Case Study

CD34 positive dysplastic giant platelets masquerading as blasts on flow cytometry

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Abstract: Giant platelets are commonly seen in myelodysplastic syndrome (MDS), however their immunophenotypic characteristics are not well studied. Here we report a patient with a history of MDS that demonstrated a dysplastic, immunophenotypically abnormal subpopulation of giant platelets. In this patient, flow cytometric analysis of the marrow aspirate showed that 59% of events were with characteristics of CD34 positive blasts, raising the possibility of acute leukemia. However, microscopic examination of the bone marrow aspirate smears showed only 8% blasts and many dysplastic giant platelets. Immunohistochemistry showed CD34 positivity in the patient's megakaryocytes. Flow cytometric analysis of the patient's peripheral blood platelets showed that the dysplastic platelets were positive for CD34. This case shows that large dysplastic platelets in MDS may express CD34 and show CD45 staining and side scatter characteristics similar to blasts and can cause a dramatic but spurious increase in the blast count detected by flow cytometry.

Keywords: Myelodysplastic syndrome, giant platelets, CD34

Introduction

Giant platelets may normally be present in the peripheral blood and usually represent young platelets, however they are more commonly seen in pathological conditions such as inherited giant platelet disorders, myelodysplastic syndrome (MDS), myeloproliferative neoplasms, aplastic anemia and immune thrombocytopenic purpura [1–9]. MDS usually causes abnormal megakaryopoiesis, which leads to the production of platelets that have intrinsic dysfunction, reduced life span, abnormal morphologic features, and/or abnormal immunophenotype [10–13].

In this article, we present the case of a patient with history of MDS with dysplastic megakaryocytes as well as a dysplastic subpopulation of large to giant platelets that abnormally express CD34. These platelets showed flow cytometry characteristics (side scatter and immunophenotype) that overlap with those of blasts resulting in a false increase in the blast count detected by flow cytometry. These findings highlight a potential pitfall in the interpretation of flow cytometry that could result in an erroneous diagnosis of acute leukemia.

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Case Report

A 71-year-old man with a long standing history of coronary artery disease and untreated MDS best classified as refractory cytopenia with multilineage dysplasia (RCMD, 2008 WHO criteria) diagnosed 18 months prior, presented with acute gastrointestinal bleeding, symptoms of severe aortic stenosis and pancytopenia. CBC showed pancytopenia with white blood cell count of 0.8 x 10^9/L, hemoglobin of 7.6 g/dL, and platelet count of 58 x 10^9/L. The differential count showed 32% neutrophils, 60% lymphocytes, 6% monocytes, 1% eosinophils and 1% basophils. Physical examination did not reveal any evidence of lymphadenopathy or hepatosplenomegaly. To evaluate for MDS progression, a bone marrow biopsy was performed.

The peripheral blood smear showed decreased platelets with many giant and hypogranular forms [Figure 1A]. Red blood cells exhibited moderate anisopoikilocytosis with occasional circulating nucleated red blood cells. Neutrophils showed dysplasia, including occasional hypogranular and hypolobated forms, as well as a mild shift to immaturity. No circulating blasts were identified.

The bone marrow aspirate showed increased megakaryocytes with marked dysplastic features including small, hypolobated nuclei and nuclear separation; many giant platelets were also present [Figure 1B, C]. The myeloid precursors showed a shift to

