube mucosal involvement, despite widespread disease with bilateral tubal serosal implants, is consistent with these tumors having arisen in the fallopian tube mucosa (88). However, a recent publication by Rabban et al. questioned the assumption that the tubal mucosa is resistant to metastasis (17). Out of 100 cases in which the fallopian tube harbored a metastasis from a non-gynecologic cancer, 29 had tubal mucosa involved. However, these 29 cases were collected over 23 years, so it could be considered that the fallopian tube mucosa is relatively resistant to metastasis. In our 90 cases of non–HGSCs only in five cases was cancer seen to involve the tubal mucosa and in all five it did not appear to be a metastasis but rather the primary site of disease; two patients had primary grade 1 endometrioid adenocarcinoma of the fallopian tube, both were associated with dysplastic/intraepithelial precursor lesions in the rest of the fallopian tube (one patient had TMI; one patient had fimbria involvement only). One patient had a mixed clear cell and endometrioid cancers involving the fallopian tube and ovary at its junction in the background of endometriosis (in this case the fimbria was involved). Two cases had low grade serous cancer involving the tubal mucosa, one of whom had extensive multifocal disease involving the peritoneum, bowel serosa and a few tubal fimbria (21 year old immunosuppressed patient), and the other with a large borderline ovarian tumor with a low grade invasive serous cancer at the tubo-ovarian junction (both cases had fimbria involvement).The high proportion of cases in which TMI was identified and reported as part of a clinical pathology report suggests that the reporting of this criteria falls within the skills of most gynecologic oncology pathologists.

Had the pathologists used their finding TMI or STIC and assigned the site of primary as per the consensus statement of Singh et al, the proportion of cases signed out as tubal cancer would have been 56% (95% C.I. 46.8-64.9). This increase of 28% would have been achieved by a shift of 38.3%, 36.4% and 50% from HGSC that had been signed out as ovarian, peritoneal, and uncertain cancers were considered as fallopian tube origin (Tab 9). Overall, 56% (70/125) cases were changed from ovarian, peritoneal, or uncertain into fallopian tube origin. The 95% confidence intervals of tubal origin assigned according to the recommended criteria were: 95% C.I. 20.3-36.7. No overlap between the two 95% C.I. is present.

Reporting the correct site of primary in the case of HGSC is not an academic exercise. The cure rates for this disease have remained stagnant for 30 years because the vast majority present it advanced stages (21). Attempts at early diagnosis have failed, largely because diagnostic strategies were directed at the ovary. However, the fallopian tube clearly is an important site of origin of HGSC and this fact needs to be brought into the consciousness of clinicians – gynecologists, primary care physicians, pathologists. This will only happen if pathology report reflects this.

My results suggest that it is feasible to adopt the proposals put forward by Singh et al. and several eminent pathologist including Crum et al., to better assign the site of origin of HGSC. I chose a retrospective study design because my aim was to evaluate surgical pathology practice as is – to determine how much extra effort or work it would entail to correctly assign cases as per proposal by Singh et al. I found that much of the components necessary are already in place.

STRENGHTS

To the best of my knowledge, this is the largest case series of consecutive patients diagnosed with HGSC compared to non-HGSCs in which the fallopian tubes were examined in *toto*. It shows what is feasible in the context of clinical practice.

LIMITATIONS

The main limitation of this study is that it was carried out in a single institution, with pathologic examinations performed by a small, specialized group of physicians. This alongside routine examinations of the tubes *in toto*- is the only requirement for implementing the suggested criteria and thus improve assignment of site of origin of high-grade serous ovarian cancer (HGSC). By design, I took reports as they were finalized. Each case was reviewed twice, once at initial reporting and the second time at Tumour Board. This could have been done by the same or a different pathologist. I did not evaluate whether different pathologists would agree in identifying tubal mucosa involvement (TMI) within the same sample. My intention was to identify what is done already done in the real world of clinical practice, and the extra workload associated with adopting the suggested criteria.

