

CD163 expression in leukemia cutis

Background: Proper diagnosis of myeloid leukemia cutis (LC) is of great clinical importance but can be difficult because no single immunohistochemical marker is adequately sensitive or specific for definitive diagnosis. Thus, a broader panel of markers is often desirable. CD163 is highly specific for normal and neoplastic cells of the monocyte/histiocyte lineage. In this study, we examined the value of CD163 in the diagnosis of acute myeloid LC.

Methods: A total of 34 cases, including 18 cases of myelomonocytic or monocytic LC, 10 cases of myeloid LC without monocytic component and 6 cases of acute lymphoblastic leukemia/lymphoma (ALL), were stained with CD163.

Results: CD163 was expressed in 8 of 18 (44%) of myelomonocytic or monocytic LC and 1 of 10 (10%) of other myeloid LC, but in none of the ALL cases (0/6). CD163 was highly specific (90%) for myeloid LC with a monocytic component, but showed low sensitivity in the diagnosis of both myeloid LC in general (24%) and myeloid LC with a monocytic component (44%).

Conclusions: Our results suggest that CD163 has utility as a specific marker for myeloid LC in conjunction with currently used immunohistochemical stains, but should not be used alone for diagnosis.

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Acute myeloid leukemia (AML) may form tumor masses in extramedullary sites, with skin being one of the most common sites.¹ The involvement of skin by myeloid leukemic infiltrate, or myeloid leukemia cutis (LC), may precede the appearance of systemic disease, occur concurrently with systemic AML, or herald disease recurrence in treated patients.^{1,2} In addition, the onset of myeloid LC may be a sign that blastic transformation of a pre-existing myelodysplastic syndrome or myeloproliferative neoplasm (previously known as chronic myeloproliferative disease) is occurring.

Recognition of myeloid LC is clinically essential, yet sometimes poses diagnostic difficulties. Particularly, the diagnosis of LC in the absence of known systemic disease (aleukemic LC), which occurs in 7% of cases is challenging.² Clinical manifestations of myeloid LC are non-specific and may present as erythematous rash, urticaria, purpura, plaques or nodules. Histopathologically, early myeloid LC may involve the dermis in an angiocentric pattern, with or

without infiltration between collagen bundles; more developed lesions show diffuse dermal infiltrate of immature granulocytes or monocytes, sparing the epidermis.² In many cases, however, histopathologic features alone are not sufficient to rule out other entities, such as cutaneous lymphoproliferative processes or acute lymphoblastic leukemia/lymphoma (ALL).³ Therefore, immunophenotyping is routinely employed for accurate diagnosis of LC. Useful markers include CD68 (KP1 or PG-M1), myeloperoxidase (MPO), CD117, CD43 and lysozyme, as well as B-cell or T-cell markers. CD68, one of the most sensitive markers, may also stain a variety of other tumor types, including melanomas and carcinomas.^{4,5} MPO is more specific for myeloid lineage, but reports vary regarding its sensitivity, particularly in LC with monocytic differentiation.^{1–3} Rarely, myeloid LC may display aberrant expression of B-cell or T-cell markers.³ In challenging cases, a more complete armamentarium of immunohistochemical markers is desirable in order to increase diagnostic accuracy.

CD163 is a transmembrane glycoprotein in the class B scavenger receptor cysteine-rich (SRCR) family. CD163 exhibits high-affinity binding and endocytosis of the haptoglobin–hemoglobin complex, thus serving as the primary effector of haptoglobin–hemoglobin clearance from circulation.⁶ CD163 expression is largely restricted to the monocyte/macrophage lineage.^{4,5} In bone marrow and lymph nodes, CD163 immunoreactivity is detected in AMLs with monocytic differentiation. Carcinomas, most melanomas and B-cell or T-cell lymphomas were found to be negative for CD163,^{4,5,7,8} although its expression has been reported in 29% of desmoplastic melanomas.⁹

Few reports have examined the expression of CD163 in myeloid LC, and none evaluated its specificity in distinguishing myeloid LC from cutaneous

ALL.^{4,10,11} Because of its specificity for the monocytic lineage and its expression in bone marrow and extramedullary sites other than skin, we explored the expression of CD163 in myeloid LC. We hypothesized that CD163 would be useful as a specific marker for myeloid LC with monocytic differentiation.

