Frequent Expression of CD99 in Anaplastic Large Cell Lymphoma

A Clinicopathologic and Immunohistochemical Study of 160 Cases

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Abstract

Originally described as a diagnostically useful marker for Ewing sarcoma, CD99 immunoreactivity has also been documented in a variety of other tumors, including hematopoietic neoplasms. By using conventional paraffin immunoperoxidase staining and tissue microarrays, we retrospectively investigated CD99 expression in a series of 160 anaplastic large cell lymphoma (ALCL) cases. Of the 160 cases, 103 (64.4%) were positive for CD99. The distribution of CD99 positivity was similar for nodal (66/103 [64.1%]), extranodal (21/32 [66%]), and primary cutaneous lesions (16/25 [64%]). CD99 expression was present in 96 (64.4%) of 149 of the common type, 4 (80%) of 5 of the small cell variant, and 3 (50%) of 6 of the lymphohistiocytic variant cases. CD99 expression was slightly more frequent in anaplastic large cell lymphoma kinase (ALK)+ cases compared with ALK− cases (43/54 [80%] vs 44/81 [54%]). With 2 exceptions, ALK+ ALCL was seen only in patients younger than 41 years. We conclude that CD99 is frequently expressed in ALCL, with a slightly increased frequency in the younger age ALK+ cases. Nodal and extranodal ALCL should be considered in the differential diagnosis when a CD99+ neoplasm is encountered.

CD99 is a membrane epitope best known for its role as a diagnostically useful marker of Ewing sarcoma/primitive neuroectodermal tumor in childhood.1 However, in adults, it has come to be recognized as present in a variety of other tumors, including some carcinomas,2-4 testicular and ovarian sex cord tumors,5-8 synovial sarcoma,9,10 and an assortment of miscellaneous tumors. Among hematopoietic neoplasms, most studies have shown CD99 immunoreactivity primarily in lymphoblastic lymphoma/leukemia11-13 and acute myelogenous leukemia,14 but some expression has been demonstrated in other non-Hodgkin lymphomas (NHLs). Data on CD99 expression in anaplastic large cell lymphoma (ALCL) is limited primarily to 2 small series (including 1 reported in abstract form) that suggest that half or more of ALCLs may show significant CD99 staining.15,16 Following our encounter with a CD99+ small cell variant of extranodal ALCL, in which the initial immunohistochemical findings were potentially misleading, we undertook a study of a larger cohort of 160 ALCL cases to better characterize CD99 expression in this tumor.

Materials and Methods

We studied 160 cases of ALCL diagnosed between 1995 and 2008. Of these cases, 28 were from Loma Linda University Medical Center, Loma Linda, CA, 131 were in tissue microarrays (TMAs) from Consultoria em Patologia, Botucatu, Brazil, and 1 was from the City of Hope National Medical Center, Duarte, CA. Patient sex, age at diagnosis, and tumor anatomic location were recorded. There were 103 nodal, 32 extranodal, and 25 primary cutaneous lesions. The diagnosis of ALCL was based on the combination of typical
morphologic features and appropriate immunohistochemical antigen expression such as CD30 and/or T-cell markers, T-cell receptor gene rearrangements by molecular studies, and/or expression of anaplastic large cell lymphoma kinase (ALK), according to the criteria of the current World Health Organization classification. For ALK– ALCL cases of T-cell/null-cell phenotype, strong CD30 expression should be seen unequivocally in more than 90% of the neoplastic cells, in addition to the typical morphologic features indistinguishable from those of ALK+ ALCL. The study cases included ALK+ and ALK– cases and a small number of the lymphohistiocytic and small cell variants of ALCL.

TMA Construction

The TMA blocks were constructed for 131 cases of ALCL using a tissue arrayer (Beecher Instruments, Sun Prairie, WI). The available H&E-stained slides for each case were reviewed by us, and representative areas were selected for TMA construction. Each individual case was represented by 3 tumor cores of 0.6 mm taken from the original paraffin blocks. Serial 3-µm sections were cut from the TMA blocks and used for immunohistochemical analysis. Appropriate positive and negative control cores for each marker were also included in the array block as a check on antibody reactivity. Reading of the TMA was performed by scanning the slides with an Aperio ScanScope GL and examination of the scanned images using the Aperio ImageScope software (Aperio Technologies, Vista, CA) to facilitate systematic study of the multiple cores present on the sections.

