



Case Report

Flow cytometry to identify bone-marrow relapse in blastic plasmacytoid dendritic cell neoplasm: a case report



Mariela Granero Farias^{a,*}, Fabiane Spagnol Pedrazzani^a,
Luis Carlos Zanandrea Contin^a, Ana Paula Alegretti^a,
Lisandra Della Costa Rigoni^a, Liane Esteves Daudt^{a,b}

^a Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil

^b Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:

Received 29 November 2016

Accepted 17 April 2017

Available online 16 May 2017

Introduction

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare acute leukemia subtype characterized by clonal expansion of dendritic-lineage cells.¹ These cells are identified immunophenotypically by weak CD45 expression and co-expression of the CD4 and CD56 antigens in the absence of other lineage-specific markers.^{2,3} Previously known as blastic natural killer (NK)-cell lymphoma or CD4⁺/CD56⁺ hematodermic neoplasm, it is currently classified by the World Health Organization (WHO) as a distinct entity, under the acute myeloid leukemia (AML) and related precursor neoplasm group.⁴

Few studies have assessed the incidence of BPDCN in the general population. The limited existing data suggest an extremely low overall incidence, representing 0.44% of all hematological malignancies^{3,5} and 0.7% of cutaneous

lymphomas.⁶ BPDCN mostly affects men, with a 3:1 male-to-female ratio, and usually occurs in patients aged 60–70 years^{7,8} although cases have been reported in children and young adults.⁹

BPDCN is particularly difficult to diagnose because of its clinical and biological heterogeneity that overlaps with other clinical malignancies, and frequent lack of chromosomal abnormalities.^{5,10} On laboratory testing, the cell morphology can be misleading, with a pseudolymphocytic appearance, abundant cytoplasm with a low nuclear-cytoplasmic ratio, strong basophilia, and no granulation. Microvacuoles are often visible, possibly representing glycogen compounds inclined to form a “pearl necklace” on the nuclear membrane, as well as cytoplasmic extensions similar to pseudopodia.^{11,12} The cell lineage must be evaluated by flow cytometry immunophenotyping, which identifies dendritic cells in their three stages of differentiation.¹⁰

* Corresponding author at: Serviço de Patologia Clínica, Unidade de Diagnóstico Personalizado, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-903 Porto Alegre, RS, Brazil.

E-mail address: mgfarias@hcpa.edu.br (M.G. Farias).

<http://dx.doi.org/10.1016/j.bjhh.2017.04.005>

1516-8484/© 2017 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

A recent study showed that, according to their CD34 and CD117 expressions, dendritic cells can be categorized into three maturational stages: (1) in BPDCN, CD34 is expressed in some of the immature blasts; (2) intermediate cells are partially CD117-positive when the expression of CD34 is absent in blast cells; and (3) mature cells do not express CD34 or CD117. These stages of maturation explain the variation in the clinical presentation of BPDCN, as well as its laboratory characteristics.¹⁰

The most differentiated stage is characterized by cells with weak expression of CD45, no expression of CD34, co-expression of CD4/CD56, presence of HLADR/CD123, and absence of specific markers of myeloid, B and T lymphoid, and NK cells.^{13,14} Co-expression of CD2, cytoplasmic CD3, CD5, CD7, CD33, nTdT, CD79a, and/or CD117 can be common.^{13,15,16}

CD123 is the α chain of the interleukin-3 receptor, and is a sensitive and specific marker of plasmacytoid dendritic cells (pDC). However, although CD123 is currently the most important marker in the diagnosis of BPDCN, it is not unique to the plasmacytoid dendritic cell lineage. CD4 and CD56 are expressed in other hematological malignancies, and are not enough to establish diagnosis.^{17,18}

Clinically, this disease manifests with isolated cutaneous involvement in the form of single or multiple lesions, and, despite initial indolent behavior, it is characterized by aggressive, rapid systemic dissemination.^{19,20} Many patients have cytopenia, particularly thrombocytopenia, as a result of extremely variable rates of dendritic-cell infiltration of the bone marrow. Although there is an initial response to systemic chemotherapy, the disease relapses as a matter of course, and survival ranges from 12 to 14 months.¹⁹