SUGGESTIONS FOR FUTURE STUDIES

Given the above limitations, future studies involving multiple institutions are needed to evaluate inter-observer agreement in identifying tubal mucosa involvement (TMI). Unlike serous tubal intraepithelial carcinoma (STIC), it should have acceptable reproducibility and inter-observer agreement, but it remains to be shown in larger study. If such studies suggest the identification of TMI is reliable across pathologists, the next step would be to investigate the logistics, in terms of cost and added burden, of introducing routine *in toto* examination of fallopian tubes for all patients diagnosed with HGSC.

CONCLUSION

The examination of the fallopian tubes *in toto* and meticulous reporting of tubal mucosa involvement (TMI) appear feasible in clinical practice and may help bridge the gap between research and clinical practice. Understanding the correct site of origin of high-grade serous ovarian HGSC is crucial to inform on effective strategies for prevention and early diagnosis and thus has immense clinical importance.

SUMMARY

Ovarian cancer is the deadliest gynecological malignancy in North America despite efforts were put into screening and early detection programs. The reason for this is because the most common type of ovarian cancer, high-grade serous (HGSC) starts in the fallopian tubes rather than in the ovaries. Traditionally, HGSC has three possible origins: the ovary, the peritoneum and the fallopian tube. Research studies have shown that the vast majority of HGSC arise in the fallopian tube. This has been shown in both the high risk and the general population (BRCA1/BRCA2 mutation positive and sporadic ovarian cancer respectively). However, in clinical practice fallopian tube cancer is still considered a rare disease. The reason for this gap is that research studies use specific criteria to assign primary in HGSC: the evidence, in fallopian tubes analyzed according the SEE-FIM protocol, of serous tubal intraepithelial carcinoma (STIC), and tubal mucosa involvement (TMI). However, there are challenges in applying these criteria to clinical practice: using the SEE-FIM protocol routinely may be perceived as unnecessary, STIC is difficult to standardize and TMI has been challenged as a reliable criterion. Therefore, we wanted to explore the experience of a tertiary care hospital to see how frequent tubal cancer is and whether there are criteria that are routinely used in clinical setting that can may be suggested for application on a larger scale. With this in mind we examined all consecutive cases reported as ovarian, primary peritoneal, tubal cancer at the McGill University Health Center (MUHC) over a seven-year period. We did a retrospective analysis of all surgical pathology reports signed out as cancer of the ovary, peritoneum, fallopian tubes, at a publically funded cancer centre relating to cytoreductive surgeries performed between January 2007 and December 2013. Of 277 cases, 215 (125 HGSC and 90 non-HGSCs), had fallopian tubes examined *in toto*. The primary was assigned as ovary, peritoneum, fallopian tube, uncertain in 48%, 17.6%, 28%, and 6.4% respectively of HGSC cases vs. 95.6%,

1.1%, 3.3%, 0%, and 0% respectively of non-HGSCs. (STIC) was seen only in 12.8% of HGSC and TMI in 56%. If TMI was used systematically as a criterion to assign tubal origin, the proportions would have been: 29.6% primary ovarian cancers, 11.2% primary peritoneal, 56 % tubal and 3.2% uncertain. The frequency of TMI in HGSC vs non-HGSCs were compared and only five cases of non-HGSCs were found to have TMI. These results suggest that TMI is a criterion that has the potential to be applied in clinical practice for correct assignment of HGSC origin. Examination of the fallopian tubes *in toto* and meticulous reporting of TMI appear feasible in clinical practice and may help bridge the gap between research and clinical practice. Understanding the correct site of origin of HGSC is of clinical importance because it has the potential to changing diagnostics and survival of HGSC.