Immunohistochemical Analysis

Primary antibodies, source, dilution, and antigen retrieval methods, as well as the institutions that performed the stains, are shown in Table 1. CD99 immunohistochemical staining in the majority of cases (n = 153) was performed at the Immunohistochemical Laboratory, Division of Pathology, of the City of Hope National Medical Center. Briefly, immunoperoxidase staining of conventional paraffin sections and TMA sections (Image 1) was performed using a heat-induced epitope retrieval technique by pressure cooker and a universal secondary antibody kit that used a peroxidase-conjugated dextran polymer (DAKO EnVision+ System, Peroxidase, DAKO, Carpinteria, CA). Commercially purchased primary antibodies directed against CD99 (O13, 1:160 dilution; Signet Laboratories, Dedham, MA) and ALK-1 protein (DAKO), in addition to terminal deoxynucleotidyl transferase (TdT; Ventana, Tucson, AZ) for the small cell variant of ALCL, were used. Diaminobenzidine was used as a chromogen. Normal rabbit and/or mouse serum was used as the negative control sample. Additional control samples omitted the primary antibody. The remaining 7 cases were stained for CD99 at Department of Pathology and Human Anatomy, Loma Linda University School of Medicine, Loma Linda, CA, on a Ventana Benchmark XT automated stainer using standard procedures and reagents as noted in Table 1, with the Ventana ultraView Universal DAB detection kit for visualization of antibody localization. Immunoreactivity for CD99 was considered positive if at least 25% of the neoplastic cells demonstrated a membranous pattern of staining.

Results

Of the cases for which demographic information was available, there were 94 males and 65 females, aged 4 to 92 years. Morphologically, most cases were of the “common” type (149/160 [93.1%]), with 5 (3.1%) of 160 diagnosed as the small cell variant and 6 (3.8%) of 160 as the lymphohistiocytic variant of ALCL.

The expression frequencies of CD99 and ALK in various groups of ALCL were summarized in Table 2 and Table 3. Of the 160 cases, 103 (64.4%) were positive for CD99. Positive staining for CD99 was observed in 66 (64.1%) of 103 nodal, 21 (66%) of 32 extranodal, and 16 (64%) of 25 cutaneous ALCLs. CD99 expression was present in 96 (64.4%) of 149 cases of the common type ALCL, 4 (80%) of 5 cases of the small cell variant, and 3 (50%) of 6 cases of the lymphohistiocytic variant. Of 103 nodal cases, 45 (43.7%) were ALK+, as were 9 (28%) of 32 extranodal cases; all 25 primary cutaneous cases were ALK–. CD99 and ALK coexpression in nodal (n = 36) and extranodal (n = 7) sites occurred more frequently than CD99 expression.
Buxton et al / CD99 Immunoreactivity in Anaplastic Large Cell Lymphoma

**Table 1**

Antibodies Used and Technical Details

<table>
<thead>
<tr>
<th>Antibody (Institution Where Tested)</th>
<th>Source</th>
<th>Clone</th>
<th>Dilution</th>
<th>Antigen Retrieval</th>
<th>No./Total Tested (%) With Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD99 (COH)</td>
<td>Signet Laboratories, Dedham, MA</td>
<td>O13</td>
<td>1:160</td>
<td>PC-EDTA</td>
<td>99/153 (65)</td>
</tr>
<tr>
<td>CD99 (LLU)</td>
<td>Ventana Medical Systems, Tucson, AZ</td>
<td>HO36-1.1</td>
<td>1:200</td>
<td>CC1 (TRIS)</td>
<td>4/7 (57)</td>
</tr>
<tr>
<td>ALK (CP)</td>
<td>Dako, Carpinteria, CA</td>
<td>ALK1</td>
<td>1:100</td>
<td>S-TRIS</td>
<td>41/131 (31)</td>
</tr>
<tr>
<td>ALK (COH)</td>
<td>Dako</td>
<td>ALK1</td>
<td>1:25</td>
<td>O-BW</td>
<td>6/16 (38)</td>
</tr>
</tbody>
</table>

ALK, anaplastic large cell lymphoma kinase; BW, bond wash (Vision BioSystems, Mount Waverley, Victoria, Australia); CC1, cell conditioning solution 1; COH, City of Hope National Medical Center, Duarte, CA; CP, Consultoria em Patologia, Botucatu, Brazil; GNZ, Genzyme, Los Angeles, CA; LLU, Loma Linda University Medical Center, Loma Linda, CA; M, microwave; O, oven baked; PC, pressure cooker; S, steamer; TRI, tris(hydroxymethyl)aminomethane (Tris).

* The Ventana clone is provided as a prediluted commercial solution.
† Antigen retrieval is automated on the Ventana BenchMark XT.

