the effusion was now haemorrhagic, and a pleural biopsy revealed an invasive mesothelioma (Fig. 2E,F).

To our knowledge, apart from the patient reported by Pulford *et al.*,¹ there are no published data on the appearance of mesothelioma *in situ* in effusion cytology specimens, and this issue was recently the subject of a multi-author position paper.³ Finding suitable cases to investigate this can be problematic, because invasive tumour may be present for some period before it becomes visible on imaging or visual inspection of the pleura, and both these modalities may not be able to pick up subtle foci of invasive tumour. Thus, it is difficult to be sure whether an effusion specimen is sampling *in situ* disease or subtle invasive disease.

This case is informative because the effusion samples are derived from a long time interval before the development of invasive mesothelioma. Even if one argues that invasive disease might have been present and not detectable for some period of time, it is extremely unlikely that invasive mesothelioma was missed on imaging/direct inspection of the pleura/biopsy over 4 years.

Figure 1 shows that there is an arguable subtle increase in cytological atypia over the 4 years of effusion sampling, but all of the samples in our view are malignant and meet guideline criteria for the diagnosis of mesothelioma in cytology specimens,⁴ even though the earlier samples in all probability are derived from mesothelioma *in situ*. Thus, these findings suggest that an effusion cytology sample from a case with visual or radiological evidence of tumour can be reliably diagnosed as invasive mesothelioma, but if there is only an effusion and no evidence of tumour on imaging or direct inspection, then a diagnosis along the line of 'at least mesothelioma *in situ*, cannot exclude invasive diffuse mesothelioma' would be appropriate. This distinction is important, since in theory mesothelioma *in situ* is potentially a treatable and curable disease.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

**Andrew Churg^{1,2}, Francoise Galateau-Salle³,
Lawrence Tan⁴, Gefei Qing⁵**

¹Department of Pathology, Vancouver General Hospital, Vancouver, BC, Canada; ²Department of Pathology, University of British Columbia, Vancouver, BC, Canada; ³Centre National Référent MESOPATH, Centre Leon Berard, Lyon, France; ⁴Division of Thoracic Surgery, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; ⁵Department of Pathology, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

Contact Andrew Churg, MD.
E-mail: achurg@mail.ubc.ca

1. Pulford E, Henderson DW, Klebe S. Malignant mesothelioma *in situ*: diagnostic and clinical considerations. *Pathology* 2020; 52: 635–42.
2. Churg A, Galateau-Salle F, Roden AC, *et al.* Malignant mesothelioma *in situ*: morphologic features and clinical outcome. *Mod Pathol* 2020; 33: 297–302.
3. Klebe S, Nakatani Y, Dobra K, *et al.* The concept of mesothelioma *in situ*, with consideration of its potential impact on cytology diagnosis. *Pathology* 2021; 53: 446–53.
4. Hjerpe A, Ascoli V, Bedrossian CW, *et al.*, International Mesothelioma Interest Group, International Academy of Cytology. Guidelines for the cytopathologic diagnosis of epithelioid and mixed-type malignant mesothelioma:

complementary statement from the International Mesothelioma Interest Group, also endorsed by the International Academy of Cytology and the Papanicolaou Society of Cytopathology. *Diagn Cytopathol* 2015; 43: 563–76.

DOI: <https://doi.org/10.1016/j.pathol.2021.04.011>

Adjunct molecular analysis in the distinction of primary ovarian mucinous carcinoma from colon carcinoma metastatic to the ovary

Sir,

It is well-recognised that the distinction of primary ovarian carcinoma from carcinoma metastatic to the ovary can be problematic, even with the use of ancillary immunohistochemistry. This is particularly the case with metastatic colorectal carcinoma (CRC) which can closely mimic endometrioid or mucinous carcinomas of ovarian origin.¹ The difficulty is compounded by the fact that many primary ovarian mucinous neoplasms exhibit a gastrointestinal phenotype morphologically and immunophenotypically. Herein, we report a problematic case in which adjuvant molecular analysis clarified the ovarian origin of a mucinous carcinoma arising in a patient with a history of CRC.

The patient was a 54-year-old female with a history of left hemicolectomy for sigmoid adenocarcinoma (pT3N0M0). At that time staging studies had shown a right ovarian cyst which demonstrated progression in size and complexity on follow-up imaging. Therefore, 2 years later she underwent hysterectomy, bilateral salpingo-oophorectomy, appendectomy and omentectomy, with peritoneal washings for cytology. The right ovarian tumour was received piecemeal 130 mm in maximum dimension and on macroscopic examination included solid and thin-walled cystic components. Intra-operative frozen section was interpreted as adenocarcinoma favoured to be of metastatic origin. Subsequent histology of the more solid tumour component demonstrated a mucinous adenocarcinoma with a predominant expansile growth pattern comprising crowded and complex glands with intraluminal papillae. The glands and papillae were lined by stratified columnar cells containing apical mucin and there were scattered goblet cells. The nuclei showed predominantly moderate and focal high grade nuclear atypia, and there was intraluminal tumour necrosis (Fig. 1). There was a minor infiltrative tumour component, focally involving the ovarian capsule surface, composed of haphazardly distributed small glands (Fig. 2). The cystic tumour was mainly lined by histologically bland mucinous epithelium but there was focal architectural and cytological atypia without invasion, suggestive of a borderline tumour component. The other specimens were negative for neoplasia. Immunohistochemistry showed cytokeratin (CK)7 and CK20 (both focal) and CDX2 (diffuse, Fig. 2) expression within the ovarian tumour while WT1, PAX8, SATB2, ER and PR were negative. Mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) expression was retained, similar to findings in the patient's previous sigmoid carcinoma. The ovarian tumour was reviewed by several experienced gynaecological pathologists and initially a diagnosis of metastatic CRC was favoured. Subsequent

