Title Page

Primary Site Assignment in Tubo-Ovarian High-Grade Serous Carcinoma: Consensus Statement on Unifying Practice Worldwide

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Running head: Primary site assignment in pelvic high-grade serous carcinoma

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Background

Strategies to improve outcomes in cancer patients are underpinned by accurate recording of high quality data in a standard format. 'Ovarian' cancer, of which by far the most common subtype is high-grade serous carcinoma (HGSC), constitutes the second commonest gynecologic malignancy and overwhelmingly the one with the highest mortality. Despite some advances in prevention, early detection, surgery and chemotherapy, the mortality rate has not decreased significantly. This makes accurate and uniform data recording essential for monitoring the impact of new strategies, both in clinical practice and trial settings.

There have been significant recent developments in our understanding and approach to the classification of HGSC. The FIGO 2014 staging system commendably unifies the staging of primary ovarian, tubal and peritoneal cancers (1). 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. In the World Health Organisation (WHO) 2014 classification of tumors of the female genital tract, site assignment is left to the 'experience and professional judgment' of the reporting pathologist/tumor board (2). The WHO classification has also altered the criteria for making a diagnosis of primary peritoneal carcinoma (PPC). Previously broadly defined by size criteria, PPC is currently defined as peritoneal HGSC when both tubes and ovaries are grossly and microscopically normal (or enlarged by benign disease), a change of which practitioners should be made universally aware (2). A further major significant development is the evidence that has accumulated over the last 15 years that the majority of non-uterine HGSC arise in the fimbrial end of the fallopian tube and not the ovary (3). However, there is variation in the awareness and acceptance of this evidence.

In the absence of a defined and agreed protocol, all of the above threaten to produce at least three areas of potential discrepancy in the way HGSC is classified by different individuals/tumor boards: one is the classification of tubal versus ovarian, the second the diagnosis of PPC and the third the likelihood of significant numbers of cases being classified as 'undesignated'. This in turn could impact on data collected by cancer registries and produce artificial regional and national differences. The authors of this document propose the adoption of a unified approach to site assignment in tubo-ovarian HGSC.

Pivotal to this approach is the evidence supporting a tubal origin in the large majority of cases. Findings in risk-reducing salpingo-oophorectomy specimens (RRSO) have established serous tubal intraepithelial carcinoma (STIC) to be the earliest lesion in familial cases (4). Insights gained from RRSO specimens rejuvenated the quest for precursors in sporadic cases of HGSC. These early microscopic lesions are almost invariably located within the epithelium covering the fimbrial end of the tube, an area that was not routinely sampled for histological examination prior to this discovery. As a result, more detailed sampling of the tube has been widely adopted using a protocol that maximizes microscopic examination of the fimbrial epithelium (SEE-FIM: **S**ectioning and **E**xtensively **E**xamining the **FIM**bria (4); an essential step in detection of these lesions.

In the past, the microscopic nature of STIC, its location requiring detailed sampling of the fimbrial end of the tube and the tendency for HGSC to present at high clinical stage when any precursors have been overgrown all made it difficult to demonstrate definite precursors or early lesions in sporadic cases. Notwithstanding these difficulties, however, there is now sufficient evidence, which has been extensively reviewed elsewhere (3), showing that STIC or a small tubal mucosal HGSC is present in the majority of sporadic HGSCs when the tubes can be identified grossly. There is also important recent evidence that STIC or a small tubal mucosal invasive lesion is present in virtually all cases of HGSC discovered as early incidental lesions following surgery carried out for unrelated indications (3).

The clonal relationship between STIC and co-existing HGSC has also been difficult to establish until recently, for the reasons outlined above. HGSC is characterized by a tremendous degree of intra-tumoral genomic diversity partially due to defects in the function of genes involved in DNA repair mechanisms, such as the homologous recombination pathway (5). Despite this diversity, which arises early during oncogenesis, the tumors involving different sites in a patient with HGSC have been demonstrated to be clonally related, not only at presentation but also in disease recurrences. Mutations in *TP53* are known to be the earliest and founding molecular event in HGSC, being present in 97% of cases (6); the demonstration of identical *TP53* mutations in tumor sampled from different sites is compelling evidence of origin from a single clone. More recent work has established that STICs harbor *TP53* mutations that are identical to the ovarian and extra-adnexal disease foci (7). Although proposed as a theoretical possibility, there is absolutely no evidence to support multicentric origin in HGSC.

A further possibility, which has been put forward to question the tubal origin of most cases of HGSC, is that the tube could be involved as a result of metastasis. However, this possibility is also allayed by multiple lines of evidence: STIC lesions have been demonstrated to show shortened telomeres in comparison to their invasive counterparts, as would be expected from a precursor-carcinoma relationship (8). An elegant high-resolution study incorporating phylogenetic mapping of lesions from different sites showed tubal mucosal tumour to be the clonal ancestor of tumor at other sites (9). In the recently reported series of incidental HGSC mentioned above, all cases confined to a single site, before any metastatic spread has occurred, are located in the fallopian tube (3).

A major impediment to our acceptance of tubal origin has been the simplistic conventional approach of assigning primary site on the basis of tumor size. However, the ovary frequently harbors metastases whose size exceeds that of the primary tumor, as classically exemplified by metastatic gastric signet-ring carcinoma (Krukenberg tumor), but also by metastases of appendiceal, colorectal, hepatobiliary and cervical origin. Tubal HGSC appears to be yet another example of this phenomenon. It is widely recognized that, like endometrial serous intraepithelial carcinoma (the postulated precursor of uterine serous carcinoma), and unlike more indolent precursor lesions such as CIN3/HSIL, this disease is capable of metastasizing while still intraepithelial, by exfoliation onto peritoneal and ovarian surfaces.

