ALK-positive anaplastic large cell lymphoma limited to the skin: clinical, histopathological and molecular analysis of 6 pediatric cases. A report from the ALC199 study

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ABSTRACT

Anaplastic large cell lymphomas are peripheral T-cell lymphomas that are characterized by a proliferation of large anaplastic blasts expressing CD30. In children, systemic anaplastic large cell lymphomas often present at advanced clinical stage and harbor translocations involving the anaplastic lymphoma kinase (ALK) gene leading to the expression of chimeric anaplastic lymphoma kinase (ALK)-fusion proteins. Primary cutaneous anaplastic large cell lymphoma is regarded as an ALK-negative variant confined to the skin and is part of the spectrum of primary cutaneous CD30-positive T-cell lymphoproliferative disorders. Thirty-three of 487 pediatric patients registered within the Anaplastic Large Cell Lymphoma-99 trial (1999 to 2006) presented with a skin limited CD30-positive lymphoproliferative disorder. In 23 of the 33 patients, material for international histopathological review was available, and the cases were studied for histopathological, immunophenotypical and clinical features as well as for breaks within the ALK gene. Five of 23 cases and one additional case (identified after closure of the trial) expressed ALK-protein. Complete staging excluded any other organ involvement in all children. Expression of ALK proteins was demonstrated by immunohistochemistry in all cases and the presence of breaks of the ALK gene was genetically confirmed in 5 evaluable cases. The histopathological and clinical picture of these skin-restricted ALK-positive lymphomas was indistinguishable from that of cutaneous anaplastic large cell lymphoma. Five children presented with a single skin lesion that was completely resected in 4 and incompletely resected in one. Three of these patients received no further therapy, 2 additional local radiotherapy, and one chemotherapy. All children remain in complete remission with a median follow up of seven years (range 1-8 years). We present 6 pediatric cases of ALK-positive primary cutaneous anaplastic large cell lymphomas. After thorough exclusion of systemic involvement, therapy confined to local measures seems to be sufficient to induce cure.

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Introduction

Anaplastic lymphoma kinase-positive (ALK-positive) anaplastic large cell lymphoma (ALCL) is characterized by a neoplastic proliferation of large pleomorphic (anaplastic) CD30-positive (CD30+) T cells with typical translocations involving the ALK gene and subsequent expression of chimeric ALK protein. This lymphoma accounts for approximately 15% of childhood non-Hodgkin’s lymphomas, but is rare in adulthood. ALK-positive (ALK+) ALCL is usually a systemic disease that frequently involves extranodal sites. In children, 18-25% of systemic ALCLs develop skin manifestations during the course of the disease and this is a poor prognostic factor. Systemic ALK-negative (ALK-) ALCL is included in the updated WHO classification as a separate preliminary entity. ALK-negative ALCL accounts for less than 5% of pediatric systemic ALCLs. However, both ALK-positive and ALK-negative ALCLs are considered potentially disseminated diseases.

Primary cutaneous ALCL (cALCL) is regarded by the WHO as a separate disease entity and belongs to the spectrum of
primary cutaneous CD30-positive lymphoproliferations (CD30+LPD), a group that also includes lymhpomatoid papulosis (LyP). CD30+LPDs share with systemic ALCL the presence of neoplastic CD30+ large T cells, but lack ALK translocations and protein expression. cALCLs remain confined to the skin, virtually never disseminate beyond local lymph nodes, and show an excellent prognosis after surgical resection without systemic therapy. Most cases of cALCL present as solitary skin lesions, but multiple skin nodules are also found. In contrast to systemic ALCL, cALCL is only rarely found in children and young adults. Recently published recommendations for the diagnosis of CD30+LPD state that immunohistochemical detection of ALK expression should be considered highly suspicious of a cutaneous manifestation of underlying systemic ALCL. In contrast, IRF4 translocations have been reported in cALCL and in ALK cALCLs but not in ALK ALCL. In the international multicenter trial ALCL99, children included with localized skin disease were not to receive systemic chemotherapy based on the assumption that their disease would be CD30+LPD. We describe a series of 6 pediatric ALCLs that clinically and histologically resembled cALCL but expressed ALK fusion proteins. These localized cutaneous ALK+ ALCL followed the typical benign clinical course of a CD30+LPD.

