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The Engraftment Kinetics of White Blood Cells and Platelets Following Peripheral Blood Stem Cell Transplantation

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Abstract

The recovery of white blood cells and platelets following high-dose chemotherapy and peripheral blood stem cell transplant is described through mathematical models. Created from a unique interaction with clinical data, these models provide a natural way to monitor patient progress throughout engraftment, providing estimates of hospital stay and early indications of difficulties with engraftment.

1. Introduction

Chemotherapy is as a standard treatment option for most types of cancer [1]. However, doses necessary to eradicate all residual cancer, especially metastatic cancer, can be lethal to the patient as the chemotherapy also destroys the blood-producing cells of the body. These hematopoietic stem cells (HSC) reside in the bone marrow but can be collected from peripheral blood after a treatment that mobilizes them [2]. Peripheral blood stem cell (PBSC) transplants have allowed for the use of near-lethal doses of chemotherapy in attempts to kill all cancer cells. The transplanted cells are able to *engraft*, or reconstitute a patient's blood cells, following the ablative effects of chemotherapy.

Currently, women with high-risk primary or metastatic breast cancer are most commonly treated with high-dose chemotherapy and an autologous (a patient's own) PBSC transplant [3]. Although this treatment provides generally favorable results, including increased disease-free and overall survival, problems such as delayed engraftment (especially with platelets) and high relapse rates indicate that a better understanding of the mechanisms of hematopoietic reconstitution, and thus engraftment, is needed. In this paper, the time course of the recovery of mature white blood cells (WBC) and platelets, the *kinetics of engraftment*, following an autologous PBSC transplant are discussed. The research was clinically motivated by two related questions:

1. *Is it possible to detect difficulties in engraftment before the consequences of this difficulty reduce treatment options?*
2. *Can individual patient estimates of the hospital stay following transplant be made?*

The results described in this work are well suited for adaptation to the clinic though a "control-chart" format.

2. Methods and Materials

This research is based on 35 stage II (n=30) and stage IIIA (n=5) breast cancer patients undergoing a PBSC transplant between 12/94 and 2/98 at St. Luke's Medical Center, Milwaukee, WI. Patient ages ranged from 26 to 64 with a mean (± standard deviation) of 46.8 (± 10.02). Patients initially receive surgery (lumpectomy, mastectomy), 4 - 6 cycles of induction chemotherapy, and possibly radiation treatment. High-dose chemotherapy follows the nationally recognized Stamp V protocol, including cyclophosphamide (1500 mg/m^2/day), carboplatin (200 mg/m^2/day), and thiotepa (125 mg/m^2/day).

PBSC are harvested prior to high-dose chemotherapy in a outpatient procedure called leukopheresis, which collects only the cells required for the transplant (CD34+ cells). If possible, a minimum of 5×10^6 CD34+ cells/kg are collected and cryopreserved for the PBSC transplant. After four days of high-dose chemotherapy, PBSC are re-infused into the patient, denoting Day 0. Data from daily lab analysis of post-transplant peripheral blood is recorded and levels of WBC and platelets are closely monitored. Transfusions of blood products, especially red blood cells and platelets, may be necessary until these populations become self-supporting. Patients are termed "engrafted" and released from the hospital when

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the absolute neutrophil count (ANC) exceeds 0.5-k/μl and platelet levels surpass 20-k/μl. These engraftment levels are not standardized and do not refer to the *rate* these cells return to peripheral blood. Patients are required to return for check-up weekly for 100 days following release then yearly thereafter.

3. Kinetics of White Blood Cell Engraftment

White blood cells, or leukocytes, are the cells of the immune system. Comprised of lymphocytes (T cells and B cells), macrophages, dendritic cells, and granulocytic cells (neutrophils, basophils, and eosinophils), WBC are responsible for identifying, remembering, and eliminating foreign invaders from the body. Neutrophils comprise the greatest percentage of the WBC compartment (50%-70%) and are generally the first cells to arrive at sites of infection [4]. When levels of WBC fall to low levels following high-dose chemotherapy, a patient is at severe risk for opportunistic infections.

In order to capture the characteristics of the WBC repopulation of peripheral blood, plots were obtained of WBC versus time for all 35 patients [Figure 1]. Similar plots were seen for all patients, with the return of WBC to peripheral blood occuring around day 6. Since we are interested in the kinetics of engraftment, we focus on the upward curve of the graph, indicating the region where WBC are actively returning to peripheral blood.

