Int J Clin Exp Pathol 2014;7(7):4498-4502
www.ijcep.com /ISSN:1936-2625/IJCEP0000851

*Case Report*

# Blasts-more than meets the eye: evaluation of post-induction day 21 bone marrow in *CBFB* rearranged acute leukemia

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Received May 20, 2014; Accepted June 28, 2014; Epub June 15, 2014; Published July 1, 2014

**Abstract:** Induction chemotherapy is often the first therapeutic intervention for acute myeloid leukemia (AML). Evaluation of post induction bone marrow provides critical information for clinical management; in general increased blast counts or increased marrow cellularity is an ominous sign, suggestive of ineffective therapy, and may warrant additional rounds of chemotherapy. However, increased blasts alone are not necessarily predictive of recurrent/persistent disease. Here we report a very unusual observation in a case of AML with a *core binding factor beta* (*CBFB*) rearrangement. In this case the day 21 post-induction marrow biopsy showed a high blast count (approximately 20%), however, subsequent fluorescence in-situ hybridization studies were negative for *CBFB* rearrangement. We compared this finding to post-induction marrows from a series of 6 AML cases with *CBFB* rearrangements, none of which showed an increased blast count. This case illustrates that increased blast counts, even those comprising 20% of cells, are not *de facto* evidence of induction failure, and that correlation with ancillary studies such as fluorescence in-situ hybridization should be used to distinguish a persistent neoplastic clone, from a brisk marrow recovery.

**Keywords:** Core binding factor beta, acute myeloid leukemia, inv(16), day 21 marrow, post induction, DUP98, t(11;20)(p15;q11.2)

## Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy resulting from clonal expansion of myeloid precursors that involves peripheral blood, bone marrow, as well as extramedullary tissues. AML is the most common acute leukemia in adults and was estimated to cause over 10,000 deaths in 2012 [1]. According to current 2008 WHO classification, a diagnosis of AML can be made when blasts exceed 20% in either peripheral blood or bone marrow.

The initial treatment of nearly all subtypes of AML is induction chemotherapy. The actual regimens are based on patients' age, performance status and prior history of myelodysplasia or cytotoxic therapy. The purpose of induction is to reduce of tumor burden and restore normal hematopoiesis of the bone marrow. Post-induction bone marrow evaluation is recommended 7 to 10 days after induction, or commonly known as "Day 14 bone marrow" or less commonly "Day 21 bone marrow", depending on various induction regimens. Important clinical decisions are based on the findings from post-induction bone marrow, and patients who do not show an appropriate reduction in bone marrow blasts or bone marrow cellularity are often given additional rounds of induction chemotherapy.

Inversion 16, or t(16;16) involves the *Core Binding Factor Beta* (*CBFB*) gene and is considered as a favorable translocation with good outcome of induction chemotherapy [2]. Here we report an unusual observation of a high percentage of non-neoplastic myeloblasts (approximately 20% of total marrow cells) in a post-induction bone marrow from an AML case with *CBFB* rearrangement.

## Case report

The patient was a 56 year-old woman with a past medical history of hypothyroidism and dia-

