SHORT COMMUNICATION

Role of Immunohistochemistry in Acute Leukemias with Myelonecrosis

Subramanian Kalaivani¹ · Gurusamy Dharma Saranya¹ · Rakhee Kar¹ ·
Debdatta Basu¹

Received: 26 October 2017 / Accepted: 2 January 2018 / Published online: 12 January 2018
© Indian Society of Hematology and Blood Transfusion 2018

Abstract Myelonecrosis is a rare antemortem finding most commonly seen in haematopoietic neoplasms, especially in acute leukemia. When myelonecrosis occurs at the time of presentation, it imposes certain diagnostic issues in subcategorization of leukemias which is necessary for therapeutic as well as prognostic purposes. Flow cytometry, though is a powerful modality, has its own limitations especially when the cells are not fresh and viable; and when the specimen is not of adequate cellularity which is usual in cases of myelonecrosis. In such situations, immunocytochemistry (ICC) or immunohistochemistry (IHC) may play a major role in lineage specification in leukemias as the necrosed marrow with the ghost cells can still retain the antigenicity for certain immunomarkers. Four such interesting cases of common B acute lymphoblastic leukemia (ALL) where IHC was used for diagnosis were included. ICC and IHC done on the necrosed marrow contributed to the diagnosis of ALL in all the four cases and contributed to subsequent management. ICC and IHC if contributory can play a major role in

identifying the primary cause of myelonecrosis as the ghost cells can retain the antigenicity despite being morphologically non-viable.

Keywords Acute lymphoblastic leukemia · Immunocytochemistry · Immunohistochemistry · Myelonecrosis · Necrobiosis

Introduction

Myelonecrosis is defined as “necrosis of the myeloid tissue and medullary stroma in large areas of hematopoietic bone marrow”, which is more common in malignancy, especially hemopoietic neoplasia [1–3]. In usual cases, acute leukemias are often classified without difficulty because of accessibility of the neoplastic cells within the peripheral blood and/or bone marrow allowing flow cytometric analysis, immunohistochemistry (IHC) and genetic studies to be carried out with ease. However, in a case of myelonecrosis bone marrow aspiration is generally unsuccessful, and multiple aspirations may be necessary and the nonviable “ghost cells” have a high propensity to shear in the flow chamber [4]. In such situations IHC is particularly useful for analyzing malignant cells that are too fragile to remain intact during specimen processing or the hydrodynamic focusing steps of flow cytometry (FC) [4]. We present here four such interesting cases of common B acute lymphoblastic leukemia (ALL) where IHC done on the necrosed marrow contributed to the diagnosis and further management.

Presentation at a meeting: Included as a part of a study in APCON 2015.

✉ Rakhee Kar
drrakheekar@gmail.com

Subramanian Kalaivani
klvnselvi@yahoo.co.in

Gurusamy Dharma Saranya
dhrmsaranya@yahoo.com

Debdatta Basu
ddbasa@gmail.com

¹ Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry 605006, India

Materials and Methods

All cases were subjected to IHC with the primary panel including CD34, CD20, CD3, CD10, TdT and MPO. If these markers were non-contributory other markers including CD19, CD22, CD79a, CD117, CD61 were done. IHC was done based on standard protocol using pressure cooker for antigen retrieval. The peripheral smear slides, imprint and the aspiration smears were treated with a mixture of ammonium chloride, tris buffer and distilled water for 1 min to lyse RBCs followed by treatment with hydrogen peroxide for 1 min to block endoperoxidase activity and then fixed in formalin vapour for 10 min and then washed in water. The slides were then subjected to antigen retrieval by pressure cooker method and was further processed similar to the IHC on paraffin sections.

The expression of these markers and its intensity in the necrotic leukemic cells were evaluated and compared with the adjacent viable leukemic cells if present. IHC in the study was not interpreted on debris or degenerated cells. Only those areas where the cell architecture was maintained were analyzed for IHC expression. Intensity was interpreted strong if it showed bright and crisp positivity and it was interpreted as weak if showed faint positivity.

Results

Case 1

A 22-year-old male presented with fever, epistaxis, fatigue, lymphadenopathy, hepatosplenomegaly and bone pain. Complete blood count (CBC) showed pancytopenia (Hb-7.2 g/dl, WBC- $2.3 \times 10^9/L$, platelets- $56 \times 10^9/L$). Peripheral smear (PS) showed leucoerythroblastic blood picture with no atypical cells. Bone marrow aspirate (BMA) was aparticle and showed suboptimal cellularity. FC was not done due to scanty material. The bone marrow biopsy (BMB) showed myelonecrosis with myelofibrosis (Fig. 1a). In view of strong suspicion a repeat marrow was advised which again showed myelonecrosis with patchy viable areas showing blasts. The viable areas as well as the ghost cells in necrotic areas were positive for CD 34, TdT, CD10 and CD20 and negative for CD3, CD61 and MPO favoring the diagnosis of Common B ALL (Fig. 1b–d). The IHC panel done in hindsight in the previous marrow showed similar positivity in the ghost cells. The patient's post-induction marrow showed persistence of blasts, viable and positive for the same IHC markers. However, the patient succumbed during re-induction chemotherapy.