Currently, there is no consensus as to the ideal treatment for BPDCN.^{1,21} Retrospective studies have evaluated different treatment strategies, including multi-agent chemotherapy according to established protocols for acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML), while a few cases have been submitted to allogeneic hematopoietic stem cell transplantation (HSCT).^{1,21} The neoplastic cells are initially sensitive to chemotherapy agents which are typically active against lymphoblasts, such as steroids, vincristine, and asparaginase. Therefore, it is recommended that ALL protocols be followed, with subsequent HSCT when appropriate.^{1,20} It is important to highlight that the effectiveness of this procedure still needs to be investigated by clinical studies, and the role of transplantation has yet to be defined.¹ The Martín-Martín et al. study demonstrated that this strategy (ALL therapy plus HSCT) was associated with the best prognosis.¹⁰

Within this context, the aim of this paper is to report a case of this rare, difficult-to-diagnose neoplasm in which flow cytometry immunophenotyping contributed to the correct identification of leukemic cell lineage.

Case report

A 51-year-old previously healthy female presented with a 3-month history of progressively disseminating cutaneous lesions, with no defined diagnosis and no clinical response to topical treatments. Immunohistochemistry skin biopsy was performed and revealed blast-appearing cells, positive

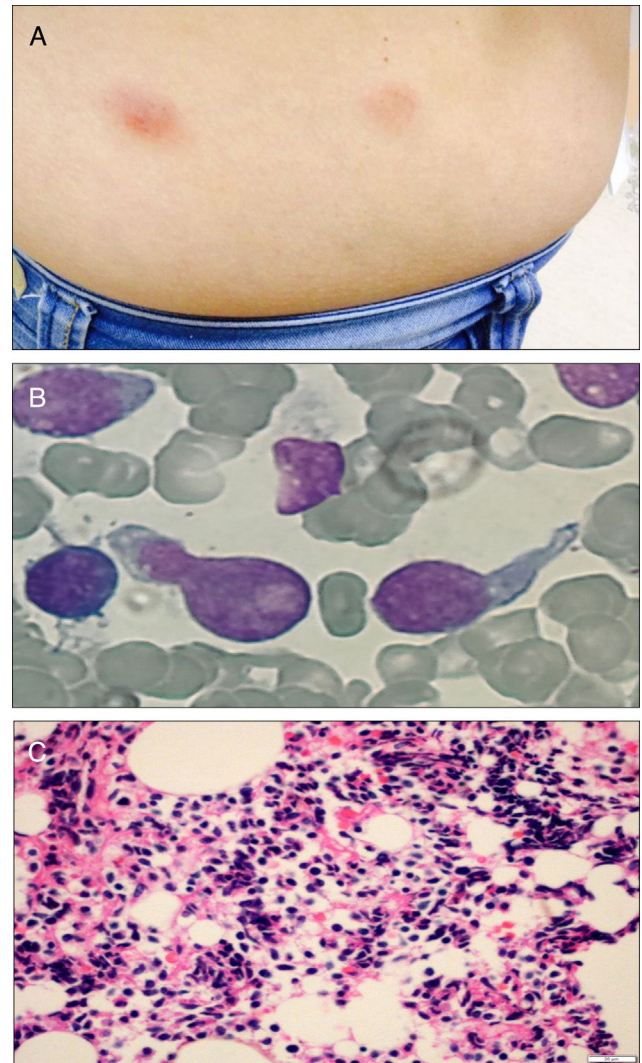


Figure 1 – Physical and morphologic features of blastic plasmacytoid dendritic cell neoplasm. Cutaneous lesions on the patient's abdomen (A); morphology of leukemic blasts in the bone-marrow aspirate smear (B); anatomopathological study showing infiltration by blast cells (C).

for CD45 and CD123, and negative for TCL1 related to the CD3 and CD20 antigens, indicating BPDCN. Bone marrow aspiration and biopsy did not show bone-marrow involvement at the time of diagnosis. Treatment was performed with cytarabine and daunorubicin (7 + 3 protocol – induction, re-induction, and three consolidations). Three months after completing high-dose cytarabine maintenance, a cutaneous relapse was detected (Figure 1A) with central nervous system (CNS) involvement. At this time, bone marrow biopsy identified 80% immature cells with lymphoblast appearance (Figure 1B) and an anatomopathological study revealed diffuse infiltration by blast cells of lymphoid origin (Figure 1C). Bone marrow immunophenotyping by flow cytometry (Figure 2) using the FACSCanto II system (Becton Dickinson, San Jose, CA, USA) showed 62% of cells with expression of the antigens CD4, HLADR, CD38, weak and heterogeneous CD56 expression,

