Anaplastic Lymphoma Kinase Immunocytochemistry in Fine Needle Aspiration Diagnosis of Anaplastic Large-cell Lymphoma

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Abstract

Background: Anaplastic large-cell lymphoma (ALCL) is a rare subtype of non-Hodgkin’s lymphoma (NHL) characterized by the presence of unusual giant cells. It is a CD30+lymphoma of T-cells lineage, which shows anaplastic lymphoma kinase-nucleophosmin (ALK-NPM) rearrangement. ALCL on fine needle aspiration cytology (FNAC) shows unusually large and bizarre tumor cells. Materials and Methods: All aspirates seen over a 6-year period from November 2009 to November 2015 in which a diagnosis of ALCL or Hodgkin’s lymphoma (HL) with bizarre giant cells were suspected on cytomorphology were prospectively selected. Twenty such aspirates were subjected to CD-30 and ALK-1 immunocytochemistry (ICC). Subsequent biopsy was available in all cases. Results: Out of 20 cases, seven cases, suspected to be ALCL on FNAC, were confirmed on biopsy. ALK-1 was positive in both cytology and biopsy of 6/7 of these. Two cases suspected to be ALCL on cytomorphology were HL(1) and diffuse large B-cell lymphoma (DLBCL) (1) on biopsy, both of which were ALK-1 negative on cytology. Eight cases of HL and three cases of large-cell NHL, which were all ALK negative on cytology, were confirmed on biopsy. Conclusion: ICC for ALK and CD30 is useful in aspiration cytdiagnosis of ALCL. One CD30 positive DLBCL and one ALK negative ALCL showed concordant results of ICC on cytology and histology.

Keywords: ALK-1 immunocytochemistry, anaplastic large-cell lymphoma, CD30 immunocytochemistry, fine needle aspiration cytology

Background

Anaplastic large-cell lymphoma (ALCL) is a subtype of T-cell non-Hodgkin’s lymphoma (NHL) characterized by the presence of CD30 positive large atypical lymphoid cells. A majority of the cases have a t (2;5) (p23;q35) translocation, which leads to fusion of nucleophosmin (NPM) gene (5q35) and anaplastic lymphoma kinase (ALK) (2p23) gene. Based on ALK gene rearrangement and protein expression, the lymphoma is classified into ALCL, ALK positive (ALK+), and ALCL, ALK negative (ALK-). We have published fine needle aspiration cytology (FNAC) features of ALCL based on a retrospective analysis of biopsy confirmed cases, describing the unusual giant cell types. However, the main role of FNAC remains the screening of lymph nodes for those patients in whom there is a suspicion of lymphoma, so that early lymph node biopsy can be performed. Because ALCL on FNAC shows unusually large and bizarre tumor cells, the appearance is unlike a lymphoma, and hence, a diagnosis of poorly differentiated carcinoma metastasize to the lymph node is frequently rendered. This can result in waste of valuable time in searching for a primary site or estimation of serum markers rather than a lymph node biopsy. The characteristic morphological features of ALCL seem sufficiently distinctive to enable cytdiagnosis. The advent of ALK-1 immunohistochemistry (IHC) has greatly facilitated the biopsy diagnosis of ALCL; however, the role of ALK-1 immunocytochemistry (ICC) in FNAC diagnosis is still anecdotal. We found ALK-1 to be positive in our previous study as either strong nuclear or cytoplasmic staining. In the present

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Materials and Methods

This was a prospective study carried out on 20 aspirates of suspected lymphomas seen over duration of 6 years from November 2009 to November 2015. All aspirates had both Papanicolaou (Pap) and May–Grunewald–Giemsa (MGG) stained smears as well as a minimum of two unstained smears for ICC available [Figure 1]. At routine sign out, a detailed cytological examination was carried out and the presence of large and bizarre tumor giant cells in an aspirate from a lymph node or soft tissue mass, where a differential diagnosis of ALCL was kept, were selected. Cases of suspected Hodgkin’s lymphoma (HL) but showing numerous Reed Sternberg (RS)-like cells and a profusion of mononuclear Hodgkin’s cells were also included, but routine aspirates of HL without the giant cells were excluded. Aspirates with overlap features of ALCL but clinically having a definite primary site for a carcinoma and aspirates with definite grouping of the tumor cells suggesting a carcinoma were excluded. The FNAC smears for ICC were fixed in 95% ethyl alcohol. ICC for ALK-1 (Spring bioscience, clone: SP144, dilution: 1:200) and CD30 (Bio SB, clone: Ber-H2, dilution: 1:300) were done in all the included cases. The antigen retrieval was done using a microwave in citrate buffer (pH = 6). Subsequent biopsy specimen was available in all cases, fixed in 10% buffered formalin and stained with hematoxylin and eosin and further characterized by IHC (LCA, CD3, CD20, CD15, CD30, ALK-1, and EMA).

