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Hazardous cross-reaction in a thyroid fine-needle aspiration.

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1. INTRODUCTION

Thyroid fine-needle aspiration cytology (FNAC) is one of the most performed medical procedures worldwide.[1] It is used as a diagnostic test to separate benign thyroid nodules (colloidal and hyperplastic nodules) from thyroid malignancies, either primary (papillary thyroid carcinoma (PTC), medullary thyroid carcinoma (MTC), poorly differentiated thyroid carcinoma (PDTC), anaplastic thyroid carcinoma (ATC)) or less often metastatic.[2] The negative and positive predictive values (NPV and PPV) of this procedure in The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) are respectively 97% and 98% for the benign and malignant categories.[3] Being a so frequent procedure, it is thus not rare in general cytopathology practice to encounter unusual lesions that pose diagnostic problems. The modern cytopathologist should also be aware of the clinical setting in which a lesion is aspirated and should interpret cytomorphology and ancillary tests results as a whole, in order to maintain high thyroid FNAC diagnostic accuracy.

We report one of such difficult cases of thyroid FNAC that required integration of clinical, morphological and immunohistochemical findings to reach a correct diagnosis.

2. CASE HISTORY

A 54-year-old man with unremarkable past medical history presented with a rapidly growing right laterocervical nodule that, as he said, became palpable within a month. However, he also presented a pharyngeal discomfort with right shoulder pain for several months. On examination, the patient was in overall good physical condition. Cervical palpation confirmed a diffusely enlarged right thyroid lobe, as well as a right cervical mass measuring approximatively 5 cm. No sign of thyroid dysfunction was noted.

A cervical ultrasound (US) confirmed the clinical appreciation and revealed not one but multiple and bilateral enlarged lymph nodes, all of them with highly suspicious features. This presentation suggested a primary thyroid malignant neoplasm with local metastatic spread. FNAC under ultrasound (US) guidance was then performed on the right thyroid lobe and the largest pathological lymph node. Following a cytopathological diagnosis of a high grade B-cell lymphoma, a lymph node biopsy was performed for precise subtyping and final diagnosis.

Meanwhile, a 18-FDG PET/CT revealed multiple pathological uptakes in the liver and spleen, as well as in the previously described cervical lesions.

3. MATERIALS AND METHODS, RESULTS

Cervical echography showed a 3.8x3.3x2.5 cm hypoechoic and hypovascular thyroid lesion with digitiform outline and heterogeneous content (Figure 1A, upper part), as well as multiple hypoechoic and partly ill-defined lymph nodes showing pathological vascularization and measuring up to 5.0x4.1x2.4 cm in the right neck levels II, III and IV (Figure 1A, lower part).

FNAC material aspirated from the thyroid lesion and the largest pathological lymph node showed similar cytomorphological features, consisting mainly of numerous malignant cells, isolated (Figure 1B) or forming loosely cohesive clusters on a background of cellular debris (Figure 1C). These cells had a high N/C ratio, a delicate cytoplasm that was often imperceptible on smears, and a large hyperchromatic nucleus with a granular chromatin and one or more nucleoli. Nuclear molding was observed. Focally, these cells were arranged in follicular or rosette-like structures sometimes surrounding a substance akin to colloid (Figure 1D). The apparent cohesiveness of tumor cells, atypical mitoses and foci of tumor necrosis were easily identified on cell block preparation (Figure 1E).

Based on a first morphological impression of a poorly differentiated malignant tumor, we performed an immunohistochemical panel from serial sections of the cell block, consisting of Thyroglobulin, TTF-1, Cytokeratins (CK) 8/18, Calcitonin, Pax-8, Chromogranin, Synaptophysin and S-100 (Table 1). Our initial differential diagnosis included a thyroid carcinoma (PDTC, ATC, MTC) metastatic to regional lymph nodes, and had to exclude secondary involvement of the thyroid gland and cervical lymph nodes by a metastatic process (in particular a melanoma and a neuroendocrine carcinoma, based on cytomorphology).

All markers tested in this first round came back negative, apart from intense and diffuse nuclear positivity of Pax-8 (Polyclonal, Lubioscience) (Figure 1F). Our initially wide differential diagnosis was then mainly narrowed to ATC and PDTC as Pax-8 is typically positive in primary thyroid carcinoma. Loss of expression of epithelial markers such as cytokeratins and of thyroid differentiation markers (Thyroglobulin and TTF-1) are also well-known findings in ATC.

