Haematologica 2000; 85:978-981
case of the month

Malignant Lymphomas

Anaplastic lymphoma kinase expression as a marker of malignancy. Application to a case of anaplastic large cell lymphoma with huge granulomatous reaction

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ABSTRACT

Anaplastic large cell lymphoma (ALCL) shows a wide morphologic spectrum, including the occurrence of reactive components obscuring the neoplastic population. This makes its distinction from hyperimmune reaction difficult. The authors describe an ALCL in a girl who had a tick bite 20 days prior to clinical presentation. She developed a huge epithelioid reaction (an unprecedented finding for this tumor). The diagnostic controversies were solved by applying the ALKc antibody against anaplastic large cell lymphoma kinase (ALK), in conjunction with reagents anti-nucleophosmin (NPM), which showed the typical staining pattern observed in ALCL carrying t(2;5). Comprised within the epithelioid component there were large anaplastic cells and small-medium sized atypical elements displaying strong nuclear and cytoplasmic positivity for ALK and NPM (N-terminal region). This pattern, never observed in normal lymphocytes, corresponds to the presence of the product of the hybrid gene NPM/ALK produced by t(2;5). Following the diagnosis, the patient - whose general conditions were critical - underwent aggressive chemotherapy, achieving complete remission.

Key words: anaplastic large cell lymphoma, chromosomal aberration, immunophenotype, chemotherapy.

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The term *anaplastic large cell lymphoma* (ALCL) is applied to a group of neoplasms characterized by a certain degree of heterogeneity in terms of clinical presentation, morphology, phenotype, and genotype.[1-5] About 60% of primary systemic ALCLs of the common type and most, if not all, lymphohistiocytic and small-cell variants carry the (2;5)(p23;q35) translocation, while the giant-cell-rich and Hodgkin's-like forms most often lack this aberration.[2-7] Molecular studies have shown that t(2;5) produces the formation of a hybrid gene, termed NPM/ALK, which encodes for a chimeric protein formed by the N-terminal region of nucleophosmin (NPM) and the entire cytoplasmic domain of the tyrosine-kinase receptor ALK.[8] Monoclonal antibodies raised against the intracytoplasmic portion of ALK and the N- and C-terminal regions of NPM (N-NPM and C-NPM) have recently been developed and applied to the study of ALCL.[2-5,9-11] The tumor shows a characteristic staining pattern in the presence of t(2;5): in fact, anti-ALK and N-NPM antibodies produce nuclear and cytoplasmic positivity, while those against C-NPM provide an intranuclear signal.[2,3,10] This pattern is due to the fact that the hybrid NPM/ALK protein is produced in the cytoplasm and partially shuttled to the nucleus by the formation of heterodimers with normal NPM.[2,10] On the other hand, normal NPM is quickly and almost entirely harvested within the nucleus in the form of homodimers.[2,10] In a small number of ALCLs, ALK-positivity is limited to the cytoplasm: this finding corresponds to the occurrence of chromosomal aberrations other than t(2;5), but involving chromosome 2 at p23, such as inv(2)(p23;q35), t(1;2)(q21-25;p23), t(2;2)(p23;q23), or t(2;3)(p23;q35).[12-17] The expression of ALK protein seems to attain prognostic relevance, since patients with primary systemic ALK+ ALCLs (also termed *ALKomas*) have a significantly better response to therapy and survival rate than negative ones.[6,18] Herein, we report on an example of ALCL, characterized by misleading clinical and morphologic findings, which was definitively diagnosed and appropriately treated thanks to the application of the new anti-ALK and NPM antibodies.

Case Report

In December 1998, a 9-year old female – who had received a tick bite on the neck 20 days previously– entered another hospital for sudden supraclavicular lymph node swelling and high fever. Physical examination and CT scan showed a mediastinal mass and hepatosplenomegaly. Serologic tests revealed a weak positivity for anti-rickettsial antibodies. Antibiotic therapy, in association with antifungal agents and corticosteroids, were started. After a transient improvement of symptoms, the patient developed severe interstitial pneumonia with pulmonary failure and was admitted to an intensive care unit. At that time, the supraclavicular lymph node was removed and a diagnosis of hyperimmune reaction was suggested based on morphologic and phenotypic findings. Because of the rapid deterioration of the patient's general conditions, the pathologic material was sent for consultation to the Service of Pathologic Anatomy and Haematopathology of Bologna University, where a diagnosis of anaplastic large cell lymphoma was made (see below). The patient was treated with an antiblastic regimen containing methotrexate, cyclophosphamide, doxorubicin, vincristine, and prednisone. After the second course she went into complete remission, which is still maintained at the time of this writing.

Pathologic findings

The lymph node biopsy had been fixed in 10% buffered formalin for 24 hours and processed according to routine procedures.[19]

At microscopic examination of hematoxylin/eosin and Giemsa stained preparations, the normal lymph node structure was totally effaced due to a diffuse growth mainly consisting of epithelioid cells with a tendency to granulomata formation (Figure 1). Occasional features of hemophagocytosis were observed. In this context, there were a few plasma cells, rare eosinophils, a moderate number of small-medium sized, irregularly shaped lymphocytes, and some mononuclear blasts with deeply basophilic cytoplasm, horseshoe-shaped nuclei and prominent nucleoli (Figure 2). Several mitotic figures were seen. P.A.S. and Ziehl-Neelsen stains did not reveal fungi or acid-fast bacilli.