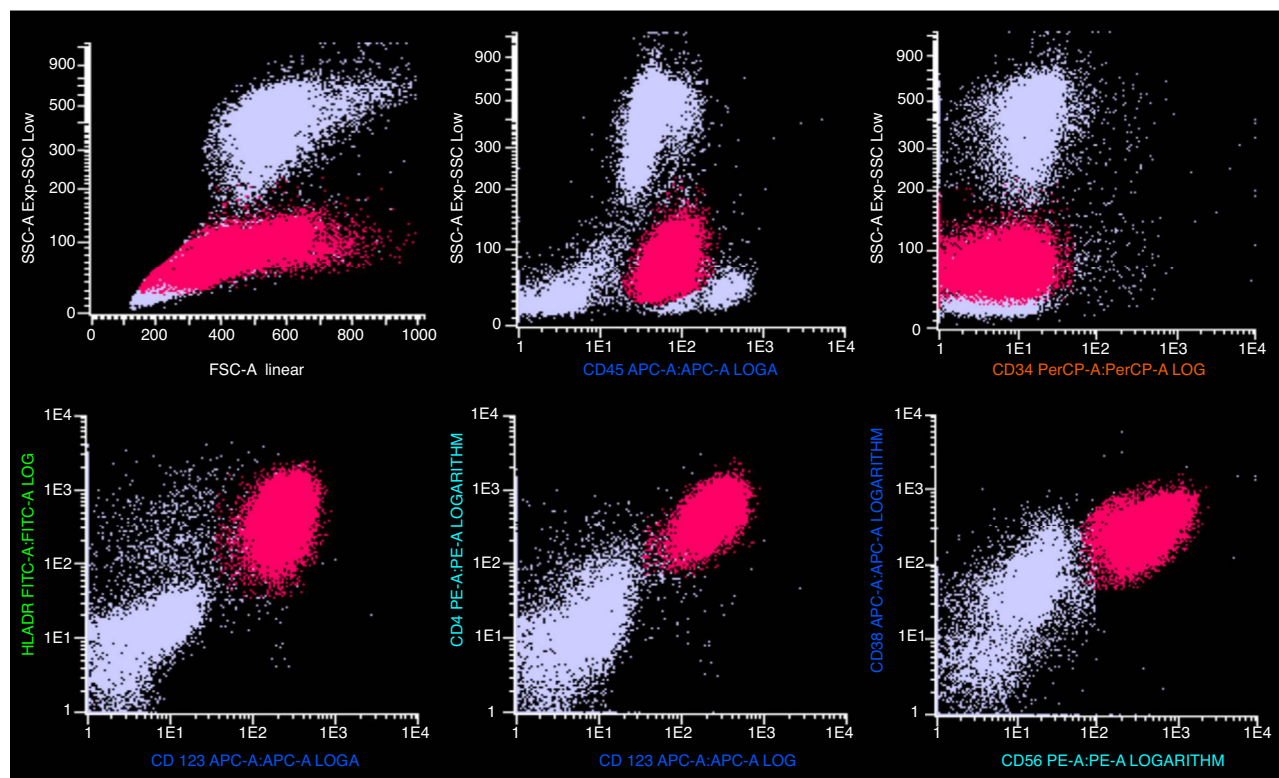


Figure 2 – Immunophenotyping by flow cytometry of a bone-marrow aspirate sample, showing the main markers that identify and characterize plasmacytoid dendritic blast cells.

strong and homogeneous CD123 and weak CD45 expression. There was no expression of CD34 or markers of lymphoid B (cCD79a, CD19), lymphoid T (CD7, CD3, CD5, CD2), NK (CD11b, CD16), or myeloid cells (cMPO, CD13, CD15, CD64, CD65) suggesting BPDCL. The patient began a HyperCVAD regimen (cyclophosphamide, vincristine, doxorubicin and dexamethasone) and underwent allogeneic HSCT. The post-transplant course was complicated by septic shock and hemorrhagic alveolitis, and the patient died 18 days after HSCT.