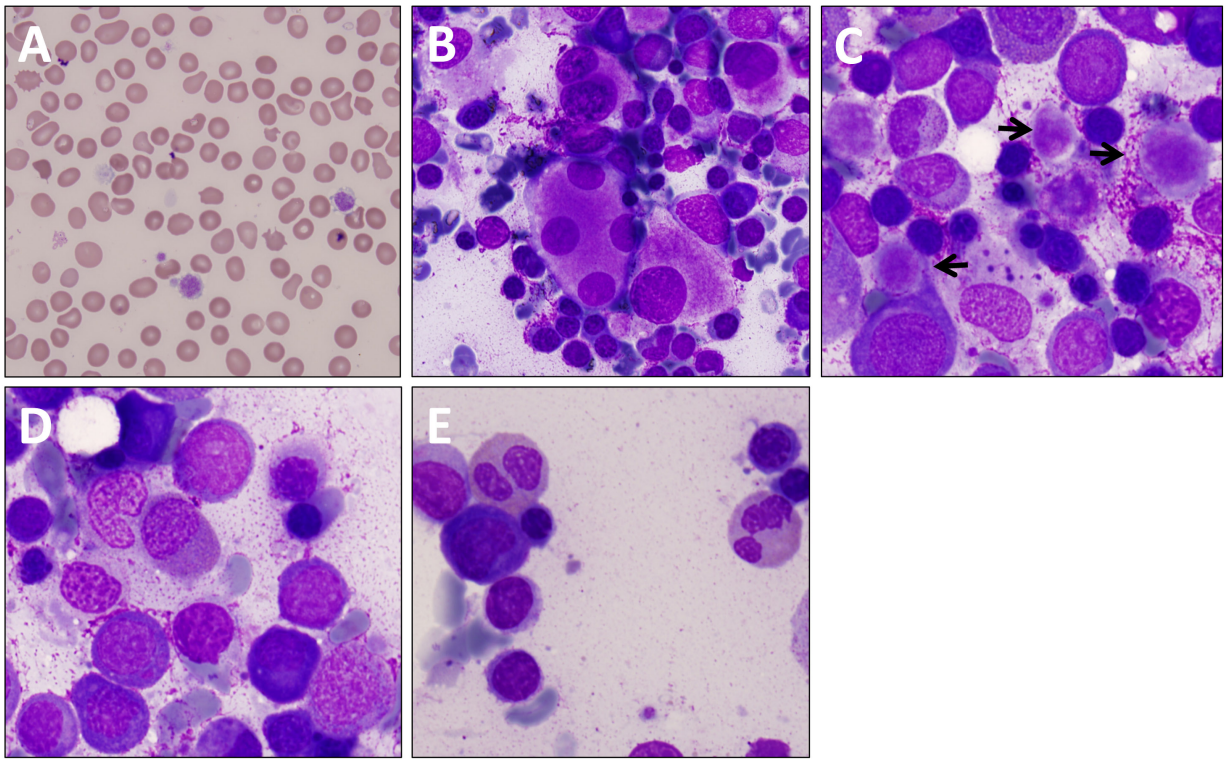

Figure 1: Peripheral blood smear (A) and marrow aspirate (B-E) findings. A, Peripheral blood smear shows circulating giant and hypogranular platelets. B, Megakaryocytes are small and hypolobated with nuclear separation. C, Many giant platelets are observed (arrows). D, Myeloid precursors show shift to immaturity with no significant number of blasts. E, Myeloid precursors show hypogranular and hypolobated forms. (A-E: Wright-Giemsa; A, C-E: 1000X, B: 600X).