**Table 2**

Expression of CD99 in 160 Cases of ALCL

<table>
<thead>
<tr>
<th>Group</th>
<th>No. (%) With Positive Staining</th>
<th>No. of Cases Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>103 (64.4)</td>
<td>160</td>
</tr>
<tr>
<td>Skin ALCL</td>
<td>16 (64)</td>
<td>25</td>
</tr>
<tr>
<td>Nodal ALCL</td>
<td>66 (64.1)</td>
<td>103</td>
</tr>
<tr>
<td>Extranodal ALCL</td>
<td>21 (66)</td>
<td>32</td>
</tr>
<tr>
<td>All ALK+</td>
<td>43 (80)</td>
<td>54</td>
</tr>
<tr>
<td>All ALK–</td>
<td>60 (56.6)</td>
<td>106</td>
</tr>
<tr>
<td>Small cell variant</td>
<td>4 (80)</td>
<td>5</td>
</tr>
<tr>
<td>Lymphohistiocytic variant</td>
<td>3 (50)</td>
<td>6</td>
</tr>
</tbody>
</table>

ALCL, anaplastic large cell lymphoma; ALK, anaplastic large cell lymphoma kinase protein.

In ALK– cases (43/54 [80%] vs 44/81 [54%]), excluding primary cutaneous ALCL. By $\chi^2$ analysis, the association between CD99 and ALK positivity was statistically significant ($P = .003$). Cases in the TMA showed 62.6% (82/131) positivity for CD99, similar to but slightly less than that observed with conventional paraffin sections (73% [21/29]). The staining pattern of CD99 was predominantly membranous Image 21. A few cases showed membranous staining combined with cytoplasmic Image 31 or, rarely, Golgi region staining Image 41. Staining for ALK-1 generally showed a diffuse nuclear and cytoplasmic pattern but was occasionally primarily membranous/cytoplasmic or cytoplasmic/granular in its localization (not shown).

**Table 3**

Expression of CD99 in Systemic ALK+ and ALK– ALCL Cases

<table>
<thead>
<tr>
<th>Group</th>
<th>CD99+/Total ALK+</th>
<th>CD99 Expression (%)</th>
<th>CD99+/Total ALK–</th>
<th>CD99 Expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodal ALCL</td>
<td>36/45</td>
<td>80</td>
<td>30/58</td>
<td>52</td>
</tr>
<tr>
<td>Extranodal ALCL</td>
<td>7/9</td>
<td>78</td>
<td>14/23</td>
<td>61</td>
</tr>
<tr>
<td>All systemic ALCL</td>
<td>43/54</td>
<td>80</td>
<td>44/81</td>
<td>54</td>
</tr>
</tbody>
</table>

ALK, anaplastic large cell lymphoma kinase protein.

In cases for which the sex of the patient was known, the CD99 expression rate was 63% (59/94) in males and 66% (43/65) in females. The average patient age in CD99-expressing cases was 37.4 years compared with 47.5 years in CD99– cases, but the SDs were wide (21-24 years; $P = .03$).

ALK+ ALCL was seen only in patients younger than 41 years, with the exception of 2 patients aged 63 and 74 years. ALK positivity was found in 45 nodal and 9 extranodal,
noncutaneous cases. In cases for which the sex of the patients was known, 33 (35%) of 94 males and 20 (31%) of 65 females had ALK+ tumors. The average age of patients with ALK+ tumors was 21.4 years (SD, 13.9 years) compared with 54.0 years in ALK− cases (SD, 18.6 years; \( P < .0001 \)). However, after adjusting for age, the association between CD99 and ALK was of only marginal statistical significance (\( P = .09 \)).

None of the 5 small cell variant cases showed nuclear expression of the TdT antigen, as they might be confused morphologically with lymphoblastic lymphoma, in which nuclear expression of TdT antigen is usually seen. TdT staining was not performed on the common type and lymphohistiocytic variant of ALCL because the morphologic features and CD30 expression were sufficient to distinguish them from lymphoblastic lymphoma.

Discussion

ALCL is a T-cell lymphoma characterized by lymphoid cells that are usually large, with abundant cytoplasm, and pleomorphic, often horseshoe-shaped, nuclei. Small cell and lymphohistiocytic variants are recognized. There are 2 clinical forms of ALCL—primary systemic ALCL and primary cutaneous ALCL. Primary systemic ALCL accounts for approximately 3% of adult and 10% to 20% of childhood NHLs, occurs more often in males during the first 3 decades of life, and in about 70% of patients manifests as advanced stage III or IV disease with B symptoms present at diagnosis. The neoplastic cells coexpress CD30 antigen and ALK protein in most younger patients, whereas ALK− primary systemic ALCL is more common in older people. ALK+ primary systemic ALCL frequently involves lymph nodes and extranodal sites, such as the skin, bone, soft tissues, lung, and liver, but rarely the gut or central nervous system, whereas ALK− primary systemic ALCL shows less frequent extranodal involvement. Cytogenetic studies may show a characteristic t(2:5)(p23;35) translocation that results in NPM-ALK fusion or other variant translocations involving the ALK gene on chromosome 5.

In contrast, primary cutaneous ALCL only rarely expresses ALK and is more common in older people.