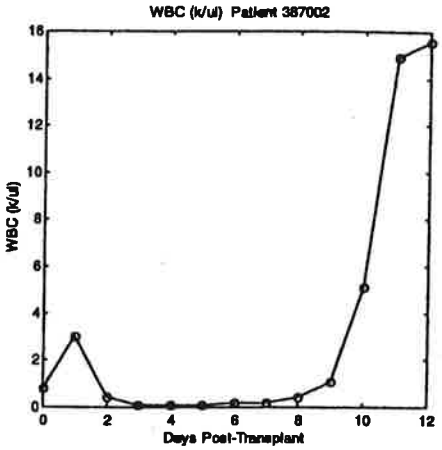

Figure 1: Plot of post-transplant WBC counts

As hematopoiesis, or the process of mature blood cell formation, is based on cellular division, an exponential relationship is expected between peripheral cell counts and time. If true, semi-log plots of WBC counts during the growth region should be linear. For nearly all patients ($n \approx 30$), upward curvature in semi-log plots indicated that this transform *failed to linearize* the WBC data. Furthermore, residual plots from linear regression fits of semi-log transformed data display a parabolic pattern. Therefore, WBC are returning to peripheral blood at a rate that is *faster than exponential*.

Another attempt at linearizing the data included the negative reciprocal (NR) transform. Plots of NR-transformed WBC data versus time did produce a linear relationship. Linear regressions of NR-transformed data provided better R^2-values (0.94 average) as well as random residual plots. The type of growth characterized by the NR-transformation is *hyperbolic growth*. This surprising rate of growth has not been identified before. A defining feature of hyperbolic growth is that cells following this pattern approach a vertical asymptote. It is the position of this asymptote that we will use to define engraftment, for no matter what level engraftment is set clinically, cell counts will have surpassed these levels by the day associated with the asymptote.

3.1. Creation of a Clinical Control Chart

Once WBC begin re-appearing following PBSC transplant, it is possible to obtain predictions of *time to engraftment* (TTE). Mathematically, we use linear regression to fit NR-transformed WBC data during the aggressive growth period:

$$\frac{-1}{wbc(t)+1} = a \cdot t + b$$

Solving for $wbc(t)$ we see: $wbc(t) \approx \frac{-1}{a \cdot t + b}$, and our estimate of TTE is thus $TTE = t = -\frac{b}{a}$ where $a > 0$ and $b < 0$. Requiring at least 3 data points for regression, if WBC begin returning on Day 6, by Day 8 we have our first prediction of engraftment. TTE is updated each day new WBC data is collected.

We can observe changes in a patient's engraftment progress by tracking the predicted TTE through a control-chart format, which uses confidence intervals to define acceptable estimates of TTE. To accomplish this, we compare the estimated time to engraftment of all available data (TTE) against the estimated time to engraftment from the previous three days of WBC data (T3). This gives us a long-term estimate and a short-term estimate, which should be comparable if the growth rate of WBC remains stable. If, however, the growth rate of WBC slows, estimates of T3 would immediately increase, indicating delayed engraftment.

To identify significant differences in TTE and T3, we create 80% confidence intervals around the estimate of TTE. Recall $TTE = -\frac{b}{a}$ where a, b are normally distributed regression parameters from linear fits of NR-transformed WBC data. Therefore, by simulating 1000 normally distributed $a-$values and 1000 normally distributed $b-$values, we calculate 1000 estimates of TTE. These estimates, when sorted and stripped of the first and last 100 values, create an 80% confidence interval (C.I.) about TTE. It is when the value of T3 falls outside of the 80% C.I. that immediate changes in a patient's engraftment are identified.

Using the control-chart format for all 35 patients, we were able to identify 2 patients that were known (by the hospital) to have engraftment problems. Two patients have been observed real-time with no engraftment problems being predicted. The use of such a tool in a clinical setting would be an invaluable tool for clinicians and doctors. Identifying problems, sometimes days before they appear in cell count numbers would allow for immediate intervention and more careful monitoring.