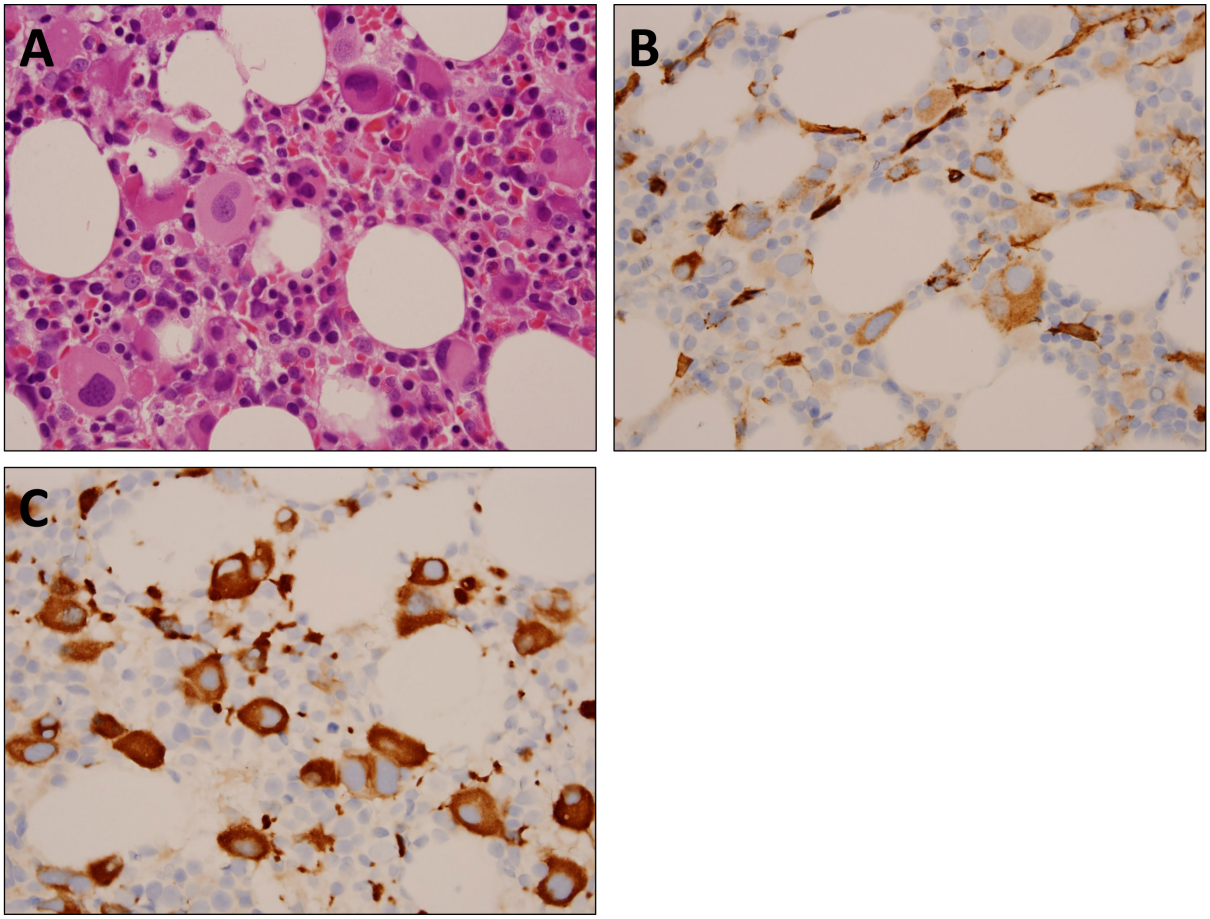

Figure 2: Bone marrow core biopsy findings. A, Megakaryocytes are increased and show clustering with many small and hypolobated forms (H&E; X600). B, Immunohistochemistry of CD34 highlights occasional megakaryocytes and rare blasts (X600). C, Immunohistochemistry of CD61 highlights the increased number of megakaryocytes (X600).

immaturity [Figure 1D] with dysplastic hypogranular and hypolobated forms [Figure 1E]. Blasts were mildly increased, 8% by manual differential count. No Auer rods were seen. Erythroid precursors showed mild dysplastic features including irregular nuclear contours and nuclear budding.

The bone marrow core biopsy was normocellular with 30-40% cellularity. Megakaryocytes were increased and showed focal clustering with many small forms with nuclear hypolobation [Figure 2A]. The myeloid elements showed shift to immaturity. The erythroid precursors were increased and showed irregular nuclear contours and nuclear budding. Immunohistochemical staining for CD34 highlighted scattered blasts (approximately 10-15%) and these cells had large nuclei with fine chromatin and scant amount of cytoplasm. Additionally, CD34 highlighted many dysplastic megakaryocytes [Figure 2B]. CD61 immunohistochemical stain highlighted markedly increased megakaryocytes [Figure 2C].

Conventional karyotying showed: 44~45,XY,-5[7],-7[6],add(17)(p11.2)[7][cp7]/46,XY[13]. Fluorescent in-situ hybridization (FISH) analysis was positive for deletion of 5q and monosomy 7.