Abundant malignant cells and necrotic material were also supporting the hypothesis of ATC, despite the absence of clear-cut cell spindling or other heterologous elements. Pax-8 expression is not restricted to thyroid malignancies, but can also be seen in thymic epithelial neoplasms (thymic carcinoma and carcinoma showing thymus-like differentiation – CASTLE) as well as in metastatic carcinoma mainly of Müllerian origin and kidney. Expression of cytokeratins 8/18 would however be expected at least focally in these carcinoma, as well as in Pax-8 negative carcinoma from other origins such as from the head and neck, lung and digestive system. Despite an expression of Pax-8 known to be restricted to carcinoma, and because of the particular cytomorphology of this tumor, additional antibodies were tested to strictly exclude a lymphoma. (Figure 1F, insets)(Table 1). To our surprise, a diffuse and strong expression of CD45 and CD20 by tumor cells was observed, thus raising some incertitude as for the expression of Pax-8 only by malignancies of epithelial lineage. Knowing that a subset of aggressive primary thyroid carcinoma shows an aberrant expression of CD20[4] and that CD45 positivity has been rarely described in undifferentiated carcinoma[5], a strong and diffuse expression of Pax-5 unequivocally confirmed the lymphomatous nature of the aspired tumor.

Based on a preliminary cytopathological diagnosis of a high grade (large cell type) B-cell lymphoma, an excisional biopsy of a lymph node was advised for more precise subtyping. Biopsy material of the previously aspirated lymph node was sent to our laboratory few days later, and allowed to confirm a highly proliferating (ki-67 about 90%) diffuse large B-cell lymphoma with diffuse and strong expression of CD20 as well as of Pax-5 and polyclonal Pax-8 (Figure 1G). As no translocation in *MYC*, *BCL2* and *BCL6* were identified by fluorescent in situ hybridization (FISH break apart probes, Zytovision, ref. Z-2090-200, Z-2192-200, Z-2177-200) the final diagnosis was that of a diffuse large B-cell lymphoma (DLBCL), NOS, infiltrating a cervical lymph node and the thyroid gland. After one cycle of a classic R-CHOP chemotherapy for a DLBCL, NOS stage IV-A, a partial response was observed on a 18-FDG PET/CT, in particular with a reduction of more than 70% of the volume of the largest laterocervical lymph node. After two additional cycles, a complete response with extinction of all initial uptakes was eventually observed.

4. DISCUSSION

Undifferentiated thyroid carcinoma can lose expression of markers of epithelial and thyroid differentiation, while keeping a clear expression of Pax-8.[6] Moreover, in presence of compatible cytological and clinical features, *i.e.* malignant cells and necrosis aspirated from a locally invasive tumor as assessed radiologically, further pathological investigations are sometimes unnecessary and a diagnosis of PDTC or ATC can be readily made.[7, 8]

In the particular case of our patient, a combination of clinical and morphological aspects, ~~along with the scientific curiosity of one of the authors (A. N.),~~ were not fully compatible with our first cytomorphological impression and prevented a diagnosis of PDTC or ATC.

Clinically, ATC usually present as a thyroid mass with wide extrathyroidal extension.[8] Laterocervial lymph node metastasis are not so frequent. Immediate symptoms related to brisk extrathyroidal infiltration often lead to a high clinical suspicion of ATC. In our case, the patient symptoms appeared progressively and the US examination of the thyroid and neck were not pathognomonic of an ATC. However, absence of cytokeratin, Thyroglobulin and TTF-1 expression coupled with retained Pax-8 expression were still favoring a diagnosis of ATC.[9] Retrospectively, additional cytokeratins could have been tested to decrease the possibility to be dealing with an ATC. Cytokeratin 7 alone or as part of a cytokeratin cocktail such as AE1/AE3 or MNF-116 would have been an appropriate addition, as it is reported that CK7 and CK18 are expressed in 84% and 80% of ATC, respectively.[10] Moreover, use of Pax-8 is encouraged in undifferentiated thyroid tumors with limited or no expression of TTF-1 and cytokeratins.[9] Clinically, a well-delineated thyroid nodule associated with regional metastatic lymph node would be more consistent with a metastatic PTC or PDTC. The first hypothesis was excluded by the absence of typical PTC nuclei and by the absence of cytokeratin and TTF-1 expression. As for PDTC, absence of papillary or follicular architecture, severe crowding, single cells, and high nuclear/cytoplasmic ratio were the most predictive diagnostic features.[11] Despite partly compatible cytomorphology, the absence of cytokeratin, Thyroglobulin and TTF-1 expression is atypical for a PDTC, as it is known to show diffuse expression of TTF-1 and at least to retain some expression of Thyroglobulin.[9, 12]

Trying to find an explanation in the literature for the expression of Pax-8 in our case, apart from numerous articles describing the well-known expression of this protein by tumors of genito-urinary origin, we identified some interesting papers.