Materials and methods

After obtaining approval from the University of Michigan Institutional Review Board for human subject research, 34 consecutive cases of LC were identified through a retrospective search of the pathology database from the Department of Pathology at the University of Michigan. All tumors examined are listed in Table 1.

Table 1. CD163 staining results for LC in comparison to other markers

Case	Diagnosis	CD163	CD68	Lysozyme	MPO	CD43
1	AML-M0	Negative	ND	ND	ND	Positive
2	AML-M1	Negative	ND	ND	Rare positive	ND
3	AML-M1	Negative	ND	Negative	Focal positive	Positive
4	AML-M1	Negative	ND	Negative	Rare positive	Positive
5	AML-M1	Negative	Negative	Negative	Negative	Weak positive
6	AML-M2	>50%	ND	ND	ND	Positive
7	AML-M2	Negative	ND	Positive	Positive	Positive
8	AML-M2*	Negative	ND	ND	ND	ND
9	AML-M3v†	Negative	ND	ND	ND	ND
10	AML-M4	>50%	Positive	Subset positive	Subset positive	Positive
11	AML-M4	>50%	Positive	Positive	Subset positive	Positive
12	AML-M4	10–50%	ND	ND	Subset positive	Positive
13	AML-M4	10–50%	ND	Subset positive	Positive	Positive
14	AML-M4	Negative	ND	ND	Rare positive	Positive
15	AML-M4	Negative	Positive	Negative	Negative	Positive
16	AML-M4	Negative	Positive	Positive	Rare positive	Positive
17	AML-M4	Negative	Positive	Positive	Subset positive	Positive
18	AML-M4	Negative	ND	Subset positive	Subset positive	Positive
19	AML-M4	Negative	Weak positive	ND	Negative	Positive
20	AML-M4	Negative	Positive	Subset positive	ND	ND
21	AML-M5b	>50%	ND	Positive	Subset positive	Positive
22	AML-M5	10–50%	ND	Negative	Negative	Positive
23	AML-M5	10–50%	Weak positive	Positive	Rare positive	Positive
24	AML-M5	Negative	Negative	Subset positive	Positive	Positive
25	AML-M5b	Negative	ND	ND	ND	ND
26	AML-M5b	Negative	Weak positive	Positive	Rare positive	Positive
27	AML-M4/M5	10–50%	Negative	Positive	Negative	Positive
28	AML-M6	Negative	ND	ND	ND	ND
29	T-ALL	Negative	ND	ND	ND	ND
30	Precursor B-ALL	Negative	ND	Subset positive	Negative	Positive
31	Precursor B-ALL	Negative	ND	ND	ND	ND
32	Precursor T-ALL	Negative	Negative	Negative	Negative	Weak positive
33	T-ALL	Negative	ND	ND	ND	ND
34	Precursor B-ALL	Negative	ND	ND	ND	ND

MPO, myeloperoxidase; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia/lymphoma; ND, not done.

*AML, t(8;21)(q22;q22) under the new WHO classification.

†Acute promyelocytic leukemia under the new WHO classification.

Immunohistochemical stains other than CD163 were performed prior to inclusion of cases in the study. The original diagnoses were confirmed by histopathologic review by two pathologists (PH and LM). All AML cases collected for this study were from our pathology archives and were classified using the French-American-British (FAB) system at the time of the original diagnoses. Therefore, we have utilized this classification in the current study. According to the current World Health Organization (WHO) guidelines,¹ we found that the majority of cases would fall under the category of AML, not otherwise specified. There were two exceptions: one case of AML-M3v fits best as acute promyelocytic leukemia and one AML-M2 would be classified as AML with t(8;21)(q22;q22) under the new WHO guidelines (see Table 1).