Surprisingly, flow cytometric analysis performed

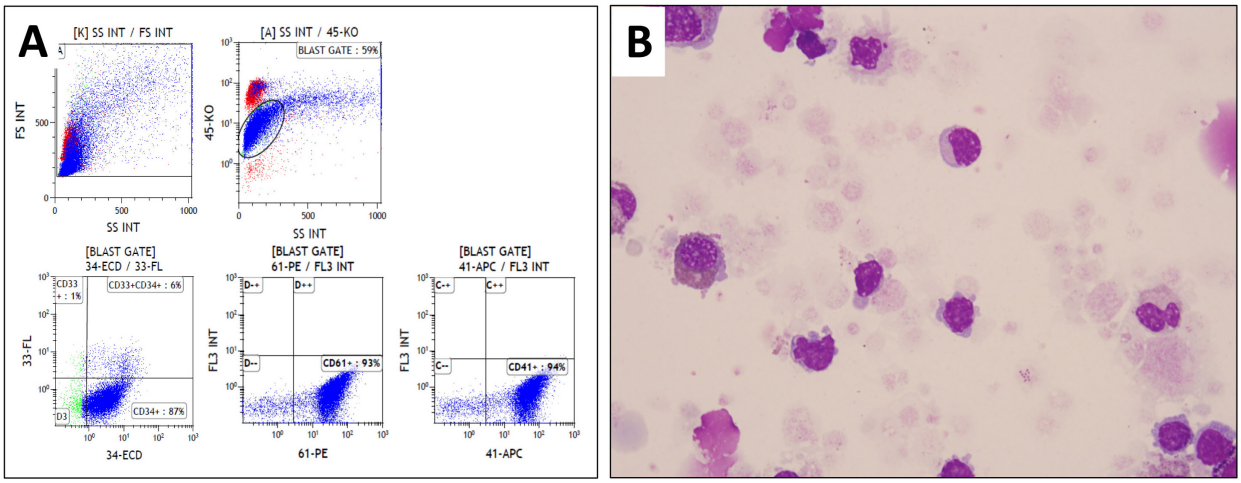

Figure 3: A, Flow cytometric analysis performed on the bone marrow aspirate shows that the events with characteristics of blasts are only positive for CD34, CD41 and CD61. B, cytospin slide of the sample used in the flow cytometric analysis shows a large number of dysplastic giant platelets with no significant number of blasts (Wright-Giemsa; X1000).