Consensus Statement

We strongly believe it is time to acknowledge the compelling evidence that the fallopian tube is the site of origin of most HGSCs and reflect this in our clinical practice. We believe it is essential to implement this worldwide because of the significant variation in current practice; some regions currently classify most HGSCs as tubal in origin while others still classify the majority as primary ovarian neoplasms. The increasing use of chemotherapy rather than surgery as the initial treatment for those with stage IIIC/IV disease can also confound disease classification as treatment decisions and classification are decided by imaging and biopsy alone, with potential for larger numbers of cases being classified as PPC. This move should be preceded by appropriate dissemination of this decision to all stakeholders including cancer registries as well as healthcare commissioners and providers, including insurers. The term 'tubo-ovarian HGSC' is recommended as a diagnostic term, to distinguish this disease clearly from uterine serous and ovarian low-grade serous carcinomas; this should take into account all clinical and pathological parameters, including history, clinical, imaging and surgical findings, and morphology with immunohistochemistry for WT1, p53 and other markers as appropriate in routine practice. We propose that the default assignment of the majority of cases of HGSC as ovarian should be discarded and recommend the use of the following criteria for site assignment in HGSC (summarized in Table 1):

- Primary site should be assigned as tubal in the presence of STIC or invasive mucosal carcinoma in the fallopian tube, or when the tube is partly or fully incorporated and inseparable from a tubo-ovarian mass.
- 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.
- 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.
- In cases diagnosed on an omental/peritoneal biopsy or a cytological sample and where primary surgery is not undertaken, the presumed primary site should be assigned as tubo-ovarian. By the 2014 WHO criteria, PPC is likely to become vanishingly rare, making it unnecessary to include this as part of the differential diagnosis.
- Chemotherapy alters disease distribution; in most cases there is sufficient disease remaining to enable categorization using the above criteria, but those with no residual disease should also be assigned as tubo-ovarian.
- Site assignment as 'undesignated' should be avoided as far as possible.

A uniform approach to site assignment in HGSC is recommended by the International Collaboration on Cancer Reporting (ICCR) (10), which has incorporated most of the criteria listed above in their dataset on ovarian, tubal and primary peritoneal cancer. Using an earlier modification of the criteria listed above, in 53 prospectively studied chemo-naive cases of HGSC with unknown genetic status, 83% were classified as tubal, 17% as ovarian and none as peritoneal primaries (3). The protocol we present here has wider application than that recommended in the ICCR dataset, including guidance for cases diagnosed on small samples and following chemotherapy. Adopting a uniform approach worldwide will ensure accurate data collection for

comparing disease outcomes in routine practice and clinical trials. This will also promote wider understanding of the nature of this disease and the use of standardized pathology protocols in specimen dissection and reporting. This may in turn pave the way for developing effective ovary-conserving preventative strategies in this fatal disease; this is less likely to happen if we continue to consider most HGSCs as being of ovarian origin.

Table 1: Criteria for assignment of primary site in Tubo-Ovarian HGSC

Criteria	**Primary site**	**Comment**
STIC present	Fallopian tube	Regardless of presence and size of ovarian and peritoneal disease
Invasive mucosal carcinoma in tube, with or without STIC	Fallopian tube	Regardless of presence and size of ovarian and peritoneal disease
Fallopian tube partially or entirely incorporated into tubo-ovarian mass	Fallopian tube	Regardless of presence and size of ovarian and peritoneal disease
No STIC or invasive mucosal carcinoma in either tube in presence of ovarian mass or microscopic ovarian involvement	Ovary	Both tubes should be clearly visible and fully examined by a standardized SEE-FIM protocol. Regardless of presence and size of peritoneal disease
Both tubes and both ovaries grossly and microscopically normal (when examined entirely) or involved by benign process in presence of peritoneal HGSC	Primary peritoneal HGSC	As recommended in WHO blue book 2014[2] This diagnosis should only be made in specimens removed at primary surgery prior to any chemotherapy; see below for samples following chemotherapy.
HGSC diagnosed on small sample, peritoneal/ omental biopsy or cytology	Tubo-ovarian	Note: this should be supported by clinicopathological findings including immunohistochemistry to exclude mimics, principally uterine serous carcinoma
Post-chemotherapy with residual disease	As above	

Post-chemotherapy with no residual disease	Tubo-ovarian	

Figure Legends

Figure 1: Conventional tubal sampling; in the absence of a specific indication only representative single sections are taken from structures that are macroscopically normal. 1A: This is an essentially normal ovary and fallopian tube. 1B: In the past only a single transverse section was taken from the mid portion of the tube.

Figure 2: SEE-FIM protocol for detailed examination of macroscopically normal fallopian tubes in prophylactic salpingo-oophorectomies and in familial and sporadic cases of HGSC. 2A: The tube is dissected away from the ovary. 2B: The fimbrial end

(arrow), comprising the distal 2cm or so, is separated from the rest of the length of the tube. 2C: The fimbrial end is sliced longitudinally in 4 or more slices. 2D: The rest of the tube is sliced in transverse slices. All of the tissue is processed and examined histologically.

Figure 3: Microscopic tubal carcinoma identified in fimbrial mucosa.

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