

THE VALUE OF CD34, MYELOPEROXIDASE AND CHLORACETATE ESTERASE (LEDER) STAIN IN THE DIAGNOSIS OF GRANULOCYTIC SARCOMA

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Background: The differentiation of extramedullary myelogenous leukemia/granulocytic sarcoma (GS) from malignant lymphoma can sometimes be difficult. In the current study, we explored the value of CD34, myeloperoxidase and nonspecific esterase (Leder) stains in differentiating GS from lymphomas.

Materials and Methods: Fifteen cases of phenotypically confirmed GS were stained for CD34, myeloperoxidase and Leder stains. The same stains were performed in 16 malignant lymphomas as controls. The GS cases were also immunostained for CD3 and CD20 to detect the incidence of aberrant T and B lymphocyte expression.

Results: CD34 was expressed in 7 of the 15 cases of GS (46%). Myeloperoxidase was expressed in 10 of the 15 cases (66%), and Leder stain was positive in 9 cases (60%). All 15 cases had expression of at least one marker; 8 cases had expression of two markers and one case had expression of all 3 markers. None of the lymphomas showed expression of any of the three markers. Five cases (35%) of GS showed T cell antigen expression and 2 (14%) showed B cell antigen expression.

Conclusion: Our findings suggest that in cases of GS, the use of the combination of CD34, myeloperoxidase and Leder stains can help reach a definitive diagnosis, especially if lymphoma is difficult to exclude. Expression of B and T cell antigens in such lesions should not rule out the diagnosis of GS.

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Key Words: Granulocytic sarcoma, immunophenotyping, CD34, myeloperoxidase, Leder.

Granulocytic sarcoma (GS) is an extramedullary neoplasm manifested by the infiltration of immature myeloid cells.¹ The disease manifests itself in any anatomical location,² including the orbit,³ male and female genital tracts,⁴⁻¹⁰ lymph nodes,¹¹ skin,^{12,13} gastrointestinal tract,^{14,15} bone,¹⁶ and soft tissue.¹⁷ The disease may precede,^{7,18} coexist with,^{19,20} or follow¹ overt leukemia, and may be the only presentation of it without peripheral blood or bone marrow involvement.²¹⁻²⁵ It can also be associated with myelodysplastic syndrome.^{13,26,27}

Phenotyping of a leukemic myeloid infiltrate can be easily achieved using flow cytometric immunophenotyping.^{5,12} This can also be confirmed using cytogenetics.²⁸ The diagnosis of GS may, however, be difficult on tissue sections.^{23,29} This is mainly due to the fact that lymphoma is usually the most common diagnosis, especially in the absence of overt leukemia. The misdiagnosis may be as high as 40%.²³ Furthermore,

granulocytic sarcomas may paradoxically express T and B cell antigens.²⁹

We reviewed 15 cases of phenotypically proven granulocytic sarcomas which were retrieved from the files of King Faisal Specialist Hospital and Research Centre (KFSH&RC) and the Riyadh Military Hospital (RKH), and stained them with myeloperoxidase and CD34 antibodies. We also used the chloroacetate esterase (Leder) stain to see if these stains would help confirm the myeloid nature of the neoplasms in tissue. The tumors were also stained for the T and B lymphocyte markers CD3 and CD20, respectively, to assess the incidence of aberrant expression of T and B cell markers in these neoplasms. Lymphomas were used as controls to see if these three markers are expressed in the neoplasms.

Materials and Methods

Fifteen cases of granulocytic sarcoma diagnosed between 1990 and 1999 were retrieved from the files of KFSH&RC and RKH. Another 16 cases of confirmed malignant non-Hodgkin's lymphoma were retrieved from the files of KFSH&RC and used as controls. All hematoxylin and eosin-stained slides were reviewed and one representative section was selected for immunohistochemical and histochemical stains. Histochemical stain

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TABLE 1. Age and sex distribution of patients with tumor location associated with leukemia or not.

