UTILIZING THE AUTOMATED RETICULATED PLATELET COUNT ON THE ABBOTT SAPPHIRE TO DETECT MARROW RECOVERY AND/OR RESPONSE IN BONE MARROW AND STEM CELL TRANSPLANT PATIENTS

By

Beth Ward

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Clinical Laboratory Science—Master of Science

2023

ABSTRACT

There is an interest in a biomarker that could predict bone marrow engraftment of megakaryocytes following bone marrow or stem cell transplant which relates to the prospect of withholding platelet transfusion support when engraftment is imminent. Reducing the number of platelet transfusions would limit the exposure of the patient to the infectious disease risk associated with them and save expense to the patient and hospital. This 43 subject retrospective study of the temporal relationship between marrow engraftment and the peripheral blood reticulated platelet percentage (%rP), following bone marrow or stem cell transplant identified the threshold value for the %rP indicating marrow engraftment. Using the Abbott Cell-Dyn Sapphire hematology analyzer and its %rP parameter which is not strictly comparable to the Sysmex immature platelet fractionation (IPF), it was found that the %rP is valuable for this predictive purpose.

TABLE OF CONTENTS

INTRODUCTION

Platelets: Formation and Production

The human body is comprised of many biological components that play distinct and invaluable roles in human survival. One of these components, the thrombocyte or platelet, begins its journey into our blood stream from a hematopoietic cell originating in our bone marrow. These small cells were first studied in 1897 when James Homer Wright altered the Romanowsky staining protocol in order to visualize these bluish/purple particles in more detail. Utilizing light microscopy, he noted that these cells were roughly one-third to one-half the size of circulating red blood cells. Continuing with this staining technique, he was able to demonstrate that platelets came from the bone marrow megakaryocytes (MKs). [1]

Thrombopoiesis is the process through which platelets are generated from megakaryocytes. Megakaryocytes are unique in the way they mature. Megakaryopoiesis involves endomitosis, wherein the nucleus divides and the cell accumulates cytoplasm without typical cell division. This process is unlike most other human cells that replicate their nuclear content and then go through a cell division process that results in two daughter cells. The megakaryocyte reaches maturation when the number of chromosomes, or ploidy, reaches 8N to 64N. The average ploidy for normal human megakaryocytes is 16N and the final ploidy is determined by a genetic component. [2] Cytoplasmic volume expands according to ploidy level, and this determines the number of platelets that will be produced. The greater the ploidy of the megakaryocyte nucleus, the more cytoplasm and specific platelet structures it will have.

In thrombopoeisis, once endomitosis stops, platelet production begins. Megakaryocytes MKs) give rise to circulating platelets (thrombocytes) once the multipotent stem cell commits to the MK lineage and finally reaches terminal differentiation were the MK can no longer proliferate. [3] This maturation process from stem cell through platelet is depicted in Figure 1 [3] and Figure 2. [2]

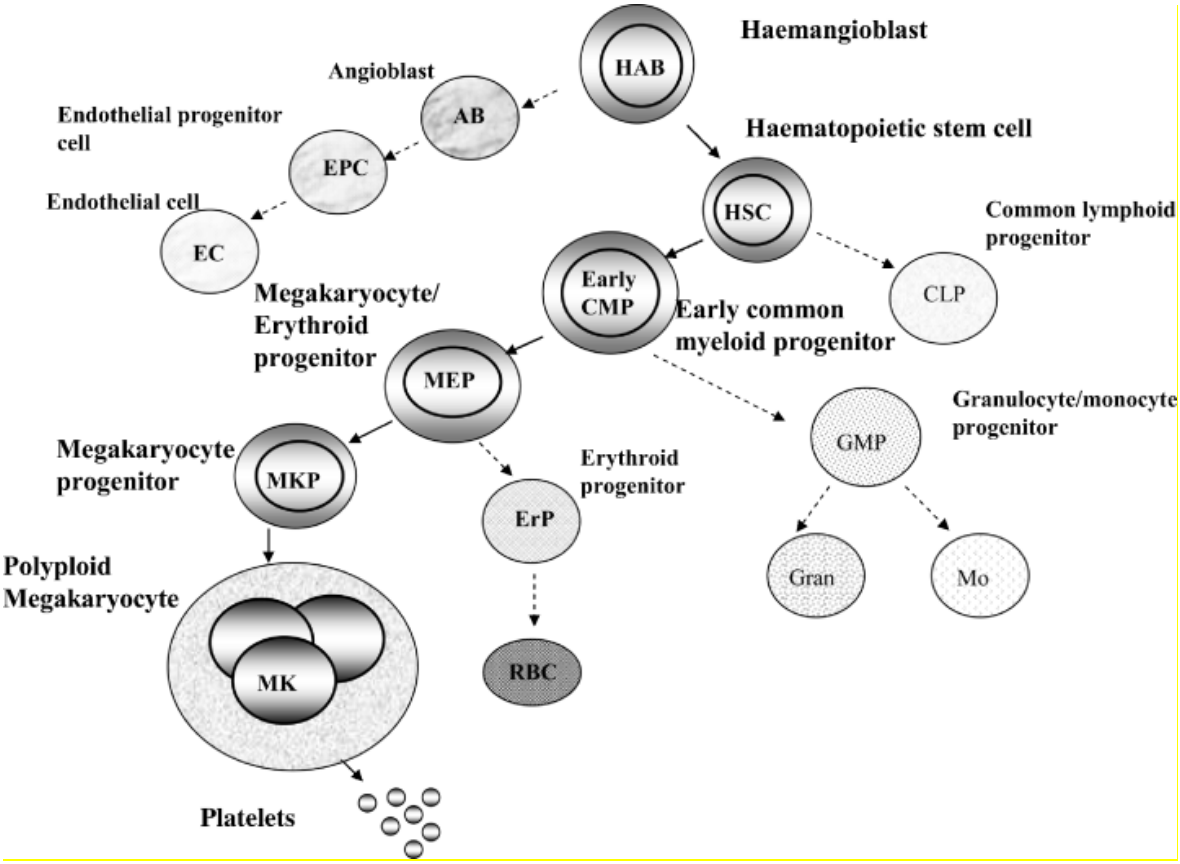


Figure 1: Haemangioblast to Platelet Maturation.

