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Cutaneous presentation of ALK-positive anaplastic large cell lymphoma following insect bites: evidence for an association in 5 cases

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ABSTRACT

Background
Skin involvement is frequent in ALK-positive ALCLs. The role of insect bite as a triggering event has been postulated but not well documented.

Design and Methods
We retrospectively investigated five cases of ALK-positive ALCL that presented with skin lesions occurring after an insect bite. Biopsies were immunostained with antibodies against CD30, ALK, T and B-cell antigens.

Results
Persistent skin lesions developed after solitary insect bites in three patients and multiple bites in two. Regional lymphadenopathy developed within weeks after the bite in three cases. In four cases diagnosis was delayed due to misdiagnosis as a reactive infiltrate in skin (2) or lymph node (2) respectively; all subsequently revealed small numbers of cells with nuclear and cytoplasmic staining for ALK. Final diagnoses were lymphohistiocytic variant (3) or composite common/ small cell type (2) of ALCL. After treatment three patients were alive at the last follow up. Two patients died, one of pneumonia and one of disseminated disease.

Conclusions
In these cases the sequence of events between the insect bites, and the occurrence of both skin lesions and satellite lymphadenopathy suggest a direct relationship between the bite and the presentation with ALCL. We postulate that insect bite associated-antigens could result in an influx of T lymphocytes, some bearing the t(2;5). The subsequent release of cytokines at the site of bite could act as a second hit to elicit the activation of the latter cells, which then would express the oncogenic NPM-ALK protein and undergo an uncontrolled proliferation.

Key words: ALK-positive, NPM-ALK, skin involvement.


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**Introduction**

Anaplastic Lymphoma Kinase (ALK)-positive anaplastic large cell lymphomas (ALCL) is recognized as a distinct entity in the 2008 World Health Organization (WHO) classification of malignant lymphomas. The majority of ALCLs are associated with a reciprocal translocation, t(2;5)(p23;q35) which juxtaposes the gene at 5q35 encoding nucleophosmin (NPM), a nucleolar-associated phosphoprotein, with the gene coding for a tyrosine kinase receptor, the anaplastic lymphoma kinase (ALK), at 2p23. However, variant translocations involving ALK and other partner genes on chromosomes 1, 2, 3, 17, 19 and 22 have been described. All result in abnormal expression of ALK chimeric proteins, with tyrosine kinase activity and oncogenic properties. ALK fusion proteins can be detected with anti-ALK antibodies that are of crucial diagnostic value to identify lesions that may resemble ALK-positive ALCL morphologically and phenotypically (i.e. CD30 expression, T/Null phenotype), such as ALK-negative ALCL and some primary cutaneous CD30-negative lymphoproliferative disorders (i.e. primary cutaneous ALCL and lymphomatoid papulosis). ALK-positive ALCLs are characterized by frequent extranodal involvement, notably skin involvement, (reported in 20%-30% of cases) which carries negative prognostic impact. Due to the broad morphologic spectrum of ALK-positive ALCL, the diagnosis ALCL presenting with cutaneous involvement is not always easy, a finding which explains the original histopathologic diagnosis of “non-malignant inflammatory disease” in some cases. In occasional cases the latter erroneous diagnosis may be suggested by a clinical history of insect bite. Interestingly, the role of insect bite as a triggering event of systemic ALCL has sometimes been postulated even though its role remains controversial.10 We report here five cases of systemic ALK-positive ALCL presenting at onset with skin lesions occurring after an insect bite.

