The Criteria for Bone Marrow Recovery
Post–Myelosuppressive Therapy for Acute Myelogenous Leukemia
A Quantitative Study

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Context.—Although the early post–myelosuppressive chemotherapy pathologic changes of the marrow have been described, the rate and the histologic definition of recovery are not defined.

Objective.—To study the rate of recovery of bone marrow in patients given myelosuppressive therapy for acute myelogenous leukemia, establish the histologic criteria of recovered marrow, and correlate the recovery pattern with those patients who received a bone marrow transplant by using histology, peripheral blood, immunophenotyping, and computerized morphometry and mathematical slope equation.

Design.—We studied the post–myelosuppression recovery of the bone marrow to determine patterns and rate of recovery in 135 serial bone marrow biopsies of 51 patients. These patients were divided into 2 groups: 1 group of 28 cases diagnosed with acute myeloid leukemia, the majority treated with cytarabine (Ara-C) infusion for 7 days and daunorubicin intravenously daily for 3 days (7+3 regimen), and the other control group of 23 cases treated with chemotherapy or allogeneic bone marrow transplantation for a variety of hematologic malignancies. All biopsies during the recovery period were obtained before consolidation regimen. We used morphometry to calculate the cellularity and myeloid to erythroid ratio and quantified megakaryocytes CD10 versus time from day 14 onward. The absolute neutrophil and platelet counts for 28 cases were related to histologic recovery.

Results.—From day 14, we noted a differential slope of recovery of these patients with no difference in male and female patients, \( P = .45 \), but a difference between younger and older patients (> 58.5 years), \( P = .03 \). After regenerative hyperplasia, the cellularity plateaus, the myeloid to erythroid ratio, and the megakaryocytes even out with platelet normalization, and the early CD10+ B cells rise from day 40 onward, \( P = .01 \). The patterns of recovery after day 60 of postchemotherapy and posttransplantation patients are similar. Complete histologic and peripheral blood recovery is noted at day 38 and thereafter.

Conclusions.—By linear equation using at least 2 trephine biopsy specimens, the projected rate of cellular recovery may be determined, and 5 histologic features are associated with complete histologic recovery.

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Bone marrow trephine biopsies, usually obtained from the posterior iliac crest, are used for the diagnosis and confirmation of acute leukemia. Hematopoietic regrowth after chemotherapy is usually monitored by evaluation of sequential peripheral blood indices only as a convenient window as to the state of the marrow. A study addressed this window by determining granulocyte recovery indicated by CD34+ granulocyte-macrophage colony-forming unit (GM-CFU). Hematogones give rise to lymphocytes and to dendritic cells, but their emergence in relation to hematopoietic recovery has not been studied. Some studies used animal models and peripheral blood reconstitution as measures of marrow recovery despite the fact that the histologic criteria of posttherapy recovery in correlation with peripheral blood recovery have not been established. Because most descriptions of the marrow recovery used the posttransplantation setting, we also compared the marrow cellular recovery in patients who received transplants in a subset of cases.

Previous studies established the sequence and frequency of histopathologic events developing after chemotherapy and transplantation, but the temporal criteria for recovery were not established. In a previous report, Witteles described in detail the initial posttherapy depletion phase, which was characterized by progressively decreasing hematopoietic cells with loss of cells uncovering the underlying stromal network. This phase is overlapped lat-
er by reconstitution. The other earlier studies of marrow recovery with immunostaining reported on qualitative changes in the marrow but did not address the quantitative changes in sequential biopsies or the histologic criteria for recovery. We quantitated the sequence of cellular recovery of posttherapy marrow and peripheral blood parameters; to define the criteria for histologic recovery.

In the literature, there is no clear definition of the time onset of recovery using the bone marrow trephine or particle section findings. To address the previously mentioned issues, the kinetics of cellular recovery of posttherapy bone marrow using a linear slope equation was studied. The objectives of our study were to evaluate the correlation between the slope of recovery and gender, age, and histologic and immunophenotypic characteristics of bone marrow and peripheral blood parameters; to define the timeline of histologic recovery in relationship to cellularity, normalization of myeloid to erythroid (M/E) ratio, stabilization of megakaryocytes number, peripheral blood recovery of neutrophil and platelets, and bone marrow emergence of early B cells or hematogones; and to compare the long-range recovery of the posttransplanted and postchemotherapy marrow.