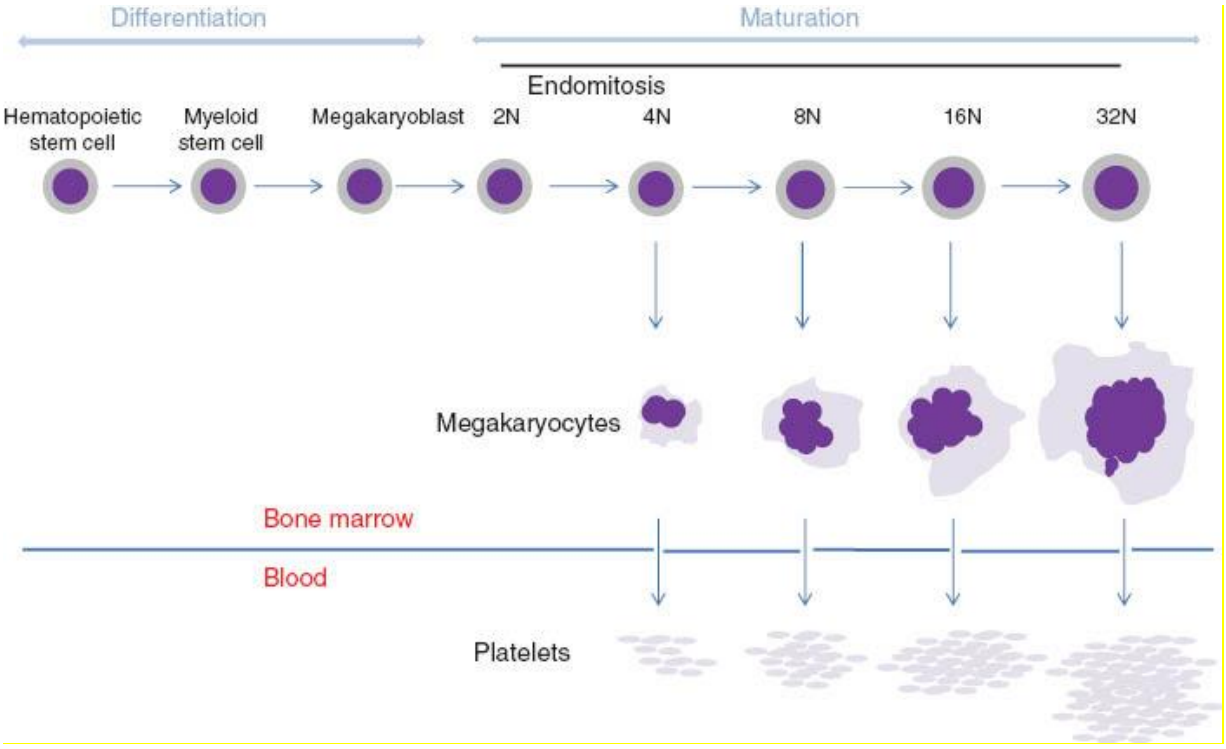


Figure 2: Hematopoetic Stem Cell to Platelet Maturation and Ploidy.

Platelets are produced when the MKs have reached maturity and release their cytoplasmic fragments. A single megakaryocyte can generate between 5,000-10,000 platelets and in steady-state conditions, the platelet production rate is aimed at keeping the total circulating platelet mass constant where platelet mass equals the platelet count multiplied by the Mean Platelet Volume (MPV). [1]

Regardless of how and why platelets are formed, the average lifespan of platelets in circulation is brief, close to ten days in humans. Then instead of being consumed by hemostatic processes, the majority of these platelets undergo a programmed cell death called apoptosis. [4]

Immature platelets, also referred to as reticulated platelets, are the platelets released from the megakaryocyte cytoplasm that contain a small amount of RNA and use this RNA for protein synthesis. The platelets containing this RNA represent the youngest

platelets in circulation, similar to the reticulated red blood cells (reticulocytes) in erythropoiesis. The reticulated platelets (rPLTs) only circulate in peripheral blood for <1 day before further maturation. [5] Because they only circulate for about one day, they can and are used as an indicator of marrow activity.

Platelet Physiology

One of the main functions of the platelet is to participate in hemostasis. The primary goal for platelets in hemostasis is to initiate the formation of a clot by activating the coagulation cascade in times of vascular distress and bleeding. Although platelets play a central role in maintaining hemostasis, they are also involved in thrombosis and atherothrombotic disease [6], inflammation, host defense, tumor biology, and the maintenance/regulation of vascular tone. [7]

Platelets have many functions as exhibited in Figure 3 [7], and a defect in either platelet function or number will result in a wide array of pathological conditions. The typical blood concentration of 150,000-450,000/uL is maintained via a daily turnover in each individual of roughly 10^{11} cells. [8] Platelet counts <150,000/uL, deemed thrombocytopenia, are a major clinical problem encountered across a number of conditions, including immune thrombocytopenic purpura (ITP), myelodysplastic syndromes (MDS), chemotherapy, aplastic anemia, human immunodeficiency virus infection, complications during pregnancy and delivery, and surgery. [9] The causes of thrombocytopenia can be divided into categories due to either decreased platelet

production in the bone marrow or increased destruction or consumption of platelets in the peripheral blood.

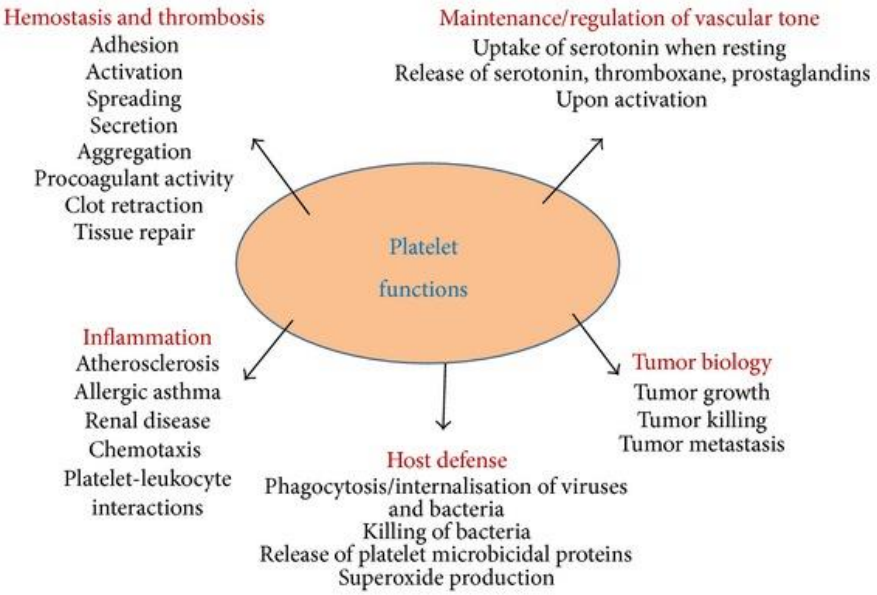


Figure 3: Categories of Platelet Functions.

Reticulated Platelets: Physiology and Clinical Utility

The clinical utility of the reticulated platelet (rPLT) has been studied extensively and has been shown to be valuable in diagnosing and monitoring many pathological conditions. Clinicians utilize the measured reticulated platelet percentage (RP%) from flow cytometry as well as the automated %rP (percent reticulated platelet) and IPF% (immature platelet fraction) performed on hematology analyzers interchangeably as these values have shown good correlation in numerous studies.

Because the circulating rPLT count indicates the level of present marrow activity it has been shown to be valuable in distinguishing between thrombocytopenias due to bone marrow failure (hypoproduction) from peripheral destruction or consumption. [10]

[11] Thrombocytopenia and falling platelet counts in pediatric intensive care units have been shown to be associated with an increased risk of mortality and length of stay, [20] making the ability to assess thrombocytopenias quickly is essential for proper patient management. The benefits of assessing platelet marrow activity by obtaining a blood specimen versus having a patient undergo a bone marrow biopsy are extensive, as bone marrow biopsies can be very traumatic in the pediatric population.