**Design and Methods**

Between 1984 and 2007, five children with similar clinical and histological features were identified among a series of more than 400 patients with ALK-positive ALCL from different institutions. The clinicopathologic features of one of these cases (case 4) has been previously reported. They all presented with a systemic ALK-positive ALCL occurring after an insect bite. Clinical data were collected from patients’ charts and included age at diagnosis, presenting symptoms, clinical stage of disease, treatment, and follow-up. Diagnosis of ALK+ ALCL was made on a skin biopsy in one case (n°3), both skin and lymph node biopsy in two cases (n°2 & 5) and on a lymph node biopsy in the two remaining cases (n°1 & 4). In case 1, a lung biopsy was also performed because of pulmonary atelectasis. Hematoxylin and eosin (H&E) and immunohistochemical-stained slides from archival material were reviewed and additional stains performed when paraffin blocks were available. In case 5, immunostaining was performed on de-stained H&E sections as previously described.11 Overall, in addition to monoclonal antibodies against CD30/BerH2 and ALK, most cases were immunostained for EMA and several T-cell (CD2, CD3, CD4, CD5, CD7, CD8, CD43) and B-cell markers (CD20, CD79a). Antibody binding was detected with Dako REAL Detection System (Code K5001).

**Results**

Clinical features at presentation, diagnosis, treatment and follow-up data are summarized in Table 1. Three patients were male and two female aged from 7 to 11 years. They received solitary (cases 1, 3 & 4) or multiple insect bites (cases 2 & 5). Four of them (cases 1, 2, 3, 5) presented with persistent or growing skin lesion at the site of the bite. Satellite lymphadenopathy developed a few weeks after the bite in two cases (cases 1 & 2). In one case (n°4), supraclavicular lymphadenopathy, one month after a neck bite, was the presenting symptom. All patients received antibiotics or steroids with no response in four cases (n°1, 2, 3 & 4) or partial response (case 5). Four patients had fever at the time of diagnosis and two of them underwent deterioration of their general condition with weight loss, asthenia, and pulmonary signs (cases 1 & 4). In one case (n°4), the nature of the insect bite was clearly identified as being a tick bite on the neck one month earlier with positive test for Rickettsia. A tick bite was also suspected in case 1 but no serologic testing was performed. In case 3, according to the patient’s family, the skin lesions appeared after a wasp bite and in the two remaining cases (n°2 and 5) the nature of the insect bites remained undetermined. However, the clinical history of case 5 left no doubt regarding the crucial role of insect bites in the presentation of the disease. Briefly, a 9 years old white male went on a camping trip in May 1984 with his boy scout troop. He received multiple insect bites. Most of these resolved but, 2-3 weeks later he developed new lesions on the back and neck, up to the size of 2 cm. After a course of steroids, all the lesions resolved except one on the left flank. The lesion was biopsied and a diagnosis of nonspecific reactive infiltrate was originally made (Figure 1A). After the first biopsy, the lesion did not heal and progressively enlarged. A new skin biopsy was performed in February 1985 and led to the diagnosis of “large cell immunoblastic lymphoma”. Approximately one week later the patient underwent a surgical resection of a left axillary lymph node confirming the diagnosis of lymphoma (Figure 1C-D).

Except for case 4, previously reported10, all the other cases were observed before the availability of anti-ALK antibodies and only CD30 and EMA stains were initially performed.4,5 All skin and lymph node biopsies were re-examined. In addition to CD30 and EMA, ALK staining and several T and B-cell markers were used. In case 1, the lymph node lesion originally interpreted as lymphadenitis rich in macrophages proved to be an ALK-positive lymphohistiocytic ALCL (Figure 2A). Scattered CD30 and EMA positive cells were positive for ALK protein (Figure 2B). Some large atypical cells with hallmark cell features were associated with a small cell component showing a nuclear-restricted ALK staining. Few months later, the patient developed pulmonary symptoms with cough and atelectasis secondary to a bronchial tumor diagnosed as Ki-1 lymphoma (i.e. CD30+). This lesion consisted of a pure population of large basophilic cells (i.e. hallmark cells) that met the criteria of ALK-positive common type ALCL. Comparable features were observed in the supraclavicular lymph node biopsy from case 4 which appeared after a tick bite on the neck one month earlier (Figure 2C). The diagnosis of hyperimmune reaction was made initially, but...
despite steroids and antibiotics, the patient developed rapid deterioration of her general condition associated with liver and spleen enlargement. Chest X rays showed a mediastinal mass with pulmonary failure. Finally, the diagnosis was revised to ALK-positive lymphohistiocytic ALCL (Figure 2D). A small cell component was also noted in this case after ALK staining (Figure 2D).