TABLES AND FIGURES

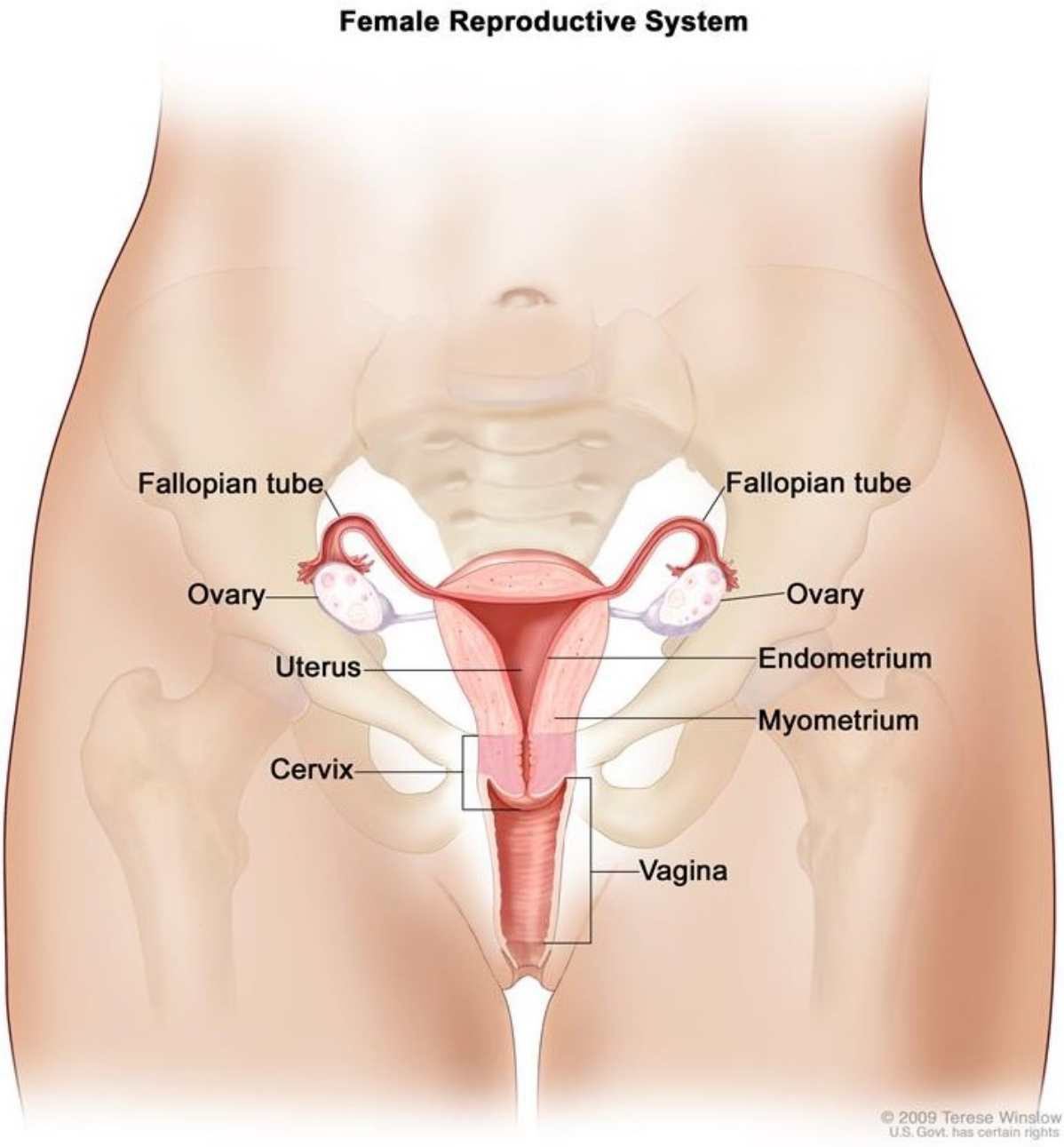

Figure 1. Normal female pelvic anatomy

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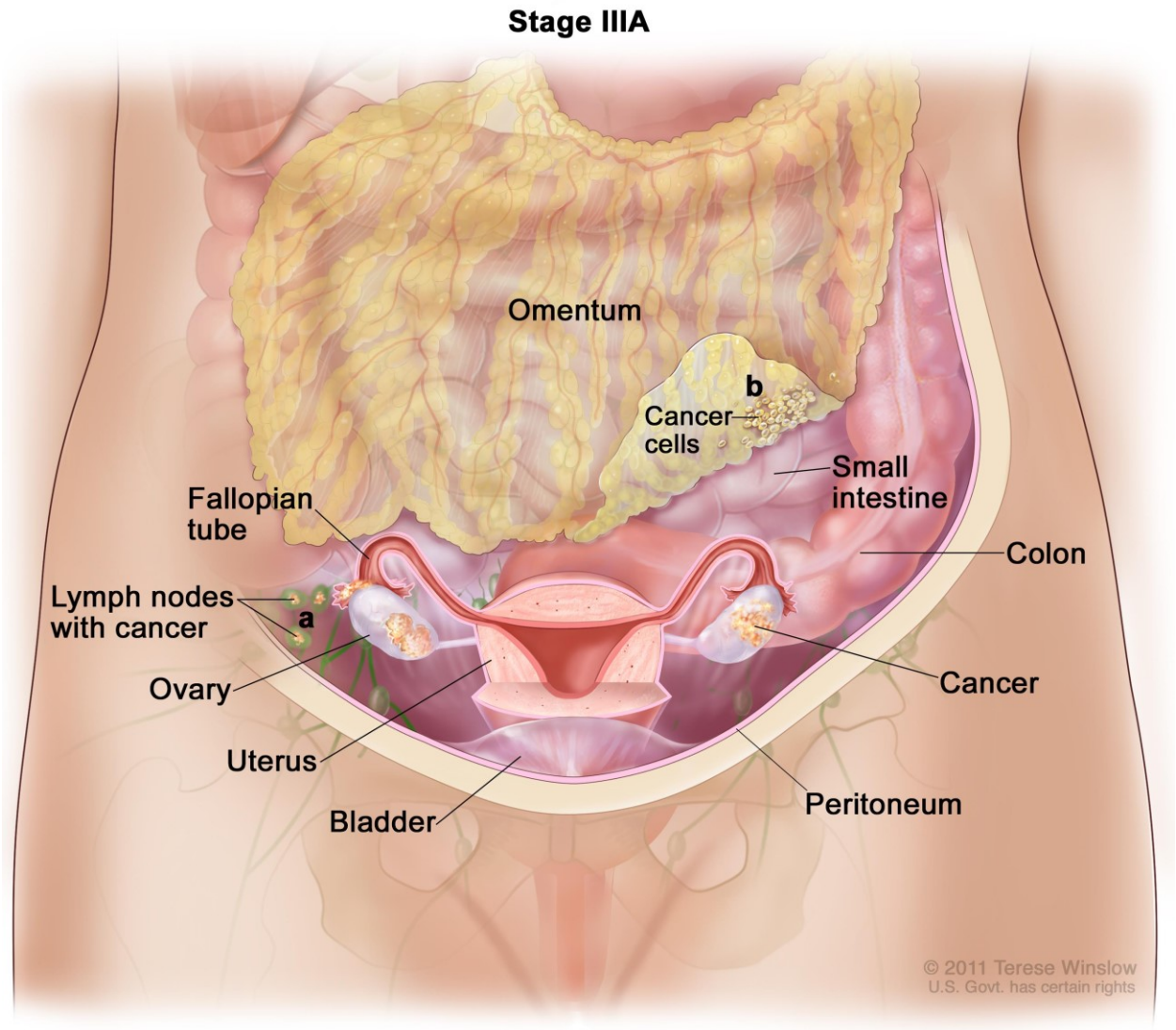