A decrease in peripheral platelet count accompanied by an elevated reticulated platelet count demonstrates that bone marrow failure is absent and can be attributed to either a destruction or consumption issue. It has also been demonstrated when based on a reference range of 1.6-10.1%, an IPF% $\geq$13 predicts a peripheral mechanism for thrombocytopenia making bone marrow aspirations unnecessary in these patients. [12] Additionally, patients diagnosed with essential thrombocytosis (ET) exhibit an increased amount of reticulated platelets [13] , similar to those with thrombocytopenia due to peripheral destruction. [14] The immature platelet count can also be used to distinguish primary immune thrombocytopenia from aplastic thrombocytopenic disorders [15] and the IPF% measurement alone has utility in both the diagnosis of ITP and identifying patients at increased risk of hemorrhage. [16]

Studies show that RP% from flow cytometery can be used to predict the recovery of platelets after hematopoietic stem cell (HSC) transplantation [17] and the IPF% can also predict platelet engraftment post hematopoietic stem cell transplant. [18] By utilizing the IPF% parameter on the Sysmex XN-1000, it also enables an earlier prediction of peripheral platelet recovery to >20,000/uL within 2.5-3.5 days. [19] Additionally, the IPF

on the Sysmex XE-2100 has been shown to be a useful marker for imminent platelet recovery in pediatric patients post chemotherapy treatment for malignant disorders. [20]

Laboratory Methods for Determining Reticulated Platelet Counts

Reticulated platelets (rPLTs) were initially discovered in 1969 by Ingram and Coopersmith during a canine study that focused on peripheral blood smears from dogs following acute blood loss. These new cells were visualized microscopically using a new methylene blue stain where course punctate condensations (reticulum) were observed. In this study it was determined that both reticulated red blood cells and reticulated platelets were increased following acute blood loss. [21]

Manual methods for enumerating reticulated cells were utilized over the next two decades until a study with the fluorescent dye, thiazole orange (TO) was conducted in 1986 by Lee, Chen, and Chiu. Thiazole orange (TO) is a cyanine compound that fluoresces when bound within nucleic acid structures and can be used to identify reticulated red blood cells by flow cytometry. A potential disadvantage was observed in the study as both DNA and RNA were stained by the thiazole orange. An advantage of TO is its ability to permeate the membrane of living cells. The study showed that leukocytes did not interfere with the analysis. However, other types of cells such as platelets may cause interference. [22] TO was then used in subsequent studies to stain reticulated platelets successfully. Kienast and Schmitz were the first to use TO identify reticulated platelets in 1990. Their study included samples from both a normal population as well as from populations exhibiting varying thrombocytic abnormalities.

The clinical utility of the percent reticulated platelet count (RP %) was first suggested in this study. [23]

Various flow cytometry methods have been introduced and performed successfully. However, inconsistencies have been observed in cytometry assays due to lack of standardization and technical error. By combining CD41/CD61 platelet enumeration with thiazole orange and an effective gating strategy, improvements in accuracy of RP% particularly in the low range were observed in a study performed in 2015. [24] A new fluorescent dye SYTO 13 was evaluated against TO in 2019 to enumerate rPLTs, and demonstrated distinct technical advantages at determining RNAlow and RNArich rPLTs that was confirmed by RNA quantification of sorted platelets. [25] Even with the improvements, the flow cytometry method still presented issues with cost and accessibility.

Automated methods were developed for use in the core laboratory's hematology departments. Current automated processes are performed on Abbott's CD-Sapphire (%rP) and Sysmex's XE-2100, XE-5100, and XN-1000 series (IPF%). Both IPF% and %rP give useful information on platelet turnover and a moderate correlation between the Sysmex XE and Abbott Sapphire was observed with a better separation of patient groups with high PLT turnover like ITP/HIT from normal controls being obtained by the Abbott Sapphire. [26] These automated systems use proprietary dyes to stain the RNA contained in the immature platelets, and then use hydrodynamic focusing and flow cytometry to gate for the respective cell populations. Table 1 [2] and Figure 4 [2] list and illustrate the key characteristics of all three methodologies and scatterplots obtained for their measured parameter. To expand on the table, an additional study has

shown that the XN reagent stains both the mitochondrial DNA and RNA and as well as the cytosolic mRNA in platelets. [27]

	Flow cytometry	Sysmex IPF	Abbott retPLT
Sample preparation	Variable: none, fixation or isolation of platelets	None	None
Fluorescent dye	Thiazole orange, acridin orange	Polymethine (XE-series); oxazine (XN-series)	CD4K530
Incubation time	Variable 15 min–2.5 h	Not specified	47 s
Precision at normal platelet count, CV	Not specified	7%–11%	<12%
Precision in thrombocytopenia, CV	Not specified	9%–36%	11%–32%
Reference range,%	Highly variable 1%–15%	1.1–6.6	0.5–6.0
Reference range, 10^9/L	Mean 3.2	2–17	1–18

Table 1: Key Characteristics of Methods and Measurements for Reticulated Platelets.

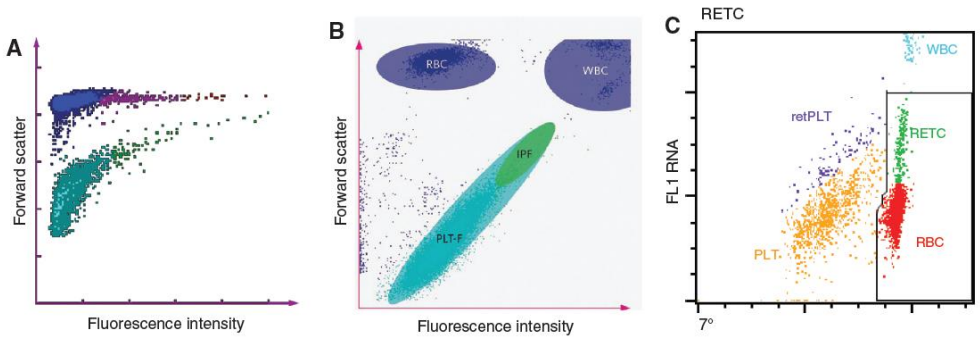


Figure 4: Scatterplots of the Automated Assays for Determining Reticulated Platelet Counts. A=Sysmex XE-series IPF%, B=Sysmex XN-series IPF%, C=Abbott CELL-DYN Sapphires %rP.

Many studies have been conducted that have shown good the correlation between the %rP from the Abbott system, IPF% from the Sysmex platforms, and RP% obtained from flow cytometry. [2] The IPF% from the Sysmex XE-2100 showed good correlation with the reticulated platelet measurement determined by flow cytometry using thiazole orange and CD61 PerCP [14] as well as the IPF% from the XN-1000 with flow cytometry. [15] The IPF% measurement on the Sysmex XE compensates for platelet size due to the automated algorithm used to detect the threshold for larger platelets that have a higher intensity of auto-fluorescence versus normal sized platelets compared to

the RP% obtained by flow cytometry, allowing the instrument to screen for macrothrombocytopenia. [28] Additionally, a significant correlation was shown between the IPF% values between the XE and the XN, however, the IPF% measurement on the XN was superior at detecting an earlier platelet recovery in patients who had undergone hematopoietic stem cell transplantation. [29] Recently, an additional parameter, “highly” fluorescent IPF percentage (H-IPF%) has been investigated on the XN-1000.The H-IPF% did not improve the differential diagnosis of thrombocytopenia when compared to the IPF%. [30] Stability studies have shown specimens collected in EDTA are stable up to 24 hours at room temperature on all platforms [31], but can be increased to 4 days at room temperature on the XE-5000 if stored in citrate-theophylline-adenosine-dipyridamole (CTAD) solution. [32] Stability studies have also shown that starting from one hour post venipuncture, the longer a specimen is exposed to anticoagulants the more unstable the automated measurement of platelet size (MPV) becomes regardless of storage temperature. [33]

Additionally, patient reference ranges varied between each of the platforms depending on age and ethnicity. For example, the IPF% on the XN for a Japanese adult population was shown to be 1.0-10.3% [34] The IPF% on the XN for an Indian adult population was 1.5-12.8% in healthy donors. [35] For a Spanish adult population, 1.6-9.6% was the normal range [36], and for a Danish adult population on the XE, the normal reference range was 1.3-9.0. [37] An IPF > 5.2% differentiated ITP from bone marrow failure [16], and IPF values >15% should be interpreted with caution. [28]

Similarly on the Abbott Sapphire, the adult normal reference range for %rP was observed at 1.0-3.8% when compared to the same samples run on the XE obtaining the

range of 0.8-7.9% [26] An additional study determined the normal adult reference range on the Abbott Cell-Dyn to be 0.3-5.4%. [38] For pediatric patients on the Abbott Sapphire, the normal reference range was further divided into the following categories: 1mo-1yr: 1.31-8.10%, 1yr-3yr: 0.95-8.93%, 3yr-6yr: 0.35-6.01%, 6yr-12yr: 0.26-7.33%, and 12-18yr: 0.33-5.22%. [38] These studies illustrate the importance of determining reference ranges based upon patient population and instrumentation.

