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LETTER TO THE EDITOR

Flow cytometric demonstration of decrease in bone marrow leukemic blasts after 'Day 14' without further therapy in acute myeloid leukemia

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In acute myeloid leukemia (AML), patients who receive standard cytarabine/anthracycline ('7 + 3') induction routinely undergo bone marrow evaluation 7–10 days after completion of chemotherapy to evaluate response ('Day 14 marrow'). If the marrow has <10–20% cellularity and <5–10% blasts by morphology, National Comprehensive Cancer Network (NCCN) guidelines recommend observation without therapy until these criteria are met or until complete remission (CR) is observed [1]. To document CR, a repeat bone marrow examination is required demonstrating <5% blasts by morphology along with peripheral blood count recovery [2]. However, multiple studies, some [3] giving 7 + 3 and others [4] using cytarabine at higher doses, have suggested that Day 14 marrow findings have limited value in predicting whether a patient will enter CR or not without further therapy. One explanation is the use of morphologic enumeration to assess blasts and the inability of morphology to distinguish normal and leukemic blasts once the blast count is <5%. In contrast, multiparametric flow cytometry (MFC) can detect leukemic blasts if present at a sensitivity of 0.1–0.01% [5]. Inaba et al. [6] and Loken et al. [7] have shown that MFC is superior to morphology in forecasting relapse in patients whose counts have recovered. In the current study, we evaluated the concordance between leukemic blasts detected by morphology and MFC on Day 14 and in a later marrow. As a corollary, we assessed the ability of blasts evaluated by morphology and MFC on Day 14 to predict CR following a single course of induction therapy. In the following, we considered AML to be persistent either if MFC detected any level of leukemic blasts (>0%) or if morphology showed >5% blasts. Hence, we were particularly interested whether morphology or MFC would best predict CR in cases where MFC showed >0% leukemic blasts ('abnormal') but morphology showed <5% blasts ('normal').

We analyzed 136 patients (median age 54 years, range 20–78) including 96 newly diagnosed and 40 relapsed AML (acute promyelocytic leukemia excluded) treated at University of Washington/Fred Hutchinson Cancer Research Center from 2008 to 2011. All patients had a Day 14 marrow (range from Day 14–17) after initiation of induction therapy and a later marrow (range from Day 21–44) without receipt of additional therapy. Induction therapy consisted of standard-dose 7 + 3 (71 patients) and high-dose regimens including cytarabine at individual doses $\geq 1\,g/m^2$ with or without other drugs (65 patients). Bone marrow blasts were evaluated by 10-color MFC using a standard panel [8,9]; leukemic blasts (blasts with an abnormal immunophenotype) were reported as a proportion of total white blood cells. A 'difference form normal' approach was used to identify leukemic blasts by recognizing immunophenotypic deviations from the patterns of antigen expression on normal hematopoietic progenitors of similar lineage and maturation stage [10]. The overall sensitivity of the assay is conservatively estimated as 0.1% but extends to 0.01% for some immunophenotypes. The age, cytogenetic characteristics (SWOG risk categories [11] 57% 'intermediate', 31% 'unfavorable', and 12% 'favorable'), and CR rates [±measurable residual disease (MRD) by MFC, 80% in newly diagnosed and 38% in relapsed/refractory patients], were similar to those seen in patients not included in the study generally because they did not have paired marrows. Follow-up data were current as of 31 December 2015.

In Day 14 marrows, 67% (91/136) patients had <5% blasts by morphology while 33% (45/136) had ≥5% blasts (Table 1). Fifty percent (68/136) patients had no leukemic blasts detected by MFC while 50% (68/136; Table 1) had residual leukemia (range 0.05–95.4%; median 6.7%). The concordance for <5% blasts by morphology (negative) and 0% abnormal blasts by MFC (negative) is 69% (61/91).

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Table 1. Blasts in Day 14 marrow assessed by morphology and MFC.

	Blasts by morphology	Leukemic blasts by MFC		
		Negative (0%)	Positive (>0%)	
Day 14 marrow	<5%	63	28	91
	≥5%	5	40	45
		68	68	136

MFC: multiparametric flow cytometry.

Likewise, the concordance for ≥5% blasts by morphology (positive) and >0% abnormal blasts by MFC (positive) is 88% (40/45). Thirty-one percent (28/91) patients with <5% blasts by morphology had leukemic blasts detected by MFC, while only 7% (5/68) patients with no disease as assessed by MFC had ≥5% blasts by morphology ($p < .001$ using McNemar's test). The cause of the discordance is that morphologic assessment cannot distinguish normal and leukemic blasts as flow cytometry. All five patients with no leukemic blasts detected by flow cytometry achieved CR.

Table 2 (Part A) summarizes the concordance between the Day 14 and later marrow results for both morphology and MFC. In the later marrow, 87% (79 of 91) patients with <5% blasts by morphology ('negative') at Day 14 continued to have <5% blasts. Of the 45 patients with persistent leukemia by morphology (>5% blasts, 'positive') at Day 14, 27 had <5% blasts and 18 had ≥5% blasts in a later marrow. Using MFC, 88% (60 of 68) patients who were negative (0% leukemic blasts) at Day 14 remained negative for residual leukemia while 65% (44 of 68) of those who were positive (>0% leukemic blasts) at Day 14 remained positive in the later marrow.

