

Impact of Day 14 Bone Marrow Biopsy on Re-Induction Decisions and Prediction of a Complete Response in Acute Myeloid Leukemia Cases

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Abstract

Background: With acute myeloid leukemia (AML), there are limited data about the accuracy of day 14 bone marrow (BM) biopsies for predicting complete remission as compared to day 28 BM biopsy results. We here aimed to estimate the correlation between, and the diagnostic accuracy of, both approaches. **Materials and Methods:** We reviewed 84 patients with AML treated with standard induction chemotherapy to evaluate the remission rate and treatment decisions based on day 14 BM biopsy from 2000-2012. **Results:** Sixty five patients (77%) demonstrated remission (CR) with less than 5% blasts on their day 14 BM. Thirteen patients (16%) had residual disease (RD), and 6 (7%) were classified as indeterminate response (IR) i.e., blasts 5-20%. Two patients with RD on day 14 underwent re-induction. Out of the 17 remaining cases with RD+IR, 14 (all 6 with IR and 8 out of 11 with residual disease with no re-induction) demonstrated a morphologic complete remission (CR) on day 28 BM. The percentages for complete remissions on days 28 and 14 were significantly different [94% versus 79.3%, respectively; $p=0.004$, (OR= 0.143, 95% CI: 0.032-0.63)]. Day 14 BM had 82% sensitivity in predicting CR on Day 28; however, it had insufficient specificity (60%) in predicting failure of CR. **Conclusions:** Induction treatment response assessment based on day 14 BM does not accurately predict the response rate on day 28 and the use of day 14 BM as a sole marker of response to therapy might expose patients to unnecessary interventions.

Keywords: Acute myeloid leukemia- AML- bone marrow biopsy- day 14

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Introduction

Acute myeloid leukemia (AML) is a disorder characterized by abnormal proliferation of blast cells in the bone marrow (BM) with suppression of normal hematopoiesis. While Blast cells share many morphological features with normal stem cells, their presence in excess of 20% in the BM or peripheral blood defines the diagnosis of AML (Cheson et al., 2003). Achieving a complete remission with the return of blast cells to < 5% with evidence of recovering hematopoiesis (i.e., WBC>1000, platelets >100,000 and transfusion independence) has been considered one of the most important end-points and correlated with overall survival and relapse free survival in most AML trials.

AML is a heterogeneous disease and attempts have been made to classify this disorder using different prognostic factors (O'Donnell et al., 2012). While one of the best studied prognostic factor is the cytogenetic abnormalities, another important factor is the disease refractoriness to chemotherapy (Kern et al., 2003; Estey

et al., 2000). The standard therapy of AML includes an anthracycline with cytarabine in the form of "7+3" regimen with possible addition of a third agent. Patients who fail to achieve complete remission on day 28 after induction chemotherapy have a poorer prognosis (Döhner et al., 2010). One way to improve their outcome is to identify this group early in their course and consider a more intensive chemotherapy before the full recovery of BM, although this strategy remains controversial (Rowe et al., 2010; Walter et al., 2014; Buchner et al., 1999).

While early response to therapy and clearance of blasts on day 7 or 14 is a strong predictor of favorable outcome in acute lymphoblastic leukemia, particularly in children (Panzer-Grümayer et al., 2000), this is not the case in AML. The role of early re-treatment based on day 14 BM interpretation and its impact on outcome remains less established in AML. Although some studies have shown the prognostic impact of day 14 or day 15 blast clearance in AML and its correlation and value in predicting remission on day 28 (Bertoli et al., 2014), other studies did not find this association (Hussein et al., 2008; Morris et al.,

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2013). Therefore, we sought to assess the value of day 14 bone marrow in predicting complete remission and its correlation with day 28 bone marrow results.

Materials and Methods

We reviewed the charts of all patients between the ages of 18 and 60 years with morphologically confirmed and previously untreated AML who received chemotherapy at our academic institution over a 13 year period.