At immunohistochemistry, which was performed on routine sections by applying previously described antigen retrieval methods,[19] the alkaline anti-alkaline phosphatase immune complexes (APAAP) technique (20) and the panel of antibodies listed in Table 1, the blasts and most lymphoid elements carried the following phenotype: CD30+, EMA+, TIA-1+, CD3±, OPD4±, CD1a-, CD8-, CD15-, CD21-, CD68-, CD79-, CNA.42-, MPO-, glycophorin A- (Figure 3). About 60% of the blasts and lymphoid elements were in the cell cycle, as shown by the Ki-67 marking. Epithelioid elements turned out to be CD68+, CD4+ and Mib-1-. A diagnosis of ALCL with a huge epithelioid cell reaction was proposed. This interpretation was confirmed by further immunostaining that showed cytoplasmic and nuclear ALK (Figure 4) and N-NPM positivity both in the blasts and lymphoid component, thus supporting the occurrence of the ALCL-associated translocation (2;5)(p23;q35).

Discussion

The diagnosis of ALCL may, at times, be difficult by conventional light microscopy. This is not surprising if one considers that the tumor was only identified in 1985, when Stein *et al.* observed the reactivity of the Ki-1/CD30 monoclonal antibody in cases that had previously been diagnosed as malignant histiocytosis or metastatic carcinoma.[21] Immunohistochemistry is

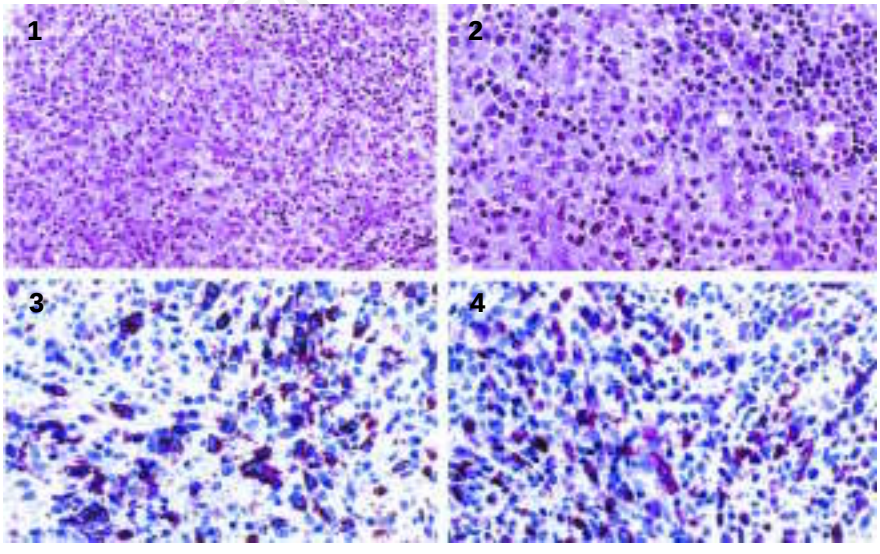

Figure 1. At low power, the normal lymph node structure is totally effaced by a diffuse growth, characterized by the huge number of epithelioid cells with tendency to granulomatous formation (hematoxylin and eosin, x100).

Figure 2. At higher magnification, large anaplastic cells and irregularly shaped lymphoid elements are seen within the epithelioid cell population (hematoxylin and eosin, x400).

Figure 3. Positivity for the CD30 molecule (Ber-H2 monoclonal antibody; APAAP technique; x400).

Figure 4. Expression of the ALK protein by large anaplastic cells and atypical lymphoid component both in the nucleus and the cytoplasm (ALKc monoclonal antibody; APAAP technique; x400).