PURPOSE OF THE INVESTIGATION

Because the clinical utility of the RP% has been demonstrated and clinicians have requested access to this parameter, its availability supports improved patient care. Interest in this biomarker to predict bone marrow engraftment of megakaryocytes following bone marrow or stem cell transplant relates to the prospect of withholding platelet transfusion support when engraftment is imminent, thus limiting exposure of the patient to the infectious disease and other risks associated with transfusion, in addition to reduced expenses for the patient and hospital.

There are currently two high-end hematology analyzers that can assay for immature platelets as potential biomarkers for megakaryocyte engraftment in the bone marrow: the Sysmex XN (FDA-approved) which analyzes immature platelet fraction (IPF) and the Abbott Cell-Dyn Sapphire which analyzes reticulated platelets percentage (%rP). For laboratories running the Abbott Sapphire, the %rP is determined on all specimens run in the RETC mode, but the parameter has not been FDA-approved to release.

There is conflicting information in the literature concerning the ability of these analyzers to predict platelet recovery following transplant: Meintker L et al, 2017 has shown that immature platelets are not reliable markers for platelet recovery following stem cell transplant or intensive chemotherapy [39]; Hennel E et al, 2012 showed that IPF did not reliably predict recovery in children following stem cell transplant [40]; In contrast, Grabek J et al. 2021 found that the Sysmex IPF assay did predict recovery following allogeneic bone marrow transplant and improving patient outcomes could be extended to labs running the Abbott Sapphire by validating this new parameter. [41]

The purpose of this investigation was to validate the %rP as a laboratory developed test (LDT) on the Abbott Sapphire automated hematology analyzer. Once validated, we would then examine if the reticulated platelet percentage in peripheral blood samples increases prior to bone marrow engraftment post-transplant. Additionally, determination of the threshold value for the %rP indicating marrow engraftment would be compared to current transfusion practices.

A retrospective study of the temporal relationship between marrow engraftment and the peripheral blood %rP following bone marrow or stem cell transplant (TX) was performed. Because this data is currently not reported, the %rP parameter is only available from archived Cell-Dyn printouts. The data was collected over a five- month period selecting subjects identified on the weekly bone marrow transplant lists. Printouts were required because the %rP parameter is not approved by the FDA, and not available in the patients' electronic medical records.

The study hypothesis is that the %rP will be successfully validated for clinical and diagnostic use on the Abbott Sapphire with a threshold value or range to predict marrow engraftment.

MATERIALS AND METHODS

This study was conducted at Cincinnati Children's Hospital Medical Center. The principal investigator was Dr. Paul Steele, the Clinical Laboratory Medical Director. The major piece of equipment utilized was the Abbott Sapphire automated hematology analyzer to obtain the following laboratory results for each subject: CBC (complete blood count), Reticulocyte, and %rP. The Abbott Sapphire utilized the following reagents in order to generate reportable lab values: Abbott Quality Control, Calibrators, and the appropriate bulk reagents for each parameter.

Subjects were identified from the list of bone marrow transplants provided weekly to the clinical lab, during the interval of 10/26/2016 and 3/21/17. The EPIC electronic medical record of these subjects was examined for the number and date of platelet transfusions, during the above interval; the total time interval for the subjects' EPIC chart study extended from two weeks prior to transplant up to two months following transplant. The %rP was available on the hematology instrument printouts. The patients with the smallest number of platelet transfusions prior to engraftment were chosen for this retrospective study, with the goal of including only those subjects whose %rP values were not unduly influenced by exogenous platelet transfusions. Forty-three subjects met the study criteria.

The study data collected include the following three parameters from the peripheral blood studies: %rP, mean platelet volume (MPV), and the platelet count. The trajectory of the %rP values prior to megakaryocyte engraftment (as evidenced by cessation of dependence on platelet transfusion) was studied and reported.

Each subject was assigned a random study ID number. A single table was maintained that linked the study ID number to the medical record number, and that table accompanying documentation was retained by Dr. Steele in his locked file cabinet. Those records will be retained for a time period extending up to two years following publication of this study, at which time the table and the data will be shredded with the HIPAA trash. No list of subjects will be maintained after the data is destroyed.

The study was limited to previously obtained clinical and laboratory data. There was no anticipated risk to the subjects from participating in the study. The waiver of consent did not adversely affect the rights and welfare of the subjects. The research cannot be conducted in the future without a waiver because the clinical lab at CCHMC's base hospital no longer possesses the instrument (Abbott Cell-Dyn Sapphire) with which the data was collected; this study was always intended to be retrospective. There was no pertinent information that emerged from the study that would be appropriate or helpful to provide to the subjects.

In order to contact each subject, it would be necessary to contact the patient's physician or to use other means such as obtaining patient phone numbers from the EPIC clinical record. This incursion on the patients' privacy was felt to outweigh privacy concerns related to the use of data in the study. The PHI use involved no more than minimal risk to privacy. Subjects may no longer be available for telephone follow up because they may have moved or because they may have succumbed to the illness for which the bone marrow or stem cell transplant was performed, thus limiting the size of the study. Without a waiver for authorization for subject use, the study could not have been conducted.

Microsoft Excel was utilized to record the data for each subject and graphs displaying the results were generated. (Appendices K-VV) Daily platelet counts, %rP, and MPV values for 43 select stem cell and bone marrow patients, pre- and post-transplant, were recorded to document the projected increase in %rP between days 5-14 post-transplant date with corresponding peripheral platelet count and MPV and correlated with clinical presentation and transfusion status. Data was recorded on a collection sheet similar to Table 2 and then transcribed into the Excel workbook.

Subject ID: Transplant type:	Platelet count	%rP	MPV	Platelet transfusion
Pre-transplant Date				
Pre-transplant Date				
Transplant Date				
Post-transplant Date				
Post-transplant Date				
Post-transplant Date				
etc.				

Table 2: CCHMC Data Collection Sheet for Study Subjects in the Investigation

Additionally, CCHMC policies and procedures for validating a laboratory developed test were followed. CCHMC's Validation Plan and Summary form depicted in Appendix A was completed prior to initiating validation studies for this assay and the Assay Verification Checklist displayed in Appendix C was completed to document the dates that the validation tasks were completed.

Daily accuracy and precision of the platelet and reticulocyte parameters was monitored and recorded utilizing the manufacturer's quality control material to verify the Sapphire was operating under acceptable conditions as stated in the manufacturer's instructions for use and quality control package inserts for the specific lots utilized. See

Appendix B for results of the acceptable accuracy and precision studies performed along with examples of passing daily quality control onboard the Sapphire.

Carryover studies were performed for the analytes included in the assay during installation with passing criteria obtained and documented.

