

## Diagnosis of Kaposi sarcoma by a modified fine needle aspiration method combining cell block in Chinese patients with human immunodeficiency virus/acquired immunodeficiency syndrome

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*To the Editor:* Kaposi's sarcoma (KS), a low-grade vascular tumor, is one of the most common neoplasms reported in patients with acquired immunodeficiency syndrome (AIDS). Based on epidemiological and clinical characteristics, four types of KS have been reported: classic KS, endemic KS (in equatorial Africa), AIDS-associated KS (AIDS-KS), and iatrogenic KS. Clinically, the presentation of KS can be variable with dark or brown macules, plaques, or nodules that may bleed or, ulcerate on the skin and mucous membranes. Besides, the lymph nodes and some visceral organs can also be involved in more advanced stages of the disease.

Fine needle aspiration cytology (FNAC) has been used as the first-line diagnostic process for breast, thyroid gland, skin, and superficial lumps as well as enlarged lymph nodes. In contrast to other diagnostic methods, FNAC has many advantages, the procedure is simple, inexpensive, less traumatic, safe, free of complications, well tolerated by a patient, and fast with high accuracy. Despite the importance of the disease, only very few publications have appeared on the diagnosis of KS by FNAC and cell block method. In this article, we evaluated the performance of FNAC combined with cell-block in the diagnosis of KS.

We recruited 771 human immunodeficiency virus (HIV)-infected patients with lymphadenopathy or superficial lump who were hospitalized in the Department of Pathology, Beijing Ditan Hospital, Capital Medical University in Beijing, between June 2009 and February 2019. FNAC was done on all of these patients after a brief explanation about the technique and informed written consents were collected. Patients' demographic details were obtained from the laboratory requisition forms submitted by the clinicians and from pathology reports.

FNAC was performed without anesthesia using an auto-vacuumed syringe, which had a latch on the tube bottom and a slot in the plunger following a pencil-grip operation. We flushed the aspirated material on slides and then spread it to make thin smears. These slides were fixed immediately with 95% ethyl alcohol and then stained by Hematoxylin and Eosin (H&E) for cytological examination. The remaining material was processed into cell blocks using ethanol coagulation and formaldehyde fixation. All cell blocks were treated similarly to surgical biopsy specimens, including formalin fixation, paraffin embedding, and sectioning at 4 to 5  $\mu\text{m}$  thickness, followed by H&E staining and immunohistochemical staining. Immunohistochemical analyses were performed by the avidin-biotin-peroxidase method with the Leica Autostaining System (Bond-Max, Leica Biosystems Inc, Buffalo Grove, IL, USA) on cell block sections. Staining was carried out with the following antibodies (Beijing Zhongshan Biotechnology, China): Cluster of differentiation 31 (CD31), CD34, factor VIII, HHV-8, smooth muscle actin (SMA), Desmin, S-100, Vimentin, and Cytokeratin (CK AE1/AE3). CD4<sup>+</sup> T cell count was determined by flow cytometry (BD FACSCalibur, BD Bioscience, Franklin Lakes, NJ, USA). All procedures performed in studies involving patients were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 *Helsinki Declaration* and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Beijing Ditan Hospital, Capital Medical University (No. 2019-049).

In this study, 16 cases (2.1%) of KS were diagnosed, which were all male, and the average age was  $37.3 \pm 10.6$  years. Eight cases had KS in lymph node (four cervical, three inguinal, and one posterior), three in the oral mucosa, and five in the subcutaneous nodule. The average age of AIDS patients with KS in lymph node was  $29.5 \pm 4.8$  years,

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which was markedly lower than that in the subcutaneous nodule and oral mucosa ( $P = 0.002$  and  $<0.001$ , respectively). The average age of AIDS patients with KS in the oral mucosa group was significantly higher than that in the subcutaneous nodule group ( $54.7 \pm 5.1$  and  $39.4 \pm 3.2$  years, respectively,  $P = 0.002$ ). The  $CD4^+$  T cell count of the peripheral blood for all patients was lower than  $200/\mu\text{L}$ , and the average value was  $83.9 \pm 58.2/\mu\text{L}$ . There was no relationship between the number of  $CD4^+$  T cells in peripheral blood and age as well as the site of KS.