In addition, we looked at the negative predictive value (NPV) and positive predictive value (PPV) of the Day 14 marrow for the outcome of morphologic CR at the later marrow [Table 2 (Part B)]. A negative test is defined as <5% blasts by morphology or no detectable leukemic blasts by MFC, and a positive test as the corresponding converse. The NPV for this outcome was 75% (68/91) for morphology and 83% (55/66) for MFC. The PPV for this outcome was 49% (22/45) for morphology and 46% (31/68) for MFC.

We also examined the NPV and PPV of the Day 14 marrow for the outcome of MRD-negative CR at the later marrow [Table 2 (Part C)]. The NPV for this outcome was 64% (58/91) for morphology and 78% (53/68) for MFC. The PPV for this outcome was 62% (28/45) for morphology and 65% (44/68) for MFC. Hence for forecasting either CR or MRD-negative CR the NPV and PPV of Day 14 marrow blast enumeration seemed quite similar regardless of whether enumeration was done by morphology or MFC.

It is well-known that a marrow with ≥5% blasts by morphology on Day 14 can decrease to <5% without therapy. We were less sure whether the specifically leukemic blasts detected by MFC on Day 14 would decrease without additional therapy. Sixty-eight patients had >0% leukemic blasts by MFC on Day 14 (Table 1). Table 2

Table 2. Decrease in blasts without additional therapy in the later marrow assessed by morphology and MFC.

A

By morphology		Later marrow blasts	
		<5%	≥5%
Day 14 marrow Blasts	<5%	79	12
	≥5%	27	18

By MFC		Later marrow leukemic blasts	
		Negative (0%)	Positive (>0%)
Day 14 marrow Leukemic blasts	Negative (0%)	60	8
	Positive (>0%)	24	44

B

By morphology		CR	No CR
Day 14 marrow Blasts	<5%	68	23
	≥5%	23	22

By MFC		CR	No CR
Day 14 marrow Leukemic blasts	Negative (0%)	55	11
	Positive (>0%)	37	31

C

By morphology		MRD-neg. CR	no MRD-neg. CR
Day 14 marrow Blasts	<5%	58	33
	≥5%	17	28

By MFC		MRD-neg. CR	No MRD-neg. CR
Day 14 marrow Leukemic blasts	Negative (0%)	53	15
	Positive (>0%)	24	44

D

By MFC		Later marrow leukemic blasts	
		Negative (0%)	Positive (>0%)
Day 14	Positive (>0%)	24	44
	≤1% (13)	6 (46%)	
	1 to ≤5% (18)	7 (39%)	
	5 to ≤10% (11)	5 (45%)	
	10 to ≤20% (9)	3 (33%)	
	>20% (17)	3 (18%)	

CR: complete remission; MRD-neg. CR: measurable residual disease-negative CR; MFC: multiparametric flow cytometry.

(Part D) indicates in 24 of these 68 (35%) the leukemic blasts detected by MFC on Day 14 disappeared in the later marrow. Hence persistent leukemic blasts detected by MFC on Day 14 may disappear, although as the proportion of Day 14 leukemic blasts assessed by MFC increased, the chance of a later marrow showing no leukemic blasts without additional therapy decreased, from 6/13 (46%) with <1% blasts to 3/17 (18%) with >20% blasts on Day 14 ($p = .063$). All 24 patients who had a decrease without further therapy from >0 to 0% leukemic blasts as assessed by MFC (including 3/3 who had >20% blasts on Day 14) entered CR (without residual leukemia). Twenty two of the 24 patients were newly diagnosed, and 12 of the 24 received high-dose and 12 standard-dose intensity induction treatment. Possible explanations for these 'false positives' in Day 14 marrow include damage to residual leukemic blasts that leads to delayed cell death and leukemic blasts that have lost leukemogenic potential through therapeutic selection or differentiation. Host mechanisms may also play a role such

as effective immune surveillance that keeps low levels of residual disease in check. The slow early response seen in some of our patients is similar to that described in pediatric acute lymphocytic leukemia/lymphoma (ALL) [12].

In conclusion, Day 14 assessment by morphology and MFC have fairly similar properties with respect to predicting achievement of CR ± residual leukemia after a first course of induction therapy. It must be kept in mind that later marrows were not obtained at a single time point but rather between Days 21 and 44. Many patients could not be included in these analyses because they did not have paired Day 14 and Day 21 marrows; hence, our conclusions may be affected by selection bias. Of course once CR is achieved, MFC is known to be a more accurate predictor of relapse than morphology [13]. This has recently prompted the European Leukemia Network (ELN) (Estey, personal communication) to recommend accounting for presence of MRD when evaluating CR previously defined purely on morphologic and blood count criteria. Nonetheless, our results suggest the limitations of MFC imposed by use of early time points (during remission induction). Because AML blasts undergo dynamic change after induction therapy, caution should be exercised in interpretation of MFC results during induction. From a clinical standpoint, decisions regarding timing of a second course of induction therapy must rest not only on the possibility of decrease in blasts without further therapy, but on the likelihood of therapeutic failure, including relapse, based on pretreatment characteristics such as cytogenetics and on the patient's fitness to begin a second course.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article online at http://dx.doi.org/10.1080/10428194.2017.1307359.

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