Inclusion criteria

Patients were included if they met the following criteria:

1) Documented new diagnosis of de novo AML; 2) Received an induction chemotherapy at the discretion of the treating physician; 3) Had two documented BM assessments; at nadir (i.e. day 14) after induction, and after recovery (i.e. day 21-42).

Patients with AML-M3 by FAB classification were excluded.

Patients' demographics, AML-FAB subtype and cytogenetic information were extracted from the patients' notes, electronic health records and pathology reports. Based on the result of day 14 BM samples, patients were classified to have either complete response (CR, ie. no evidence of leukemia), or a suboptimal response (SOR). Repeated BM examinations were done at the time of blood count recovery following induction chemotherapy between days 21 and 42.

Definitions for response criteria

We adopted the international working group (IWG) criteria to assess the response to induction treatment which includes the assessment of early treatment response and morphological complete remission (Cheson et al., 2003). Although post-treatment early BM biopsy is likely to be hypocellular, it provides an indication of anti-leukemic activity (O'Donnell et al., 2012; Estey et al., 2000). The morphological complete remission (CR) is defined as a blast percentage of <5% and no Auer rods visualized at the time of marrow recovery with an absolute neutrophil count of more than 1,000/ μ L, platelets of \geq 100,000/ μ L and independence from blood transfusions.

Since a blast count of more than 20% defines acute leukemia or (definite) residual disease (RD) and less than 5% defines remission (CR), we defined indeterminate response (IR) for blast count between 5-20%. All cases who did not achieve CR were assigned to have

a suboptimal response (SOR) which combines IR with RD groups (Table 1). In post-chemotherapy state, the pre-mature cells in the BM might represent either normal recovering precursors or proliferating leukemic blasts and based on morphology, distinction is difficult despite many suggested morphological features. We did not use flow cytometry or cytogenetics to define remission as the data were incomplete for some of the included patients and because of the technical difficulty in interpreting the results as reported previously (Paietta., 2012).

Statistical analysis

The primary aim of our study was to examine the correlation of day 14 BM biopsy with the remission at day 28 BM based on morphological criteria. We plotted a 2X2 table and used the McNemar's test to determine the level of significance (Agresti., 2013). We estimated the odds ratio with its corresponding 95% confidence intervals (CI) for the day 14 BM to predict remission status on day 28. In addition, we calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of day 14 BM to predict day 28 BM biopsy result using the standard methods (Altman and Bland,1994).

Results

Patient demographics

Of the 100 patients evaluated, 84 patients met our inclusion criteria for evaluation. The median age of included patients was 43 years (range: 18–77) and 44 (51.2%) were females. Cytogenetic data at the time of diagnosis were known for 73% of them; 32 patients (53%) had good- or intermediate-risk cytogenetics, the remaining 29 (47%) patients had poor-risk or complex cytogenetics. Table 2 summarizes the patients' baseline characteristics, FAB classification and cytogenetic data.

Predictive value of day 14 BM biopsy

Sixty five patients (77%) had remission (CR) with less than 5% blasts on their day 14 BM biopsy. Thirteen

Table 1. Day 14 Bone Marrow Biopsy Classification

Day 14 response criteria	Bone marrow cellularity	Bone marrow blasts (%)	Comment
No evidence of residual disease	Hypocellular	\leq 5	No Auer rods
Indeterminate response (IR)	Sub-optimal reduction	5–20	Blast % by morphology
Residual disease (RD)	Sub-optimal reduction	$>$ 20	Blast % by morphology
Sub-optimal response(SOR)	IR + RD	$>$ 5	Considered a positive screen

Table 2. Baseline Characteristics of the Patients

Patients' characteristics	Number
Total number	84
Age in years, median (range)	43 (15-60)
FAB classification	
M0	15
M1	23
M2	15
M4	16
M5	12
M6	3
M7	0
Cytogenetic profile	
Good/ Intermediate risk	32
Poor-risk/ Complex	29
Unknown/Not available	23