The unstained slides were stained with CD163 antibody, as previously described.⁹ Briefly, 4 μ m thick unstained sections were pretreated with Ventana CC1 solution (pH 8.0) for antigen retrieval and were incubated with NCL-CD163 monoclonal

antibody (clone 10D6, 1:50 dilution; Vision Biosystems, Norwell, MA, USA) using a Ventana Benchmark XT system (Ventana Medical Systems, Tucson, AZ, USA). The UltraVision System and a 3,3'-diaminobenzidine chromagen were used for detection.

The staining pattern and intensity of CD163 were assessed on each slide. Cytoplasmic and/or membranous staining for CD163 in at least 10% of tumor cells was considered positive. Entrapped dendritic cells or macrophages were not considered when determining positivity. The percentage of tumor cells labeled was recorded as one of three categories: <10% (negative), 10–50% and >50%.

Results

As previously described, CD163 labeled dermal dendritic cells in normal skin (Fig. 1A).^{9,12} The results of CD163 staining in LC cases are summarized in Tables 1 and 2. In many cases, additional markers for

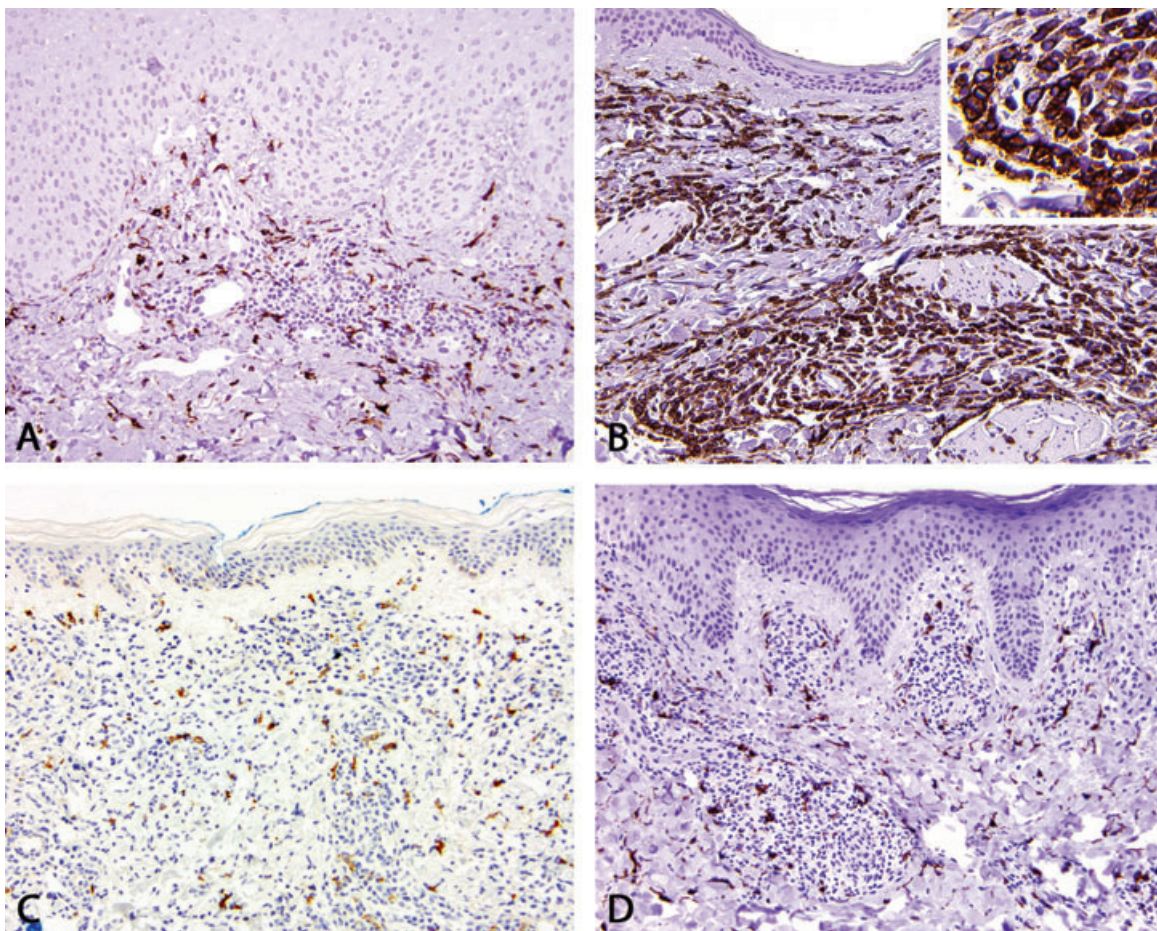


Fig. 1. CD163 expression in leukemia cutis (LC). A) CD163 labels dermal dendritic cells in normal skin. B) CD163 expression in myeloid LC with monocytic differentiation. C) Myeloid LC without monocytic differentiation normally does not express CD163. D) CD163 is absent in LC associated with ALL.

Table 2. Summary of immunohistochemistry results in LC by various markers*

Diagnosis	CD163	CD68	Lysozyme	MPO	CD43
AML-M4	4/11 (36%)	7/7	7/8	8/10	10/10
AML-M5	3/6 (50%)	2/3	4/5	4/5	5/5
AML-M4–5	8/18 (44%)	9/11	12/14	11/16	16/16
AML, other than M4–5	1/10 (10%)	0/1	1/4	4/5	6/6
AML, all	9/28 (32%)	9/12	13/18	15/21	22/22
ALL	0/6 (0%)	0/1	1/2	0/2	2/2

MPO, myeloperoxidase; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia/lymphoma.