### Data on the 28 Patients With Peripheral Blood and Bone Marrow Findings*

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* Plat indicates platelets × 10³/μL; ANC, absolute neutrophil counts; C, cellularity percentage; MEGS, megakaryocytes; M/E, myeloid to erythroid ratio; AML, acute myelogenous leukemia; FAB, French-American-British; DATE, daunorubicin, Ara-C, topotecan, etoposide; HiDAC, high-dose Ara-C; 7+3, Ara-C and daunorubicin; MDS, myelodysplastic syndrome; NA, not available; ATRA, all-trans retinoic acid; MEC, mitoxantrone, etoposide, cytarabine; s/p, status post; BM, bone marrow; and BMT, bone marrow transplant.

**DESIGN**

### Materials

**Group 1.**—We studied the post–myelosuppression recovery of the bone marrow to determine patterns and rate of recovery in patients with acute myelogenous leukemia (AML) consisting of 125 serial bone marrow determinations. The average number of posttherapy biopsies per patient was 5 (range, 2–13). There were 98 adequate biopsies of 28 patients (27–86 years; mean, 55.8 years) diagnosed with AML, 24 were treated with conventional Ara-C and daunorubicin (7+3) induction regimen (2 with added all-trans retinoic acid [ATRA] and 2 with added Hydrea) and 3 with daunorubicin, Ara-C, topotecan, etoposide (DATE) and 1 with mitoxantrone, etoposide, cytarabine (MEC) regimen (Table). Granulocyte colony-stimulating factor (Neupogen) was given only to 2 patients and therefore was not a significant player in cellularity recovery. The French-American-British classifications included 10 with M4, 8 with M5, 4 with M1, 4 with M2, and 2 with M3. There were 19 men and 9 women with M/E ratio of 2:1. The 27 excluded biopsies did not have adequate core or clot particles because of inadequate specimen, subcortical location, or extremely fibrotic or crushed tissue. All patients had pretherapy, immediate follow-up from day 14 to day 25, and preconsolidation follow-up biopsies, with
Figure 1. A series of digital images (640 × 480 pixel resolution) per patient were obtained on periodic acid-Schiff–stained bone marrow biopsy (A, day 14; B, day 40) showing the morphometric result of segmentation highlighting the nuclei in red for day 14 (C) and day 40 (D) bone marrow of 2 index cases (original magnification ×20).

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the estimated cellularity in the pathology report indicated a high concordance suggesting validity of the technique. For quantifying the M/E ratio, the erythroid islands were outlined freehand and totaled for the erythroid fraction. This value was subtracted from the total cellularity, giving an estimate of the myeloid area and the M/E ratio was then calculated. A correlation coefficient of $r = 0.91, P < 0.001$ between marrow M/E ratio obtained by morphometry and the estimated M/E ratio in the pathology aspirate report indicated a high concordance. Because cellularity varies with age, the cellularity obtained as discussed previously was converted and adjusted to a normalized value for age by the formula: Normalized Cellularity = (Actual Cellularity $\times 100 - \text{Age}) \times 100$. The normalized cellularity was compared with the peripheral blood findings to determine the definition of histologic recovery.

Immunohistochemistry studies for myeloperoxidase and hemoglobin were performed using Ventana automated Benchmark LT instruments (Tucson, Ariz) according to standard protocol. The tissues for flow cytometry were prepared using a Coulter Epics Profile (Beckman Coulter, Fullerton, Calif), which is a safe, standardized flow cytometer. Cell count and viability using trypan blue were performed for assessment of the sample. The cell concentration was adjusted to $5 \times 10^5$ or $1 \times 10^6$ cells per mL. Cells were stained by a direct method, which treated the patient’s cell population with monoclonal antibodies conjugated to a variety of different fluorescent tags. The antibodies were added directly to the tubes containing the appropriate cell concentration and incubated at room temperature in the dark for 20 minutes. The tubes were then washed with 2 mL of 1% PBS for 5 minutes and centrifuged at 1500 rpm. The supernatant was decanted, and the pellet was resuspended by adding 0.5 mL of 1% paraformaldehyde. The tubes were gently vortexed and placed at 2°C to 8°C until ready to be run on the flow cytometer. Analysis was performed using logical gating on lymphocyte gate counting 5000 events. A Coulter Epics (Hialeah, Fla) was used for 4-color acquisition and analysis. A membrane reactive antibody to CD10 (CALLA) was used.

**Definition of Recovery Groups**

The postchemotherapy AML patients were divided into 2 groups for early and late slope: early at 10 to 25 days postchemotherapy and late at 25 to 50 days postchemotherapy. Given the observed initial regenerative hyperplasia postchemotherapy, we defined presumptive recovery using actual cellularity as more than 40%, near recovery as 30% to 40%, and nonrecovery as less than 30%. A contingency matrix of the groups according to recovery was compared as to sex and age using 1-way analysis of variance (SAS System, SAS Inc, Cary, NC). The slope of early recovery was compared with the slope of postrecovery, and the correlation between these 2 slopes was determined.