Fig. 1 (A) Expansile-type invasion in ovarian tumour comprising complex, crowded glands with intra-cystic papillae and luminal necrosis (centre field). (B) There is focal high-grade nuclear atypia with prominent mitotic activity, and necrosis is also evident (upper field).

immunohistochemical analysis of the sigmoid carcinoma demonstrated CK20, CDX2 and SATB2 staining, while CK7 was negative. While the ovarian tumour exhibited partial morphological and immunophenotypic overlap with the prior colonic carcinoma, the ambiguous findings promoted additional molecular studies comparing the somatic mutation profile of both tumours.

Samples from the sigmoid carcinoma and from three different areas of the ovarian tumour including the histologically benign/borderline, expansile invasive, and infiltrative invasive components were extracted. Somatic sequence variant (mutation) analysis was performed using next generation sequencing (NGS) as previously described,² and the results are summarised in Table 1. The invasive components of the ovarian tumour (expansile and infiltrative) demonstrated identical *TP53* sequence variants whereas the histologically benign/borderline areas demonstrated a *KRAS* sequence variant. The colonic carcinoma exhibited *APC*, *PIK3CA* and *KRAS* sequence variants; the *KRAS* variants in the ovarian and colonic tumours differed (Table 1). The distinct mutational profiles of the two tumours argued against a clonal relationship and the final pathological diagnosis was amended to primary ovarian mucinous carcinoma (FIGO stage 1C2).

Clinical and radiological findings together with pathological features (macroscopic, microscopic and

immunophenotypic) usually resolve tumour origin in patients presenting with mucinous carcinoma of the ovary.¹ Indeed, simple algorithms incorporating tumour size and unilateral versus bilateral distribution provide accurate diagnosis in the majority of cases.³ However, there remains a subgroup of tumours in which conventional assessment provides conflicting or ambiguous data. This is illustrated by the case presented herein which initially was favoured to be of metastatic origin, mainly based upon the history of CRC, the presence of focal infiltrative tumour invasion including ovarian capsular involvement, and intraluminal necrosis characteristic of metastatic CRC. Although bilateral ovarian involvement strongly favours metastasis in non-serous ovarian carcinomas, approximately one-third of metastatic tumours present with unilateral ovarian involvement.⁴ Furthermore, while the presence of an apparently benign or borderline tumour component is also generally supportive of primary ovarian origin it is well-recognised that metastatic carcinomas may demonstrate deceptively bland cytological appearances (so-called ‘maturation phenomenon’).¹

It should be noted that a minority of tumours arising in many anatomical sites demonstrate an apparently anomalous and potentially misleading immunohistochemical profile, and there may be differences between primary tumours and corresponding metastases.⁵ Furthermore, as noted previously, many primary ovarian mucinous neoplasms exhibit intestinal

Fig. 2 (A) Ovary capsule cleft showing tumour involvement and partly detached neoplastic epithelium overlying the capsule surface. (B) The tumour cells show diffuse, strong CDX2 expression.

Table 1 Summary of next generation sequencing analysis in the colonic and ovarian carcinomas

Tumour site	Gene	Variant	Allele frequency
Colon	<i>APC</i> <i>PIK3CA</i> <i>KRAS</i>	c.4012C>T, p.(Gln1338Ter) c.3140A>G, p.(His1047Arg) c.35G>A, p.(Gly12Asp)	17.1% 18.6% 29.5%
Right ovary			
- benign/borderline	<i>KRAS</i>	c.34G>C, p.(Gly12Arg)	7.5%
- expansile invasion	<i>TP53</i>	c.746G>T, p.(Arg249Met)	67.8%
- infiltrative invasion	<i>TP53</i>	c.746G>T, p.(Arg249Met)	5.4%

differentiation including expression of markers such as CK20 and CDX2, as in the present case, while the Müllerian marker PAX8 is often negative. Although SATB2 staining is considered more specific for lower gastrointestinal origin, a minority of primary ovarian tumours also express this marker.⁶ Immunohistochemistry for β -catenin may also be helpful in this diagnostic scenario. In one study nuclear β -catenin expression was demonstrated in 19/23 (83%) metastatic CRCs compared to 4/43 (9%) primary ovarian mucinous carcinomas.⁷ However, aberrant activation of the Wnt signalling pathway, in some cases due to *APC* or *CTNNB1* mutations, has been reported in a minority of primary mucinous carcinomas.^{8,9}