The normal pediatric reference range study was completed at CCHMC and documented in a poster presentation given during the 2014 American Association for Clinical Chemistry (AACC) convention. The values obtained in that study were utilized in the validation plan and are displayed in Table 3.

Group	1	2	3	4	5
N	52	134	92	216	268
Parameter					
RETC (x10^3/uL)	23.56 - 123.60	18.3 - 101.00	22.58 - 99.86	29.74 - 121.63	27.45 - 105.92
pRETC (%)	0.66 - 3.24	0.41 - 2.24	0.51 - 2.23	0.64 - 2.50	0.66 - 2.21
IRF (fraction)	0.12 - 0.40	0.13 - 0.37	0.10 - 0.30	0.10 - 0.31	0.11 - 0.34
MCV (fL)	74.08 - 101.83	74.13 - 87.56	76.06 - 88.39	78.27 - 91.94	79.62 - 94.29
MCVr (fL)	81.76 - 108.27	80.10 - 100.11	81.78 - 100.51	86.01 - 102.94	89.19 - 107.66
MCH (pg)	24.27 - 35.81	25.14 - 30.22	25.70 - 31.29	26.63 - 32.04	26.59 - 32.51
MCHr (pg)	20.07 - 31.66	22.10 - 30.56	23.38 - 31.50	24.94 - 31.94	25.35 - 32.39
MCHC (g/dL)	32.14 - 36.13	32.55 - 35.77	32.63 - 36.58	32.54 - 36.40	32.48 - 35.97
CHCr (g/dL)	25.57 - 31.39	26.64 - 31.73	27.30 - 32.57	27.51 - 32.17	27.33 - 31.56
PLT (x10^3/uL)	194 - 541	152 - 506	171 - 470	172 - 442	152 - 407
prP (%)	1.31 - 8.16	0.95 - 8.93	0.35 - 6.01	0.26 - 7.33	0.33 - 5.22

Table 3: 2014 CCHMC Pediatric Normal Reference Range Study (0.33-8.93%) for %rP.

It was noted by Ferreira et al. that the vast majority of studies evaluating the %rP/IPF counts do not include a parallel flow cytometry analysis due to the challenges of standardizing platelet flow cytometry analyses compared to a CBC. [42] According to the study by Roemer, Nebe, and Scott, the Abbott Sapphire is capable of accurately

determining the %rP. [43] Taking these factors into consideration, we did not include a correlation study as part of the validation protocol.

To determine the threshold value for %rP indicating marrow engraftment we evaluated and correlated the %rP values with date of platelet transfusion succession and date the platelet count rose within Cincinnati Children's Hospital Medical Center's pediatric normal reference range of 135-466 X 10^3/uL.

RESULTS

Of the original 43 subjects, nine subjects were removed from the study due to either lack of sufficient data or transplant (TX). Data and statistics were collected and calculated for the remaining 34 subjects. Graphs were produced to display the temporal relationship between the patients' daily platelet count, %rP, and platelet transfusions received during the course of treatment leading up to transplant day (Day Zero) and post-transplant. The graphs and accompanying statistical analysis for individual patients are displayed in the Appendix D for each subject listed Patient #1-43 (Graphs and statistics for Patients #10, 12, 16, 42, and 43 have been excluded due to no transplant received or lack of data). See also Appendix C for the CSV file utilized in R studio for statistical analysis.

The subjects included in the study encompassed the full pediatric age range. 7 patients fell in the 1 month-1 year range where an expected normal reference range for %rP was 1.31-8.10%. 5 patients were in the 1-3 year range with an expected normal %rP in the 0.95-8.93% range. 9 patients were in the 3-6 year age range with an expected normal reference range for %rP of 0.35-6.01%. 8 patients were in the 6-12 year age range with an expected normal reference range for %rP of 0.26-7.33, and six patients were in the 12-18 year age range with an expected normal reference range for %rP of 0.33-5.22. The distribution of the study subjects by age and gender is further demonstrated in Figure 5.

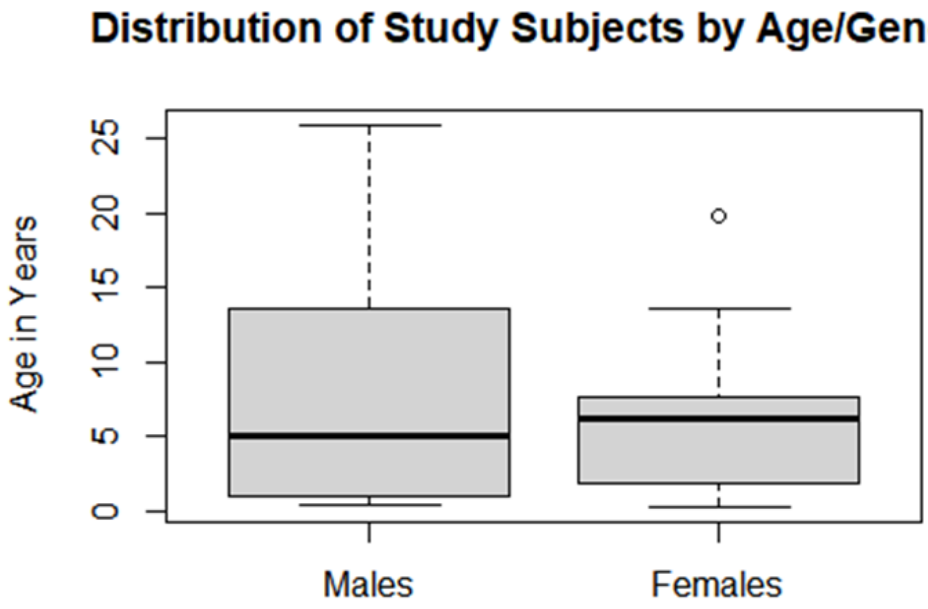


Figure 5: Distribution Boxplot. Males=20, Females=23, Mean Age in Years=6.995. Shapiro-Wilk Normality Test: W=0.86357, p-value=5.088e-05, yes>0.05= data is normal.

The mean value was calculated for the peak %rP observed for all 39 patients post-transplant indicating marrow response. The mean observed was 10.28 with a range of 5.80-18.30. The standard deviation calculated was 2.66. Results are summarized in the histogram depicted in Figure 6.