Figure 2. Clinical presentation of ovarian cancer

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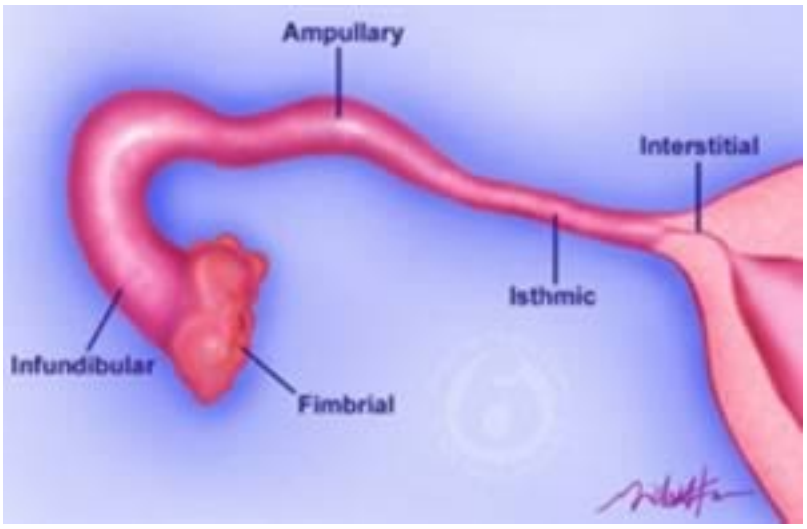