Case 2

A five and half year-old male child with history of surgery and chemotherapy for retinoblastoma during first year of life, presented with bilateral lower limb pain and ecchymoses and bony tenderness. CBC and PS showed leucocytosis (WBC- $55 \times 10^9/L$), thrombocytopenia (platelets- $15 \times 10^9/L$) and anemia (Hb-7.3 g/dl) with 83% blasts of lymphoid morphology which were block positive for Periodic Acid Schiff (PAS) and negative for Sudan Black B (SBB). BMA was diluted and the imprint was paucicellular with amorphous granular material and many degenerated cells with presence of blasts (Fig. 2a, b). Immunocytochemistry (ICC) attempted on the PS, BMA and imprint smears showed CD79a, CD10 positive, CD34 weakly positive and CD3, CD20, TdT, MPO negative. Thus a diagnosis of Common B-ALL was made. FC also showed positivity for CD10, CD19, CD22, TdT and HLA-DR concurring with the diagnosis. BMB received subsequently showed predominantly myelonecrosis with patchy viable tumor area where the similar IHC panel was tried and showed strong CD10 positivity in the necrosed ghost cells and other markers were negative (Fig. 2c, d). The patient was started on chemotherapy and the post-induction marrow was in remission with regenerative haematopoiesis.

Case 3

An 18-year-old male presented with fever and generalized lymphadenopathy for 2 months. CBC and PS showed leucocytosis with 85% blasts. BMA was diluted with few scattered blasts and eosinophilic granular material in the background. Cytochemistry for PAS and SBB were negative. However, ICC attempted in aspirate showed positivity for CD34, CD10, CD79a and TdT. BMB showed myelonecrosis with patchy viable areas. IHC showed variable positivity. CD79a was positive only in the viable cells, CD10 was strongly positive in necrotic and viable blasts, TdT was strongly positive in viable blasts and weakly positive in necrotic cells (Fig. 3a–d). FC was positive for CD10, CD19, CD22, CD79a and TdT. A diagnosis of Common B-ALL was given and the patient was started on induction chemotherapy during the course of which he succumbed to the disease and chemotherapy related complications.

Case 4

A 25-year-old male presented with recurrent fever for 4 months, jaundice, hepatosplenomegaly and bilateral palpable kidneys. Blood counts showed pancytopenia with leucoerythroblastic blood picture and 1% blast in the PS.

Fig. 1 **a** Bone marrow trephine biopsy showing myelonecrosis (H&E, $\times 400$); **b–d** CD 34, CD10 and Tdt positivity in the ghost cells respectively (IHC, $\times 400$)

BMA was diluted with scattered blasts, suspicious of acute leukemia. BMB showed patchy cellularity with increased blasts and large areas of myelonecrosis. IHC showed positivity for CD10, TdT and CD20. CD34, MPO, CD117 and CD3 were negative favouring the diagnosis of Common B-ALL. CD10 was positive in the viable blasts as well as in the areas of necrosis. The patient was started on chemotherapy and on follow up.

Discussion

The exact pathogenesis of myelonecrosis is unclear. In leukemia, it is considered that the ischemic infarction of bone marrow due to infiltration of medullary nutrient vessels by blasts could be a probable cause [5]. Hematopoietic malignancies, especially, acute leukemia has been found to be the most common underlying disease [2, 3]. Other malignant causes of myelonecrosis are lymphomas, chronic myeloproliferative neoplasms and solid tumors [6]. Hence malignancy should always be excluded

in a patient with MN without a clear cause [7]. Case 1 also presented with myelonecrosis of unknown cause, a strong suspicion of leukemia/malignancy led to repeat a marrow which clinched the diagnosis.

Myelonecrosis is characterized by necrosis of the myeloid tissue with a background of amorphous eosinophilic material and cells that lose their normal staining characteristics. In spite of necrosis, it is seen that the ghost cells still retain their antigenicity which can be explained by the theory of necrobiosis which is the state of transition between necrotic and viable cells; and the antigenicity can be demonstrated by IHC markers which has been highlighted by all these cases [8]. In addition, IHC reveals architectural features and estimates tumor cellularity in paraffin sections. Thus when acute leukemia is suspected in presence of myelonecrosis, one should look at the ghost cell morphology for monomorphic population and the lineage assessment can be done by IHC.

Antigens of various tissues are frequently preserved in the infarcted necrotic organs and can be detected by immunohistochemical techniques. There are studies that

Fig. 2 **a, b** Bone marrow aspirate showing many degenerated cells and blasts with eosinophilic granular material (Leishman, $\times 100$, $\times 400$); **c** bone marrow trephine biopsy showing myelonecrosis (H&E, $\times 400$); **d** CD10 positivity in the ghost cells (IHC, $\times 400$)

found immunostainings of both necrotic tissues and viable tissues yield similar results and were used in the diagnosis of lesions [9]. Mori et al. [10] studied the expression of IHC markers, CD3, CD20, CD30, CD15, LMP1, etc. and found expression of immunomarkers in the infarcted areas and concluded that at least on some occasions IHC can be informative for accurate diagnosis in lymphoid neoplasms.

