CD71 (Transferrin Receptor)

An Effective Marker for Erythroid Precursors in Bone Marrow Biopsy Specimens

Derek K. Marsee, MD, PhD, Geraldine S. Pinkus, MD, and Hongbo Yu, MD, PhD

Key Words: CD71; Transferrin receptor; Glycophorin A; CD235a; Erythroid precursors; Bone marrow biopsy; Hemoglobin; Hematopoietic diseases

DOI: 10.1309/AJCPCRK3MOAOJ6AT

Abstract

Accurate analysis of the erythroid lineage is essential in evaluating bone marrow biopsy specimens and can be particularly challenging in the setting of dyserythropoiesis. Transferrin receptor (CD71) mediates the uptake of transferrin-iron complexes and is highly expressed on the surface of cells of the erythroid lineage. Although CD71 has been used for flow cytometric analysis, its usefulness in paraffin-embedded bone marrow biopsy specimens has not been examined. This study defined the immunohistochemical profile of CD71, as compared with glycophorin A (CD235a) and hemoglobin, in 65 bone marrow biopsy specimens, including normal marrow specimens and cases of myelodysplastic syndrome, acute myeloid leukemia, acute lymphoblastic leukemia, plasma cell neoplasm, and metastatic carcinoma. Immunoreactivity for CD71 was restricted to erythroid precursors in normal and dyspoietic marrow samples and exhibited a membranous and cytoplasmic staining pattern. The vast majority of mature erythrocytes lack expression of CD71, greatly facilitating interpretation. CD71 is a highly effective marker for the detection of cells of erythroid lineage in bone marrow biopsy specimens.

The transferrin receptor (CD71) is an integral membrane protein that mediates the uptake of transferrin-iron complexes.1 Two transferrin receptors have been cloned (TfR1 and TfR2); however, TfR1 is considered the major protein responsible for iron uptake owing to its higher affinity and expression pattern.2 CD71 is a homodimeric glycoprotein containing 760 amino acids, and it binds to diferric transferrin at the cell surface.3 This binding is followed by internalization, a process that is regulated in part by interaction with the HFE protein, which is mutated in patients with hereditary hemochromatosis.4 Diferric iron is subsequently released from transferrin owing to endosomal acidification.

The transferrin receptor is most highly expressed on placental syncytiotrophoblasts, myocytes, basal keratinocytes, hepatocytes, endocrine pancreas, spermatocytes, and erythroid precursors.5 The level of transferrin receptor expression is highest in early erythroid precursors through the intermediate normoblast phase, after which expression decreases through the reticulocyte phase.6-8 The maturation to erythrocytes results in loss of transferrin receptor expression, in concert with down-regulation of the machinery for hemoglobin synthesis. The high level of transferrin receptor within erythroid precursors makes it an excellent potential marker for evaluating erythroid elements within the bone marrow.

CD71 has been studied for use in the assessment of leukemias and myelodysplastic syndromes (MDSs) in flow cytometric studies, in which expression serves as a lineage marker for the diagnosis of erythroid leukemia.9 In addition, several studies have demonstrated that CD71 expression is decreased in dysplastic erythroid precursors.10,11 However, the usefulness of CD71 expression has not been examined...
in paraffin-embedded bone marrow biopsy specimens. In paraffin-embedded tissue, particularly in dysplastic marrow samples, erythroid precursors may not be easily differentiated from other types of precursors on H&E-stained and Giemsa-stained sections, and immunohistochemical analysis is useful for this distinction. Traditional markers for erythroid lineage, such as hemoglobin and glycophorin A (also referred to as CD235a), may be difficult to interpret owing to reactivity of both markers for abundant mature nonnucleated erythrocytes in the marrow and the cytoplasmic staining pattern of hemoglobin.12 The goal of this study was to evaluate the distribution and specificity of CD71 immunoreactivity for erythroid precursors in a variety of clinical settings in paraffin-embedded bone marrow biopsy specimens, in comparison with immunohistochemical studies for hemoglobin and CD235a.