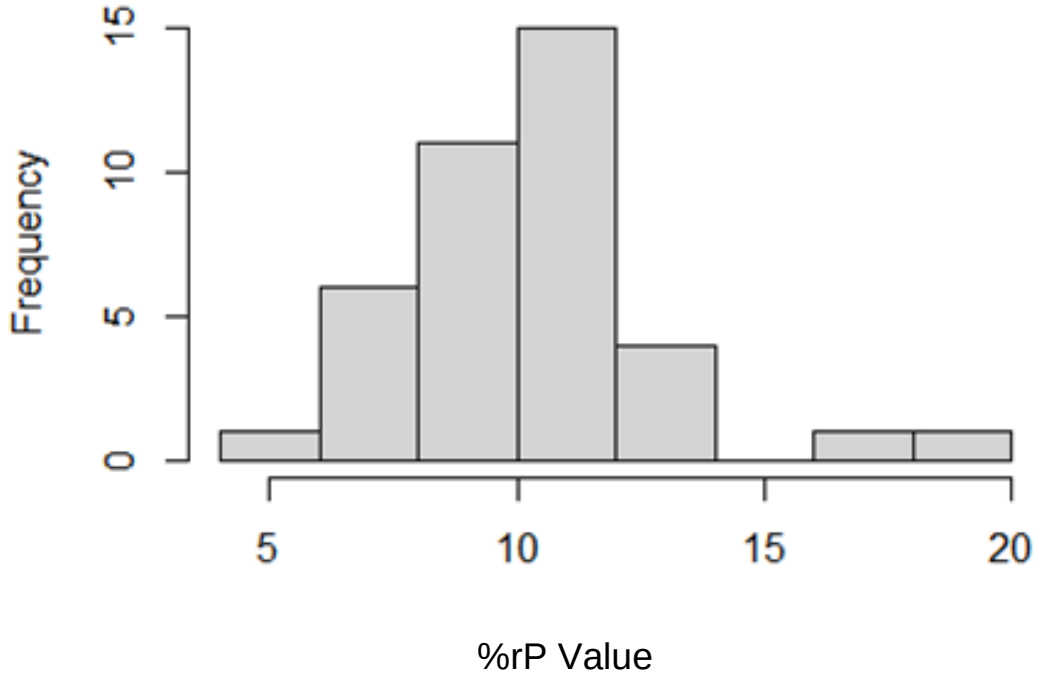


Figure 6: Distribution of Peak %rP Observed Post-Transplant: N=39
Min. 1st Qu. Median Mean 3rd Qu. Max. NA's
5.800 8.605 10.500 10.284 11.400 18.300 9
SD= 2.661744: 2 SD range: 4.96-15.52
Shapiro-Wilk normality test: W = 0.95213, p-value = 0.09694, yes>0.05= data is normal.

The mean number of days from day zero (day transplant was received) until a rise in the %rP outside of the normal reference range indicating marrow response was 9.49 days with a range of 2-18 days. The date indicating a rise or spike in %rP corresponded to the first day post-transplant that the %rP rose above the normal reference range for that patient's age.

Since we had a small sample size (<50), it was important that we determine the distribution of days to peak %rP in order to choose an appropriate statistical method. A Shapiro-Wilk test was performed and did not show evidence of non-normality (W= 0.95434, p-value=0.1151). Based on this outcome and after visual examination of the

histogram for number of days from transplant to peak %rP (Figure 7) we decided to use a parametric test for further data analysis.

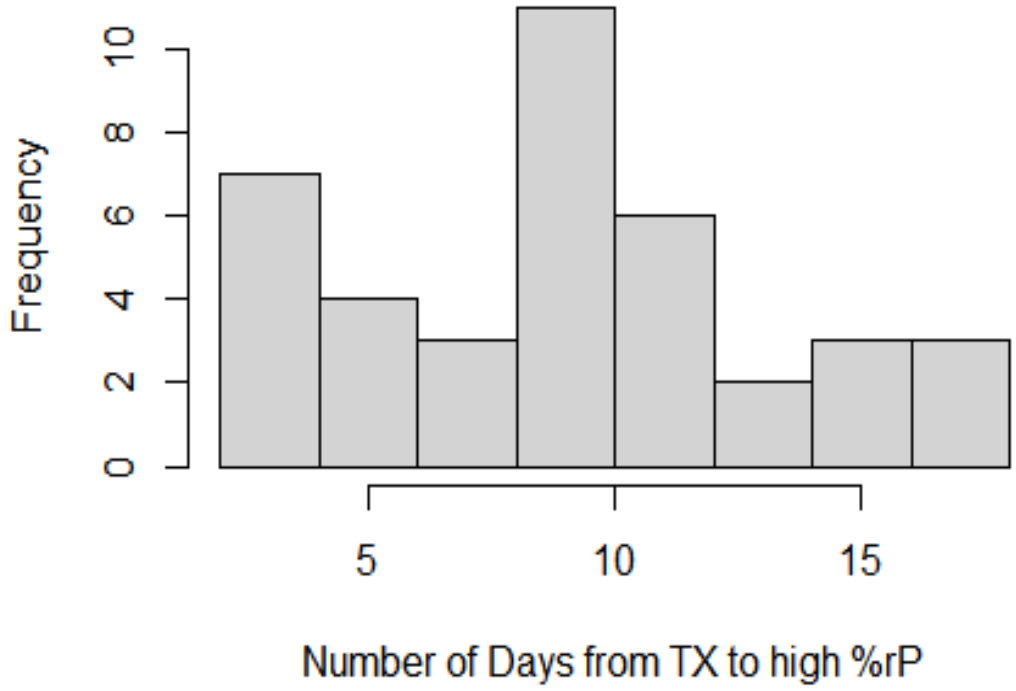


Figure 7: Distribution of Number of Days from Transplant to High %rP: N=39

Min.	*1st Qu.*	*Median*	*Mean*	*3rd Qu.*	*Max.*	*NA's*
2.000	*5.500*	*9.000*	*9.487*	*12.000*	*18.000*	*9*

Shapiro-Wilk normality test: W = 0.95434, p-value = 0.115, yes>0.05= data is normal.

A Pearson Correlation Test was computed to assess the relationship between the number of days from transplant to peak %rP and the patients' age. A scatterplot summarizes the results for 39 samples in Figure 8. There was a negative correlation between the two variables, r= -0.39, n=39, p=0.01.

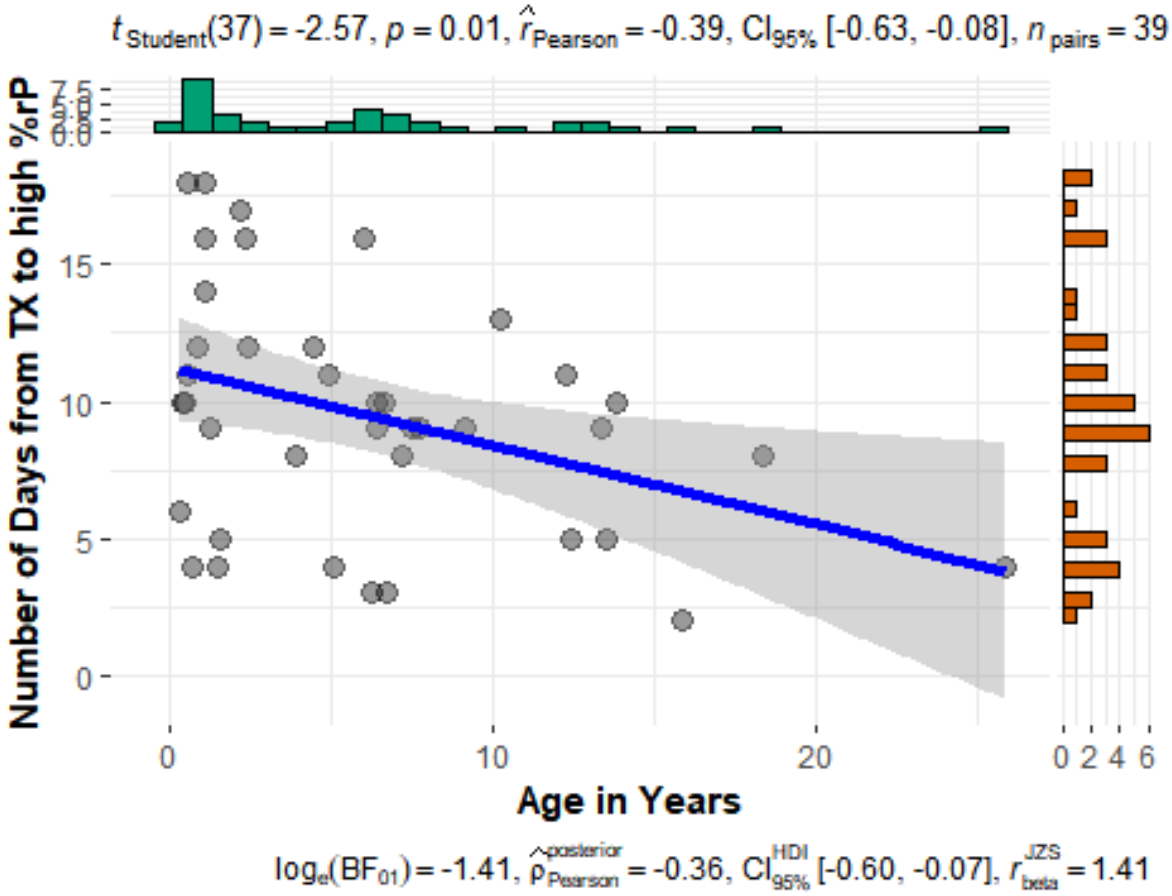


Figure 8: Pearson Correlation between Number of Days from TX to high %rP vs Age in Year; Absolute t (2.57)> Critical t (1.686) df=38: Reject null
Negative correlation= the older a patient is the faster the marrow response post-transplant.