Case No.	Age	Sex	Location	Presence of overt leukemia
1	37	M	Hip	Yes
2	84	F	Stomach	No
3	19	M	Cervical LN	Yes
4	1	F	Inguinal LN	Yes
5	3	F	Cervical LN	Yes
6	8	M	Cervical LN	Yes
7	18	M	Cervical LN	Yes
8	33	F	Cervical LN	No
9	20	F	Lumbar vertebrae	Yes
10	23	M	Frontal mass	Yes
11	52	M	Maxillary sinus	No
12	33	M	Scalp	Yes
13	38	F	Cervical LN	Yes
14	44	F	Endometrium	Yes
15	40	F	Cervical LN	Yes

TABLE 2. Phenotype of the 15 cases of granulocytic sarcoma.

Case No.	CD34	Myeloperoxidase	Leder	CD3	L26
1	-	-	+	+	-
2	-	+	+	-	+
3	+	-	+	-	-
4	+	-	+	-	-
5	+	-	-	-	-
6	-	+	-	+	-
7	-	+	+	-	-
8	-	-	+	-	-
9	+	+	-	+	-
10	-	+	+	-	-
11	+	+	-	+	-
12	+	+	-	-	-
13	+	+	+	-	+
14	-	+	-	-	-
15	-	+	+	-	-

for the chloroacetate esterase (Leder) stain was performed.³⁰ Immunohistochemical stains were performed for CD3, CD20, myeloperoxidase (DAKO) and CD34 (Navocastra) on the above-mentioned selected slides. The immunoperoxidase technique with microwave antigen retrieval was used.³¹ Assessment of the cell type and the architectural pattern was performed. The cases were proven to be leukemia by flow cytometric immunophenotyping, cytogenetic and/or molecular genetic analysis of the same or subsequently obtained tissue.

Results

The 15 patients (7 male and 8 female) had an age range of 3 to 84 years, with a median of 33 years. Eleven of the patients were younger than 40 years. Seven patients had extramedullary involvement prior to the development of leukemia, 5 patients had extramedullary involvement subsequent to the leukemia and 3 patients had no leukemic

manifestation prior or subsequent to the diagnosis of granulocytic sarcoma. Table 1 shows the age and sex distribution and the association with leukemia.

Two of the seven cases where granulocytic sarcoma preceded the leukemia were originally diagnosed as non-Hodgkin's lymphoma. Two of the three cases without leukemia were diagnosed as lymphoma. The erroneous diagnosis was rendered because aberrant expression of T or B cell antigens were expressed in the malignant cases. All four cases were proven to be acute myelomonocytic leukemias (M4). The remaining cases were M2 (n=5) and M3 (n=6). Two of the patients received chemotherapy for non-Hodgkin's lymphoma with no clinical response. They subsequently developed leukemia and were adequately treated.

Histological examination of the involved organs, especially lymph node, showed a diffuse infiltrate mainly in the paracortical region. The infiltrates were mainly composed of small-to-intermediate cells with immature chromatin, irregular nuclear membrane and prominent small nucleoli (Figure 1).

CD34 was expressed in 7 of the 15 cases of GS (46%), and ranged from 10%-60% (Figure 2). Myeloperoxidase was present in 10 cases (66%), with expression similar to that seen with CD34 (Figure 3). The Leder stain was positive in 9 cases (Figure 4). All 15 cases were positive for at least one marker, 10 cases showed expression of two markers (66%) and only one case showed expression of three markers (Table 2). Four cases (26%) of GS showed expression of T cell antigen, and 2 cases (13%) expressed the B cell antigen. None of the lymphomas expressed any of the three myeloid markers.

Discussion

The differentiation of GS from malignant lymphoma is sometimes difficult, even more so when GS is expressing B or T cell markers. The differentiation of these two types of malignancy is essential, as the treatment of GS is significantly different from that of malignant lymphoma.³² We had four cases in our files that had been erroneously diagnosed as non-Hodgkin's lymphoma. Two of the patients had received treatment for non-Hodgkin's lymphoma without demonstrable clinical response. Both cases showed T or B cell antigen expression. A large study has shown that the diagnosis of granulocytic sarcoma is missed in a large proportion of cases.²³

In our study, we explored the value of CD34, myeloperoxidase and Leder stain in differentiating GS from malignant lymphoma and in confirming the myeloid nature of the neoplasm. CD34 is a monoclonal antibody directed against the surface glycoprotein on normal myeloid and mixed colony-forming cells.⁷ It helps differentiate GS from malignant lymphoma, acute lymphocytic leukemias, and would help recognize early myeloid cells.¹² The antibody is



FIGURE 1. Case of granulocytic sarcoma showing a diffuse infiltrate of intermediate-sized cells with irregular nuclear membrane, small nucleoli and high mitotic rate (H&E, 40x).



