would normally be present, although were not seen in our melanoma case. A single layer of large cells with abundant cytoplasm and preserved nuclear/cytoplasmic ratio may also be seen in instances of endocervical gland radiation induced atypia. The nuclei in this situation may appear 'smudged', accompanying stromal and vascular changes would be expected to be present, and no mitoses seen. Endocervical Arias-Stella change may also enter the differential diagnosis, as a single layer of enlarged, occasionally 'hob-nail' cells with abundant cytoplasm, and nuclei which may contain pseudoinclusions.

The pattern of tumour recurrence in this unique case also raises awareness of the phenomenon of epidermotropic metastasis in melanoma, and the difficulty in separating this entity from a separate primary malignancy, a well documented occurrence in melanoma with differing management implications.

In summary, this is an unusual vaginal melanoma which colonised endocervical glands, potentially mimicking endocervical adenocarcinoma *in situ* on histological grounds, on a background of recurrent, multifocal, metastatic and primary disease.

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

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DOI: <https://doi.org/10.1016/j.pathol.2020.08.023>

Atypical lymphoid hyperplasia of cervix with clonal *IGH/IGK* and *BCL6* gene rearrangements

Sir,

Florid reactive lymphoid hyperplasia or lymphoma-like lesion (LLL) of the uterine cervix is characterised by a superficial, polymorphous lymphoid infiltrate with intermixed large B-cells, resembling diffuse large B-cell lymphoma (DLBCL). It is important to distinguish between LLL, a benign and reactive process, and DLBCL to avoid unnecessary chemotherapy. Here, we report a clinically indolent case of an atypical lymphoid proliferation of the cervix with clonal *IGH/IGK* gene rearrangements by polymerase chain reaction (PCR) and *BCL6* gene rearrangement by fluorescence *in situ* hybridisation (FISH). The atypical lymphoid proliferation was absent in the subsequent cone biopsy by loop electrosurgical excision procedure (LEEP) and the patient remains asymptomatic without any treatment.

A 21-year-old healthy female (gravida 0) presented to her gynaecologist for an annual exam. The Pap smear detected a low grade squamous epithelial lesion with human papilloma virus (HPV) effect. The subsequent colposcopy directed biopsies showed a superficial, non-infiltrative, polymorphous lymphoid proliferation under the epithelium (Fig. 1A). The lymphoid proliferation was composed of large centroblasts, small lymphocytes and plasma cells (Fig. 1B). Necrosis was absent. The large cells were B cells positive for CD20, PAX5, BCL6 and partial CD10 (Fig. 1C–E), and negative for CD5, cyclin D1, CD43 and BCL2. CD23 showed the absence of a follicular dendritic cell meshwork. Ki-67 showed a high proliferation index of 90% (Fig. 1F). Plasma cells were polytypic by kappa and lambda light chain *in situ* hybridization (ISH). EBER ISH was negative. Molecular studies identified clonal *IGH* (frameworks I, II and III) (Fig. 2) and *IGK* (V-J) gene rearrangements by PCR. Rearrangement of the *BCL6* (3q27) locus was detected by FISH analysis in 69.5% of analysed cells. Positron emission tomography–computed tomography (PET-CT) imaging performed after the biopsy detected cervical thickening with a standardised uptake value (SUV) max of 8.8, no mass and no lymphadenopathy. Fifty-one days after her initial biopsy, a cone biopsy by LEEP showed no significant lymphoid infiltrate.

The case was initially diagnosed as DLBCL. However, given the absence of symptoms, the non-infiltrative nature of the lymphoid infiltrate, and subsequent negative LEEP biopsy, the diagnosis of DLBCL was withdrawn and the patient underwent surveillance every 4 months without chemotherapy. The patient remained asymptomatic with negative cervicovaginal Pap tests at 6 and 10 month follow-ups.

Distinguishing between LLL of the uterine cervix and lymphoma can be difficult as the histological features and molecular findings often overlap. Ramalingam and colleagues summarised 43 cases of LLL reported in the literature and a diagnosis of lymphoma was either made or considered in 25 (58%) cases.¹ LLLs are examples of exuberant lymphoid hyperplasia and have been found in association with infections, including *Chlamydia* spp.,

Fig. 1 Histological and immunohistochemical stains of the atypical lymphoid proliferation of the uterine cervix. (A) A non-infiltrative, polymorphous lymphoid proliferation is under the epithelium. (B) The lymphoid proliferation is composed of large centroblasts, small lymphocytes and plasma cells. (C) The large cells are B cells expressing PAX5, (D) partial CD10, and (E) BCL6. (F) Ki-67 shows a high proliferative index.

Epstein–Barr virus (EBV) and HPV related squamous dysplasia.² Typically, LLLs are superficial, non-invasive, polymorphous lymphoid lesions that do not form masses. Large cells can be present in clusters or sheets, which often raises concern for large cell lymphoma. *IGH* gene rearrangement studies can be further misleading as monoclonal *IGH* gene rearrangements can be detected by PCR analysis in 29% of reported LLL cases.^{1–3} In such instances, demonstrating clonal *IGH* gene rearrangement by PCR does not unequivocally confirm the diagnosis of lymphoma.^{2,3} Clonal B-cell proliferation in reactive conditions is considered a non-malignant process driven by antigen stimulation. Awareness of LLL of the female genital tract, a non-invasive lymphoid proliferation characterised by an

indolent clinical course, is key to avoid the over-diagnosis of this entity as an aggressive lymphoma.