**Design and Methods**

**Identification of cases and histopathological review**

In the ALCL99 multicenter study, 487 children and young adults with the diagnosis of ALCL were registered from 1999 to 2006, including 33 patients with a CD30+ lymphoproliferative disorder limited to the skin. Patients with isolated skin lesions diagnosed by complete staging procedures were to be followed after resection by ‘watchful waiting’ without further systemic therapy regardless of the ALK status. For 23 of these, skin limited lymphoma material was available for an international histopathological review. One additional case reported here was identified after completion of registration in 2006. The histological review of the cases was performed by members of the international pediatric lymphoma pathology panel (JO, LL, AN, EdA, UH, KH, ISK, JM, LM, MT) using hematoxylin and eosin (H&E) stained slides as well as slides stained immunohistochemically in various laboratories (see below). The registered clinical data from the study center were reviewed and additional details were obtained by contacting the attending pediatric oncologist. The study was part of the scientific projects accompanying the ALCL99 study, for which informed consent was obtained. The study was carried out according to the local ethical guidelines and in accordance with the ethical guidelines of the studies in which the patients were treated.

**Immunohistochemistry and fluorescence in situ hybridization**

All immunohistochemical stainings were performed on whole tissue sections. The stainings were scored semiquantitatively as negative, weak (<30% positive tumor cells or all tumor cells weakly positive), positive (>30% positive tumor cells) or not interpretable. The minimal staining panel for each lymphoma included CD20, CD3, CD30, and ALK. Additional stainings for granzyme B, perforin, TIA1, EMA, CD2, and CD5 were available for individual cases. Due to the retrospective nature of the study, the staining procedures and antibody sources for these markers varied between the participating countries but had been previously established within the group as part of the ALCL99 study. Fluorescence in situ hybridization (FISH) for chromosomal breaks in the ALK gene or at the IRF4/DUSP22 locus was performed as previously described.

**Results**

**Identification of the 6 ALK-positive cases limited to the skin**

Among the 23 cases with ALCL or CD30+ lymphoproliferations confined to the skin registered into the ALCL99 study and available for international histopathological review, 5 patients with expression of ALK protein were identified. During the preparation of the manuscript, another case of ALK+ ALCL limited to the skin was identified by the NHL-BFM study center and included in this series.

**Table 1. Histopathological and immunohistochemical features of 6 pediatric cases of ALK-positive primary cutaneous anaplastic large cell lymphoma.**

<table>
<thead>
<tr>
<th>Case n.</th>
<th>Epidermal change</th>
<th>Epidermotropism</th>
<th>Histological features</th>
<th>CD30 (pattern)</th>
<th>Immunohistochemistry</th>
<th>EMA</th>
<th>CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hyperplastic, ulceration</td>
<td>+</td>
<td>+</td>
<td>ni</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>ni</td>
<td>ni</td>
<td>+</td>
<td>neutrophils</td>
<td>++</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>normal</td>
<td>–</td>
<td>+</td>
<td>lymphohistiocytic</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>normal</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>hyperplastic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>hyperplastic</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Scoring of histopathological features: +: present; -: absent; ni: not interpretable. Scoring of immunohistochemistry: - : negative staining; +: weak staining or <30% of cells moderate to strong staining; ++: moderate to strong staining in >30% of cells; n+cyt: nuclear and cytoplasmic staining; cyt: cytoplasmic staining only. *In all evaluable cases dermal involvement was superficial and deep.
Histological and immunohistochemical features

The main histological and immunohistochemical features of the 6 cases are summarized in Table 1. In most cases, a superficial and deep cutaneous infiltration extending into the subcutis was observed (3 of 4 cases in which all skin layers were included in the biopsy specimen). The lesions were rather poorly demarcated. In one case, an isolated subcutaneous nodule without dermal involvement was seen. In 5 cases, the epidermis was included in the specimen and was either normal in appearance (n=2), showed hyperplastic changes (n=2), or hyperplastic changes with additional focal superficial erosion (n=1). A large number of CD30+ neoplastic blasts forming cohesive sheets were detectable in 5 of 6 cases. However, one case displayed only scattered blasts. In 3 of 6 lesions, the growth pattern of the blasts was perivascular. Reactive inflammatory bystander cells were composed of a moderate number of neutrophils (1 of 6) or lymphohistiocytic cells (2 of 6). No inflammatory bystander cells were detectable in 3 of 6 lymphomas. Figure 1 shows one representative example of an ALK+ ALCL confined to the skin.