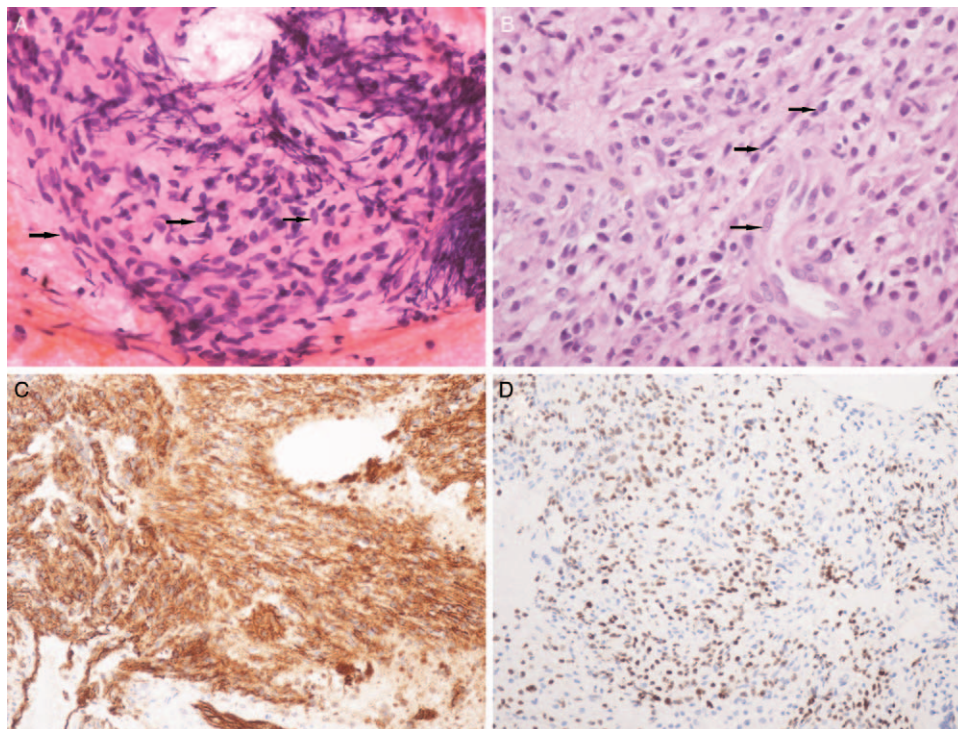
On the smears, KS exhibited hypercellular characteristics with plump spindle cells in a hemorrhagic context. The cells had a moderate amount of eosinophilic cytoplasm having oval to spindle nuclei with mild pleomorphism, inconspicuous nucleoli, and finely granular chromatin [Figure 1A]. Few mitotic figures were seen that were mimic as low-grade spindle cell neoplasm. However, other histological features, such as erythrocyte exosmosis and hemosiderin deposition were not easily to be identified because of hemorrhagic background.

The vasoformative architecture could be recognized easily with the cell block section. The tumor contained proliferating blood vessels arranged in slit-like spaces and fascicles that were full of red blood cells. These spaces were separated by spindled cells having a moderate amount of eosinophilic cytoplasm and mild to moderate

nuclear atypia [Figure 1B]. At the periphery of the tumor, inflammatory cells, mainly lymphocytes, were observed. The morphological features of the cellblock were similar to histopathology and of valuable for a definitive diagnosis.

According to the results of immunohistochemical stains, the tumor cells were highly positive for CD31, CD34, factor VIII, Vimentin, and HHV-8 [Figure 1C, and 1D]. We performed SMA, Desmin, S-100, and CK AE1/AE3 immunostains and achieved negative results. The immunohistochemical study showed expression of CD31 (15 cases), CD34 (16 cases), factor VIII (13 cases), Vimentin (16 cases), and HHV-8 (14 cases) in the atypical spindle cells.

