Evaluation of Early Post-transplant Leukocyte Recovery Using the Undiluted Erythrocyte Lysing Technique

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Abstract. The undiluted erythrocyte lysing technique was evaluated to see if it provides more accurate total leukocyte counts and differential leukocyte counts of severely leukopenic blood samples, in order to detect the onset of hematopoietic recovery after stem cell transplantation. Leukocyte counts using the conventional automated cell counting technique were found to be inaccurate, especially in blood samples with total leukocyte counts <500/µl. In cases where the difference between results by the two methods was >100/µl, a positive correlation was found between the difference value and the blood reticulocyte count (r = 0.39, p = 0.002). Hematopoietic recovery after stem cell transplantation in a group of patients with chronic myelogenous leukemia (CML) was different from that of non-CML groups. In the CML group, the initial leukocyte counts were higher and the number of days until neutrophil recovery was higher than in the non-CML groups. Also, the day on which the absolute neutrophil count (ANC) exceeds 100/µl could serve as an indicator of neutrophil recovery. This study shows that the undiluted erythrocyte lysing technique can be used to count leukocytes accurately, especially in severely leukopenic samples. This new method can detect neutrophil recovery at ANC >100/µl, as well as at an earlier date than the conventional method. (received 7 December 2002, accepted 22 December 2002)

Keywords: Undiluted erythrocyte lysing technique, blood leukocyte count, leukocyte differential cell count, absolute neutrophil count, stem cell transplantation, leukocyte recovery post-transplantation

Introduction

Leukopenia is a common event during the treatment of hematologic malignancies. The leukopenic period is critical, owing to the patient’s heightened susceptibility to infectious and bleeding disorders. The frequency of infection correlates with the severity and duration of neutropenia, with a higher risk of infection when the absolute neutrophil count (ANC) is <100/µl [1]. The degree of neutopenia needs to be accurately determined, even when the ANC is <500/µl [2]. Usually the blood total leukocyte count and ANC are determined using an automated cell counter and/or by performing a manual differential cell count. However, it is questionable if such counts of severely leukopenic samples are accurate, because the automated cell counter measures the leukocytes in a small volume of blood.

In this study, we evaluated the undiluted erythrocyte lysing technique to see if it provides more accurate total leukocyte counts and differential cell counts. We estimated the accuracy of this new method in comparison to automated cell counting in severely leukopenic blood samples. Also, we used the undiluted erythrocyte lysing technique to detect the onset of leukocyte recovery after stem cell transplantation.

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Methods and Materials

Total leukocyte counts and differential cell counts using the undiluted erythrocyte lysing technique. We studied 210 samples of peripheral blood from patients who underwent stem cell transplantation (n = 48) and chemotherapy (n = 30) due to hematologic diseases. The samples (2 ml) of EDTA-anticoagulated blood were collected and analyzed within 2 hr after collection. The leukocytes were routinely determined with an automated cell counter (Coulter Corp., Miami, FL, USA). Leukocyte counting using the undiluted erythrocyte lysing technique was limited to samples with total leukocyte counts <1000/µl.

Leukocyte counts by the undiluted erythrocyte lysing technique were performed as follows: 500 µl of blood was mixed with 9.5 ml of lysis solution (0.1 N HCl) and centrifuged at 2100 rpm for 10 min. The supernatant was discarded and the final sample volume adjusted to 500 µl. The leukocyte pellet was carefully resuspended and the leukocytes counted using an Improved Neubauer Counting Chamber. The leukocyte suspension was introduced into both sides of the chamber. After leukocytes had settled, they were counted in a minimum of 10 squares, but often in all 18 squares, by two different, experienced observers. The results were expressed as mean leukocyte count per µl. After the total leukocyte count was performed, the remaining leukocytes were concentrated on slides using a cytospin apparatus (Shandon Ltd., Astmoor, UK). Following Wright staining, differential leukocyte counts were performed by microscopic examination (Fig. 1).

To evaluate the accuracy of the undiluted erythrocyte lysing technique, two samples of peripheral blood with total leukocyte counts of approximately 5000/µl were used. After the samples were diluted serially, the leukocyte counts were compared using the two methods (ie, automated cell counting versus the undiluted erythrocyte lysing technique).

Patients who underwent stem cell transplantation. Forty-eight patients who underwent hematopoietic stem cell transplantation were included. EDTA-anticoagulated blood samples were collected daily beginning on the day before transplantation. The patients’ median age was 28 yr (range 2 to 48 yr). Twenty-three patients received grafts from HLA-matched siblings, 14 from HLA-matched unrelated donors, 8 from autologous bone marrow, 2 from cord blood, and 1 from an HLA-mismatched related donor. The patients’ hematological disorders were: chronic myelogenous leukemia (CML, n = 11), acute myeloid leukemia (AML, n = 10), acute lymphoblastic leukemia (ALL, n = 9), severe aplastic anemia (SAA, n = 9), myelodysplastic syndrome (MDS, n = 3), paroxysmal nocturnal hemoglobinuria (PNH, n = 2), plasma cell myeloma (PCM, n = 1), congenital agranulocytosis (n = 1), myelofibrosis (n = 1), and Fanconi anemia (n = 1).