Discussion

BPDCL is rare, aggressive, and difficult to diagnose. Knowledge about this condition has been improving in recent years because of the increasing number of reported cases and a better comprehension of its biological characteristics.²² Given the clinical and biological heterogeneity of this neoplasm, correct identification and classification require the experience of the clinician and histopathologist, as well as appropriate diagnostic tests.

Diagnosis of BPDCL by flow cytometry may be challenging in some cases because of the different maturational stages or because cells do not exhibit the distinctive profile due to either the absence of the main markers or the presence of additional markers indicative of other lineages.^{10,13} The advantage of flow cytometry in the diagnosis of BPDCL is that it is a quantitative and qualitative method, in addition to being more sensitive and specific, as it not only demonstrates

antigen co-expression but also shows the density of each antigen quantitatively. This technique also has better ability to detect numerous antigens simultaneously, including some not routinely tested by immunohistochemistry.^{12,23}

Expression of CD123 is typical of BPDCL, but this marker can be found in basophils and in many cases of acute leukemia of other lineages.²⁴ Intensity of expression of this antigen can vary during dendritic cell maturation, although strong, homogeneous expression is an important indicator of differentiated plasmacytoid dendritic origin; therefore, it must be evaluated together with other markers.¹³

It is important to distinguish BPDCL from other malignancies that express CD4 and CD56, such as aggressive NK-cell leukemia/lymphoma, nasal NK-cell lymphoma, and AML, which can feature aberrant expressions of these markers and cutaneous involvement.²⁵

We reported the case of BPDCL in a female patient with an indolent clinical presentation, cutaneous and CNS infiltration, no initial bone marrow involvement, and no response to AML treatment. During relapse, a high percentage of plasmacytoid dendritic-lineage cells was identified in the bone marrow by flow cytometry. The patient died 18 days after HSCT.

We emphasize the importance of performing flow cytometry with a panel of monoclonal antibodies suitable to identify BPDCL, especially in patients presenting with lesions and neoplastic cells showing morphological characteristics indicative of the lymphoid lineage. This technique can establish the correct diagnosis and define the most appropriate treatment.²⁵

Funding

HCPA Research and Event Promotion Fund (FIPE-HCPA).