*For all listed markers, numbers of positive cases are given as a fraction over total cases stained.

myeloid LC were used alongside CD163 (Tables 1 and 2).

Among cases of myeloid LC, CD163 positivity was observed in 44% (8/18) of myeloid LCs with a monocytic component (AML-M4 or AML-M5 in the FAB classification system) (Fig. 1B). AML-M5 showed a higher probability of CD163 expression compared with AML-M4 (50 and 36% positivity, respectively). Among positive cases, there was no significant difference between AML-M4 and AML-M5 with respect to staining pattern or intensity. With one exception (1/10), CD163 was negative in myeloid LC without a monocytic component (FAB classification of AML M0-M3 or M6) (Fig. 1C). Of note, one case of AML-M2 showed strong and diffuse CD163 positivity. In addition, all six cases of T-ALL or B-ALL were negative for CD163 expression (Fig. 1D).

In our dataset, CD163 was 100% specific for the purpose of distinguishing myeloid LC from LC associated with ALL. The expression of CD163 was also highly specific (90%) for myeloid LC with a monocytic component when compared with myeloid LC without monocytic differentiation. CD163 showed low sensitivity (24%) for all myeloid LC and a sensitivity of 44% for myeloid LC with a monocytic component.

Discussion

Myeloid LC often poses a diagnostic challenge, particularly in cases of aleukemic LC. Because morphologic features are rarely adequate for diagnosis, a precise immunophenotyping is critical. Standard markers for this purpose include CD68, MPO, lysozyme or CD43. As the most common types of myeloid LC are AML-M4 or AML-M5, finding a reliable monocytic marker will be of particular utility. The reported sensitivity of CD68 (KP1 or PG-M1) for AML varies in the literature from 33