The histologic definition of recovery as a normalized cellularity based on age was correlated with the conventional peripheral blood criteria of recovery: neutrophils greater than 1500/µL and platelet count greater than 50 000/µL thresholds, respectively.

The subset of 23 patients with postchemotherapy and posttransplantation were plotted as to M/E ratio, cellularity, megakaryocytes, and CD10 versus days in the x-axis, respectively.

**RESULTS**

**Slope of Early Recovery**

Using morphometry, our data showed that the recovery kinetics of the bone marrow cellularity were similar to a positive slope of a line on a Cartesian plane: the higher the slope, the faster the recovery (Figure 2). From day 14, (Figure 1, A) we noted a differential slope of recovery of these patients and assigned them to 3 groups: early, mid, and late slope with a slope of 3.3, 1.8, and 1.2, respectively (slope $m = [c - c_1]/[\text{day} - \text{day} 1]$, where the differences between later and earlier cellularity values were plotted against the corresponding days of recovery).

**Sex and Gender Difference**

Men and women did not differ significantly with regard to early slope, $P = .45$. Furthermore, patients older than
58.5 years had an average recovery slope of \( m = 2.33 \). The group of patients younger than 58.5 years had an average recovery slope of \( m = 2.65 \). Older patients (\( >58.5 \) years) had significantly lower early slope than younger patients, \( P = .03 \).

**Regenerative Hyperplasia**

All of the patients with recovering marrow (Figure 1, B) showed an initial regenerative panhyperplasia and then tapered down to a normal range of cellularity. The patients with higher early cellularity slope had significantly lower later slope, \( P = .009 \). Early and late slopes were negatively correlated \( (r = -0.50) \), implying normalization of regenerative hyperplasia as a recovery phenomenon. The normalization applied to M/E ratio and megakaryopoiesis as well. The M/E ratio was noted to be initially high at the early posttherapy period day 20 onward with stabilization of M/E ratio, megakaryocytes, and cellularity beginning from day 40 and mostly completed by day 75.

**Peripheral Blood Recovery**

The histologic findings were compared with ANC and platelet count on 28 cases that had data to correlate recovery. This group showed a mean of 39 days (range, 22–73 days) with resulting mean ANC of 5565/\( \mu \)L (3642–7488/\( \mu \)L, 95% confidence interval) and a mean platelet count of 177 000/\( \mu \)L (124 000–229 000/\( \mu \)L, 95% confidence interval). After day 38 none of the patients had lower than the cut-off threshold platelets and ANCs. Before day 38, 18% of patients had platelets less than 50 000/\( \mu \)L and 13% had ANCs less than 1500/\( \mu \)L. Before day 30, platelets were less than 50 000/\( \mu \)L in 9% and ANC was less than 1500/\( \mu \)L in 13% of patients. After day 30 but before day 38, all patients had ANCs greater than 1500/\( \mu \)L, but 9% still had platelets below threshold indicating lag in productive megakaryopoiesis compared with granulopoiesis.

**Histologic Definition of Recovery**

The histology of recovering marrow was studied in the time frame expected to show recovery, between 4 to 6 weeks.16 The localization and distribution of elements had a predictable pattern from an early prerecovery pattern of discrete and delineated islands of erythroid and myeloid precursors. The marrow from day 30 to day 52 was characterized by progressive localization of myeloid precursors adjacent to bone trabeculae and replacement of pockets of paraosteal abnormally localized erythroid colonies with layers of myeloid precursors (Figure 3, A and C).

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**Figure 3.** Panels of day 28 (A) and day 38 (C) bone marrow showing repopulating marrow with myeloid and erythroid elements in relation to the trabeculae (hematoxylin-eosin, original magnification ×20). Corresponding myeloid-specific immunostains for day 28 (B) and day 38 (D) highlighting the recovery myeloid localization to paratrabeculae (myeloperoxidase, original magnification ×20).
**Figure 3.** A series of acute myelogenous leukemia patients with recovering blood and marrow histologic parameter data were plotted against the day of determination with total cellularity versus days and all parameters versus days, respectively. The y data were converted relative values to fit the display.

**Figure 4.** A series of acute myelogenous leukemia patients with recovering blood and marrow histologic parameter data were plotted against the day of determination with total cellularity versus days and all parameters versus days, respectively. The y data were converted relative values to fit the display.