Materials and Methods

Cases were retrieved from the surgical pathology files of Department of Pathology, Brigham and Women’s Hospital, Boston, MA. This study was approved by the Brigham and Women’s Hospital Institutional Review Board. Representative H&E-stained and Giemsa-stained sections were reviewed to confirm the diagnoses. The neoplastic diagnoses were classified according to the World Health Organization classification13; however, for simplicity of categorization, the cases of acute myeloid leukemia (AML) were designated by the former French-American-British (FAB) classification14 in Table 1.

Paraffin sections of 58 bone marrow biopsy specimens fixed in Zenker solution were analyzed: 10 normal marrow samples, 11 cases of MDS with excess blasts (refractory anemia with excess blasts [RAEB-1 or RAEB-2]), 24 cases of AML, including 2 each of FAB subtypes M0-M5, 7 cases of acute erythroid/myeloid leukemia (FAB AML-M6a), 5 cases of acute pure erythroid leukemia (FAB AML-M6b), and 5 cases of acute lymphoblastic leukemia; 3 cases of plasma cell neoplasm; and 5 cases of metastatic carcinoma. In addition, 4 cases of MDS and 3 cases of AML that had been fixed in formalin were also examined. In all cases, immunoreactivity was evaluated for erythroid precursors, mature erythrocytes, blasts, and background tissue.

Immunohistochemical studies were performed on 4-μm-thick paraffin-embedded tissue sections. Following deparaffinization, mercury salts were removed from Zenker-fixed tissues and slides were treated for 5 minutes with 3% hydrogen peroxide to eliminate endogenous peroxidase activity. Studies for CD71 were performed following heat-induced epitope retrieval in a steamer using Retrieve-All-2 (Covance Research Products, Dedham, MA). Studies for glycophorin A and hemoglobin did not require antigen retrieval. Slides were incubated for 1 hour at room temperature with antibodies against CD71 (clone H68.4, dilution 1:1,000; Invitrogen, South San Francisco, CA), glycophorin A (CD235a, clone JC159, dilution 1:1,000; DAKO, Carpinteria, CA), and hemoglobin (rabbit polyclonal, dilution 1:3,000; DAKO). Detection was performed using the EnVision+ system (DAKO) with DAB+ (DAKO) as the chromogen. Sections were counterstained with hematoxylin, dehydrated, and mounted. The pattern and intensity of staining for each antibody were evaluated.

Table 1: Comparison of Immunohistochemical Staining Patterns for CD71, Hemoglobin, and CD235a in Normal and Neoplastic Bone Marrow biopsy Specimens

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total No. of Cases</th>
<th>CD71</th>
<th>Hemoglobin</th>
<th>CD235a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>10/0</td>
<td>10/10/0</td>
<td>10/10/0</td>
</tr>
<tr>
<td>MDS</td>
<td>11</td>
<td>11/3/0</td>
<td>11/11/0</td>
<td>11/11/0</td>
</tr>
<tr>
<td>AML</td>
<td>24</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>FAB M0</td>
<td>2</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>FAB M1</td>
<td>2</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>FAB M2</td>
<td>2</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>FAB M4</td>
<td>2</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>FAB M5</td>
<td>2</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>FAB M6a</td>
<td>7</td>
<td>7/2/0</td>
<td>7/7/0</td>
<td>7/7/0</td>
</tr>
<tr>
<td>FAB M6b</td>
<td>5</td>
<td>5/0/5</td>
<td>5/5/5</td>
<td>5/5/5</td>
</tr>
<tr>
<td>B-ALL</td>
<td>3</td>
<td>3/0/0</td>
<td>3/3/0</td>
<td>3/3/0</td>
</tr>
<tr>
<td>T-ALL</td>
<td>2</td>
<td>2/0/0</td>
<td>2/2/0</td>
<td>2/2/0</td>
</tr>
<tr>
<td>Myeloma</td>
<td>3</td>
<td>3/0/0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>5</td>
<td>5/0/0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; FAB, French-American-British; MDS, myelodysplastic syndrome; T-ALL, T-cell acute lymphoblastic leukemia.