to 100%.^{4,11,13,14} Although PGM1 is regarded as more specific than KP1, both have been shown to stain melanomas and other malignancies.⁴ In addition, they often stain AMLs without a monocytic component.^{4,11,13} Although highly specific for the myeloid lineage, MPO has been reported to be only 58% sensitive for all myeloid LC,² and is negative in many cases with monocytic differentiation.^{1,3,4} This is to be expected as MPO is generally strongly expressed only in leukemias with a prominent granulocytic component (i.e. AML-M2, AML-M3 and AML-M4). The monocyte/granulocyte marker lysozyme is highly sensitive, with expression in 93% of myeloid sarcomas;¹⁵ however, it is frequently negative in immature monoblasts.^{1–3,7,13,14,16,17} CD43 is sensitive for AML, but is also expressed in a large percentage of B-cell and T-cell lymphomas.^{1,18} Ultimately, because no marker or combination of markers is completely sensitive or specific, additional markers are often desirable to increase diagnostic confidence in cases of myeloid LC.

CD163 is an endocytic receptor responsible for scavenging haptoglobin–hemoglobin complexes⁶ and its expression is largely restricted to cells of the monocytic/histiocytic lineage.⁵ Recent studies showed the expression of CD163 in tumors derived from monocytes/histiocytes, including AML with monocytic differentiation.^{4,5} Moreover, Garcia et al. showed that CD163 was less sensitive (49%) than CD68 (81%), but was slightly more specific (89%) than CD68 (83%) for the presence of monocytic differentiation in AMLs of bone marrow.⁷ Recently, Cronin et al. presented a large study of CD163 in myeloid LC¹¹ and reported 25% sensitivity for myeloid LC. Among seven positive cases, three were acute monocytic leukemia and two were associated with chronic myelomonocytic leukemia. In agreement with their observations, we found that the overall sensitivity of CD163 for myeloid LC in general was 32%. The slight difference in sensitivity between Cronin et al.'s study and ours could be because of the higher proportion of cases with monocytic differentiation in our study group.

We showed that CD163 had a sensitivity of 44% for detecting myeloid LC with monocytic differentiation, which correlates well with Garcia et al.'s findings.⁷ The sensitivity we observed is lower than the sensitivity (60%) reported by Cronin et al., in part because those authors included only acute monocytic leukemia in the calculation.¹¹ Moreover, we found that CD163 was slightly more likely to be expressed in AML-M5 than AML-M4, probably as a result of greater monocytic component in the AML-M5. Similarly, a previous study in leukemic blasts from peripheral blood has shown that CD163 is more frequently expressed in leukemic cells

of AML-M5 than AML-M4, although this study showed a much greater difference between the two (84 and 25%, respectively) than ours.⁸

Interestingly, we found 1 of 10 myeloid LCs without monocytic differentiation stained strongly for CD163. CD163 immunoreactivity has been previously reported in occasional AMLs without monocytic differentiation.^{7,8,11} Flow cytometry of bone marrow in this case showed a CD13+/CD14+ monocytic cell population. As monocytic differentiation is associated with a greater propensity for extramedullary tumor formation in AML, it is possible that the skin infiltrate in this case was enriched for cells with monocytic differentiation.

As indicated in Table 2, no single marker was positive in all cases of myeloid LC. Given the high specificity of CD163 for myeloid LC with monocytic differentiation, as well as the observation that no marker is 100% sensitive, an optimal approach toward immunophenotyping potential cases of myeloid LC would be to combine CD163 with CD68, MPO, lysozyme and CD43 (with additional markers as needed), in order to maximize both specificity and sensitivity.

It is important to note that CD163 may be expressed in other diseases, including Langerhans cell histiocytosis.⁹ A true diagnostic dilemma arises when attempting to distinguish myeloid LC from histiocytoid Sweet's syndrome.¹⁹ Our preliminary data (unpublished observation) indicates that CD163 may not be helpful for this purpose. The finding is unsurprising, as CD163 does not distinguish between mature and immature cells of the monocytic/histiocytic lineage.

In summary, CD163 represents an important addition to an immunohistochemical panel for diagnosing myeloid LC with monocytic differentiation, given its high specificity for cells of monocytic lineage. However, CD163 is only modestly sensitive for myeloid LC and therefore should not be used alone for diagnosis.

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