All patients presented with skin lesions that were clinically related to insect bites and were biopsied in three instances (n°2, 3 & 5). In one case (n°3) a skin biopsy of a tumor that developed at the site of a wasp bite led to the accurate diagnosis of Ki-1 lymphoma based on morphologic features and reactivity of neoplastic cells with antibodies against CD30, EMA and CD3. ALK staining revealed areas consisting of large cells associated with a dominant population of small to medium sized cells suggesting an ALK-positive ALCL with composite pattern (Figure 3 A-B).

Table 1. Clinical features, histopathologic diagnosis and follow-up of the 5 patients included in the study. 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years)/Sex</td>
<td>11/M</td>
<td>7/F March 1986</td>
<td>7/M</td>
<td>9/F December 1998</td>
</tr>
<tr>
<td>History</td>
<td>Insect bite (tick) on the right shoulder</td>
<td>Undetermined insect bite on the wrist</td>
<td>Clavicular wasp bite</td>
<td>Neck insect bite (tick) (+ serologic test for Rickettsia)</td>
</tr>
<tr>
<td>Chief complaint</td>
<td>Persistent skin lesion on the shoulder after insect bite associated with ipsilateral axillary lymphadenopathy</td>
<td>Wrist skin lesions (n=3)</td>
<td>Supravclavicular subcutaneous mass at the site of wasp bite</td>
<td>Supravclavicular lymphadenopathy</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Supravclavicular LN: reactive lymph node</td>
<td>First skin biopsy: reactive lymphoid hyperplasia</td>
<td>Subcutaneous mass: ALCL Composite pattern (common plus small cell patterns)</td>
<td>LN biopsy: reactive lymph node</td>
</tr>
<tr>
<td></td>
<td>Lymphohistiocytic pattern (later proved to be positive for ALK)</td>
<td>Lymphohistiocytic pattern</td>
<td>Positive for ALK1)</td>
<td>Lymphohistiocytic pattern</td>
</tr>
<tr>
<td></td>
<td>April 1986: lung atelectasis with bronchial tumor: “Ki-1 lymphoma”</td>
<td>First skin biopsy: reactive lymphoid hyperplasia</td>
<td>(later proved to be positive for ALK1)</td>
<td>First Skin biopsy: reactive lymphoid hyperplasia, later shown to contain ALK+ cells</td>
</tr>
<tr>
<td>B symptoms</td>
<td>Fever, weight loss</td>
<td>Fever</td>
<td>Fever</td>
<td>Fever</td>
</tr>
<tr>
<td>Stage according to Ann Arbor classification</td>
<td>IV (bone marrow positive)</td>
<td>IV</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>Skin lesions at the time of ALCL diagnosis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Time from insect bite to onset of disease</td>
<td>1 week</td>
<td>3 months</td>
<td>4 months</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Treatment</td>
<td>Methotrexate, doxorubicin, vincristine, prednisone</td>
<td>Methotrexate, doxorubicin, vincristine, prednisone</td>
<td>Methotrexate, doxorubicin, vincristine, prednisone</td>
<td>Methotrexate, doxorubicin, vincristine, prednisone</td>
</tr>
<tr>
<td>Response to treatment</td>
<td>Failure (autologous bone marrow autograft)</td>
<td>Progression</td>
<td>Complete remission</td>
<td>Complete remission</td>
</tr>
<tr>
<td>Overall survival</td>
<td>144 months</td>
<td>4 months</td>
<td>21 months</td>
<td>127 months</td>
</tr>
<tr>
<td>Current status</td>
<td>Alive</td>
<td>Dead (cytomegalovirus)</td>
<td>Dead</td>
<td>Alive</td>
</tr>
</tbody>
</table>