Neutrophil recovery was analyzed in patients who survived ≥ 21 days after transplantation. Primary graft failure was defined as an ANC <100/µl in patients who survived ≥21 days post-transplant [3]. An ANC of ≥ 500/µl for 3 consecutive days was considered an indication of neutrophil recovery [4]. Two of the patients developed graft failure. Both received repeat stem cell transplants from the initial donor at >21 days after the first transplant.
Results

Comparison of automated versus manual leukocyte counts. The leukocyte counts of patients' blood samples using the automated cell counter correlated well with those obtained by the undiluted erythrocyte lysing technique ($r = 0.946, p < 0.001$) (Fig. 2). But the leukocyte counts were relatively inaccurate in blood samples with leukocytes <500/µl ($r = 0.780$). In cases where the difference between the total counts by the two methods was >100/µl, a positive correlation was observed between the difference value and the blood reticulocyte count ($r = 0.391, p = 0.002$) (Fig. 3). On the other hand, the dilution study revealed excellent correlation between the two methods ($r = 0.994, p < 0.001$), even when total leukocyte counts were <500/µl ($r = 0.956, p < 0.001$).

When the automated cell counter was used, the differential leukocyte count was often unavailable, if we were unable to find an adequate number of leukocytes to count on the blood smears.

Hematopoietic recovery after transplantation. The onset of hematopoietic recovery after transplantation in the CML group of patients was different from the non-CML group of patients (Fig. 4).

In the non-CML group of patients, the median total leukocyte count was 44/µl on the day of transplantation. The total leukocyte count increased slightly on post-transplant days 1 and 2; it then decreased gradually until it reached the lowest level on day 6 (median 11/µl). The total leukocyte count began to rise on post-transplant day 8.

In the non-CML group of patients, the ANC showed a pattern similar to the leukocyte count, with a transient increase of neutrophils detected on post-transplant days 1 and 2. Neutrophils decreased to the lowest level on post-transplant day 6 (median 5/µl) and then began to rise.

The ANC values by the undiluted erythrocyte lysing technique reached 100/µl on post-transplant day 12 (range 8 to 30 days), which averaged 2 days earlier than the onset of neutrophil recovery by the conventional automated method. Once neutrophil counts reached >100/µl, they continued to increase. When the undiluted erythrocyte lysing technique was used, all patients showed an ANC of >100/µl on the same day or earlier (median 2 days, range 0 to 6 days), compared to the conventional automated
method. One PNH patient who developed graft failure did not reach ANC >100/µl. The median percentage of neutrophils was 88% on the day of transplantation. This value gradually decreased until day 8 and then began to increase.

In the CML group of patients, the median leukocyte count on the day of transplantation was higher than in the non-CML group (median 2067/µl, p = 0.001). The leukocytes decreased slowly, reaching the lowest level (median 34/µl) on day 7. The leukocyte counts of the CML group began to rise later than the non-CML group (day 11).

The ANC values showed a pattern similar to the leukocyte counts. In the CML group, the median onset of ANC >100/µl was day 14, which was later than the non-CML group (day 12, p = 0.01). One CML patient developed graft failure and did not reach ANC >100/µl. The median percentage of neutrophils in the CML group was 94% on the day of the transplantation. This value decreased steadily until day 11 and then began to increase.

Discussion

In many laboratories, manual counting of diluted blood samples has been the preferred method to determine low white cell counts. It has been recognized that the lower the concentration of leukocytes in the specimen, the greater the volume of specimen that must be analyzed to count a reasonable number of cells [5]. Although the Coulter automated cell counter has been reported to be effective for counting low concentrations of white cells [6], our results suggest otherwise. We observed that when total leukocyte counts were <500/µl, the automated cell counts were not very accurate, especially for analysis of blood samples from patients who have undergone stem cell transplantation and chemotherapy for hematologic diseases.

Interestingly, our dilution study showed a good correlation between the results of the automated and manual methods, even when the leukocyte counts were <500/µl. We sought to explain the apparent difference of accuracy between the results of the
dilution test of blood samples with normal leukocyte counts, compared to measurements of leukopenic samples. In animals irradiated with γ-rays, Mackova and Misurova [7] noted that automated leukocyte counts appeared artefactitiously increased in the post-irradiation period, probably due to higher resistance of reticulocytes to the lysing agent. We found that blood reticulocyte counts were positively correlated with the differences between the results of the manual and automated leukocyte counts. Therefore, we speculate that the automated cell counter may give inaccurate leukocyte counts in leukopenic patients with high reticulocyte counts.

Using the undiluted erythrocyte lysing technique, we were able to obtain informative data on blood leukocyte and neutrophil counts of patients during the early post-transplant period. In CML patients, the early post-transplant leukocyte recovery pattern differed from that of non-CML patients. The day when the neutrophil count exceeded 100/µl occurred later in the CML group than in the non-CML group. We believe that, although the therapeutic regimens were the same in the two groups, the previous diagnoses affected the hematopoietic recovery. Using the undiluted erythrocyte lysing technique, we were able to document an onset of neutrophil recovery when the ANC reached 100/µl. After their neutrophil count had reached 100/µl, all of the patients showed a gradual increase of neutrophil counts. Two cases of graft failure never reached an ANC >100/µl. Therefore, we consider that the day post-transplant when the ANC becomes >100/µl, rather than 500/µl, can serve as an indicator of neutrophil recovery, for both the non-CML and CML groups of patients.

In conclusion, these results suggest that the undiluted erythrocyte lysing technique can be used to count blood leukocytes accurately, especially in severely leukopenic samples. We could not reliably determine the day post-transplant when the ANC reached 100/µl by using the automated cell counter and examining the blood smears. However, using the undiluted erythrocyte lysing technique, we could detect engraftment at an average of two days earlier. Based on that finding, we could decide when to boost the treatment sooner than when we used the conventional neutrophil recovery criteria.

### References