Results

Twenty cases meeting the inclusion criteria in the 6-year period were selected. Most of the patients (85%) had a lymphadenopathy, cervical lymphadenopathy (55%) being the most common presentation followed by periportal, axillary, and inguinal lymph nodes. Nodal aspiration was done in 17 cases and extra-nodal in 3 cases [Table 1].

Of these 20 aspirates, the diagnosis suspected was ALCL in 7 cases, ALCL versus large-cell lymphoma in 1 case, ALCL versus HL in 1 case, HL in 8 cases, and large-cell lymphoma in 3 cases [Table 1]. In the seven aspirates where the primary suspicion was ALCL (cases 1-7), ALK-1 immunopositivity was nuclear in five cases while cytoplasmic in one [Figure 2a and 2b] and all seven cases were positive for CD30 [Figure 2c]. Subsequent biopsy confirmed all seven cases. One case (case 2) which was CD30 positive but ALK-1 negative showed the same immunoprofile on biopsy as well and was diagnosed as ALCL, ALK- on the basis of histopathological features, immunopositivity for LCA, CD43, CD30, EMA and immunonegativity for ALK-1, CD3, CD20, CD15, CD138, MUM-1, and EBV-LMP-1.

One aspirate from cervical lymph node (case 8) showed a profusion of RS-like and Hodgkin’s-like cells as well as atypical giant cells where features overlapped between HL and ALCL. CD30 was positive while ALK-1 was negative. Subsequent biopsy showed it to be HL, mixed cellularity type with ALK-1 being negative in the biopsy.

In an aspirate from periportal lymph node (case 9) where cytological features overlapped between ALCL and large-cell lymphoma, CD30 was positive while ALK-1 was negative. Cytological diagnosis was not possible because extra smears for CD20 and other markers were not available. Subsequent biopsy showed it to be a diffuse large B-cell lymphoma.
lymphoma (DLBCL). CD30 was positive in the biopsy as well.

Eight aspirates during routine sign out were suspected to be HL but had excess of RS and Hodgkin’s cells. The main diagnosis was thought to be HL, but cases were included in the study. All showed CD30 to be positive and ALK-1 to be negative [Figure 2d]. The biopsy from all these 8 cases showed the final diagnosis to be HL.

Three aspirates (two from lymph node and one from abdominal wall nodule) showed a diffuse population of large lymphoid cells where many giant cells and atypical large cells were interspersed. The main possibility on cytology was considered to be large-cell lymphoma but the presence of the giant cells prompted a differential diagnosis of ALCL. All three showed CD30 and ALK-1 to be negative. On subsequent biopsy, they were diagnosed as DLBCL with CD30 and ALK-1 being negative.

**Discussion**

The main role of FNAC remains as an initial screening test for lymph nodal enlargements, more so in developing countries where infective etiologies like tuberculosis and numerous reasons for reactive nodal enlargements predominate. Hodgkin’s lymphoma is an important differential diagnosis in countries where Epstein-Barr virus in childhood is common, including India. FNAC cytodiagnosis, since prospective studies for role of ALK1 and CD30 on FNAC samples is scant in the literature. Cases of suspected ALCL, aspirates of HL with excess of Hodgkin’s and RS cells and aspirates of suspected DLBCL with pleomorphic and giant cells were included. Out of 20 such aspirates displaying anaplastic cells, 7 cases were confirmed to be ALCL on histopathology and were similarly characterized on both aspirates and biopsy [Figure 1a-c]. ALK-1 was positive in six possible on limited material available from aspirates, the main role of FNAC is screening nodal swellings for an early biopsy diagnosis.