4. Kinetics of Platelet Engraftment

Platelets, which are fragments of much larger cells called megakaryocytes, play a major role in blood clotting. Platelet levels in adult blood are approximately 250-k/μl with a normal range of 140 to 400 (k/μl). Anytime platelet levels fall below 20-k/μl a patient is at risk for fatal uncontrollable bleeding [5]. Thus, careful monitoring of platelet counts is necessary during the recovery from high-dose chemotherapy and transfusions are required when platelet levels fall below 10-k/μl.

Plots of daily post-transplant platelet counts were created to observe engraftment characteristics. These pictures showed a completely different engraftment nature than WBC [Figure 2]. This is due, in part, to the fact that platelets are not capable of cellular division. As chemotherapy attacks cells that can divide, platelets are not immediately destroyed, simply dying according to an exponential decay process with a half-life of about 1.5 days.

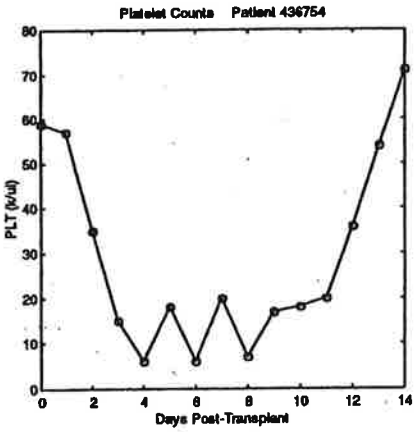

Figure 2: Plot of post-transplant platelet counts

The effects of transfusions on the data are evident [see Figure 2, Days 4-10], which make it difficult to identify the characteristics of platelet engraftment using the approach taken for the WBC data. Until new platelets are being produced, transfused platelets will simply disappear exponentially as discussed above. Using this fact, we can identify the exact day when new platelets begin to re-appear in peripheral blood *independent of transfusions*. Creating a new data set of actual platelet production, we find that initial platelet engraftment follows the cubic growth model:

$$P(t) \approx \frac{K}{6} \cdot t^3$$

where K is a combination of model parameters. This growth, which was initially assumed to be quadratic, was suggested by a compartmental model of platelet production, including stem cells, blast-type cells, megakaryocytes, and platelets.

A consequence of using a compartmental model is the ability to predict the asymptotic level of platelets in a steady state. From this, as $t \to \infty$, we find that

$$P(t) \to \frac{K}{d_p \cdot G}$$

where K is the same combination of parameters determined in the initial growth curve, G is a collection of model parameters that do not change among individual patients, and d_p is the normal half-life of platelets. This engraftment level of platelets can be predicted once the initial growth rate is known, giving valuable information to doctors about final platelet levels, especially if this level is too low.

Many patients will experience delayed platelet engraftment, with some requiring transfusions well past the time of WBC engraftment and release from the hospital. One possible reason for this is the use of certain growth factors, administered before stem cell collection and after PBSC transplant. These particular growth factors, known as granulocyte colony stimulating factor (G-CSF) and granulocyte-monocyte colony stimulating factor (GM-CSF), encourage the stem cell to develop (differentiate) down the various pathways leading to white blood cells. It has been observed that administering these growth factors to encourage WBC engraftment are doing so at the expense of platelet engraftment [6]. Additional work can be done in this area to determine optimal doses of growth factors that create a balance between both platelet and WBC engraftment.

5. Conclusion

Mathematical models created in a clinical setting can offer valuable information to clinicians, including predictions of biological systems and insights to underlying mechanisms. The model of WBC, whose form is suggested by the data, has identified a surprising growth rate following PBSC transplant, and naturally leads to the development of a clinical control chart. This model allows for prediction of hospital stays and with the control chart, can identify patients who may be experiencing problems with engraftment. The model of platelet population, which follows a cubic growth rate initially, may be used to predict ultimate levels of platelets in peripheral blood. This provides doctors with information about patients who may need additional transfusions well after release from the hospital.

6. Acknowledgments

This work is part of a collaboration that included two undergraduate students (at the time) Lexi Antonchorgy and Felisha Harris, both working in the summer McNair Scholars program. Lexi provided the initial work on WBC to identify the NR-transform while Felisha's work suggested the cubic form of the platelet model. Finally, this research would be impossible without the clinical data provided by Dr. Ann LeFever and her lab.

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