This finding suggests that the older a patient is at time of transplant, the faster the patient's marrow response will be post-transplant.

Furthermore, the average number of days in which the patients' platelet count returned to normal following the observed rise in %rP was computed. The CCHMC normal pediatric reference range for platelets used in this analysis was 135,000-466,000 per microliter. A histogram summarizes these results and displays 10.33 as the average number days until normal platelet count was observed with a range of 0-28 days (Figure 9).

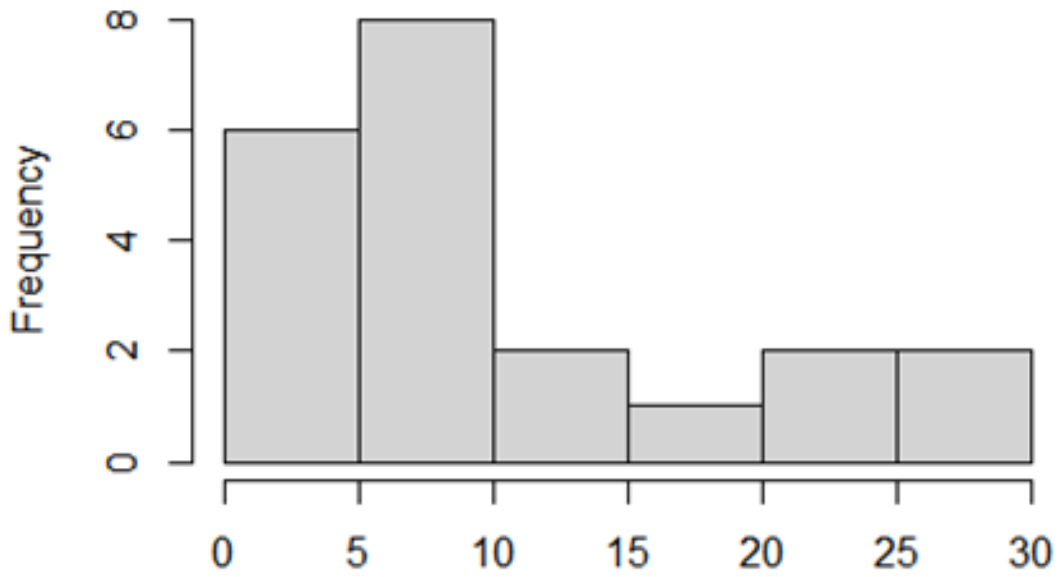


Figure 9: Distribution of Days from Peak %rP to the Day the Patient's Platelet Count Returned to the Normal Reference Range: N=21

Min.	*1st Qu.*	*Median*	*Mean*	*3rd Qu.*	*Max.*	*NA's*
0.00	*5.00*	*8.00*	*10.33*	*15.00*	*28.00*	*27*

Shapiro-Wilk normality test: W = 0.89686, p-value = 0.03043, p<0.05= data not normally distributed.

A Pearson Test was computed to assess the correlation between the number of days from peak %rP post-transplant to the number of days it took the patients' platelet count to return to normal. A scatterplot summarizes these results for the 20 samples in Figure 10. There was negative correlation between the two variables, r= -0.43, n= 20, p= 0.05.

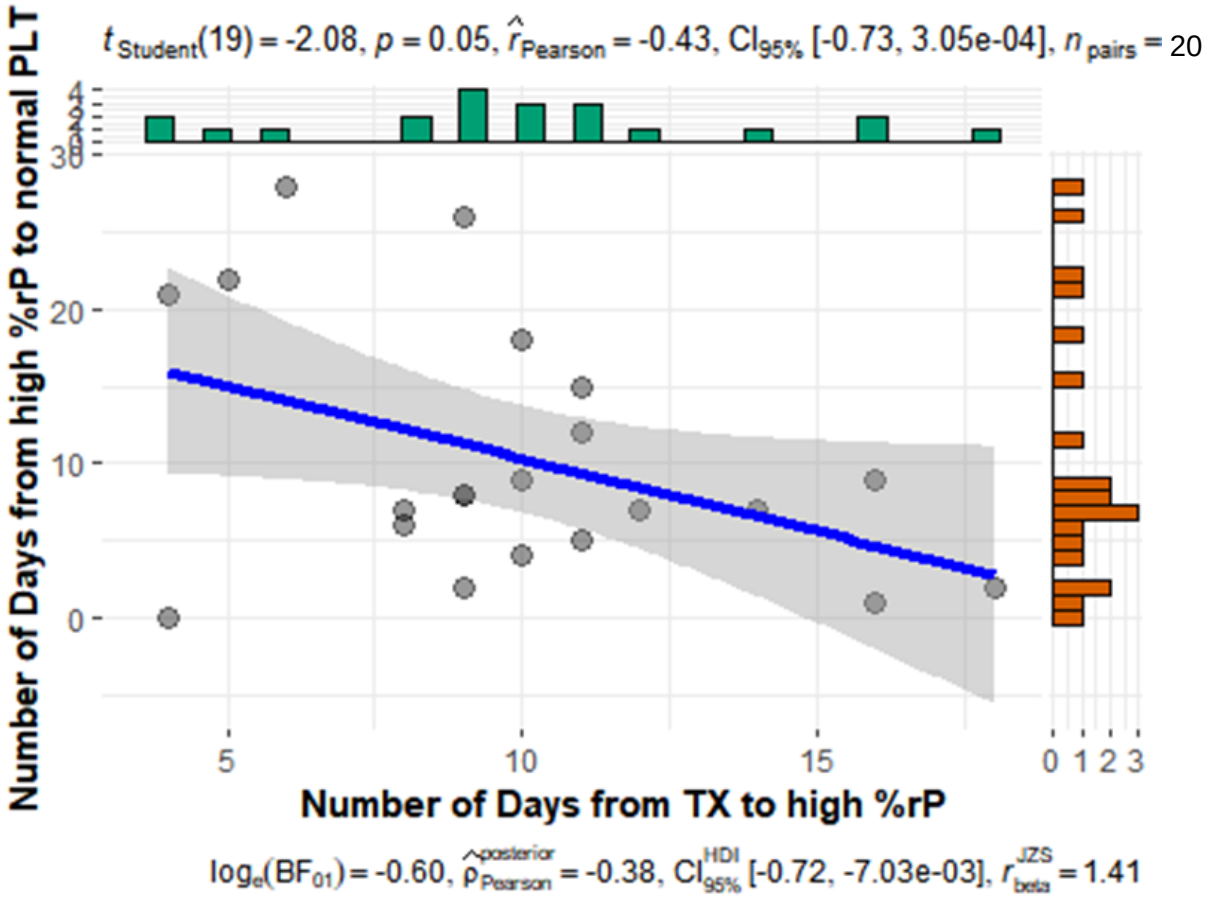


Figure 10: Pearson Correlation Test: Number of Days from High %rP to Normal PLT vs Number of Days from TX to High %rP
Absolute t (2.08) > Critical t (1.729) and (df=19): Reject null.

