Table 2 DNA damage events: analysis of variance multiple comparisons between autoantibodies and with normal healthy control subjects

(I) Autoantibody	(J) Autoantibody	Mean difference (I–J) ^a	Std error ^b	Sig	Confidence interval	
					Lower bound	Upper bound
CENP	Topo1	17.878	4.57616	0.000	8.7808	29.9751
	RNAP3	16.0996	4.73046	0.001	6.6958	25.5035
	Other	10.5906	3.91879	0.008	2.8004	18.3809
	Control	11.2856	3.49612	0.002	4.3356	18.2358
Topo1	RNAP3	–1.7783	5.49139	0.747	–12.6848	9.1382
	Other	–7.2873	4.80989	0.133	–16.8491	2.2745
	Control	–6.5923	4.47225	0.144	–15.4828	2.2982
RNAP3	Other	–5.509	4.95692	0.270	–15.363	4.345
	Control	–4.814	4.63001	0.301	–14.0182	4.3902

Dependent variable: number of micronuclei in binucleated cells (BNCs)/1000 BNCs.

CENP, centromere antibody; Other, other antibodies; RNAP3, RNA polymerase 3 antibody; Topo1, anti-topoisomerase antibody.

^a Least significant difference, based on observed means.

^b The error term is mean square (error) = 157.957.

the possibility of a causal association with the known striking female predominance of CENP positive scleroderma.

Experimental strengths in this present study include (1) the fact that all samples were blinded for analysis, (2) the scientist performing the reading of the CBMN-cyt assay had no prior knowledge of the clinical status or origin of the blood sample, and did all the readings for both patients and controls, (3) the control group was well matched for age, gender, ethnicity and B-vitamin status, (4) the patient cohort was well characterised both clinically and serologically. Limitations included the relatively small numbers of patients and controls studied allowing the possibility of a type II error.

In conclusion, elevated levels of MNi and NBUDS were observed in the female predominant CENP positive scleroderma subgroup. These findings did not appear to be related to disease duration nor deficiency of serum vitamin B12 and/or folate. Other serological scleroderma subgroups did not show elevated levels of these DNA damage biomarkers. The high levels of MNi and/or NBUDS in this selective CENP positive subgroup support the hypothesis that chromosomal instability may be an additional risk factor for autoimmunity in women.

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Cytological diagnosis of mesothelioma *in situ* versus invasive mesothelioma

Sir,

We read with interest the article by Pulford *et al.*¹ on mesothelioma *in situ*. One of the cases described in that paper

was a 74-year-old woman with recurrent pleural effusions, the development of balls of mesothelial cells that had lost BAP1 in an effusion specimen, and an eventual biopsy showing mesothelioma *in situ* but thus far no evidence of invasive disease.

An important question that arises from this scenario is whether effusion cytology can separate mesothelioma *in situ* from invasive mesothelioma in the absence of radiological or direct visual evidence of tumour. Here we present a case that sheds light on this question.

Fig. 1 Cytological appearances of pleural fluid samples over time. (A) Numerous balls of cells. This appearance was present in all specimens from 2014 to 2018 (the illustrated specimen is from 2015). (B) Sample from 2014. (C–E) Sample from 2015. Stain for BAP1 (E) shows loss in the tumour cells and a positive internal control. (F) Sample from 2016. (G) Sample from 2017. (H,I) Samples from 2018.

This case was originally described by us as mesothelioma *in situ*² on the basis of two pleural biopsies, but on further investigation we were recently able to determine that the patient had multiple pleural fluid cytological investigations over a 4-year period and these form the basis of the present report.

The patient is deceased and the University of British Columbia Research Ethics Board does not require permission to publish or ethical approval in this circumstance.

The patient initially presented in 2014 at age 70 with a right pleural effusion of unknown aetiology; the effusion was repeatedly drained over the course of 4 years but rapidly recurred after each thoracentesis. Until 2018 imaging studies showed only a pleural effusion with no evidence of pleural thickening. All of the pleural fluid cytology specimens were similar, with multiple balls of slightly atypical cells that marked as mesothelial cells (Fig. 1A).

The initial 2014 specimen was not well preserved (Fig. 1B), but the subsequent specimens showed good cytological detail (Fig. 1C–I). Cell block material was available for a specimen from 2015 and this was stained retrospectively and showed loss of BAP1 (Fig. 1E). Anti-BAP1 clone C-4 (Santa Cruz Biotechnology, USA; dilution 1:50, incubation time 20 min at 31°C) was used after Dako retrieval buffer pH 9.0 for 30 min at 97°C. Detection was carried out with the Envision Flex+ detection system (Agilent Technologies, USA). All processes were run on a Dako Omnis stainer (Agilent Technologies).

Pleural biopsies were performed in 2016 and 2017. In both instances the operator reported the pleura as visually normal. In both instances the pleural biopsies showed a pattern of mesothelioma *in situ* with a single layer of slightly atypical cuboidal mesothelial cells that had lost BAP1 (Fig. 2A–D). In 2018 imaging showed pleural thickening for the first time,

Fig. 2 Corresponding tissue biopsies. (A,B) 2016: pleural biopsy showing a single layer of slightly enlarged mesothelial cells that have lost BAP1; this is the typical appearance of malignant mesothelioma *in situ*. (C,D) 2017: pleural biopsy again showing a picture of mesothelioma *in situ* with loss of BAP1. (E,F) 2018: pleural biopsy showing invasive mesothelioma; BAP1 is again lost.

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.

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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