CD99 is a 32-kDa integral transmembrane glycoprotein produced by the MIC2 gene, a pseudoautosomal gene located on the X and Y chromosomes. Functionally, CD99 has a role in mediating T-cell interactions and apoptosis. CD99 is found on endothelial cells and is involved in the migration of monocytes. CD34+ progenitor cells also show CD99 expression that correlates with the differentiation of these cells, their proliferative potential, and their ability to migrate. In lymph nodes, activated T cells or memory B cells may show CD99 up-regulation. In thymocytes, CD99 is involved in the up-regulation of surface molecules that are associated with maturation in the cell, while absence of this up-regulation leads to apoptosis. In normal tissue, CD99 is often expressed in Sertoli cells, granulosa cells, and ependyma. In tumor cells, such as in Ewing sarcoma, CD99 is linked with the ability of these cells to migrate and with tumor differentiation.
Although best known for its role as a useful marker in the diagnosis of Ewing sarcoma,\(^1\) the list of tumors known to express CD99 has expanded with time and includes metaphasic breast carcinoma,\(^2\) pleomorphic lung,\(^3\) and stomachcarcinomas, testicular and ovarian sex cord tumors,\(^5,6\) synovial sarcoma,\(^9,10\) chondrosarcoma,\(^31\) ependymoma,\(^28\) solitary fibrous tumor,\(^32\) neuroendocrine tumors,\(^33-36\) lymphoblastic lymphoma/leukemia,\(^11-13\) and acute myelogenous leukemia.\(^14\) The reported staining pattern in these neoplasms includes membranous and cytoplasmic staining and can be focal or diffuse, but in Ewing sarcoma and lymphoblastic lymphoma, where this antibody is most useful, only a membranous pattern is considered diagnostic.\(^3-15\)

Recently, CD99 expression has also been demonstrated in other NHLs, including diffuse large B-cell lymphoma, Burkitt lymphoma, and CD30- peripheral T-cell lymphoma, as well as ALCL in 2 relatively small series.\(^15,16\) In the study by Sung et al,\(^15\) a wide range of lymphomas were examined, with CD99 expression most often seen in lymphoblastic lymphoma, diffuse large B-cell lymphoma, Burkitt lymphoma, and ALCL (8/15 [53%]). A study by Gurevich et al\(^16\) focused on pediatric lymphomas and also demonstrated frequent CD99 expression in ALCL (13/18 [72%]) and in CD30- peripheral T-cell lymphoma. Our study confirms the frequent expression of CD99 in a larger cohort (160 cases) of ALCL (103/160 [64.4%]). Furthermore, we demonstrated that this finding is relatively independent of the antibody used because the DAKO antibody for CD99 was used in the studies by Sung et al\(^15\) and Gurevich et al,\(^16\) whereas we obtained similar results using antibody clones from Signet (64.7% positive) and Ventana (57% positive).

Our findings also confirm the observation of Sung et al\(^15\) that CD99 expression is more common in ALK+ cases than ALK- cases, although the difference was not as marked in our study (Sung et al,\(^15\) 70% vs 20%; our study, 80% vs 54%) and appears to likely mostly reflect the younger age of ALK+ patients, as CD99 expression and ALK expression are more frequent in younger patients. This may explain the higher frequency of CD99 expression observed by Gurevich and associates\(^16\) as well, as their population consisted of pediatric patients in whom ALCL more frequently expresses ALK. Our study also demonstrated that CD99 expression can be observed in the small cell and lymphohistiocytic variants of ALCL, as well as in the classic form.

The frequent expression of CD99 in the small cell variant of ALCL is of particular interest, even though the number of such cases in our series was small. The morphologic differential diagnosis of the small cell variant is rather broad, and failure to recognize that this variant can be CD99+ might result in misdiagnosis as lymphoblastic lymphoma or extraosseous Ewing sarcoma.

The relatively high frequency of CD99 expression in ALCL observed by us and others\(^15,16\) is intriguing with regard to a potential role of CD99 in the pathogenesis and/or development of ALCL. CD99 has been shown to have a role in mediating T-cell interactions and inhibiting apoptosis,\(^22,23\) and to be up-regulated in activated T cells. Thus, the biologic role of CD99 in ALCL may be worthy of further investigation.

Finally, when using antibodies such as CD99 for diagnostic purposes, the pattern of staining may be as important as the presence or absence of staining. In our experience with Ewing sarcoma, cytoplasmic and/or Golgi staining for CD99 is much less specific than a membranous pattern of staining. It was for this reason that we insisted on at least a component of membranous staining before scoring a case as positive in this study. Even with careful attention to the staining pattern, it is clear that ALCL, including the small cell variant, must be included in the differential diagnosis of CD99+ neoplasms.

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References


