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Case Report

Case reports are accepted under this heading. These reports should be short and concise and contain a minimum of figures, tables and references.

PEDIATRIC SMALL CELL VARIANT OF Ki-1 (CD30)+ T-CELL LYMPHOMA WITH GERM-LINE CONFIGURATION OF THE T-CELL RECEPTOR GENE

Children presenting with a progressive clinical picture of fever, weight loss, rash, lymphadenopathy and hepatosplenomegaly are sometimes difficult to diagnose by both the clinician and the pathologist. Apart from various possible infectious agents other events should be taken into account: 1) A reactive infection associated hemophagocytic syndrome (IAHS), or familial erythrophagocytic lymphohistiocytosis (FEL), where macrophages reacting to an unknown antigenic or infectious stimulus and erythrophagocytosis characterize the histological picture (1). 2) Malignant histiocytosis which is a true neoplastic process of the mononuclear phagocytes, typically characterized by an involvement of the lymph nodes' sinuses (1). 3) A virally induced clinical syndrome like fatal infectious mononucleosis in families with the X-linked lymphoproliferative (XLP) syndrome. 4) Ki-1 neoplastic large cell lymphoma which is defined as a lymphoma containing pleomorphic large, CD30+ lymphocytes, prominent involvement of the nodal sinuses, and frequently demonstrating the t(2;5) (p23; q35) chromosomal translocation (2). Most cases have T-cell immunophenotype, and the T-cell cases often express the epithelial membrane antigen (EMA) (3). The correct diagnosis in such cases must, therefore, be based on extensive laboratory work.

We describe a child who presented with a dramatic clinical picture with evidence of a severe immunological deficiency. Based on pathological, conventional immunophenotyping studies and Ig and TCR genes rearrangement studies, it was impossible to make a diagnosis of lymphoma. The working diagnosis was a florid reaction to an infectious agent and he was treated symptomatically. Only staining with a monoclonal antibody (Ab) to CD30, and mainly cytogenetic demonstration of the t(2;5), enabled the correct diagnosis of small cell variant of Ki-1 T-cell lymphoma.

Case report. An 8 year-old Ashkenazi Jewish, previously healthy, boy was hospitalized in another hospital 2 weeks before admission to our department with spiky fever, enlarged cervical and supraclavicular lymph nodes, hepatosplenomegaly, enlarged mediastinal and hilar nodes with progresssive bilateral interstitial findings on chest x-rays. Fine-needle aspiration (FNA) from a submandibular gland was consistent with reactive changes. The working diagnosis of infectious disease was made and broad spectrum antibiotics were administered. He was referred to us because of respiratory deterioration and lack of response to antibiotics. Physical examination on arrival revealed a child in poor general condition, dyspnea, tachypnea and hypoxemia, necessitating mask delivery of high oxygen percentage. There was a maculo-papular rash on the neck and chest, progressive generalized lymphadenopathy and hepatosplenomegaly. Computerized tomography (CT) of the chest demonstrated mediastinal and hilar lymphadenopathy, bilateral progressive interstitial disease and a small right pleural effusion. Abdominal CT showed huge hepatosplenomegaly with no filling defects, enlargement of the retroperitoneal nodes, and a moderate amount of ascitic fluid. Blood cell counts showed WBC of $10-18 \times 10^9$/l with neutrophilia and lymphopenia with no blasts. The Hb level decreased from 12.2 g/l to 8.2 g/l and the platelet count from 256×10^9/l to 96×10^9/l. A bone marrow aspiration showed a reactive marrow with a shift to the left with no increase of blasts, no storage cells and no signs of hemophagocytosis. The triglyceride and fibrinogen levels were normal. Serology was negative for HIV, toxoplasmosis, CMV and EMV. Immunological studies revealed normal immunoglobulin levels. However, there was an inversion of the CD4/CD8 ratio, with CD4 cells reaching a level of 100 cells/mm^3. Erythrocyte sedimentation rate was normal. A biopsy of a large cervical node showed replacement of the normal architecture by a diffuse, polymorphic lymphoproliferative process consisting of small and medium to large cells with rich clear cytoplasm and a moderate amount of mitoses. In addition, reactive lymphocytes and a large number of reactive histiocytes, some of which demonstrated erythrophagocytosis were viewed (Fig. 1). The majority of the lymphocytes stained positive for CD3 and Ki-1 (CD30) (Fig. 2), and negative for CD16 and CD56. CD30 positive cells were also found in the peripheral blood (30%). Gene rearrangement studies were performed by the Southern blotting technique, employing the Hind III restriction enzyme, the probes for the genes encoding for the T-cell receptor β and γ chains. The T-cell receptor probe used was a human genomic 420bp fragment and the Jh probe was a 5.6 fragment. Both were obtained from Oncor, Inc., Gaithersburg, MD. No clonal rearrangement was detected neither in the genes coding for the T-cell receptor chains nor in the genes coding for the Ig chains; they were in the germline configuration. Cytogeneric studies revealed t(2;5) (p23;q35) in 24 out of 27 metaphases (Fig. 3). The child was treated with acyclovir, broad spectrum antibiotics, corticosteroids and IV gammaglobulins but he developed disseminated intravascular coagulation (DIC), his repiratory status deteriorated and he was treated by mechanical ventilation in the intensive care unit (ICU). Later on he developed Gr^- sepsis and candidemia that were complicated by hepatosplenic candidiasis. As lymphoma was diagnosed, chemotherapy was initiated according to the MACOP-B protocol (14), followed later by a modified National Institute of Health (NIH) 7704 protocol (5). The child is in complete remission 24 months after diagnosis. Bone marrow was harvested and cryopreserved as a back-up in the event of a possible future relapse.