Figure 3, A and C, show a panel of repopulating marrow with myeloid and erythroid elements in relation to the trabeculae and corresponding immunostains for myeloperoxidase (Figure 3, B and D) that highlight the myeloid elements’ localization to the paratrabeculae as sign of recovery. The sequence of recovery is characterized by 2 paraosteal findings: (1) abnormal localization of regenerative erythroid islands and (2) its replacement by myeloid bands and increase in thickness of these bands. The abnormal localization of erythroid islands is defined as those cells arraying and abutting the endosteal lining. Myeloid cells slowly replace this abnormal localization initially as a single layer in a flattened cuboidal stack, which is later transformed to clusters made up of rounded myeloid cells between 2 to 5 layers. Interosteal findings comprise 2 ends of the spectrum: discrete well-defined islands of erythroid and myeloid precursors on onset of recovery and an even mixture of these two on recovery completion. This timing and sequence of recovery was also observed in the control group.

Based on the findings shown in the Table and the study of histology from day 30 to day 52, the histologic findings fulfilling the criteria for recovery are composed of the following: (1) cellularity is more than 40% for age-expected normalized cellularity, (2) M/E ratio is stabilized to near normal level (2–3:1), (3) erythroid islands are normally localized away from bone, (4) there are admixed myeloid and erythroid elements and normal localization of myeloid bands, and (5) megakaryocytes are evenly distributed and are not clustered.

**Correlated Blood and Histology Findings**

A series of 28 patients with blood and histologic parameters were plotted against the posttherapy day to determine total cellularity versus days and all other parameters versus days, respectively (Figure 4). Results showed that the pattern of recovery of cellularity in this subset and the rest of the cases with available data and control group were similar. Interosteal findings comprise 2 ends of the spectrum: discrete well-defined islands of erythroid and myeloid precursors on onset of recovery and an even mixture of these two on recovery completion. This timing and sequence of recovery was also observed in the control group.

**Figure 5.** CD10+ B cells greater than 3% were detected only in bone marrows with actual cellularity more than 45%.

**Comparison of Posttransplant and Postchemotherapy Recovery**

The posttransplant and postchemotherapy recovery group had a similar pattern of change in terms of M/E ratio, cellularity, megakaryocytes, and emergence of hematogones (Figure 6, A through C). In this control group, similar to the main group, the M/E ratio was significantly higher (2.5 ± 1.2 vs 1.6 ± 0.5, \( P = .002 \)) in the first 100 days posttherapy and remained constant at a lower value thereafter. Bone marrow cellularity recovered to normal for age from day 40 to day 60 posttherapy for most patients in the study, both those who received transplants and postchemotherapy patients. Cellularity was significantly higher (54.7% ± 15.0% vs 42.8% ± 15.7%, \( P = .03 \)) in the first 60 days posttherapy and remained constant at a lower value thereafter. Megakaryocytes were significantly higher (12.6% ± 6.3% vs 4.4% ± 2.8%, \( P < .001 \)) in the first 60 days posttherapy and remained constant at a lower value thereafter.

**COMMENT**

Sequential examination of the trephine core biopsy of the bone marrow tissue from patients with AML posttherapy is the clinical standard of practice because aspirate smears and blood may not reflect the pathology or etiology of delayed recovery and because some marrows are inapplicable owing to hypocellularity or fibrosis.

We evaluated a cohort of cases with serial biopsies to quantify and define the recovery of hematopoietic cells in patients who were postchemotherapy and went into re-
mission and another cohort of cases with both posttransplant and chemotherapy data for early and late recovery follow-up. Earlier studies such as the one published by Islam described the very earliest findings from 1 to 6 weeks of biopsy of 20 patients after chemotherapy for AML. He noted an early hypocellularity period of 1 to 3 weeks and a recovery period of 4 to 6 weeks. The onset of posttransplant recovery is similar to postchemotherapy recovery with early phase noted from 7 to 14 days up to 28 days. We extended this window of observation beyond the immediate recovery period to more than 100 days and found that at 60 days on average and day 100 at the latest the recovery should be completed.

We also performed correlative histology blood findings during the recovery period to more precisely define the timing of histologic recovery. The criteria for histologic recovery of marrow elements in postchemotherapy have not been defined despite previous studies defining the sequence of recovery of marrow cellular elements. In this study, we quantified the sequence of cellular recovery with morphometry and discovered a linear equation that reflects the rate of recovery and correlated these data with histologic bone marrow parameters in a time-wise manner and came up with criteria for histologic recovery. We observed a sequence of abnormal histopathology that normalizes after recovery. The earliest finding is substitution of a linear collection of immature erythroid with myeloid cells in a paraosteal location. This histologic observation is confirmed by immunohistochemistry for myeloid and erythroid cells.