A first study showed that the N-terminal regions of Pax-8 and Pax-5 have a high sequence homology, and consequently, that polyclonal Pax-8 antibody can cross-react with Pax-5 N-terminal epitope present in reactive and neoplastic B-cells.[13] This was further demonstrated by the absence of Pax-8 mRNA in the B-cell lines studied. Knowing of the possible pitfalls of CD20 and CD45 expression in poorly differentiated epithelial malignancies reported in the cervical region necessitated additional specific B-cell markers.[4,5,14] The demonstration of a diffuse and strong nuclear expression of Pax-5 in tumor cells using a monoclonal antibody (1EW, Leica biosystems) in cytological (cytoblock from the FNAC) and histological (lymph node biopsy) materials confirmed the hypothesis of a polyclonal-Pax-8-antibody-to-Pax-5-epitope cross-reaction. Eventually, a monoclonal Pax-8 (API438AA, Biocare Medical, Table 1) was tested in both materials in another laboratory, and as expected, came back entirely negative.

Few case reports documenting similar cross-reactivity with polyclonal Pax-8 antibody in various adverse situations were also found, one of them describing a patient known for a metastatic renal cell carcinoma and a Pax-8 positive adrenal gland lesion that turned out to be a B-cell lymphoma in lieu of a Pax-8 positive metastasis from his kidney cancer.[15] Common to these challenging cases is the knowledge of possible antibody cross-reactivity and thus the use of extended immunohistochemistry panels in poorly differentiated tumors.

Concerning makers of lymphoid lineage, as already mentioned, there are some known overlapping stainings among lymphoma and carcinoma that can be troublesome in some situations[4,5]. CD20 is expressed in 14.8% and 8.2% of the encapsulated and infiltrative variants of PTC, respectively, as well as in metastatic PTC.[14] CD45 can even be exceptionally expressed by undifferentiated carcinoma. As for Pax-5, apart from positivity reported in few Merkel cell carcinoma[16] (along with co-expression of TTF-1), the monoclonal Pax-5 antibody we tested seems to be the most discriminant marker in the differential diagnosis between an ATC and a high grade B-cell lymphoma.

In conclusion, we would like to draw the attention of the reader on certain aspects of this case that we found interesting. Identifying the origin and nature of a poorly differentiated

tumor cannot rely only on immunohistochemical panels and has to be considered along with detailed clinical information and an appropriate morphological analysis. Polyclonal antibodies are easy to produce and thus cheaper, but they're less specific than their monoclonal counterparts and thus have to be interpreted carefully, above all in situations like the one we presented where a single antibody could be responsible of an entire diagnosis. Inner and outer controls are of prime importance in immunohistochemistry: back to the polyclonal Pax-8 staining we performed, a clue to cross-reactivity can be appreciated as it is possible to identify Pax-8 positive small round cells that are actually non-neoplastic B-cells. Finally, in our case, it has been of prime importance to avoid misdiagnosing an ATC, because the patient would have been treatable surgically and would have thus received a wrong and delayed treatment.

5. FIGURES LEGENDS

Figure 1. Ultrasound aspects of the thyroid nodule and a laterocervical lymph node, and cytopathological fine-needle aspiration features. **1A**: **upper part:** transversal view of the right lobe of the thyroid gland centered on a 3,8x3,3x2,5cm hypoechoic and hypovascular lesion with digitiform outline and heterogeneous content (arrowheads: thyroid lesion; SCM: sterno-cleido-mastoid muscle; C: carotid artery); **lower part:** right level III pathological lymph node measuring 5.0x4.1x2.4 cm. **1B, 1C:** isolated and loosely cohesive groups of malignant cells on a necrotic and hemorrhagic background. Nuclei were hyperchromatic, irregular in shape and size, partly with an evident nucleoli. Cytoplasm and cell membrane were often imperceptible. (Papanicolaou stain, background 200x, inset 400x). **1D:** follicular and rosette-like structures of malignant cells encircling a substance akin to colloid (Papanicolaou stain, 400x). **1E:** cellblock preparation with apparently cohesive malignant cells, partly atypical mitosis and foci of cellular necrosis (H&E stain, background 200x, inset 400x). **1F**: cellblock preparation with diffuse and intense nuclear positivity of polyclonal Pax-8 (immunostaining, background 200x); insets show intense and diffuse expression of CD45 (immunostaining, left inset, 200x) and CD20 (immunostaining, right inset, 200x) thus supporting the final cytological diagnosis of a high grade B-cell lymphoma. **1G:** material from the resected lymph node showing malignant cells with a diffuse and strong expression of CD20 (upper right corner, immunostaining, 40x), Pax-5 (lower right corner, immunostaining, 40x), polyclonal Pax-8 (upper left corner, immunostaining, 200x) and monoclonal Pax-8 (lower left corner, immunostaining, 200x).