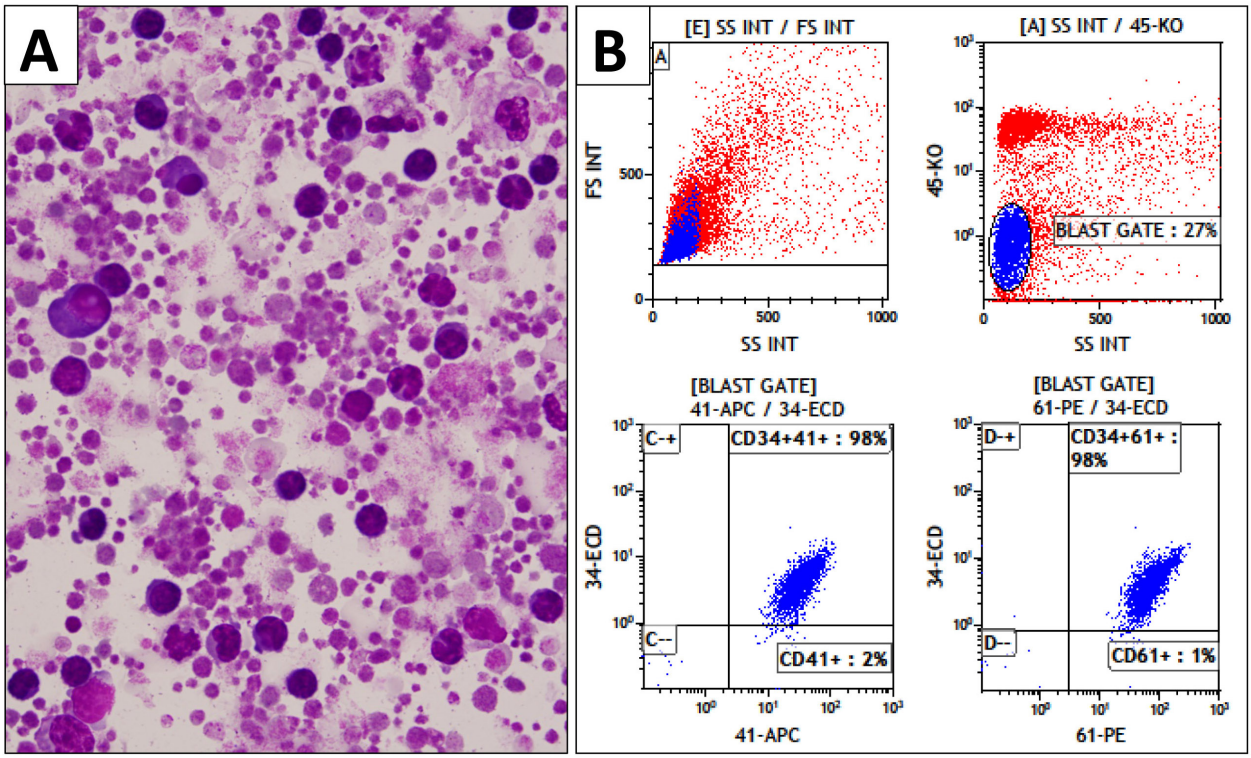

Figure 4: A, Cytospin slide of peripheral blood sample shows numerous giant platelets admixed with occasional granulocytes, lymphocytes and few micromegakaryocytes. No blasts are identified (Wright-Giemsa; X1000). B, Flow cytometric analysis shows that the vast majority of platelets are CD34 positive.

on the bone marrow aspirate showed that 59% of events had the characteristics of blasts (dim CD45+ and low side scatter) that were positive for CD34, CD41, CD61, and negative for CD117, CD33, CD13, CD11b, CD14, CD64, HLA-DR, CD56, CD2, CD3, CD19, TdT, CD79a, and CD22 [Figure 3A].

To investigate the discrepancy in the blast count between (1) the morphology and immunohistochemistry and (2) flow cytometry, the cytospin slide of the marrow aspirate sample used in the flow cytometric analysis was reviewed. The cytospin slide showed a polymorphous cell population, consisted of myeloid elements, lymphocytes, monocytes, occasional megakaryocytes, as well as many dysplastic giant platelets [Figure 3B]. There were approximately 4% blasts by manual count. Since the dysplastic megakaryocytes present in the core biopsy were positive for CD34, the possibility of a spurious increase in blast count caused by dysplastic giant platelets was entertained. To explore this possibility, the patient's peripheral blood, which had many giant platelets, was evaluated by flow cytometry. The peripheral blood specimen was stored in an upright position for 3 hours. Then, the buffy coat was harvested and sedimented by centrifugation. Review of a cytospin slide prepared from the buffy coat showed predominantly large and giant platelets with an admixture of cells including granulocytes and lymphocytes [Figure 4A]. Flow cytometric analysis showed 27% of events in the blast gate, the majority (98%) of which were positive for CD34, CD61 and CD41 [Figure 4B]. These results confirmed that the platelets were positive for CD34.

In view of these findings, the bone marrow biopsy was diagnosed as a normocellular bone marrow with multilineage dysplasia, megakaryocytic hyperplasia and increased blasts. Clinically, the overall assessment was consistent with a high grade MDS with increased blasts such as refractory anemia with excess blast type-2 (RAEB-2). Since the patient was not a candidate for a clinical trial or bone marrow transplantation, he was started on azacitidine 75 mg/m^2 per day for five days every 4 weeks. At the 2-month follow up he was doing well with no new complaints.

Discussion

Giant platelets are usually larger than 7 um while normal platelets range from 1.5 to 3.0 um [14]. Giant platelets are classically seen in several inherited platelets disorders (giant platelet disorders) [6, 7]; however, they can also be seen in other acquired benign and neoplastic conditions such as immune thrombocytopenic purpura, MDS and myeloproliferative neoplasms [15]. While many studies investigated the immunophenotypic abnormalities seen in the myeloid, erythroid and megakaryocytic elements in MDS patients [16–19], only one study explored the potential immunophenotypic alterations that could be present in the platelets of MDS patients [11]. In this study, dysplastic platelets showed aberrant expression of CD34 in one case (2% of all studied cases). Interestingly, this was a patient who had RAEB-2 with thrombocytopenia and megakaryocytic dysplasia; findings that are shared with our case.