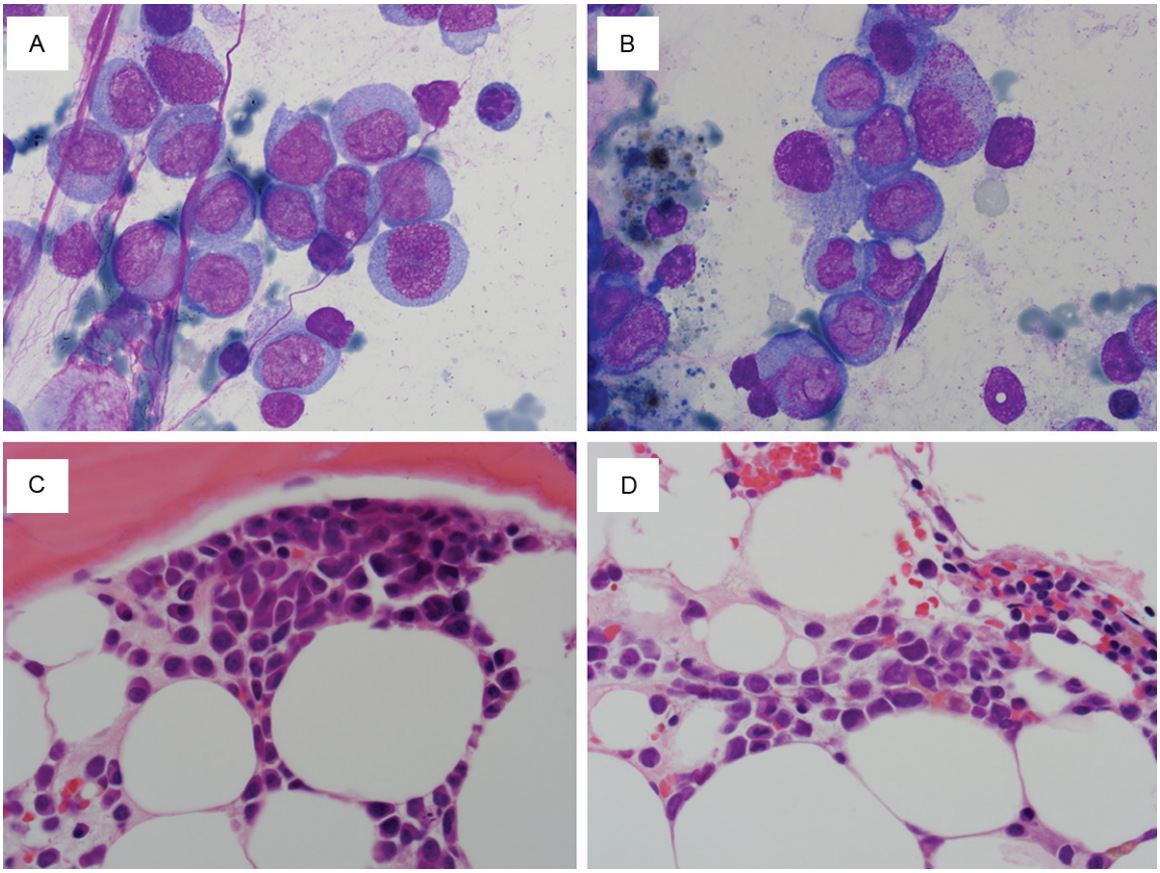


**Figure 1.** Increased blasts in the aspirate and core biopsy of the Day 21 bone marrow. A, B. The Wright-Giemsa stained marrow aspirate contains increased number of immature precursors, characterized by slightly irregular nuclear contours, fine chromatin, prominent nucleoli, and moderate amounts of light basophilic cytoplasm. (Original magnification 1000x). C, D. Hematoxylin-Eosin stained core biopsy shows pockets of immature precursors with very little maturation of granulocytic lineage (Original magnification 600x).

betes mellitus presented to an outside hospital with progressive fatigue. Laboratory tests showed severe pancytopenia, including a hemoglobin level of 4 g/dL. A bone marrow aspirate was obtained, which showed "acute leukemia".

The patient was transferred to our institution and her CBC showed the following: WBCs 2.5 x $10^9$/L, RBCs 3.08 x $10^{12}$/L, Hemoglobin 10.3 g/dL, Mean corpuscular volume (MCV) 96.4 fL, Platelets 43 x $10^9$/L. An in-house bone marrow biopsy showed 12% blasts and eosinophils with abnormal granulations. Flow cytometry showed that 31% of analyzed events were CD34-positive blasts with co-expression of CD13, CD117, CD33, HLA-DR, and negative for CD14, CD56, CD64, CD2, or CD5. The full cytogenetic karyotype was: 46XX,t(11;20)(p15;q11.2),inv(16)(p13.1q22)[9]/47,idem,+8[7]/46XX[4] and Fluorescent In Situ Hybridization (FISH) performed on metaphase cells confirmed presence of *CBFB* rearrangement and trisomy 8. These findings were diagnostic of a AML with inv(16)/*CBFB* rearrangements, and the patient was enrolled in CALGB 10801, a phase II clinical trial, and received 7+3 induction plus 13 doses of dasatinib.