In several studies, KS was found to be more prevalent in males than that in females, with a male to female ratio of approximately 10:1 to 15:1.<sup>[1]</sup> In this study, the AIDS-related KS patients were all males, which is in agreement with other studies. Although KS affects all age groups, lymph node involvement is more frequent in children and adolescents. Our results concord with this fact, the average age of AIDS patients with KS in the lymph node was  $29.5 \pm 4.8$  years in our study, which was markedly lower than that in other studies. The average age of all AIDS-related KS patients in this study was found to be  $37.3 \pm 10.6$  years. This is probably because HIV is to be most prevalent among those aged 20 to 40 years, as they



**Figure 1:** Representative image of the patient. (A) Fine needle aspiration cytology of Kaposi sarcoma. the smears show hypercellular features consisted of plump spindle cells in a hemorrhagic background. The cells have a moderate amount of eosinophilic cytoplasm having oval to spindle nuclei with finely granular chromatin that mimics as low-grade spindle cell neoplasm, which are shown by arrows (Hematoxylin and Eosin, original magnification  $\times 400$ ). (B) The cell block section of Kaposi sarcoma. the vasoformative architecture is easy to recognize. slit-like spaces are separated by spindled cells having a moderate amount of eosinophilic cytoplasm and mild to moderate nuclear atypia that is shown by arrows (Hematoxylin and Eosin, original magnification  $\times 400$ ). (C) The immunohistochemical study showed the expression of CD31 in the cytoplasm of atypical spindle cells (Immunohistochemistry, original magnification  $\times 400$ ). CD31: Cluster of differentiation 31. (D) The immunohistochemical study showed the expression of human herpesvirus 8- in the nuclear of atypical spindle cells (Immunohistochemistry, original magnification  $\times 400$ ). HHV-8: Human herpesvirus 8.

show a high-risk behavior and immunosuppression in this age group.

KS is a multicentric angioproliferative tumor of mesenchymal origin that can present with multiple bluish-red nodules on all extremities, chest, back, oral mucosa, and cervical lymphadenopathy. The lower extremity has been reported to be the most common site.<sup>[2]</sup> The data in the present study revealed that 3/5 (60%) of these patients with skin lesions have involved the lower extremities. In AIDS-KS, oral involvement is common and may lead to dysphagia and secondary infection. We observed three cases (18.8%) of oral lesions in the 16 cases. The presence of oral involvement is highly indicative of AIDS-related KS; one study revealed that 38 of 41 patients with oral lesions had AIDS-associated KS.<sup>[3]</sup>

Surgical biopsy is the gold standard for the diagnosis of KS. Nevertheless, it often requires hospitalization and is costly, time-consuming, and not always free of complications. FNAC gives a good alternative that has only a little bleeding. In our hospital, an optimized approach of FNAC and cell block preparation was adopted. The key modification is using an auto-vacuumed syringe that benefits the sampling of sufficient material to make a cell block. The aspirated material is flushed on slides and spread to make smears. The remaining material is processed into cell blocks, which not only provides morphology and partial histological structures but can also be sectioned for immunohistochemistry staining, allowing for immunohistological evaluation. Using cell smears, we could diagnose a low-grade spindle cell neoplasm, and cell block for a definitive diagnosis.

Most spindle cells of KS can be stained for endothelial cell markers such as CD34, CD31, and factor VIII, suggesting their vascular nature. KS is attributed to the infection with HHV-8, which is exclusively detected in KS spindle cells despite the clinical KS subtype and is the most diagnostically specific immunostaining to differentiate KS from other spindle cell tumors because it is specific to KS. In this study, most of the cases (14/16, 87.5%) of HHV-8 immunohistochemical staining conducted on KS cell blocks were positive, over 64% positivity was recorded by Ramos da Silva and colleagues.<sup>[4]</sup> HHV-8 immunohistochemistry is regarded as positive only when a dot-like nuclear pattern is observed, whereas a negative stain is not likely to rule out KS because false negatives could be caused by technical artifacts.

FNAC has high diagnostic accuracy, as high as 98.9% reported in some studies.<sup>[5]</sup> Further, if FNAC is combined with cell block, the diagnostic accuracy increases up to 100%.<sup>[5]</sup> FNAC has more diagnostic accuracy when performed by experienced pathologists than other clinicians. This finding, agreeing with other studies, suggests that the success of FNAC depends largely on the training and experience of the individuals implementing the procedure. An experienced pathologist could perform on-site adequacy assessment and repeat the operation until he or she obtains adequate material.

In conclusion, our results indicated FNAC combined with cell blocks is a reliable and useful method for the diagnosis of KS. Cytomorphology, morphology, and immunohistochemical studies of cell blocks could offer high accuracy in the diagnosis of KS in AIDS patients.

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### Conflicts of interest

None.

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