Figure 3. The anatomy of the fallopian tube

With Dr. Monteith's permission

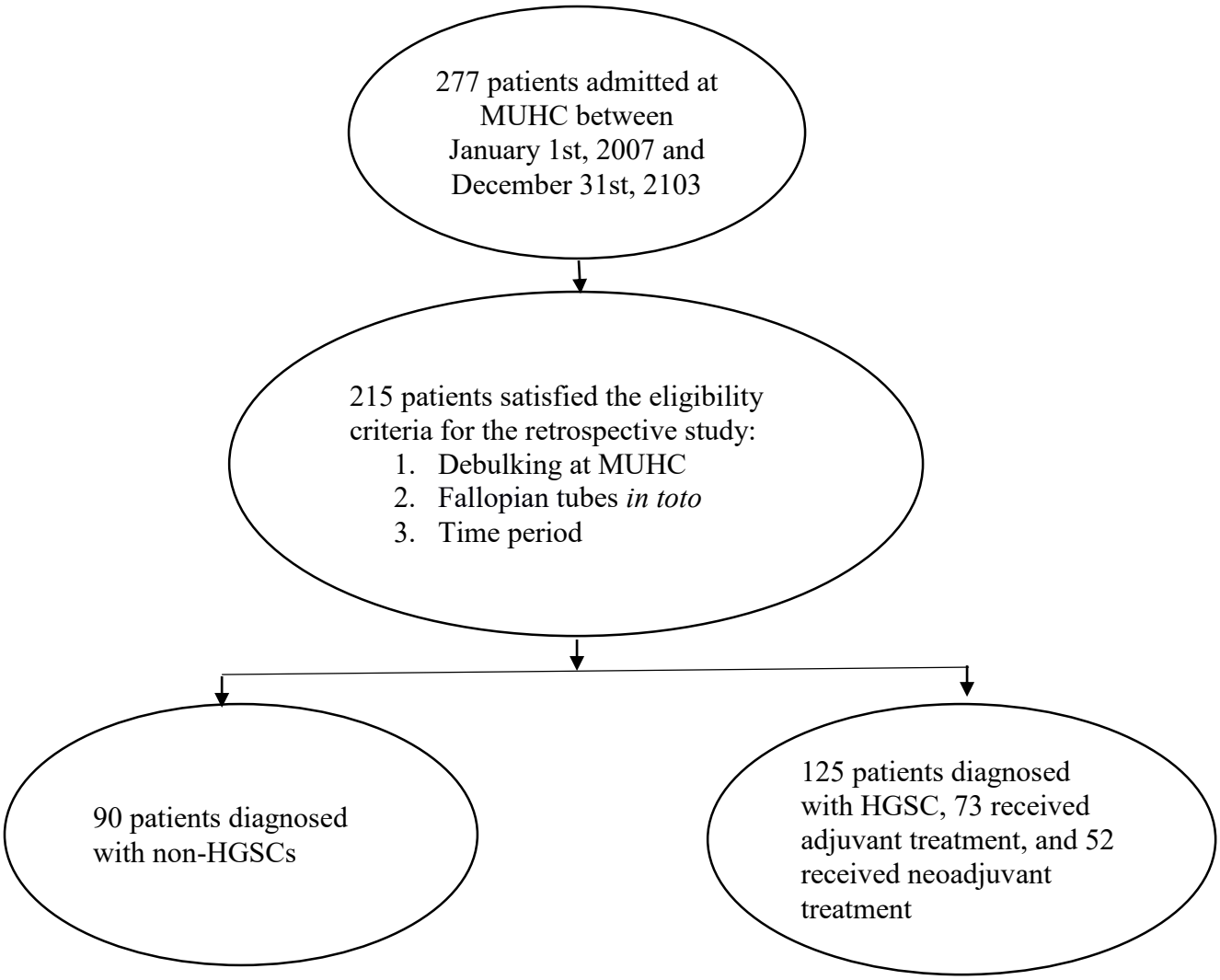

Figure 4. Identification of study population

Table 1. Methods for analyzing the fallopian tubes *in toto*

University of California	**SEE-FIM protocol**
Serial sectioning of entire fallopian tubes and ovaries at 2-mm intervals and microscopic examination of all sections (58).	2 mm serial sections along the whole length of the fallopian tube and longitudinal sectioning of the fimbria (13).