While clonal *IGH* gene rearrangement is frequently seen in LLL, cytogenetic abnormality is uncommon and has not been reported in LLL to our knowledge. Though *BCL6* rearrangement is one of the frequent cytogenetic/FISH abnormalities detected in reactive lymphoid proliferations,^{4,5} it is debatable if our case should be diagnosed as LLL or an indolent lymphoma. It may be acceptable to diagnose as a low grade B-cell lymphoma of follicle centre origin, which represents a clonal indolent process similar to primary cutaneous follicle centre lymphoma.

Our case illustrates a clinically indolent LLL of cervix with clonal *IGH/IGK* and *BCL6* gene rearrangements and

Fig. 2 *IGH* gene rearrangement studies by polymerase chain reaction and capillary electrophoresis. Clonal peaks are detected in (A) framework I, (B) framework II, (C) and framework III.

emphasises the importance of recognising this entity to avoid the over-diagnosis of DLBCL which would result in unnecessary chemotherapy.

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

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DOI: <https://doi.org/10.1016/j.pathol.2020.08.024>

Change of blue ink margin colour to black in immunohistochemistry stained slides: a potential pitfall for pathology cancer margin assessment

Sir,

In the course of our clinical practice we have become aware that some inks applied to tissue margins may change colour after immunohistochemical (IHC) staining. In evaluating this occurrence systematically across multiple breast cancers, different IHC stains, and two different IHC platforms at two different IHC laboratories, we have observed fidelity of the ink colour during routine processing and on H&E sections but a consistent change in the colour of the blue ink to black occurs after IHC staining. Awareness of this artefact of IHC processing will assist pathologists avoid a potentially serious pitfall with significant implications for patient care.

For invasive breast carcinoma, the finding of tumour at an inked margin defines a positive margin,¹ while for women with ductal carcinoma *in situ* treated with breast conserving surgery (BCS) with whole breast irradiation, a minimum clearance of 2 mm is recommended.² Because positive surgical margins are the most important modifiable risk factor for local recurrence of breast cancer, further surgery is often undertaken in an attempt to achieve acceptable margins. Surgeons and pathologists orient specimens in three dimensions to permit assessment of each surgical margin. Inking helps identify true margins on histology, while the application of a different colour indelible ink to each margin allows pathologists to specify the anatomical location of involved margins, so re-excisions may be targeted appropriately.

When epithelial proliferations occur close to margins, IHC may be helpful in distinguishing malignant lesions from benign processes. In the course of our clinical experience, we have become aware that some inks change colour after IHC staining. This colour change may lead to the erroneous anatomical designation of the involved margin, potentially impacting decisions regarding further surgery. In this brief report we wish to highlight this potential pitfall, to be mitigated by pathologists.

Following the identification of an index case, we systematically evaluated ink colour preservation in breast specimens from its application to the gross specimen, through routine processing, H&E morphology and IHC, using two different IHC platforms. Seventeen clinical breast samples and three additional composite blocks created for this quality assurance purpose were assessed.

We use blue, green, black, orange, red and yellow inks manufactured by the Davidson Marking System (DMS; Trajan Scientific and Medical, Australia). DMS inks are widely used in histology laboratories. For clinical samples,

breast cancer resections with corresponding IHC slides were included, if the IHC slides included margins inked with different colour dyes.

For further quality assurance evaluation, we prepared three composite blocks of surplus breast tissue, each comprised of three strips of cancer and benign tissue. The two long surfaces of each strip were painted with different inks, such that the three strips in each block included all six ink colours. These blocks were processed routinely for H&E assessment. Routine IHC evaluation was carried out for P63 or CD45 at two laboratories, one using the Ventana platform with the ultraView Universal DAB detection kit (product code 760-500; Ventana, USA), and the other the Dako Autostainer Link 48 IHC platform (Dako, Denmark), with the Ultravision LP detection system (product code TL-125-HL; Thermo Scientific, USA) and Dako Liquid DAB/substrate chromagen system (product code K3468; Dako). We recorded the colours of the inks at the edges of the tissue at each step in the process, including at the time of prosection, in the processed block, the H&E section and on the corresponding IHC slide.

Per standard protocols, quality assurance evaluations are exempt from formal evaluation through the institutional human research ethics committee.

The index case was an invasive breast cancer which included an atypical epithelial proliferation at the blue inked superior margin, necessitating IHC evaluation to distinguish invasive carcinoma from adenosis. IHC for P63 showed absence of a myoepithelial cell layer, confirming the impression of invasive carcinoma. When reviewed for a multidisciplinary meeting, it was noted that the involved margin was black. In this case black ink had been applied to the deep margin. This distinction was critical for management as our surgeons frequently leave no breast tissue at the superficial and deep aspects of their wide local excisions for breast re-excisions to be attempted, whereas cancer at the four circumferential margins is an indication for further breast surgery. After careful correlation with the H&E sections, it was determined that the lesion was indeed at the superior margin and the reason for the discrepancy was a change of ink colour from blue to black during IHC staining.

In our formal analysis of 20 further samples, we found fidelity of the ink colours through routine processing and H&E staining. However, in all 20 cases analysed, the blue ink changed colour to black following IHC (Fig. 1). This was observed at two IHC laboratories, on the Ventana and the Dako platforms and independently of the antibody used. The remaining ink colours were maintained after IHC processing.

Histological assessment of surgical margins of cancer resections is central to clinical care. Depending on the precise anatomical location of the margin, positive margins may be an indication for further surgery. The extent of disease at margin and margin of clearance may also influence decisions regarding radiation therapy.

Various difficulties have been described in the assessment of breast surgical margins.³ These include sampling errors, inadvertent inking of non-marginal surfaces and difficulties in distinguishing different colours, such as the orange and red ink combination.⁴ The observation of a consistent change of the blue ink colour to black following IHC staining signifies a potentially serious pitfall. Apart from the colour change, the tissue reveals no structural or tinctorial clues to alert the pathologist, such that without prior knowledge of this