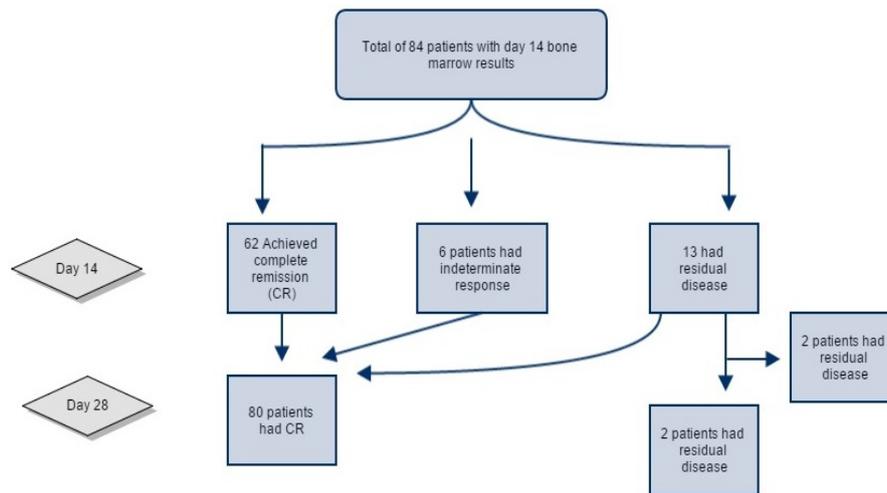


Figure 1. Flow Chart of 84 Patients with AML Included in the Study and Their Outcomes Based on Day 14 and Day 28 Bone Marrow Biopsy

Table 3. A 2x2 Table Showing the Results of Day 14 and Day 28 BM Biopsies (patient number is 82 as 2 expired patients not included in the analysis)

		BM status at 28 days (remission)		Total
		Yes	No	
Day 14 BM status	Remission	63	2	65
	SOR	14	3	17
Total		77	5	82

patients (16%) had residual disease (RD), and 6 patients (7%) were classified as indeterminate response (IR). Sixty three out of 65 patients with morphological remission on day 14 had complete response on day 28 BM biopsy. For the remaining 19 cases, 2 patients with residual disease underwent re-induction chemotherapy on day 14, developed severe sepsis and died in spite of intensive therapy. The cause of death was considered to be related to therapy. The remaining 17 patients who did not achieve CR were observed until count recovery without any re-induction therapy. Out of the 17 cases, 14 patients (All 6 patients with IR and 8 out of 11 patients with residual disease with no re-induction) had a morphologic complete remission (CR) on day 28 BM.

We performed McNemar's test at day 14 and day 28 with yate's continuity correction for cells below 5 (Table 3). Only 82 patients were included in the final analysis as 2 patients underwent re-induction and died. The percentage of complete remission reported at day 28 was significantly different from the reporting of complete remission at day 14 (94% versus 79.3%, respectively), $\chi^2=9$, $p=0.004$, (OR= 0.143, 95% CI: 0.032-0.63). The Day 14 BM had 82% sensitivity in predicting CR on Day 28 BM biopsy. However, failure to show complete remission (i.e., cytoreduction <5% blasts) on Day 14 BM biopsy had only 60% specificity in predicting the failure of CR on Day 28 BM biopsy. The PPV of the Day 14 BM followed the same pattern and was estimated to be 97% with 95% CI (91.5-98.9) as cytoreduction on Day 14 BM biopsy led to CR on Day 28 BM biopsy. On the

other hand, NPV of Day 14 BM biopsy was only 18% with 95% CI (8.3-33.9) as majority of patients without Day 14 cytoreduction achieved CR on Day 28 BM biopsy. The positive and negative likelihood ratio for day 14 BM is 2 with 95% CI (0.7 to 6) and 0.3 with 95% CI (0.13-0.71), respectively.