Table 2. Criteria for assigning origin of HGSC

Origin of HGSC	Traditional FIGO and WHO Criteria	Newly proposed criteria based on Singh et al. 2016, (9)
Ovary	*"Tumors were classified as ovarian in origin if none of the above criteria […]"* to assign peritoneal origin *"[…] were fulfilled. In addition, tumors that were >5mm on the ovarian surface (unless the tumor was imbedded in a desmoplastic plaque characteristic of secondary ovarian involvement)[…]"* are *"[…] classified as primary ovarian, even if no tumor was present in ovarian." (10)* (Bloss JD et al.1993, (10))	*"Primary site should be assigned as ovarian only when there is ovarian involvement and the tubes are clearly visible, have been dissected away from the surface of the ovaries, fully examined by a standardized SEE-FIM protocol and neither STIC nor invasive mucosal carcinoma is present in either tube."*
Peritoneum	Since 2014 primary peritoneal cancer is defined as HGSC in the peritoneum when fallopian tubes and ovaries are not enlarged or are enlarged due to benign conditions. (WHO 2014, (8))	*"Primary site should be assigned as peritoneal only when both tubes and both ovaries are grossly and microscopically normal; this diagnosis should only be made on cases undergoing primary surgery and after complete examination of both tubes and both ovaries using a standard protocol."*
Fallopian tube	*"The tumor arises from the endosalpinx. The histological pattern reproduces the epithelium of the tubal mucosa. Transition from benign to malignant epithelium is found. The ovaries are either normal or with tumor smaller than that of the tube."* (Sedlis et al. 1961, (49, 50))	*"Primary site should be assigned as tubal in the presence of STIC or invasive mucosal carcinoma in the fallopian tube, or when part or all of the tube is inseparably incorporated within a tubo-ovarian mass. […]. In cases where HGSC occurs following previous removal of tubes and ovaries which were not fully sampled, and uterine origin has been excluded, the primary site should be assigned as 'tubo-ovarian."*

Table 3. Histological subtypes of non-HGSCs included in the study

Histological type	90 n (%)
Clear cell high-grade	18 (20.0)
Endometrioid high-grade	14 (15.6)
Endometrioid low-grade	20 (22.2)
Granulosa cell tumor	5 (5.6)
Mixed clear cell mixed endometrioid high-grade	6 (6.7)
Mucinous high-grade	4 (4.4)
Mucinous low-grade	6 (6.7)
Serous low-grade	12 (13.3)
Other *	5 (5.6)

***Other:**

Germ cell, once case

Mixed clear cell and endometrioid low grade, one case

Small cell/hypercalcemic, one case

Struma *ovarii*, malignant, one case

Yolk sac tumor, one case

Table 4. Site of origin signed out by gynecologic oncology pathologist

Attributed origin of HGSC	Site of origin signed out by gynecologic oncology pathologist 125 n (%)
Ovarian	60 (48)
Peritoneal	22 (17.6)
Tubal	24 (19.2)
Tubo-ovarian	11 (8.8)
Uncertain	8 (6.4)

Table 5. Stage at diagnosis

Stage at diagnosis	HGSC 125 n (%)	non-HGSCs 90 n (%)
Early stages (I and II)	16 (12.8)	73 (81.1)
Advanced stages (III and IV)	109 (87.2)	17 (18.9)