Discussion. Childhood non-Hodgkin's lymphomas (NHL) are usually high-grade aggressive tumors of one of three histologic types: lymphoblastic, small non-cleaved cell (SNCC), and large cell lymphoma (LCL) (6). LCL constitutes 20–25% of childhood NHL (6). Among the LCL, Ki-1 anaplastic LCL (ALCL) constitutes roughly 30%, or 8% of all pediatric NHL cases (7).

In the past, Ki-1 ALCL cases were erroneously diagnosed as malignant histiocytosis, undifferentiated carcinoma, malignant fibrous histiocytoma, malignant melanoma, sarcoma or Hodgkin's disease (8). Only closer morphological, immunohistochemical (mainly the use of Ki-1 staining), cytogenetic and molecular examinations of these tumors revealed a common lymphoid derivation and were thus regarded as a new entity. This has been included in the updated Kiel classification in 1988 (9), and recently in the Revised European American Lymphoma Classification (10). CD30 which has recently been reported to be homologous to the TNF receptor (11) is encoded by a gene localized at chromosome 1p36 (12).

ALCL has a primary cutaneous form which occurs predominantly in adults, and a systemic form which is prevalent amongst children and young adults. The systemic form is characterized by aggressive clinical course, systemic symptoms, and multiple peripheral lymphadenopathy. Stage III/IV disease is observed in more than 50% of cases. Extranodal disease (especially skin involvement and bone lytic lesions) occurs in 20–30% of the cases. Bone marow and central nervous system are rarely involved (12). The familiar and better studied histologic variants of ALCL are

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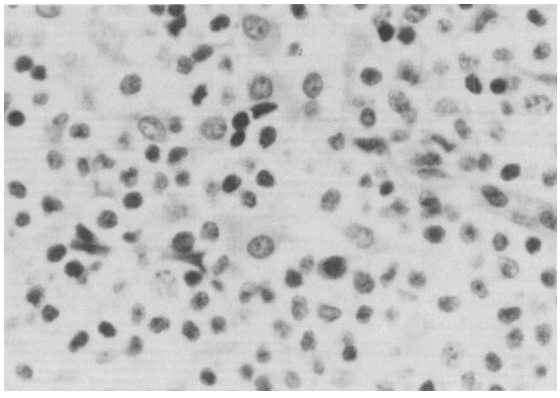

Fig. 1. A lymph node in which the normal architecture has been largely replaced by small and medium to large anaplastic lymphoid cells with a considerable amount of clear cytoplasm. There is a moderate amount of mitoses. Interspread among the cells are many reactive histiocytes, some of them exhibiting erythrophagocytosis. (Hematoxylin and eosin; magnification ×400).

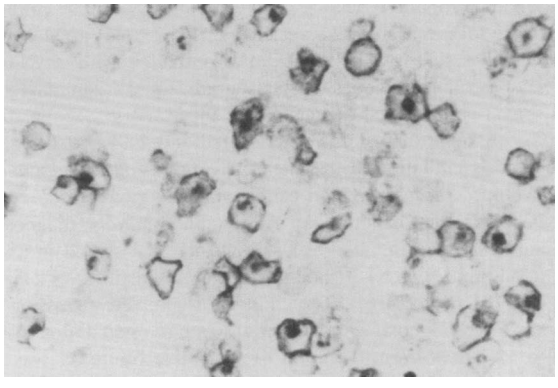

Fig. 2. Immunoperoxidase staining of paraffin sections with anti Ki-1 showing predominance of $CD30^+$ cells. The stain is intense and double (membranous and paranuclear) in the larger cells. In the smaller lymphoid cells in the background the stain is only membranous and weak.

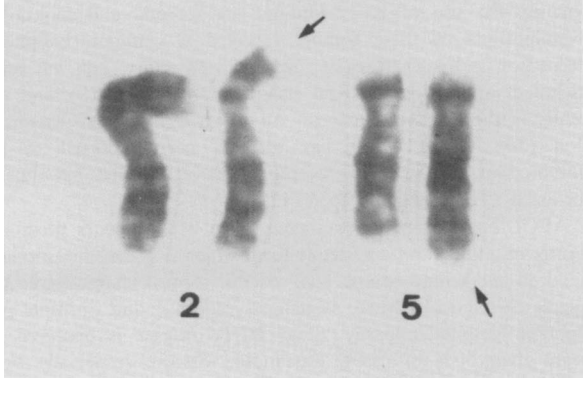

Fig. 3. G-banded karyotype showing 46xy t(2;5) (p23;q35). Arrowheads show translocated chromosomes.

the common type, and the lymphohistiocytic type which, in addition to the same cytologic features of the common types, is characterized by a large amount of benign-looking histiocytes (12).