ALK expression was immunohistochemically detectable in all cases with nuclear and cytoplasmic staining in 5 of 6 cases, indicating an underlying NPM-ALK fusion due to a t(2;5) translocation. In one lymphoma, diffuse cytoplasmic ALK staining without nuclear positivity was noted. Interestingly, in 4 of 6 lymphomas, a small cell component was detectable, as indicated by predominately nuclear staining of small lymphoma cells (Figure 2). All 5 cases tested for epithelial membrane antigen (EMA) were strongly positive. CD3 was negative (4 of 6) or weakly expressed (2 of 6). All lymphomas expressed at least one cytotoxic protein, such as granzyme B, TIA1 or perforin with the characteristic granular staining pattern (data not shown).

Fluorescence in situ hybridization

Material for fluorescence in situ hybridization was available for 4 lymphomas. Breaks in the ALK gene were detectable in all 4 analyzed cases (Figure 1). In the additional patient with multicentric skin disease NPM-ALK-transcripts were detected in the bone marrow and blood by polymerase chain reaction (data not shown) so that the ALK-translocation was confirmed molecularly in 5 of 6 patients. In contrast, breaks affecting the IRF4/DUSP22 locus in 6p25 recurrently involved in cALCL were not detectable in the 3 cases studied.

Clinical characteristics, therapy and outcome

Table 2 summarizes the clinical characteristics of the patients reported in this series. Median age was 10.8 years.
(range 7.5–13.8 years). Three patients were male and 3 female. None of the children had a clinically documented history of lymphomatoid papulosis (LyP) or mycosis fungoides. The lymphomas presented clinically as papulonodular skin lesions (5 of 6) and/or subcutaneous nodules (3 of 6). One patient displayed multiple skin lesions (case 4) which were described as multiple pink nodules on the trunk, arms and neck. The isolated lesions in the other 5 patients involved the thigh (n=5), neck (n=1) or knee (n=1). Figure 1 shows the clinical presentation of one case with a solitary lesion on the thigh (case 6). None of the children suffered from B symptoms. All patients underwent a complete initial staging procedure to exclude systemic disease according to the ALCL99 protocol, including imaging of the abdomen and thorax, full blood cell count and bone marrow cytology. Lumbar puncture was performed in 5 of the 6 patients. In one patient, minimal disseminated disease (MDD) was detectable, measured by polymerase chain reaction for NPM-ALK transcripts in the bone marrow and blood (case 4, Table 2 and data not shown). The single skin lesion was surgically completely resected in 4 of the 5 patients. One patient received addi-
Distinguish primary cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \) from secondary cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \), which show overlappping histological features. Both diseases are characterized by a neoplastic infiltrate of anaplastic \( \text{CD}^{30+} \) T cells with a variable admixture of reactive inflammatory cells. Single nodular skin lesion or, less frequently, multiple nodules that do not undergo spontaneous regression are the typical presentation of \( \text{cALCL} \).\textsuperscript{1,13} Distinguishing a primary cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \), such as LyP and \( \text{cALCL} \), from secondary involvement of the skin by systemic \( \text{ALCL} \) is clinically relevant. Treatment of systemic \( \text{ALCL} \) consists of risk-adapted polychemotherapy. Secondary skin involvement is regarded as a clinical risk factor, often utilized to stratify patients to a more aggressive treatment regimen.\textsuperscript{15,20} In contrast, primary cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \), which is limited to the skin and rarely disseminates, usually either resolves spontaneously or is treated locally, e.g. by surgical excision.\textsuperscript{13}

All of our cases fulfilled the clinical and histological criteria of a primary cutaneous anaplastic large cell lymphoma with predominantly solitary skin lesions, no history of LyP, no extracutaneous dissemination and response to local therapy, but all cases were \( \text{ALK}^+ \). Given the higher incidence of \( \text{cALCL} \) in adults, most published series analyzing \( \text{ALK} \) expression have included predominately adult patients.\textsuperscript{23} There have been only single case reports and small series of pediatric \( \text{cALCL} \), and in these \( \text{ALK} \) staining was inconsistently performed.\textsuperscript{24-28} We assume that our series is not population-based as cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \) are diagnosed and treated either by dermatologists or pediatric oncologists. Nevertheless, our data suggest that \( \text{ALK}^+ \) \( \text{cALCL} \) might be more frequent than anticipated within the pediatric population, and recommend that all \( \text{CD}^{30+} \) \( \text{LPD} \) of the skin in children should be carefully analyzed for \( \text{ALK} \) expression.