~~**Figure 2.** Immunohistochemical panels performed on cytological and biopsy materials. **2A**: on cellblock preparation, Thyroglobulin (left), Cytokeratins 8/18 (middle) and TTF-1 (right) were clearly negative (immunostainings, 100x). **2B**: on the contrary, on the same material, polyclonal Pax-8 showed diffuse and intense nuclear positivity (immunostaining, background 200x, inset 400x), narrowing down the differential diagnosis to primary or metastatic Pax-8 positive carcinoma. **2C**: additional markers to rule out a lymphoma during diagnostic process: CD45, Pax-5, CD20 (immunostainings, 100x) were diffusely positive, thus supporting the final cytological diagnosis of a high grade B-cell lymphoma. **2D**: tumor cell morphology from the resected lymph node was identical to that observed on the cellblock (H&E, background 200x). Malignant cells expressed diffusely and strongly CD20 (**2D, upper inset,** immunostaining, 40x),~~

~~Pax-5 (**2D, lower inset,** immunostaining, 40x) and polyclonal Pax-8 (**2E,** immunostaining, background 40x; inset 200x).~~

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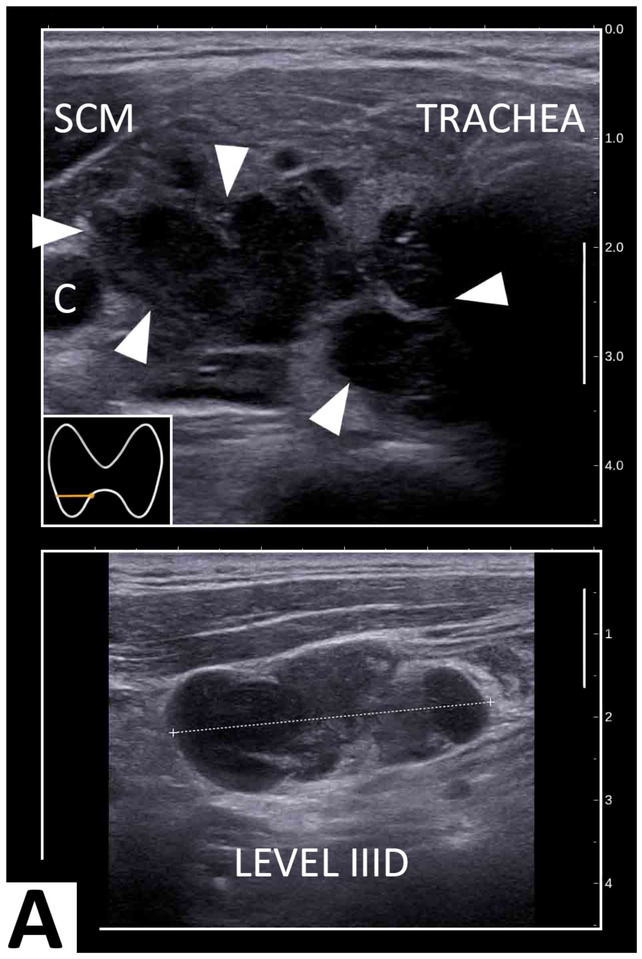
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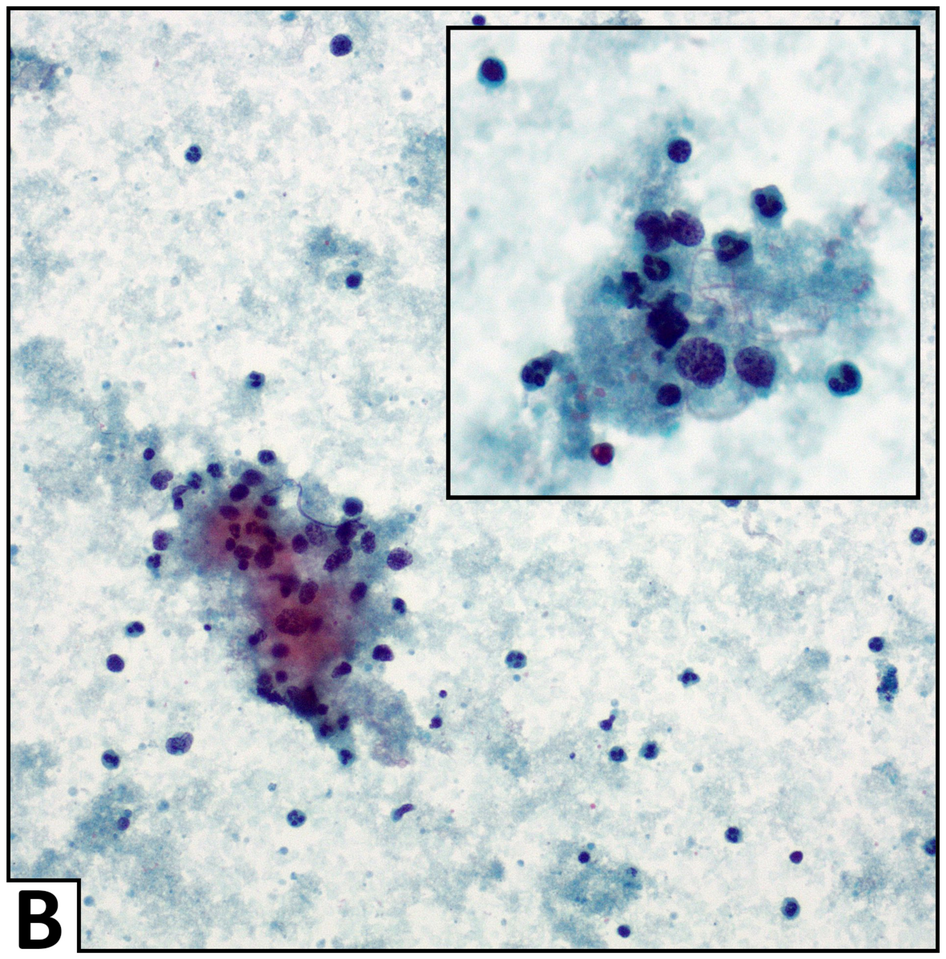
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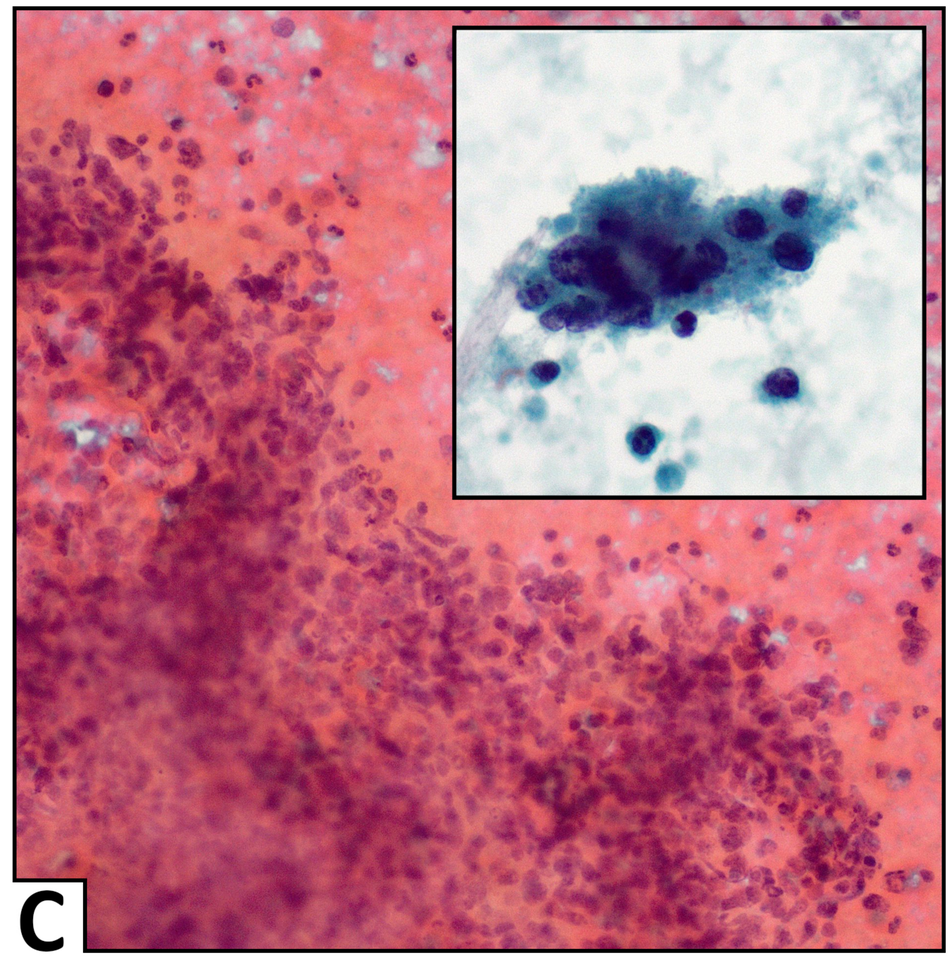
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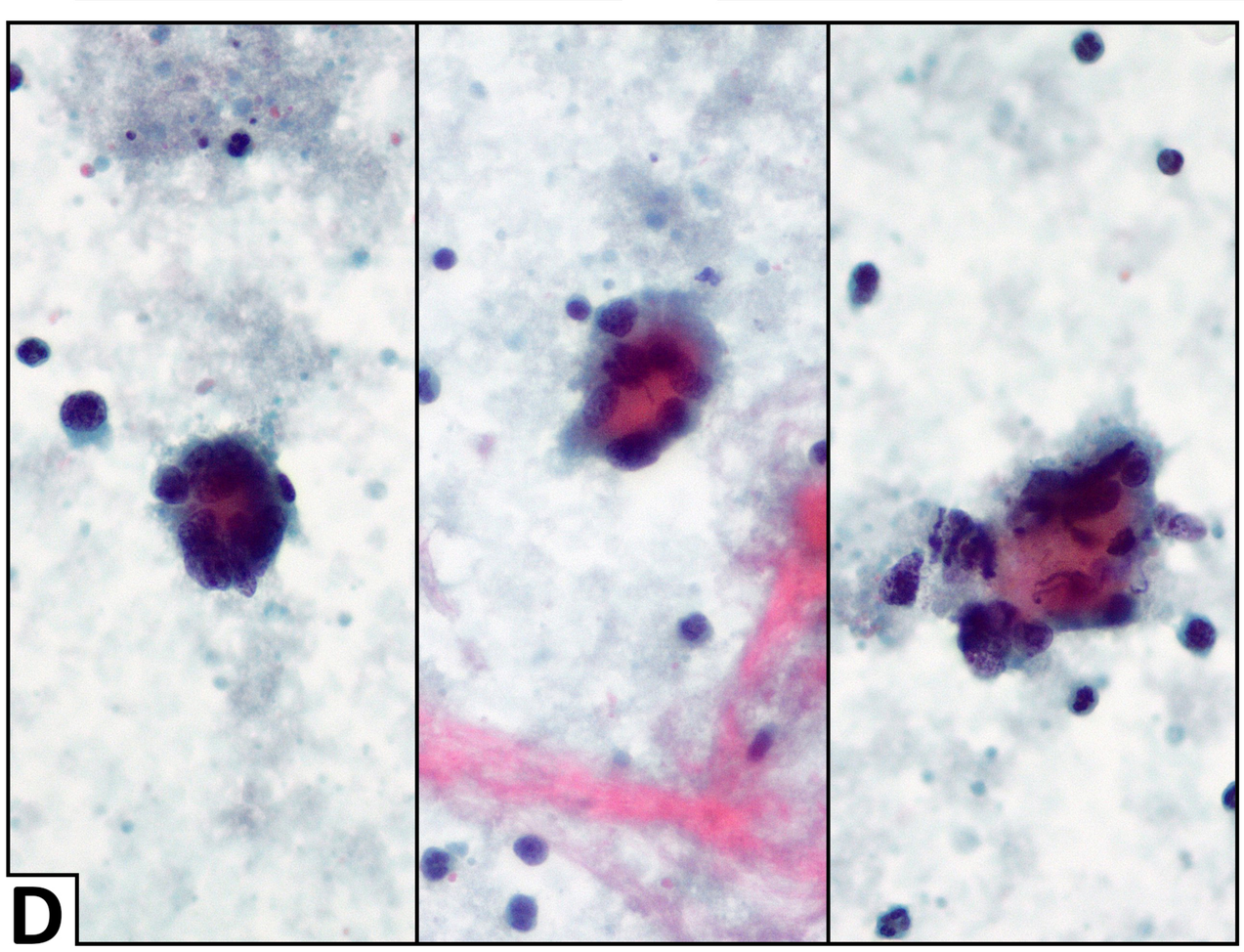