Discussion

Early bone marrow assessment is considered the standard measure of response evaluation following induction chemotherapy in patients with AML. Although, the conventional measure of response following induction chemotherapy is to assess the blast percentage on day 28 BM biopsy (Döhner et al., 2010), the assessment of BM biopsy between days 12 and 16 after commencement of induction therapy is utilized by many study groups and is based upon data showing improved early outcomes for patients with leukemic blast clearance at this time point (Kern et al., 2003., Buchner et al., 1999). In this study, we sought to determine the ability of day 14 BM biopsy in predicting CR and its relation with day 28 BM results.

Our study showed a significant statistical difference between the results on day 14 and day 28, which suggests that the day 14 BM biopsy result does not have a significant relation with day 28 result; however, it may serve as a sensitive indicator (Sensitivity: 82%). Furthermore, 17% of patients changed their status to favorable outcome (CR) on day 28, hence the low NPV of 18%. This significant shift of the sample from SOR to CR indicates that day 14 BM biopsy result may not guide to appropriate treatment decisions.

The International Working Group (IWG) guidelines for clinical response in AML allow for the early morphological assessment of the BM (Cheson et al., 2003); while this has been shown to be a possible prognostic factor as the clearance of the blasts reflects better anti-leukemic activity, this has not been proven across all the studies (Kern et al., 2003., NCI SEER stat fact sheet., 2013). There are limited data from randomized studies to prove the value of day 14 BM biopsy in decision-

making. Several studies have looked at the value of day 14 or earlier BM biopsy and its role in determining the need for re-induction therapy. Cassileth et al., (1984) found that patients with hypocellular BM and focal residual leukemia may readily achieve remission without additional chemotherapy but have an increased likelihood of early relapse. Recently, Morris et al., (2013) evaluated the accuracy of day 14 BM biopsy in determining the need for re-induction chemotherapy in a cohort of newly diagnosed de novo AML patients and concluded that the use of day 14 BM biopsy may have suboptimal sensitivity for the detection of residual leukemia and it should not be used as a sole marker of recovery. Luger (2011) reviewed the evidence on this topic and concluded that it is still unclear whether a nadir BM biopsy after induction is the best way to identify patients with failure of treatment who may require an early re-induction. It appears from the above discussion that a good number of these patients would have achieved CR without additional therapy. We found that, although, attaining major cyto-reduction on day 14 BM biopsy is sensitive (82%) in predicting remission on day 28 BM biopsy, it is not sufficiently specific (60%) to predict failure. Two of our patients who were considered to have failed induction, received re-induction with intense-dose regimen, had febrile neutropenia with fulminant sepsis and died in spite of intensive support.

Morphological assessment of residual leukemia is liable for mistakes, especially in the presence of treatment-induced hypoplastic changes in the BM. Many children with acute lymphoblastic leukemia display hematogones which are normal hematopoietic recovering stem cells and can be confused as residual leukemic blasts. The guidelines define CR state to have less than 5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells (Cheson et al., 2003). There should be no blasts with Auer rods or persistence of extramedullary disease. While slow clearance of blast cells has been suggested to be a poor prognostic marker, there is no evidence that chemotherapy will be able to change the outcome. This has been typically illustrated in the elderly with AML where many patients attain complete remission but long term survival and cure are limited (Luger., 2010).

This study has certain strengths as well as limitations. Our results are consistent with some of the previous studies and lend further support to the suboptimal nature of day 14 BM assessment in predicting complete remission (Hussein et al., 2008; Morris et al., 2013). Most of the earlier studies either used Fisher's exact test or Chi-square test and this may represent a potential limitation since it ignores the fact that the analysis includes a paired observation from day 14 and day 28 for the same sample. Using the paired observation is more robust as it examines the true change of the sample. On the other hand, lack of survival data and retrospective design are the limitations of the study that restrict our ability to make strong recommendations.

In conclusion, assessment of BM biopsy on day 14 post induction treatment for AML does not accurately predict the response rate on day 28. Thus, the use of day 14 BM result as a sole marker of response to therapy might expose the patients to unnecessary interventions.

AML patients with residual disease, particularly an indeterminate response on day 14 BM biopsy, may benefit from careful observation.

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