Kojima et al. used the panel included L26 (CD20), 56C6 (CD10), 124 (BCL-2), and polyclonal BCL-6 antibody and found that CD20 (91%) and CD10 (80%) antigen appear to be well preserved even in infarcted lymphoma tissues and provide useful information regarding the infarcted material [11].

Strauchen et al. studied the role of immunohistochemistry in the evaluation of lymphoma. He observed preservation of lymphoid antigens in 4 of 6 cases of lymph node infarction associated with malignant lymphoma, including 3 of 5 cases of diffuse large B cell lymphoma and 1 case of peripheral T-cell lymphoma. In 1 case, in which an infarcted lymph node showed a benign pattern of lymphoid antigen expression, lymphoma had not developed after 5 years. He did not observe nonspecific staining in any case and concluded that lymphoid antigens are frequently preserved in cases of lymph node infarction, and

immunohistochemical study of infarcted lymph nodes may provide clinically useful information [12].

Now, there arises a question whether all antigens will be preserved in the necrotic cells in all cases of leukemias, as there was variability in expression of markers. CD10 was the only marker which was consistently positive with strong intensity in all the four cases presented in this case series. CD34, CD20 and TdT were the other markers which showed positivity. TdT showed variable intensity of staining.

FC was successful in two of the cases which could be explained by the patchy viable tumor cells present in the marrow. Otherwise in an extensively necrotic marrow the role of FC is questionable. This series primarily aims to highlight the fact that if IHC is positive in the necrotic cells it could be contributory to the diagnosis especially in conditions of extensive necrosis with no viable cells.

Conclusion

IHC if contributory plays an important role in identifying the primary cause of myelonecrosis as the ghost cells can retain the antigenicity despite being morphologically non-

Fig. 3 **a** CD79a showing focal positivity only in the viable blasts (H&E $\times$ 400), **b** strong CD10 positivity in necrotic blasts (H&E, $\times$ 100); **c** strong TdT positivity in viable blasts (H&E, $\times$ 100); and **d** weak TdT positivity in necrotic blasts (H&E, $\times$ 400)

viable. And the markers should be selected diligently based on the clinical suspicion and the ghost cell morphology.

Compliance with Ethical Standards

Conflict of interest None.

Research Involving Human Participants and/or Animals Humans; Routine diagnostic material.

Ethical Approval Study presented is as per ethical guidelines. It describes a series of four cases where all work up was part of routine diagnosis with de-identified patient details.

References

- Habboush HW, Hann IM (1987) Bone marrow necrosis in acute lymphoblastic leukemia. *Scot Med J* 32:177–180
- Rekha JS, Kar R, Basu D (2014) Myelonecrosis: a clinicopathological study from a tertiary care center in south India over a twelve-year period. *Bone Marrow Res*. <https://doi.org/10.1155/2014/890510>
- Paydas S, Ergin M, Baslamisli F, Yavuz S, Zorludemir S, Sahin B et al (2002) Bone marrow necrosis: clinicopathologic analysis of 20 cases and review of the literature. *Am J Hematol* 70(4):300–305
- Keren DF, McCoy JP, Carey JL (2001) Flow cytometry in clinical diagnosis, 3rd edn. American Society of Clinical Pathology Press, Chicago, p 739
- Cassileth PA, Brooks JSJ (1987) The prognostic significance of myelonecrosis after induction therapy in acute leukemia. *Cancer* 60:2363–2365
- Jassens AM, Offner FC, Van Hove WZ (2000) Bone marrow necrosis. *Cancer* 88:1769–1780
- Granot H, Polliack A, Matzner Y (1980) Bone marrow necrosis as the only manifestation of disseminated carcinomatosis. *Acta Haematol* 64:232–235
- Itoh T, Orba Y, Takei H, Ishida Y, Saitoh M, Nakamura H et al (2002) Immunohistochemical detection of hepatocellular carcinoma in the setting of ongoing necrosis after radiofrequency ablation. *Mod Pathol* 15(2):110–115
- Ioachim HL, Medeiros LJ. Lymph node infarction. In: Ioachim's lymph node pathology, 4th edn. Lipincott Williams & Wilkins, pp 260–262
- Mori E, Enomoto Y, Nakamine H, Kasai T, Takeda M, Takano M et al (2012) Lymph node infarction in classical Hodgkins lymphoma. *J Clin Exp Hematopathol* 52(1):35–39
- Strauchen JA, Miller LK (2003) Lymph-node infarction. An immunohistochemical study of 11 cases. *Arch Pathol Lab Med* 127:60–63
- Kojima M, Nakamura S, Yamane Y, Murayama K, Shimizu K, Itoh H et al (2004) Antigen preservation in infarcted nodal B-cell lymphoma, with special reference to follicular center. *Cell Mark* 12(3):251–255