FIGURE 2. Case of granulocytic sarcoma showing expression of CD34 in approximately 40% of the cells (immunostain, 40x).

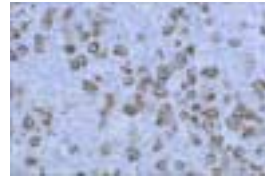


FIGURE 3. Case of granulocytic sarcoma showing expression of myeloperoxidase in approximately 70% of the cells (immunostain, 40x).



FIGURE 4. Case of granulocytic sarcoma showing expression of nonspecific esterase (Leder) in approximately 10% of the cells (histochemistry, 40x).

specific to very early progenitor (blast) cells, and shows high specificity but low sensitivity. CD34 was expressed in less than half of our cases. If the leukemic infiltrate is predominantly differentiated, expression of CD34 would be less likely. A second marker is therefore needed to identify more mature myeloid cells. The marker used in this study was myeloperoxidase. This antimyeloperoxidase is the lysosomal enzyme localized in the azurophil granules of neutrophils and monocytes.⁵ These azurophil granules are known to represent the large electron-dense granules (primary granule) seen on electron microscopic studies. The granules are absent in the lymphoid cells (normal or neoplastic). The marker is very sensitive,⁵ and was seen in approximately two-thirds of our cases. Myeloperoxidase is considered the most sensitive marker for the detection of granulocytic sarcoma.³ Although it may be more sensitive than CD34, it will not be expressed on the surface of blasts. The third stain used in this study was the histochemical

reaction involving chloroacetate esterase (Leder) present in granulocytes and mast cells, and is different from the one seen in monocytes and megakaryocytes.^{5,19,30} Leder stain also exhibited high sensitivity but low specificity. CD34 would therefore help identify early progenitor cells, whereas myeloperoxidase and Leder stain would help identify more mature myeloid cells.

The GS cases reviewed in the current study showed positivity of all cases for at least one marker. In contrast, none of the lymphoma cases showed positivity for any of the three stains. The combination of these stains would therefore help identify 100% of the cases of GS, despite the occasional expression of T or B cell markers and even if the histological features are suggestive of malignant lymphoma. Four of our cases were originally given the diagnosis of lymphoma. This paradoxical expression of B or T cell antigens may be explained by the fact that myeloid leukemia arises from a pluripotential stem cell capable of

differentiation along many lines.^{7,29} Cases where T cell antigens were expressed were myelomonocytic leukemias. Other markers that are useful in the identification of granulocytic sarcoma are CD15 and CD68.

Some histological clues on hematoxylin and eosin-stained sections favor the diagnosis of granulocytic sarcoma. These include the presence of eosinophilic myelocytes, small-to-medium-sized cells with irregular nuclear membrane, and small prominent nucleoli and immature chromatin pattern. The immature cells, if present in a lymph node, would form paracortical sheets with abundant mitotic activity. Cytoplasmic granules can be identified in Giemsa-stained sections. This cyto/histological pattern usually does not fit any known entity of malignant lymphoma and should raise the suspicion of granulocytic sarcoma. Malignant lymphomas, on the other hand, would show mature nuclei, for the most part with or without prominent nucleoli if the lymphoma cells are of small or intermediate size. The distinction may, however, not be

easy in some cases based solely on routine histological examination

In conclusion, CD34, myeloperoxidase and Leder stains may help confirm the diagnosis of GS. Overall, the diagnosis of GS should be suspected if it is difficult to categorize the neoplasm in a well-known lymphoma group. This should lead to the performance of immuno- and histochemical stains for CD34, myeloperoxidase and Leder to confirm the diagnosis.

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