The findings in our case are interesting in that the dysplastic giant platelets not only showed aberrant expression of CD34, but also showed CD45 and side scatter characteristics very similar to those of blasts. Such findings could cause confusion or a misdiagnosis of acute leukemia, particularly in laboratories where flow cytometric findings are reported separately from morphology. In our case, the CD34 expression as well as CD61 and CD41 positivity of the events within the blast gate raised the possibility of acute megakaryoblastic leukemia (AML-M7). However, there was a clear discrepancy between the flow cytometric findings and the percentage of blasts identified by morphology. This led to further investigation that identified CD34-positive dysplastic giant platelets as the source of the discrepancy. This case emphasizes the need to always correlate flow cytometric findings with morphology.

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References

1. Amorosi E, Garg SK, Karpatkin S. Heterogeneity of Human Platelets: IV. Identification of a young platelet population with [^{75}Se]Selenomethionine. British Journal of Haematology 1971; 21(2): 227-232.
2. Menke DM, Colon-Otero G, Cockerill KJ, Jenkins RB, Noel P, Pierre RV. Refractory thrombocytopenia. A myelodysplastic syndrome that may mimic immune thrombocytopenic purpura. Am J Clin Pathol 1992; 98: 502-510.
3. Latger-Cannard V, Fenneteau O, Salignac S, Lecompte TP, Schlegel N. Platelet morphology analysis. Methods Mol Biol 2013; 992: 207-225.
4. Garandeau C, Pautas E, Andreux M, Andreux J, Gaussem P, Siguret V. [Myelodysplastic syndromes]. Ann Biol Clin (Paris) 2000; 58: 405-416.
5. Elghetany MT, Hudnall SD, Gardner FH. Peripheral blood picture in primary hypocellular refractory anemia and idiopathic acquired aplastic anemia: an additional tool for differential diagnosis. Haematologica 1997; 82: 21-24.
6. Mhawech P and Saleem A. Inherited giant platelet disorders. Classification and literature review. Am J Clin Pathol 2000; 113: 176-190.
7. Kunishima S and Saito H. Congenital macrothrombocytopenias. Blood Rev 2006; 20: 111-121.
8. Garg SK, Amorosi EL, Karpatkin S. Use of the megathrombocyte as an index of megakaryocyte number. N Engl J Med 1971; 284: 11-17.
9. Steiner M and Schuff-Werner P. Giant platelets in idiopathic thrombocytopenic purpura. Wien Klin Wochenschr 2002; 114: 897.
10. Handin RI, Lux SE, Stossel TP. Blood: principles and practice of hematology. Philadelphia, Pa.: Lippincott Williams & Wilkins, 2003.
11. Sandes AF, Yamamoto M, Matarraz S, et al. Altered immunophenotypic features of peripheral blood platelets in myelodysplastic syndromes. Haematologica 2012; 97: 895-902.
12. Rodak BF. Myelodysplastic Syndromes. In: Rodak BF, Fritsma GA, Keohane EM, editors. Hematology: Clinical Principles and Applications. 4th. St. Louis, MO: Elsevier Saunders; 2012. p. 533 - 545.
13. Mittelman M and Zeidman A. Platelet function in the myelodysplastic syndromes. Int J Hematol 2000; 71: 95-98.
14. Hematology and Clinical Microscopy Glossary. College of American Pathologist 2013.
15. Cazzola M, Malcovati L, Invernizzi R. Myelodysplastic/myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program 2011; 2011: 264-272.
16. Eidenschink Brodersen L, Menssen AJ, Wangen JR, et al. Assessment of erythroid dysplasia by "Difference from normal" in routine clinical flow cytometry workup. Cytometry B Clin Cytom 2015; 88(2): 125-135.
17. Reis-Alves SC, Traina F, Harada G, et al. Immunophenotyping in myelodysplastic syndromes can add prognostic information to well-established and new clinical scores. PLoS One 2013; 8: e81048.
18. Mathis S, Chapuis N, Debord C, et al. Flow cytometric detection of dyserythropoiesis: a sensitive and powerful diagnostic tool for myelodysplastic syndromes. Leukemia 2013; 27: 1981-1987.
19. Sun JF, Yang B, Zhang L, Huang Y, Yang J. [Analysis of immunophenotypic features of blasts in patients with myelodysplastic syndrome by flow cytometry and its diagnostic significance]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2012; 20: 632-635.