still of paramount importance for the recognition of the tumor, as well as for its differentiation from other neoplasms – such as Hodgkin's disease – which also regularly express the CD30 molecule.[1,4] Immunophenotyping is indeed mandatory in diagnosing the lympho-histiocytic variant of the tumor (LH), which mainly occurs in children or young individuals.[22] In fact, this form of ALCL is often misinterpreted as a hyperimmune reaction with detrimental results for the patients, who – all potentially curable with present therapies – are instead invariably lost because of the ineffective approaches employed or the delay in therapy administration.[22] The difficulties in making the diagnosis of ALCL-LH are due to the fact that the neoplastic cells are obscured by a huge number of benign quiescent histiocytes ($CD68^+$/$Ki\text{-}67^-$). Therefore, the pathologist focuses his attention on the reactive component and regards the $CD30^+$ anaplastic cells scattered throughout as expression of the lymphoid activation, which regularly occurs in florid immune responses.[23] Careful analysis, however, shows that the blasts are $CD30^+$, EMA^+, $CD3^+$, $CD45^{+/-}$, $CD20^-$, $CD79a^-$, $CD15^-$, and $LMP\text{-}1^-$.[4,22] In hyperimmune reactions, there are numerous $CD30^+$ blasts, but they are of mixed (B- and T-cell) nature, do not express EMA, and often carry LMP-1, due to the occurrence of EBV infection.[22,23] Recently, extensive application of the newly developed anti-ALK antibodies has revealed that most if not all ALCL-LH do carry t(2;5), as shown by the strong positivity observed both in the nucleus and cytoplasm.[2-5,11] Interestingly, immunohistochemical assays have shown that the translocation is not limited to the large anaplastic cells, but is also carried by small to medium-sized lymphoid elements, thus demonstrating that ALCL can display a much wider cytologic spectrum than originally thought.[2-4] The ratio between the large anaplastic cells (also termed hallmark cells) and the small/medium-sized ones – which can vary from case to case and also within the same case at presentation and relapse – allows the subclassification of the tumor. In the common and giant-cell types hallmark cells prevail, in lympho-histiocytic ALCL large and small elements are admixed with a huge number of reactive histiocytes, and finally in the small cell variant small/medium-sized neoplastic cells predominate over the large ones, which more often assume a perivascular distribution.[2-4] Last but not least, ALK-positivity definitively proves the neoplastic nature of the process: in fact, normal lymphocytes – irrespectively of whether they are activated or not – never contain amounts of ALK protein detectable by immunostaining, even employing the most sensitive techniques.[2,10]

The present case shows strong similarities with the LH variant of ALCL, as far as the diagnostic difficulties are concerned. The neoplastic population was, indeed, obscured by the reactive cell component. However, conversely to ALCL-LH, the latter did not consist in histiocytes, but in epithelioid elements. This finding, which – to the best of our knowledge – has never been mentioned in previous reports – made the diagnosis even more problematic, since epithelioid reactions can lead to almost complete effacement of normal lymph node structure and may be sustained by different pathologic conditions, including bacterial, viral or protozoal infections.[24] The occurrence of a tick bite a few weeks before the onset of our patient's disease and the equivocal results of serologic tests had further strengthened the hypothesis that an infective agent had produced the histologic picture. In this respect, it should be underlined that the development of ALCL following an insect sting has never been the object of a specific communication in the literature, although it was matter of discussion at a workshop on peripheral T-cell lymphomas (Barcelona, May 30-June 1, 1997) without any conclusion on its significance being achieved.[25] Immunophenotyping played a basic role in making the correct diagnosis. In particular, the detection of ALK positivity gave the definitive proof of the malignant nature of the process, thus ruling out the possibility of a hyperimmune reaction, as well as of other pathologic conditions characterized by a huge granulomatous reaction, such as epithelioid-rich mixed-cellularity Hodgkin's disease and Lennert's lymphoma.[26] The latter conditions are, in fact, both negative for the NPM/ALK hybrid gene product.[2-4]

In conclusion, our report underlines the practical usefulness of applying the newly developed anti-ALK antibodies to the detection of an occult ALCL population, both in cases with a prominent reactive component and in samples – such as the bone marrow[2-4] – with minimal amounts of tumor at disease presentation or following therapy. Under these circumstances, the immunohistochemical test, which is also quite cheap and easy to use, allows correct therapeutic management and possible salvage of patients, who would otherwise be lost or undertreated.

Table 1. List of the antibodies used for the present study.

Antibody	*Reactivity*	*Source*
CD1a	CD1a	Immunotech (France)
CD3 (polyclonal)	CD3	Dako (Denmark)
OPD4	CD45R0	Dako (Denmark)
144B	CD8	Prof. D.Y. Mason
C3D1	CD15	Dako (Denmark)
IF8	CD21	Dako (Denmark)
Ber-H2	CD30	Prof. H. Stein
PG-M1	CD68	Prof. B. Falini
JCB117	CD79a	Prof. D.Y. Mason
TIA-1	TIA-1	Coulter (California)
JC159	Glycophorin A	Dako (Denmark)
Myeloperoxidase (polyclonal)	Myeloperoxidase (MPO)	Dako (Denmark)
CNA.42	Follicular dendritic cells	Prof. G. Delsol
E29	Epithelial membrane antigen (EMA)	Dako (Denmark)
ALKc	ALK protein	Prof. B. Falini
376	N-terminal region of NPM	Prof. B. Falini
338	C-terminal region of NPM	Prof. B. Falini
Mib-1	Proliferation-associated nuclear antigen Ki-67	Prof. J. Gerdes

Contributions and Acknowledgments

PPP: drafting the manuscript. SA: morphologic analysis. GFO: review of the literature. MP: immunohistochemical

analysis. APJr: clinical analysis. BF: revising the manuscript. SAP: design of the study.

The authors thank Mr. Luigi Chilli and Ms. Federica Sandri for their skillful technical assistance.

Funding

This paper was supported by grants from A.I.R.C. (Milan) and MURST, Rome, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received March 3, 2000; accepted July 6, 2000.

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