We further noted a differential recovery of bone marrow cellularity onward from postchemotherapy day 14 on the basis of age (>58.5 years, <58.5 years), but we did not find any statistical difference according to gender. By analysis of variance, the significant difference was noted at the stated cut-off year. Younger patients had a more rapid rate of recovery and reached a higher final cellularity compared with older patients. We agree with some of the observations of Wittels but differ from his finding that adults had no difference in recovery between age groups.
In contrast, we found a difference in recovery between older adults (age, >58.5 years) and those who were younger. This finding of age as independent variable is consistent with what was previously reported in postautologous and transplant engraftment and recovery of platelets. This observation is consistent with the slower hematopoietic stem cell renewal expected for older individuals because of loss of telomeric DNA, a lag likewise noted with marrow mesenchymal stem cells.

The observation of Wittels of 2 phases of an initial cellular depletion followed by cellular recovery is confirmed in our series corresponding to day 7 to 14 postchemotherapy. We further defined the cellularity in relation to blood recovery and localization and immunohistologic distribution of marrow elements. We noted early on a panhematopoietic loss of both myeloid and erythroid precursors and megakaryocytes. The initial high M/E ratio at onset of recovery suggests more erythroid than myeloid loss. In reverse, erythroid proliferation exceeds myeloid recovery at the onset of recovery. This observation agrees with a previous report that indicated that after chemotherapy erythroid precursors proliferate more rapidly than myeloid cells.

We further extended Wittels' observation of regenerative hyperplasia by looking at sequential repopulation by bone marrow histology to determine the criteria for recovery. The linear slope statistic supported an evidence of overshoot and valleys of cellularity in relation to blood recovery. We agree with the previous observation that early megakaryopoiesis has prominent overshoot and clustering hyperplasia before day 38 just to maintain platelets above normal. Platelets slowly plateau after day 38. Similarly, our data show that there is erythroid hyperplasia followed by granulocytic hyperplasia with increased M/E ratio close to recovery at day 38 with a tendency to normalize thereafter. We agree with the previous observation that erythroid recovery is the earliest, which is followed and overlapped with myeloid recovery, and then the megakaryocytic normalization, the last to recover.

The transient phase of panhyperplasia was described to range from 21 to 60 days. We refined this finding and noted that this recovery is not homogeneous but is characterized by oscillatory peaks and normalization. A rebound erythroid recovery early on is noted by inversion of the M/E ratio. This rebound overshoot and stabilization is observed in all parameters studied including megakaryocytes, cellularity, and the M/E ratio. This pattern parallels ANC and platelet overshoot. The initial and second peaks appear at day 30 and day 40, respectively, with gradual stabilization thereafter. A smaller peak corresponds to day 60 with normalization of ANC and platelets and emergence of CD10+ hematogones.

Longacre et al noted an increase in hematogones in postchemotherapy bone marrow from children. We noted emergence of hematogones in adults, which were timed at day 40 to day 60 postchemotherapy. We observed that bone marrow recovery is followed by the emergence of early B cells or hematogones characterized by a distinct rise in CD10+ CD19+ early B cells. We observed the criteria for defining recovery by histology based on peripheral blood recovery. Recovery at days 38 and beyond is characterized by a set of findings that correlated with peripheral blood component recovery of ANC and platelets.

We also noted a correlation and similarity of recovery of the marrow cellularity, M/E ratio, megakaryocytes, and hematogones between posttransplant and postchemotherapy groups to 100 days and thereafter. This group also included a heterogeneous disease category and implied a similarity in marrow repopulation kinetics with those that had 7+3 chemotherapy for AML. The recovery patterns observed were also observed in post marrow ablation and engraftment.

We only looked at allogeneic posttransplant cases, but based on the literature, it is noted that there are differences in recovery between different transplant groups. For example, the earliest recovery appears to be from autologous and peripheral blood stem cell transplants and later recovery in allogeneic bone marrow transplant, which may explain why our data on posttransplant show later recovery than those published.

We also agreed with the observation of Wittels that after 90 days, in our data, between an early range of 60 to a late range of 100 days, the recovery should be completed and nonrecovery thereafter means delayed recovery or delayed engraftment.

In conclusion, the practical application of mathematical tools and the linear modeling equation may be used to predict future bone marrow cellularity and to observe any patterns based on age or gender. The side benefit of knowing the marrow recovery kinetics may set guidelines for biopsies. This study also helped uncover a set of histopathologic criteria to determine recovered marrow postchemotherapy.

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References


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