ALCL is a rare subtype of T-cell NHL, which has a distinct, characteristic cytodiagnosis. It has a diagnostic ALK-1 translocation; however, cases with variant translocations will be negative by real-time polymerase chain reaction (RT-PCR), because the RT-PCR technique uses primers that are specific for the ALK and NPM genes. Despite this, cases with variant translocations are also positive for ALK-1 by IHC. Therefore, IHC has superseded molecular tests for the diagnosis of ALCL, based on its sensitivity and specificity. Normal lymphoid cells do not express ALK-1 protein. Among lymphomas, immunopositivity for ALK-1 protein is never seen in non-ALCL cases, with the rare exception of ALK-1 large B-cell lymphoma. Hence characteristic cytodiagnosis and ICC for ALK-1 and CD30 on FNAC can suggest a diagnosis of ALCL as published previously from our laboratory.

The present study was a prospective analysis based on routine sign out cases, the purpose of which was to qualitatively evaluate whether cytodiagnosis and ICC can achieve cytodiagnosis, since prospective studies for role of ALK1 and CD30 in FNAC samples is scant in the literature. Cases of suspected ALCL, aspirates of HL with excess of Hodgkin’s and RS cells and aspirates of suspected DLBCL with pleomorphic and giant cells were included. Out of 20 such aspirates displaying anaplastic cells, 7 cases were confirmed to be ALCL on histopathology and were similarly characterized on both aspirates and biopsy [Figure 1a-c]. ALK-1 was positive in six
of these. The seventh case was ALCL, ALK+, which is rare and impossible to diagnose using ICC.

Two aspirates were suspected to be ALCL on cytomorphology, but ALK-1 was negative. One was HL and the other was DLBCL. Hence in 78% of aspirates, a clear diagnosis of ALCL can be made while in 22% of aspirates where ALCL is suspected on cytomorphology, the biopsy shows HL or DLBCL. Since both cases, however, were negative for ALK-1 on ICC, it is possible to refine the cytdiagnosis using ICC. We found 100% concordance for CD30 and ALK-1 in ICC performed on aspirates and IHC performed on biopsies in all cases included in the study.

The remaining 11 aspirates were not suspected as ALCL but as either HL (8 cases) or DLBCL (3 cases). The main diagnosis was therefore not considered as ALCL although pleomorphic giant cells were present [Figure 1d]. ICC for ALK-1 was negative in all and in combination with CD30 helped in proper characterization. RS-like cells can be seen in ALCL, while Hallmark cells can be seen in HL. Other features of HL-like dichotomous population, sparse presence of atypical cells, and presence of eosinophils were, however, seen in these nine aspirates.[9] Immunonegativity for ALK-1 in such cases is therefore useful to demonstrate. Lymphocyte-depleted Hodgkin’s lymphoma and nodular sclerosis classical Hodgkin lymphoma (syncytial variant) which can have a predominant pattern of numerous pleomorphic, anaplastic, and RS cells can be difficult to differentiate from ALCL solely based on cytomorphological grounds.[10,12] It is likely that ICC for ALK-1 would be useful in these situations. Biopsy with extended panel of IHC for CD3, CD20, CD30, ALK-1, PAX-5, CD15, and EBV-LMP-1 plays an important role to differentiate these entities. Such cases were not included in the present study where HL was either mixed cellularity (4 cases) or nodular sclerosis (5 cases).

It is interesting to note that germ cell tumor and metastatic carcinoma were not included in this prospective analysis as suspicious for ALCL. [12,13] Despite a large oncology load in our department, all aspirates from these tumors were found to have cohesive tumor cells and the typical giant cells were not found. Melanoma is uncommon in our country due to the protective skin pigmentation, and amelanotic melanoma, therefore, is correspondingly infrequent. These differentials are therefore likely to be rare and more theoretical than practical. Hence utility of ICC for cytokeratins, PLAP, SALL-4, CD117, Oct-4, Melan A, and HMB45 are limited in ALCL cytdiagnosis. [14,15] Embryonal carcinoma is positive for CD30 but this issue was not a practical problem in this study.

The main differential diagnosis for ALCL turned out to be DLBCL with pleomorphic morphology. An ICC panel of ALK-1, CD30 as well as CD20 is, therefore, more appropriate for distinction of aspirates where ALCL is suspected. In this study, only two slides were kept unstained and hence CD20 could not be performed on most of the cases. This study demonstrates that ALCL can be easily diagnosed on FNAC with ICC for CD30 and ALK-1.

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Conflicts of interest

There are no conflicts of interest.

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