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- Pagano L, Valentini CG, Pulsoni A, Fisogni S, Carluccio P, Mannelli F, et al. Blastic plasmacytoid dendritic cell neoplasm with leukemic presentation: an Italian multicenter study. *Haematologica*. 2013;98(2):239-46.
- Jacob MC, Chaperot L, Mossuz P, Feuillard J, Valensi F, Leroux D, et al. CD4+ CD56+ lineage negative malignancies: a new entity developed from malignant early plasmacytoid dendritic cells. *Haematologica*. 2003;88(8):941-55.
- Bueno C, Almeida J, Lucio P, Marco J. Incidence and characteristics of CD4+/HLA DRhi dendritic cell malignancies. *Haematologica*. 2004;89(1):58-69.
- Facchetti F, Jones DM, Petrella T. Blastic plasmacytoid dendritic cells neoplasm. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC Press; 2008. p. 146-7. World Health Organization Classification of Tumours, vol. 2.
- Riaz W, Zhang L, Horna P, Sokol L. Blastic plasmacytoid dendritic cell neoplasm: update on molecular biology, diagnosis, and therapy. *Cancer Control*. 2014;21(4):279-89.
- Ng AP, Lade S, Rutherford T, McCormack C, Prince HM, Westerman DA. Primary cutaneous CD4+/CD56+ hematodermic neoplasm (blastic NK-cell lymphoma): a report of five cases. *Haematologica*. 2006;91(1):143-4.
- Garnache-Ottou F, Feuillard J, Saas P. Plasmacytoid dendritic cell leukaemia/lymphoma: towards a well defined entity? *Br J Haematol*. 2007;136(4):539-48.
- Facchetti F, Ungari M, Marocolo D, Lonardi S, Vermi W. Blastic plasmacytoid dendritic cell neoplasm. *Haematol Meet Rep*. 2009;3(3):1-3.
- Feuillard J, Jacob MC, Valensi F, Maynadié M, Gressin R, Chaperot L, et al. Clinical and biologic features of CD4(+)CD56(+) malignancies. *Blood*. 2002;99(5):1556-63.
- Martín-Martín L, López A, Vidriales B, Caballero MD, Rodrigues AS, Ferreira SI, et al. Classification and clinical behavior of blastic plasmacytoid dendritic cell neoplasms according to their maturation associated immunophenotypic profile. *Oncotarget*. 2015;6(22):19204-16.
- Jegalian AG, Buxbaum NP, Facchetti F, Raffeld M, Pittaluga S, Wayne AS, et al. Blastic plasmacytoid dendritic cell neoplasm in children: diagnostic features and clinical implications. *Haematologica*. 2010;95(11):1873-9.
- Tsagarakis NJ, Kentrou NA, Papadimitriou KA, Pagoni M, Kokkini G, Papadaki H, et al. Acute lymphoplasmacytoid dendritic cell (DC2) leukemia: results from the Hellenic Dendritic Cell Leukemia Study Group. *Leuk Res*. 2010;34(4):438-46.
- Garnache-Ottou F, Feuillard J, Ferrand C, Biichle S. Extended diagnostic criteria for plasmacytoid dendritic cell leukaemia. *Br J Haematol*. 2009;145(5):624-36.
- Saaddeh D, Kurban M, Abbas O. Plasmacytoid dendritic cell role in cutaneous malignancies. *J Dermatol Sci*. 2016;83(1):3-9.
- Pagano L, Valentini CG, Grammatico S, Pulsoni A. Blastic plasmacytoid dendritic cell neoplasm: diagnostic criteria and therapeutical approaches. *Br J Haematol*. 2016;174(2):188-202.
- Facchetti F, Cigognetti M, Fisogni S, Rossi G, Lonardi S, Vermi W. Neoplasm derived from plasmacytoid dendritic cells. *Mod Pathol*. 2016;29(2):98-111.
- Marafioti T, Paterson JC, Ballabio E, Reichard KK, Tedoldi S, Hollowood K, et al. Novel markers of normal and neoplastic human plasmacytoid dendritic cells. *Blood*. 2008;111(7):3778-92.
- Wang W, Li W, Jia JJ, Zheng Y, Wang H, Gao XM, et al. Blastic plasmacytoid cell neoplasm: a case report. *Oncol Lett*. 2015;9(3):1388-92.
- Chen J, Zhou J, Qin D, Xu S, Yan X. Blastic plasmacytoid dendritic cell neoplasm. *J Clin Oncol*. 2011;29(2):e27-9.
- Laribi K, Denizon N, Besançon A, Farhi J, Lemaire P, Sandrini J, et al. Blastic plasmacytoid dendritic cell neoplasm: from origin of the cell to targeted therapies. *Biol Blood Marrow Transplant*. 2016;22(8):1357-67.
- Heinicke T, Hütten H, Kalinski T, Franke I, Bonnekoh B, Fischer T. Sustained remission of blastic plasmacytoid dendritic cell neoplasm after unrelated allogeneic stem cell transplantation – a single center experience. *Ann Hematol*. 2015;94(2):283-7.
- Brody JP, Allen S, Schulman P, Sun T, Chan WC, Friedman HD, et al. Acute agranular CD4-positive natural killer cell leukemia: comprehensive clinicopathologic studies including virologic and in vitro culture with inducing agents. *Cancer*. 1995;75(10):2474-83.
- Shi Y, Wang E. Blastic plasmacytoid dendritic cell neoplasm: a clinicopathologic review. *Arch Pathol Lab Med*. 2014;138(4):564-9.
- Toba K, Koike T, Shibata A, Hashimoto S, Takahashi M, Masuko M, et al. Novel technique for the direct flow cytometric analysis of human basophils in unseparated blood and bone marrow, and the characterization of phenotype and peroxidase of human basophils. *Cytometry*. 1999;35(3):249-59.
- Reimer P, Rüdiger T, Kraemer D, Kunzmann V, Weissinger F, Zettl A, et al. What is CD4+CD56+ malignancy and how should it be treated? *Bone Marrow Transplant*. 2003;32(7):637-46.