1M: male; F: female; LN: lymph node; ALCL: anaplastic large cell lymphoma; with a minor small cell component; ‘B symptoms: fever (i.e., temperature >38°C [>100.4°F]) for 3 consecutive days; weight loss exceeding 10% of body weight for 6 months; drenching night sweats.
wrist in March 1986. A skin biopsy was performed and on conventional examination disclosed a dense lymphoid infiltrate rich in histiocytes (Figure 3C) initially considered to be non-specific reactive infiltrate. The patient was treated with antibiotics but the skin lesion persisted and 3 months later, the patient developed axillary lymphadenopathy that was resected. Immunostaining of the skin biopsy showed scattered medium-sized to large cells highlighted by CD30 and ALK stains in a lymphohistiocytic background (Figure 3D). The lymph node was massively involved by ALK-positive ALCL, lymphohistiocytic pattern. In case 5, the first skin biopsy was also interpreted as reactive infiltrate possibly related to insect bite. Given that no paraffin block was available, the H&E stained section was used to perform ALK staining and revealed scattered strongly positive cells of small to medium size with hallmark feature (arrow) characteristic of ALK-positive lymphohistiocytic ALCL. ALK staining performed later shows some large atypical cells with cytoplasmic, nuclear and nucleolar ALK staining suggesting NPM-ALK protein expression. Figure 2. (A) (hematoxylin-eosin stain, Leika DMD108, magnification x 400). Case 1, the normal lymph node architecture is effaced by a diffuse proliferation containing numerous histiocytes and scarce large cells with hallmark feature (arrow) characteristic of ALK-positive lymphohistiocytic ALCL. (B) (ALK1, Leika DMD108, magnification x 630) ALK staining performed later shows some large atypical cells with cytoplasmic, nuclear and nucleolar ALK staining suggesting NPM-ALK protein expression. (C) (hematoxylin-eosin stain, Leika DMD108, magnification x 400) Morphologic appearance of lymph node biopsy of case 4 initially considered a hyperimmune reaction and finally diagnosed lymphohistiocytic ALCL with hallmark cells (arrow). (D) (ALK1, Leika DMD108, magnification x 200) ALK staining showing scattered large malignant cells and some small cells with nuclear-restricted staining.

All patients were finally treated with multi-agent chemotherapy associated with radiotherapy and autologous bone marrow transplant in one case (n°1). Three patients (cases 1, 4, 5) achieved a complete remission and were alive without any evidence of disease at the last follow-up. Two patients died, one of a Cytomegalovirus associated pneumonia, during an aplastic phase following treatment (case 2) and the remaining patient (case 3) of disseminated disease 19 months after diagnosis.
Discussion

The cases reported here raise the question of whether the insect bite is coincidental or plays a more direct role in the development of some ALCLs. We cannot exclude a coincidental association. However, the clinical context and the sequence of events between the insect bites, and the occurrence of both skin lesions and lymphadenopathy in the corresponding anatomic areas suggest a more direct relationship. In addition to the paper previously reported by Piccaluga et al., similar cases can be found in the literature even though the authors did not stress the potential role of insect bites in the clinical presentation or evolution of ALCL. In the study by Kadin et al., in four of their six cases, there was a clinical diagnosis of insect bite and in two cases initial differential diagnoses of pathologists included non-malignant inflammatory disease. However, it is not clear from their report whether the ALCL skin lesions followed or had been mistaken for arthropod bites. Nevertheless, as in our cases, this study underscores the fact that some skin involvement in ALK-positive ALCL may closely resemble non-specific inflammatory infiltrate, as in cases 2 and 5 of the present study, and that an accurate diagnosis cannot be made without ALK staining which may reveal scarce positive neoplastic cells. ALK staining also may be of crucial diagnostic value in the recognition of some variants of ALCL involving lymph nodes, particularly the lymphohistiocytic subtype. It is noteworthy that the latter ALCL pattern, which is relatively rare (<10% of ALCL), was observed in three (cases 1, 2, 4) of the five cases. All these diagnostic uncertainties were responsible for delayed treatment for weeks to months.