A distinct subgroup of Ki-1 ALCL, defined as a small-cell predominant variant of Ki-1 (CD30) T-cell lymphoma, has recently been described in nine patients by Kinney et al. (13). These tumors were characterized histologically by the predominance of a population of small lymphocytes accompanied by large Ki-1 tumor cells, and clinically by young age of appearance (median 14 years), presence of B symptoms (56%), and frequent involvement of mainly skin (78%) and lymph nodes (67%). The actuarial 2-year disease-free survival rate was 14%, and the overall survival rate was 51%, significantly worse than those obtained in the common ALCL.

The stormy clinical picture in our case, and in most of the previously described cases, as well as the histological picture of predominance of small cells, may mislead the clinician and even the experienced pathologist to an erroneous diagnosis of a florid-reactive process which is typical to non-restrictive viral infections and syndromes like VAHS, XLP or FEL. Only a closer morphological examination, Ki-1 positive staining of the cells, and cytogenetic finding of t(2;5) can accurately establish the diagnosis.

This case demonstrates several diagnostic difficulties: (1) The morphology of a mixture of small and large cells, as well as the presence of hemophagocytosis, may mislead to a reactive process. (2) The Ki-1 antigen expression is now recognized to be non-specific, indicative of an activated state with a strong expression not only on HD, ALCL or ALCL with predominance of small cells, but also to be found on activated T, B, or null cells, activated macrophages, lymphoid cells transformed by virus (HTLV − 1 and −2 and EBV) and even some embryonal carcinoma cells (14). Undoubtedly, the existence of a clonal translocation and/or Ig or TCR gene rearrangement in the setting of Ki-1 positivity strongly supports monoclonality and malignancy. (3) The β and τ light chains of the TCR were not rearranged in our case. In a note added to the proof of Kinneys' series, a description of the rearrangement of the TCR gene in one patient is outlined. Indeed, in most lymphatic malignancies, a clonal rearrangement of the TCR or Ig gene can be demonstrated. Several explanations may be offered for the lack of rearrangement in the observed case. A) The malignant clonal population carrying the rearranged gene may represent only a minority of the analysed cell population and hence is below the level of detection by Southern blotting. The fact that most of the cells stained positive for CD30 disclaims this theory. B) The malignant cells could be derived from true natural killer (NK) cells lacking the TCR molecules. This is possibly excluded by the negative staining for CD16 and CD56 in the majority of the cells. C) The transforming event, probably t(2;5) occurred at the early stages of the T-cell differentiation before TCR rearrangement took place. In such cases, however, a few bands, representing multiple independent rearrangement processes similar to the situation in some infantile acute lymphatic leukemia cases (ALL) (15), are expected to be seen. No such bands were demonstrated in our patients. D) The most plausible explanation is that the malignant cell is the result of aberrant differentiation, leading to the expression of cell surface antigens present in normal situations only on cells that have already rearranged their TCR genes. Several cases of Ig and TCR gene rearrangement negative ALL were described (16). Whatever the cause, germline configuration of both TCR and Ig genes does not rule out the possibility of lymphatic malignancy. 4). The cytogenetic studies provided the most meaningful contribution to the diagnosis in this case. However, one must bear in mind that chromosomal aberrations have been described in situations that have not developed to overt malignancies, remaining in the hyperplastic phase (17, 18). Even

the t(2;5) translocation is not pathognomonic for lymphoma (19). 5) The lack of response to broad spectrum antibiotics, anti-viral agents, corticosteroids, high dose IV immunglobulins, and the impressive response to chemotherapy seem to be convincing evidence of malignancy, although response to chemotherapy has also been described in the reactive histiocytic syndromes (1).

The molecular basis of the t(2;5) translocation was recently thoroughly studied. By cloning and characterization of the t(2;5) breakpoint sequences, Morris et al. (20) demonstrated that this translocation leads to the fusion of the tyrosine kinase receptor ALK to a gene encoding the nucleophusmin nucleolar protein. This fusion transcript can be identified by RT-PCR. Recently, immunohistochemical studies using the P80 product of the hybrid gene in diagnosis of Ki-1 lymphoma were described (21). The use of such techniques can simplify the diagnosis of atypical cases of this entity.

The present case illustrates the need for a thorough investigation of problematic biopsies, employing all resources including immunohistochemistry, cytogenetics and molecular studies. Without these facilities, even experienced pathologists may fail to make a correct diagnosis concerning a tumor, and may not differentiate between the normal proliferative process and the malignant one. The limitation of FNA in reaching a diagnosis of a primary malignant tumor was again confirmed in this case.

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