*For each marker, the number of cases exhibiting relevant immunoreactivity is indicated. For the normal category, data are given as erythroid precursors/mature RBCs/background staining; for MDS, AML, B-ALL, and T-ALL, as erythroid precursors/mature RBCs/blasts; and for myeloma and carcinoma, as erythroid precursors/mature RBCs/tumor. In 3 cases of MDS, weak reactivity for CD71 was noted for erythrocytes.

Results

The results of immunohistochemical studies for CD71, CD235a, and hemoglobin are summarized in Table 1. In normal bone marrow samples, reactivity for CD71 was specific and restricted to erythroid lineage, with the highest intensity of membranous and cytoplasmic staining seen in early erythroid precursors and the lowest level in late normoblasts. The vast majority of mature erythrocytes were negative for CD71, greatly facilitating interpretation. Studies for hemoglobin and CD235a also detected immature erythroid elements; however, interpretation was compromised by strong reactivity of the background admixed abundant mature erythrocytes. Compared with CD235a and hemoglobin, the stain for CD71 highlighted occasional mature erythrocytes, but the level of staining was very faint. The staining pattern for CD235a was largely membranous, and the staining pattern for
hemoglobin was mainly cytoplasmic. Of the 3 markers, CD71 provided the most distinct and clean staining pattern, followed by CD235a, and then hemoglobin. Studies for hemoglobin exhibited the highest background.

In cases of MDS, AML, plasma cell neoplasm, and metastatic carcinoma, CD71 served as the most effective marker for the detection of admixed erythroid precursors owing to their distinct staining pattern and lack of reactivity in mature erythrocytes. CD71 was negative in mature erythrocytes in the majority of the cases, except 3 cases of MDS, in which weak CD71 expression was detected in mature erythrocytes. In these 3 cases, however, studies for CD235a and hemoglobin showed much stronger reactivity in mature erythrocytes. In all cases evaluated, myeloblasts, lymphoblasts, and nonerythroid marrow elements, as well as neoplastic plasma cells and carcinoma cells, were negative for CD71.

In cases of acute erythroid leukemias, CD71 was also the most sensitive marker in demonstrating the erythroid precursors and erythroblasts. CD71 was the most useful marker in assessing the percentage of erythroid elements in
acute erythroid/myeloid leukemia (FAB AML-M6a). CD71 was more effective than the other markers owing to highest expression in immature erythroid elements and its lack of reactivity in admixed erythrocytes (Image 3A). In cases of acute pure erythroid leukemia (FAB AML-M6b), reactivity for CD71 clearly demonstrated that the blasts were of erythroid lineage (Image 3B). Compared with results for CD71, studies for CD235a and hemoglobin were weaker in early erythroblasts.

The majority of cases in the study were fixed in Zenker fixative, which is routinely used at our institution because it provides superior cytologic detail. However, the major limitation of Zenker-fixed material is that immunohistochemical studies for some markers may be difficult, requiring antigen retrieval or other technical steps, including dezenkerization. However, for many markers (eg, CD235a, hemoglobin, myeloperoxidase, lysozyme, cytoplasmic immunoglobulin light and heavy chains, and CD138), excellent immunohistochemical results may be achieved without antigen retrieval. We also examined CD71 expression in 7 formalin-fixed marrow samples, including 4 cases of MDS and 3 cases of AML, to ensure that the same conditions would apply. In formalin-fixed bone marrow samples, the intensity, specificity, and pattern of CD71 expression were comparable to those with Zenker fixative, including an absence of reactivity for mature erythrocytes. Since completion of this study, many additional
cases of formalin-fixed marrow samples have been successfully evaluated for CD71. Thus, CD71 staining is a robust, specific marker for erythroid precursors in Zenker- and formalin-fixed bone marrow biopsy specimens.