Lamant \textit{et al.}\textsuperscript{22} recently reported 5 children with systemic \( \text{ALK}^+ \) \( \text{ALCL} \) that presented as skin lesions at the site of preceding insect bites, often with involvement of the draining local lymphnode. Thus, the skin might not only present a preferred microenvironment for \( \text{ALK}^+ \) \( \text{ALCL} \) but might even be the primary site of lymphomagenesis. At the moment, no reliable histopathological features are known to distinguish secondary skin involvement by a systemic \( \text{ALCL} \) from primary cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \). EMA has been reported to be positive in most systemic \( \text{ALK}^+ \) and \( \text{ALCL} \),\textsuperscript{20} but negative in \( \text{cALCL} \).\textsuperscript{15,16} \( \text{ALK} \) protein expression, as well as the underlying \( \text{ALK}\)-gene translocation, are considered indicative of systemic \( \text{ALK}^+ \) \( \text{ALCL} \) and are seen in nearly all pediatric systemic \( \text{ALCL} \) cases.\textsuperscript{20,21} In contrast, \( \text{cALCL} \) is considered \( \text{ALK}^+ \) both at the molecular and the protein level.\textsuperscript{15,16} Our cases were \( \text{ALK} \) and EMA.

**Table 3. Literature review of reported \( \text{ALK}^+ \) cutaneous large cell lymphomas and findings in this series.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Localization</th>
<th>( \text{ALK} ) expression pattern</th>
<th>Therapy of first lesion</th>
<th>Local dissemination</th>
<th>Distant dissemination</th>
<th>Number of recurrences reported</th>
<th>Treatment of recurrent lesions</th>
<th>Systemic dissemination</th>
<th>Outcome</th>
<th>Observation period in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan \textit{et al.}\textsuperscript{56} 33 m</td>
<td>multiple: trunk, head, leg</td>
<td>nuclear and cytoplasmic</td>
<td>6 cycles of chemotherapy\textsuperscript{1}</td>
<td>no</td>
<td>yes</td>
<td>2</td>
<td>excision, chemotherapy</td>
<td>systemic relapse 2 years after diagnosis</td>
<td>CCR</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Kadin \textit{et al.}\textsuperscript{57} 57 m</td>
<td>single lesion leg</td>
<td>cytoplasmic\textsuperscript{2}</td>
<td>surgical</td>
<td>no excision</td>
<td>yes</td>
<td>6</td>
<td>surgical excision and radiotherapy</td>
<td>no</td>
<td>CCR</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td>Sasaki \textit{et al.}\textsuperscript{57} and Hosoi \textit{et al.}\textsuperscript{57} 57 f</td>
<td>single lesion forehead</td>
<td>cytoplasmic</td>
<td>spontaneous regression without treatment</td>
<td>no</td>
<td>yes</td>
<td>several</td>
<td>total excision and radiotherapy</td>
<td>systemic relapse 2.5 years after diagnosis, 3 years later systemic relapse and DOD</td>
<td>DOD</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Beylot-Barry\textsuperscript{39}</td>
<td>1/26 reported primary cutaneous ( \text{CD}^{30+} ) lymphomas\textsuperscript{1}</td>
<td>nuclear and cytoplasmic</td>
<td>6 cycles CHOP\textsuperscript{1}</td>
<td>no</td>
<td>no</td>
<td>0</td>
<td>no</td>
<td>no</td>
<td>all CCR</td>
<td>mean: 65 (range 12-96)</td>
<td></td>
</tr>
<tr>
<td>Su \textit{et al.}\textsuperscript{33} 57 f</td>
<td>multiple lesions: trunk</td>
<td>cytoplasmic</td>
<td>6 cycles CHOP\textsuperscript{1}</td>
<td>no</td>
<td>no</td>
<td>0</td>
<td>no</td>
<td>no</td>
<td>all CCR</td>
<td>mean: 65 (range 12-96)</td>
<td></td>
</tr>
</tbody>
</table>

\( \text{ALK} \) expression have included predominately pediatric cases, and in these \( \text{ALK} \) staining was inconsistently performed.\textsuperscript{24-28} We assume that our series is not population-based as cutaneous \( \text{CD}^{30+} \) \( \text{LPD} \) are diagnosed and treated either by dermatologists or pediatric oncologists. Nevertheless, our data suggest that \( \text{ALK}^+ \) \( \text{cALCL} \) might be more frequent than anticipated within the pediatric population, and recommend that all \( \text{CD}^{30+} \) \( \text{LPD} \) of the skin in children should be carefully analyzed for \( \text{ALK} \) expression.


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