Two other reports mention the notion of an insect bite in the clinical history of patients with ALCL. In 2000, Gould et al. reported the case of a 4-year old girl presenting with a primary cutaneous ALK-positive ALCL that first appeared 6 months earlier coincidental to multiple urticarial arthropod bite reactions. Although, clinical details are missing, this case has some similarities with case 5 of the present study. However, the evolution was different from the latter case since surgical resection was performed, no extra-cutaneous disease was found and the patient has been free of disease for more than 44 months after surgical resection. The final diagnosis was solitary primary cutaneous ALK+ ALCL. The other case was reported in 2007 by Rannan-Eliya and coworkers. A 14 year-old female received an insect bite near umbilicus. Six months later she presented with a fluctuant lesion in the same region and excision and drainage revealed granulomatous tissue without apparent evidence of malignancy. Biopsy of a persistent skin induration revealed an ALK-positive ALCL with further dissemination and fatal relapse in the CNS.

In all these cases the nature of the arthropod is not clearly demonstrated except for the patient reported by Piccaluga et al. who received a tick bite 20 days prior to the diagnosis of ALK-positive systemic ALCL. Serologic tests revealed a weak positivity for anti-rickettsial antibodies; a tick bite was also suspected in case 1 of the present study, with persistent skin lesion and lymphadenopathy but no serologic test was performed. In the literature and in our cases the elapsed time between insect bite and the diagnosis of ALCL varies from few weeks to 6 months.

Ticks carry many pathogens the most common being Borrelia Burgdorferi, the spirochete responsible for Lyme disease that has been implicated in the pathogenesis of at least a subset of cutaneous marginal-zone B-cell lymphomas and in one case of angioimmunoblastic T-cell lymphoma. Several reports in the literature have been published on the puzzling relationship between insect bites and lymphoma. In 1965, Weed and coworkers described the occurrence of unusual cutaneous lesions secondary to insect bites in certain patients with chronic lymphocytic leukemia (CLL). These lesions were attributed to an exaggerated immune response to mosquito antigens maybe due to a greater local mobilization of lymphocytes in these patients. Afterwards, this phenomenon has been reported not only in patients with CLL but also in patients with other lymphoproliferative diseases. However, as only few patients recalled arthropod assaults the role of insect bite to initiate the eruption has remained controversial and the term insect bite-like reaction has been proposed.
Another example of the role of insect bites in lymphoproliferative disorders is the syndrome of hypersensitivity to insect bites associated with chronic active Epstein Barr virus infection (CAEBV). In this syndrome, CD4+ T cells stimulated by mosquito antigens may reactivate latent EBV-infection in NK/T cells and contribute to their unregulated expansion within the inflammatory skin lesions and in the peripheral circulation. A significant proportion of these patients die of EBV-positive NK/T-cell lymphoma or haemophagocytic syndrome. A comparable scenario can be proposed regarding the possible role of insect bite as a triggering factor in the presentation of ALCL. Insect bite associated-antigens could result in an influx of CD4+ T lymphocytes, some of them bearing the ALK1/B. Blood 1997;89:1394-404. bearing the t(2;5) translocation. The presence of the NPM-ALK fusion transcript has been detected by real time RT-PCR in reactive lymph nodes and normal peripheral blood samples of healthy individuals. The subsequent release of cytokines at the site of insect bite could act as a second hit to elicit the activation and proliferation of the latter cells, which then would express the oncogenic NPM-ALK fusion protein and undergo an uncontrolled proliferation. Alternatively, these children might have occult disease at the time of the bite, with the release of cytokines precipitating influx of neoplastic cells to the site of inflammation via chemotaxis.

Authorship and Disclosures

GD, SP, EJ recruited the patients. LL, GD and EJ wrote the paper. The authors reported no potential conflicts of interest.

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