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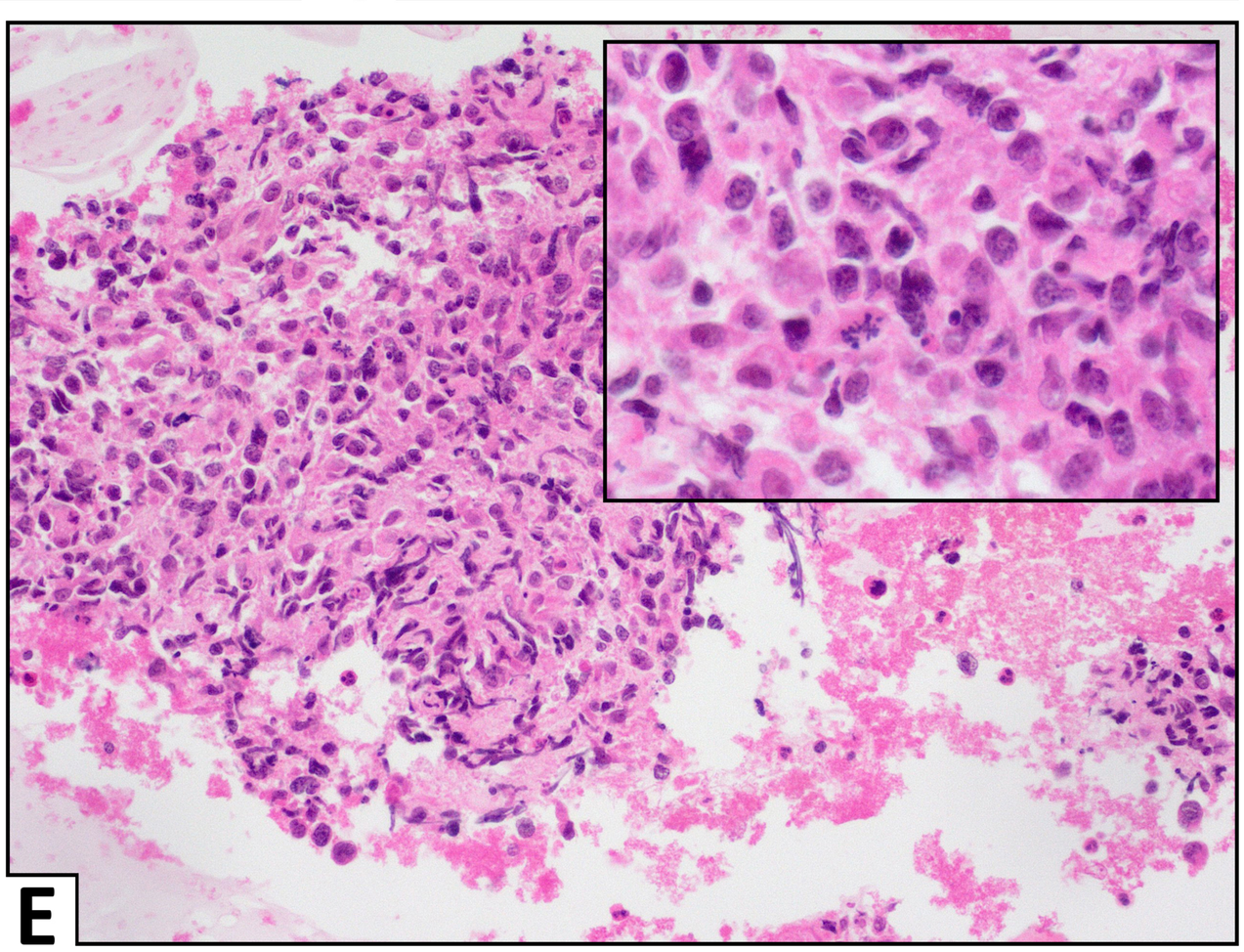
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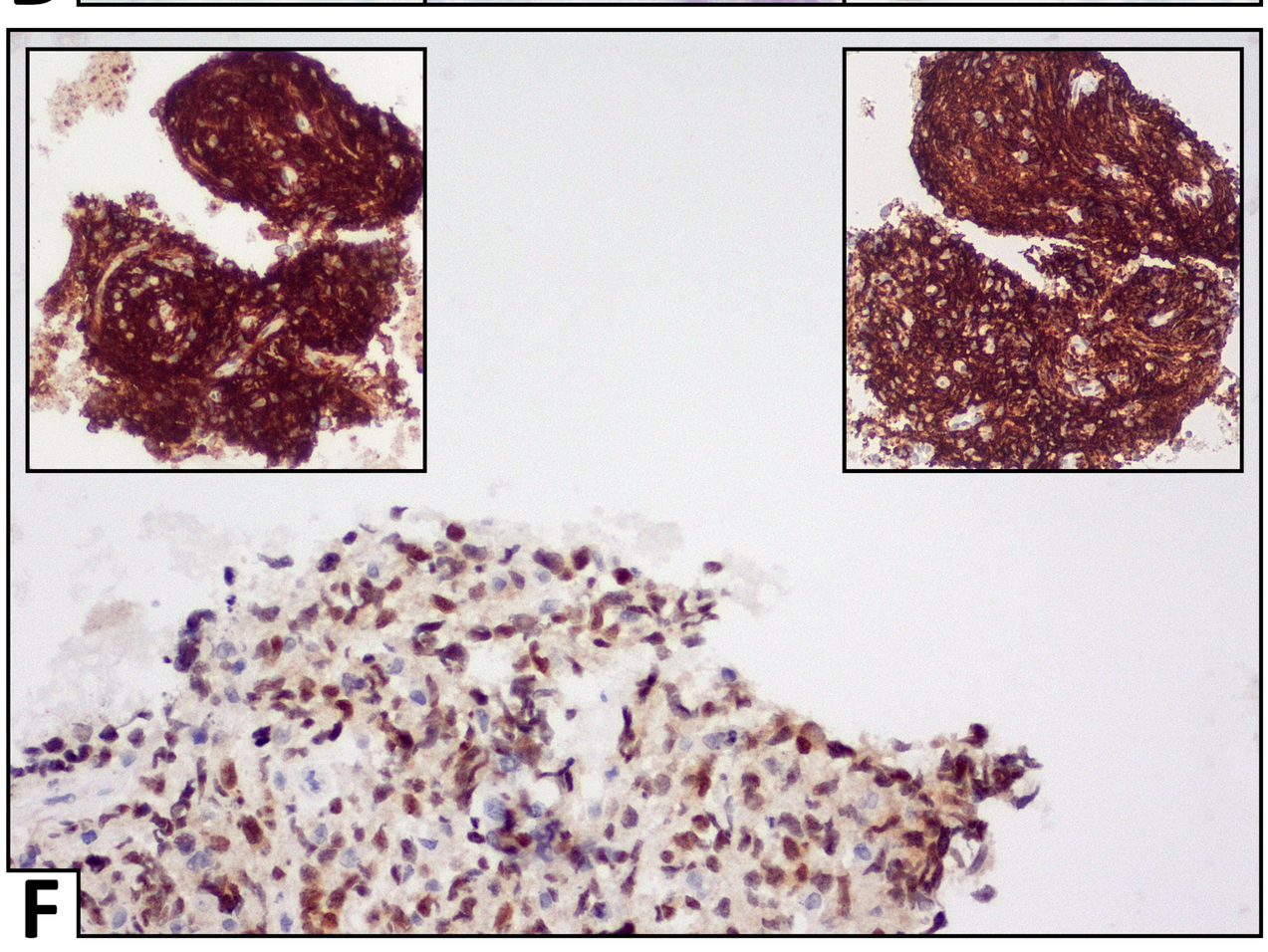
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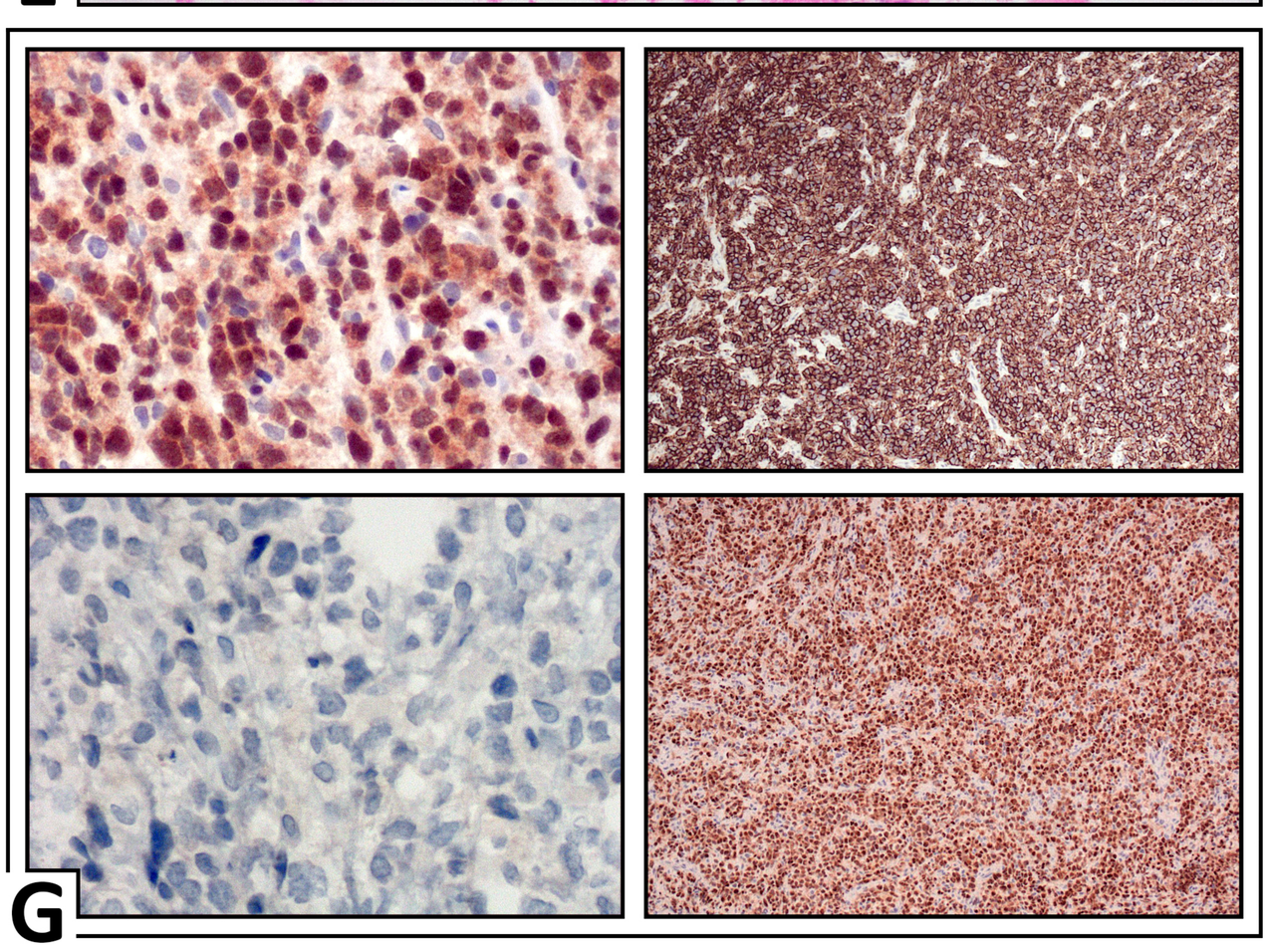
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