**Discussion**

In this study, we demonstrated that CD71, compared to CD235a and hemoglobin, is a highly effective marker for highlighting erythroid precursors in bone marrow biopsy specimens for several reasons. First, it has a distinct membranous and cytoplasmic staining pattern, which is easily recognizable. Second, CD71 expression is restricted to erythroid lineage within bone marrow biopsy specimens. Third, expression of CD71 decreases with maturation, with the highest level seen in early forms and the lowest level in late normoblasts. Finally, a feature that makes CD71 superior to CD235a and hemoglobin is that mature erythrocytes do not express CD71, which greatly facilitates analysis. Although an aspirate count may be preferred over immunohistochemical staining of biopsy specimens for determining the percentage of erythroid elements, immunostains on bone marrow biopsy specimens are sometimes necessary if adequate aspirate smears are not available and in cases with marked dyserythropoiesis. It is important to remember, however, that CD71

D, Neoplastic cells also are negative for CD71 in a case of plasma cell neoplasm (left) and metastatic prostatic adenocarcinoma (right). Residual erythroid precursors are CD71+ and demonstrate variation in staining intensity, with strongest immunoreactivity noted for early erythroid precursors and more delicate membranous reactivity for the late erythroid (left and right, immunoperoxidase, ×600).
expression decreases during erythrocyte maturation, and careful examination is required to prevent underestimation of the erythroid population.

Several markers can be used to demonstrate erythroid lineage in paraffin sections, including CD71, CD235a, hemoglobin, and spectrin. In the current study, we demonstrated that CD71 is a superior marker compared to CD235a and hemoglobin. A prior study showed that spectrin is also superior to CD235a for detection of erythroid precursors.15 Spectrin was most highly expressed in early erythroid precursors (cytoplasmic), with decreasing expression in maturing forms (membranous), including mature erythrocytes. Because mature erythrocytes express spectrin, it is most likely that immunohistochemical studies for spectrin will have high background compared with CD71. However, future studies may be warranted to compare these markers of erythroid elements in paraffin sections in order to determine which is preferable.

Although prior studies have demonstrated that CD71 is expressed in numerous types of neoplastic and proliferating cells,16,17 in our cases, we found that CD71 was not detected in neoplastic cells of cases of AML, acute lymphoblastic leukemia, metastatic carcinoma, or plasma cell neoplasm. The specificity of CD71 for erythroid precursors is likely related to

---

**Image 3** Bone marrow biopsy specimen in acute erythroid leukemia. **A**, The CD71 staining pattern demonstrates that the majority of the cells are erythroid precursors in a case of acute erythroid/myeloid leukemia. **B**, In a case of acute pure erythroid leukemia, greater than 80% of cellularity is composed of erythroblasts, which are positive for CD71 (A and B, left, H&E, ×600; right, immunoperoxidase, ×600).
a higher level of expression owing to a high level of hemoglobin synthesis in these cells. However, the potential expression of CD71 in nonhematopoietic malignancies is an important caveat. When analyzing a CD71+ neoplastic process of uncertain lineage involving the bone marrow, confirmation of erythroid lineage using additional markers such as CD235a and hemoglobin is advised.

Previous flow cytometric studies have demonstrated that CD71 expression is decreased in nucleated erythroid cells in patients with MDS. However, in our bone marrow biopsy specimens, we did not observe any decrease in CD71 expression among erythroid precursors in cases of MDS, suggesting that the decreased expression seen in flow cytometric studies may be the result of increased sensitivity of that method. In addition, we found that a subset of cases of MDS showed weak expression of CD71 in mature erythrocytes. The significance of this is unclear, and more detailed studies will be required to determine if CD71 expression is altered in this clinical setting.

CD71 (transferrin receptor) is a highly effective marker for the detection of cells of erythroid lineage in paraffin sections of bone marrow biopsy specimens.

From the Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA.

Address reprint requests to Dr Yu: Dept of Hospital Laboratories and Department of Pathology, UMass Memorial Medical Center, One Biotech Park, 365 Plantation St, Worcester, MA 01605.

Acknowledgment: We are grateful to Mark Fleming, MD, PhD, for scientific contribution to this study and to Alyson Campbell for expert technical assistance.

References