Ultimately, the integration of NGS analysis in the present case led to a revised diagnosis of primary ovarian mucinous carcinoma, further illustrating the value of adjunct molecular analysis in determining tumour origin in problematic cases.^{10,11} However, it should be noted that molecular findings also have to be carefully interpreted since tumours may show mutational heterogeneity and there is the possibility of 'clonal evolution' such that metastases may demonstrate a different mutation profile to the corresponding primary tumour.¹² In the current case the complete absence of sequence variant overlap (including different *KRAS* mutations) favoured independent neoplasms.

In summary, the distinction of primary versus metastatic origin for ovarian mucinous carcinoma continues to present a diagnostic dilemma, particularly in patients with a prior history of gastrointestinal malignancy. Accurate diagnosis is important since patients with two independent low-stage tumours generally would be expected to have a more favourable prognosis than those with metastatic carcinoma, and the pathological interpretation could also affect decisions regarding the requirement for and nature of adjuvant therapy. The case presented here demonstrates the value of adjunct molecular analysis in cases with indeterminate or ambiguous clinicopathological findings.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Daniel Ching¹, Anita Soma¹, Tze Sheng Khor², Ganendra Raj Mohan³, Colin J. R. Stewart⁴

¹Department of Histopathology, King Edward Memorial Hospital, Perth, WA, Australia; ²Division of Anatomical Pathology, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Perth, WA, Australia; ³Department of Gynae-Oncology, King Edward Memorial Hospital, Perth, WA, Australia; ⁴School of Women's and Infants' Health, University of Western Australia, Perth, WA, Australia

Contact Dr Daniel Ching.

E-mail: daniel.ching@health.wa.gov.au

- Young RH. From Krukenberg to today: the ever present problems posed by metastatic tumors in the ovary: part I. Historical perspective, general principles, mucinous tumors including the Krukenberg tumor. *Adv Anat Pathol* 2006; 13: 205–27.
- Stewart CJR, Bigby S, Giardina T, et al. An immunohistochemical and molecular analysis of papillary proliferation of the endometrium. *Pathology* 2018; 50: 286–92.
- Yemelyanova AV, Vang R, Judson K, et al. Distinction of primary and metastatic mucinous tumors involving the ovary: analysis of size and laterality data by primary site with reevaluation of an algorithm for tumor classification. *Am J Surg Pathol* 2008; 32: 128–38.
- Lee KR, Young RH. The distinction between primary and metastatic mucinous carcinomas of the ovary: gross and histologic findings in 50 cases. *Am J Surg Pathol* 2003; 27: 281–92.
- McCluggage WG. Immunohistochemistry in the distinction between primary and metastatic ovarian mucinous neoplasms. *J Clin Pathol* 2012; 65: 596–600.
- Aldaoud N, Erashdi M, Alkhatib S, et al. The utility of PAX8 and SATB2 immunohistochemical stains in distinguishing ovarian mucinous neoplasms from colonic and appendiceal mucinous neoplasm. *BMC Res Notes* 2019; 12: 770.
- Chou Y-Y, Jeng Y-M, Kao H-L, et al. Differentiation of ovarian mucinous carcinoma and metastatic colorectal adenocarcinoma by immunostaining with beta-catenin. *Histopathology* 2003; 43: 151–6.
- Babaier A, Ghatage P. Mucinous cancer of the ovary: overview and current status. *Diagnostics* 2020; 10: 52.
- Nguyen VHL, Hough R, Bernado S, et al. Wnt/ β -catenin signalling in ovarian cancer: insights into its hyperactivation and function in tumorigenesis. *J Ovarian Res* 2019; 12: 122.
- Meyniel JP, Cottu PH, Decraene C, et al. A genomic and transcriptomic approach for a differential diagnosis between primary and secondary ovarian carcinomas in patients with a previous history of breast cancer. *BMC Cancer* 2010; 10: 222.
- Kubeček O, Laco J, Špaček J, et al. The pathogenesis, diagnosis, and management of metastatic tumors to the ovary: a comprehensive review. *Clin Exp Metastasis* 2017; 34: 295–307.
- Priestley P, Baber J, Lolkema MP, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature* 2019; 575: 210–6.

DOI: <https://doi.org/10.1016/j.pathol.2021.03.011>

An unusual case of invasive lobular carcinoma with abundant extracellular mucin

Sir,

Accurate classification of breast cancer subtype is important as subtypes may show clinically distinct behaviours. Invasive lobular carcinoma (ILC) accounts for 5–15% of invasive breast carcinomas. Compared to invasive breast carcinoma of no special type (IBCNST), ILC is more likely to have a multicentric growth pattern, be larger, present at a more advanced stage and show distinctive patterns of distant