Table 6. Volumes and site of dominant tumor mass, HGSC vs non-HGSC

	HGSC **125** **n (%)**		**non-HGSCs** **90** **n (%)**	
	No-NACT 73 (58.4)	NACT 52 (41.6)	No-NACT 88 (97.8)	NACT 2 (2.2)
Median size of largest tumor mass	293.9 cm^3	32.5 cm^3	431 cm^3	30 cm^3
Location of largest tumor mass	Ovary 43 (58.9)	Ovary 19 (36.5)	Ovary 81 (92)	Ovary 1 (50)
	Omentum 20 (27.4)	Omentum 24 (46.2)	Omentum 2 (2.3)	Omentum 0 (0)
	Fallopian tube* 8 (11)	Fallopian tube 7 (13.5)	Fallopian tube 2 (2.3)	Fallopian tube 0 (0)
	Other 2 (2.7)	Other 2 (3.8)	Other 3 (3.4)	Other 1 (50)
Median size of largest ovary	222.8 cm^3	8.2 cm^3	384 cm^3	30 cm^3
Median size of largest fallopian tube	3.6 cm^3	3.3 cm^3	4.13 cm^3	2.26 cm^3

*Three cases (4%) tumor mass in the fallopian tube and five cases (6.8%) tubo-ovarian mass

Table 7. Presence of STIC and TMI in HGSC divided by No-NACT vs NACT

	HGSC		
	No-NACT **73** **n (%)**	**NACT** **52** **n (%)**	**Total** **125** **n (%)**
STIC	11 (15)	5 (9.6)	16 (12.8)
TMI	47 (64.4)	23 (44.2)	70 (56)

Table 8. Site of origin in HGSC as signed out by gynecologic oncology pathologist vs research criteria

Attributed origin HGSC	Site of origin signed out by gynecologic oncology pathologist n (%) 125 (100)	Reserach criteria based on Singh et al. (9) n (%) 125 (100)
Ovarian	60 (48)	37 (29.6)
Peritoneal	22 (17.6)	14 (11.2)
Tubal	24 (19.2)	70 (56)
Tubo-ovarian	11 (8.8)	0 (0)
Uncertain	8 (6.4)	4 (3.2)

Table 9. Cross-tabulation of site of origin assigned based on research criteria and as signed out.

		Site of origin signed out by gynecologic oncology pathologist				
		OC	PP	FTC	Uncertain	Total
Research criteria	OC	37	0	0	0	37
	PP	0	14	0	0	14
	FTC	23	8	35	4	70
Shifted*	Uncertain	0	0	0	4	4
	Total	60	22	35	8	125
	%	38.3%	36.4%	0%	50%	

*proportion of cases shifted to fallopian tube origin from other assignments as a result of applying the research criteria.

Table 10. Frequency of TMI+ and/or STIC+ vs. TMI- and STIC- among HGSC and non-HGSCs cases

	HGSC **125** **n (%)**	**non-HGSCs** **90** **n (%)**
TMI + and/or STIC +	70 (56.0)	5 (5.6)
TMI – and STIC -	55 (44.0)	85 (94.4)

***p-value <0.0001, Chi-squared test**

GLOSSARY

- Cystadenoma: benign ovarian tumor
- Endometriosis: ectopic endometrial tissue
- Endosalpingiosis: ectopic tubal tissue
- Mullerian tract: either of a pair of ducts giving rise in the female to the fallopian tubes, uterus, cervix, and upper portion of the vagina
- Ovarian cortical inclusion cysts: result of the ovarian surface epithelium going into the underlying stroma with the formation of inclusion cysts
- p53 signatures: a p53 signature is a SCOUT with p53 mutation and is defined as a linear, p53 immunopositive segment of tubal cells with at least 12 consecutive secretory cell nuclei.
- Secondary mullerian system: microscopic structures lined by mullerian epithelium that can be found at extraovarian sites or in the ovary
- STIC/TIC: serous tubal intraepithelial cancer/tubal intraepithelial cancer, defined as a proliferation of non-ciliated cells with cytological atypia and high-cytoplasmic ratio, exhibiting epithelial stratification, loss of polarity, cellular exfoliation, and epithelial fractures.
- Stroma: the supportive tissue of an epithelial organ, tumor, gonad, etc., consisting of connective tissues and blood vessels.
- Surface ovarian epithelium: a layer of cells covering the ovaries.

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