A post-induction bone marrow biopsy was performed at Day 21. Concurrent CBC was as follows: WBCs 0.7 x $10^9$/L, RBCs 2.41 x $10^{12}$/L, Hemoglobin 7.7 g/dL, Hematocrit 21.5%, MCV 89.3 fL, Red cell distribution width (RDW-CV) 13.9%, Platelets 19 x $10^9$/L. Review of peripheral blood showed pancytopenia with rare circulating blasts. The marrow aspirate and core biopsy revealed a hypocellular specimen with 19% blasts by manual differential count (**Figure 1**). The blasts were characterized by fine chromatin, large nucleoli, and moderate amount of light basophilic cytoplasm. Flow cytometric

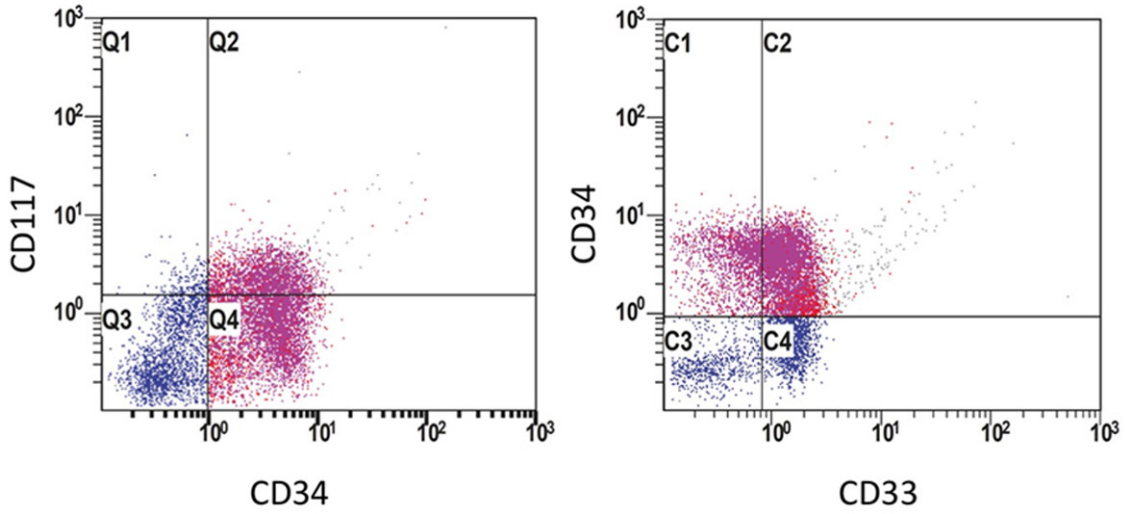


**Figure 2.** Increase of blasts identified by flow cytometry. Twenty-one percent of total cells are blasts that are positive for CD34, CD117, and CD33.

**Table 1.** Blast Counts of Day 21 Marrows of AML with *CBFB* rearrangements

| No | Age/Sex | D21 Blast% | D21 FISH | F/U FISH (interval) |
|---|---|---|---|---|
| 1[$] | 56/F | 21 | Negative | Negative |
| 2 | 62/F | 2 | 0.33% | Negative (+12 days) |
| 3 | 24/F | 2 | N/A | Negative |
| 4 | 71/F | 1 | Negative | Negative |
| 5 | 55/M | 4 | Negative | Negative |
| 6 | 48/M | 2 | N/A | Negative |
| 7* | 29/M | 0 | 1.50% | 0.30% (+22 days) |

D21: Day 21; FISH: Fluorescent In Situ Hybridization; F/U: follow-up; N/A: Not applicable. [$]The currently reported case. *This patient had allogeneic stem cell transplantation and post-transplant FISH result (143 days after the initial induction chemotherapy) was negative.

immunophenotyping showed that 21% of total cells were blasts with partial co-expression of CD34, CD33, CD64 and CD117 (**Figure 2**). Based on these findings the case was called 'persistent AML' and the patient received re-induction chemotherapy with 5+3 per protocol. However, in the days following morphologic review of the bone marrow, FISH studies were performed and showed no evidence *CBFB* rearrangement. These latter results are most consistent with increased blasts due to a recovering marrow as they did not harbor the clonal *CBFB* rearrangement identified at diagnosis.

Following induction, the patient received three cycle of high dose cytarabine and dasatinib consolidation chemotherapy followed with dasatinib maintenance therapy. All subsequent bone marrows (up to 14 months post induction) showed no evidence of recurrent leukemia by morphology and cytogenetics/FISH. These findings further support that the increased bone marrow blasts seen in the post induction marrow were not part of the AML clone.

Marked increases of non-neoplastic myeloblasts in post-induction is a rare observation. A review of the English literature showed a single report of increased non-neoplastic blasts in the peripheral blood around Day 21 post induction [3]. Interestingly, this case is also an AML patient with *CBFB* rearrangement [3]. One possible explanation for increased non-neoplastic blasts post-induction is that, compared to the Day-14 marrows, Day 21 marrows have higher percentage of non-neoplastic blasts in AML cases with *CBFB* rearrangements. To test this hypothesis, we collected all the consecutive Day 21 marrows from AML with *CBFB* rearrangements at Washington University in last three years. There were 7 cases in total with 3 male and 4 female (**Table 1**). Except for the current case (Case 1), the other 6 cases did not show increased blast counts and the blast percentage ranged from 0-4% with an average of 1.8%. In comparison, Day 14 marrows from eleven cases of AML with *CBFB* rearrangements during the same time period had blast counts ranging from 0% to 4% (specifically, 5 cases with 0%, 3 cases with 1%, 1 case with 2%, 1 case with 3% and 1 case with 4% blasts, respectively). The average blast count of the

eleven Day 14 marrows was 1.1%. The blast percentages between Day 21 and Day 14 marrows were not significantly different ($p = 0.30$). Even though limited by the number of available cases, our data argue against the hypothesis that in general, Day 21 bone marrows show higher blast counts than that of Day 14 marrows in AML cases with *CBFB* rearrangements.

## Discussion

CALGB 10801 is an on-going phase II clinical trial to assess the safety and tolerability of dasatinib with induction, consolidation and maintenance chemotherapy in newly diagnosed acute myeloid leukemia (http://www.clinicaltrials.gov/, Identifier: NCT01238211). The induction therapy is one course of 7+3 (daunorubicin, cytarabine) plus dasatinib. If Day 21 bone marrow shows cellularity ≥ 20% and leukemic blasts ≥ 5%, a re-induction therapy with 5+3 (cytarabine, daunorubicin) plus dasatinib would be given. Unfortunately, our patient had received the re-induction before FISH results were available. Consolidation therapy is 4 courses of high dose cytarabine + dasatinib. Our patient had been treated with only 3 courses due to neutropenia and thrombocytopenia. Maintenance therapy is 12 courses of dasatinib.

AML with inv(16) or t(16;16) involves *CBFB* and *MYH11* genes and usually shows both monocytic and granulocytic differentiation with the characteristic abnormal eosinophils containing basophilic granules. Inv(16) or t(16;16) is favorable translocation and the group has a complete remission rate of approximately 85%, which is not affected by additional cytogenetic abnormalities [2].

Secondary cytogenetic abnormalities are reported in approximately 40% cases of AML with *CBFB* rearrangements, common forms including +22, +8, del(7q), and +21 [4]. t(11;20)(p15;q11.2) is a rare recurrent nonrandom translocation, and was reported to result in *NUP98/TOP1* fusion protein [5]. It has been rarely reported in AML and other myeloid neoplasms. Our case is the first reported t(11;20)(p15;q11.2) case in an AML with *CBFB* rearrangement. *NUP98* (Nucleoporin 98 gene) on chromosome 11 is a component of nuclear pore complex. To date, translocations involving *NUP98* have been reported with 28 different partner genes in hematologic malignancies, predominantly myeloid neoplasms [6]. NUP98 was believed to have roles in transcription, and cell cycle regulation, and Murine models of *NUP98* fusion proteins have shown evidence of leukemogenicity [6].

A marked increase of non-neoplastic blasts is a rare observation in post-induction bone marrow, and occurs due to an unknown mechanism. Such cases could represent a diagnostic pitfall, if cytogenetic results are not available, and could result in unnecessary chemotherapy. If unexpectedly high blast counts are seen in a post-induction bone marrow biopsy from patients with favorable cytogenetic abnormalities, such as t(8;21) and inv(16), close observation of the blast count trend and correlation with cytogenetic findings should be considered.

## Disclosure of conflict of interest

None to declare.

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