This Pearson Test suggests that the quicker the marrow response occurred post-transplant, the longer it took for the patient's platelet count to return to normal.

Finally, additional statistics were calculated using an Excel workbook (Appendix C). The average number of days it took before platelet transfusions were discontinued for the patients who were receiving platelet transfusions was 3.1 days post rise in %rP with a range of 16 days prior to spike in %rP through 23 days post spike in %rP.

For 34 subjects included in the study, a significant rise in %rP was observed post – transplant suggesting marrow engraftment. Of these 34 subjects, 19 patients reported a

return to normal platelet count post spike in %rP. 14 patients displayed a spike in %rP post-transplant, however, their platelet counts did not return to normal. Of these 14 subjects, 5 patients became transfusion dependent, and 9 patients had their transfusions discontinued despite their platelet counts never reaching the lower limit of the normal reference range of 135 $x10^3$/uL. These 9 patients had platelet counts above the transfusion limit, but still below the normal reference range.

Additionally, four subjects in the study completed their treatment plan pre- and post-transplant without receiving any platelet transfusion products. For these four patients, the average spike in %rP was 9.66 within an average of 8.5 days. For patients in this group, their platelet counts returned to normal within an average of 4.67 days.

DISCUSSION

Review of the results from this study indicated that the %rP performed on the Abbott Sapphire passed CCHMC's initial validation study requirements. Final approval would still need to be granted by the Medical Director after reviewing the studies once an acceptable passing criterion (CV%) for the %rP values obtained running the Abbott provided quality control material was determined.

There is a significant advantage in being able to report out the Abbott Sapphire %rP value to clinicians as a lab developed test. It would be more economical for the patient and laboratory to obtain an automated %rP in the general hematology laboratory versus sending the sample to the flow cytometry department. This test method would also be quicker and more standardized. By including this parameter in the patients' records, the clinicians would be able to evaluate the results and use them in their decision-making process. This could lead to an improvement in patient care and the patient's outcome.

Many clinicians may not be fully aware of the clinical utility of the %rP. It may be necessary to provide an online seminar or information session aimed at detailing the %rP and its clinical utility as well as instructions for ordering the test to its providers. CCHMC may also want to consider adopting a reflex policy for reporting out the %rP. Criteria warranting a reflex %rP may include thrombocytopenia and/or diagnosis.

Additionally, the results indicated that subjects receiving either a bone marrow or stem cell transplant would display a spike in %rP on average 9.49 days post-transplant suggesting marrow engraftment. This confirmed the findings by both the Jaing [18] and Park [19] studies done by flow cytometry and performed utilizing the Sysmex XN-2000 respectively. The spike in %rP above the normal reference range would lead to a return

in normal platelet count on average within 10.33 days which confirmed the study done on the Sysmex XN series by Grabek [41]. These results further confirm that the %rP obtained on the Abbott Sapphire can be utilized to monitor the platelet marrow status in these patients and future patients post-transplant. Clinicians could use this data to predict an expected target window for marrow engraftment. Additionally, they could use this data to possibly predict a timeframe for a patient's platelet count to return to normal. Using results from this study, the clinicians could assume a spike in %rP indicates marrow activity.

Confirming the Abbott Sapphire's comparable performance to flow cytometry and Sysmex methodologies indicated that determining a threshold value for %rP that suggested marrow engraftment from this study would be a significant finding. From this study a %rP value of 10.28 within a 9.49 average day window post-transplant suggested a platelet count recovery to the normal range within an average of 10.33 days. Platelet transfusions would then be discontinued on average within 3.1 days once the threshold value was observed.

During the statistical analysis, some unexpected results were seen relating to the number and frequency of platelet transfusion products that some patients received. Some values for Subjects #6 and #29 were removed from the study due to the number of transfusions that appeared to invalidate the %rP values. This finding would suggest additional research is needed regarding the various platelet transfusion products and the interference factors they may have on the accuracy of the %rP. Both pooled and apheresis products should be evaluated for interfering properties as study subjects #6 and #29 received many of each. In addition, clinicians must consider the frequency and

type of platelet transfusion product administered when evaluating the %rP value for their patients.

It appears from the data obtained from Subject's #6 and #29 that the platelet products administered may have contributed to falsely elevated %rP results. Additional research may include investigating the %rP values of the transfusion products. Do these products contain abnormally high values of donor %rP? Do pooled products contain higher values of %rP than apheresis products? Do frequent donors have a naturally higher %rP due to turnover? Can an accurate %rP value be measured from donor products? Automated hematology analyzers usually flag samples with low red blood cell counts with aspiration errors. Subsequently, platelet transfusion products may not produce valid results on these analyzers, thus indicating another research question. How would we test these products accurately?

Finally, the MCV values were obtained and charted for each patient during this study. Once graphs were generated to display the temporal relationship of the MCV in correlation to the %rP, it was decided that a separate study would need to be completed to evaluate the significance of this parameter. The MCV was then hidden from the patient graphs, but still available for future analysis if selected from the data view option.

CONCLUSION

Based on the results of this study, the %rP was demonstrated to be a valid reportable parameter when analyzed on the Abbott Sapphire. A threshold %rP value of 10.28 with a 2 SD range of 4.96-15.52 was determined to predict marrow engraftment following bone marrow or stem cell transplant in the 34 patients studied utilizing the results obtained on the Abbott Sapphire hematology analyzer.

The platelet transfusion product itself must be considered as a potential interfering property until further research has been completed. Our study demonstrated that a spike in %rP of 9.66 for those patients who did not receive any platelet products throughout the course of their treatment suggested marrow engraftment and an earlier return to normal platelet count on average within 4.7 days. By eliminating the potential interfering property (platelet product), we were able to observe the expected results that confirmed our hypothesis in all four of these patients.

Data analysis of the samples in this study would suggest future studies focusing on the interfering factors that platelet products may contain are necessary. Evaluating the products for %rP may reveal high numbers of reticulated platelets present. It would also be imperative to investigate the %rP of both pooled and apheresis products. Questions regarding the donors of each type of product and how that relates to the number of reticulated platelets in those products arise. One may want to determine if apheresis platelet products contain a higher number of reticulated platelets versus pooled products and vice versa. Depending on the %rP value obtained when assessing these products, it may be possible to determine the level of interference each type of product produces.

Additionally, it would be of excellent value for both Sysmex and Abbott to develop quality control products that lists the acceptability criteria for reticulated platelets and cover the entire normal reference range. Currently, the Abbott quality control files collect and statistically analyze the %rP data however the package insert does not list a mean or range. The laboratory must establish their own mean and range instead of verifying the manufacturer's. For Sysmex quality control, the mean and range are established by the manufacturer, however, all three levels of quality control have identical means and ranges and do not cover the entire normal or reportable reference ranges.

REFERENCES

[1] D. J. Kuter, "The physiology of platelet production," *Stem Cells,* vol. 14, no. Suppl 1, pp. 88-101, 1996.

[2] J. J. Hoffman, "Reticulated platelets: analytical aspects and clinical utility," *Clin Chem Lab Med,* vol. 52, no. 8, pp. 1107-1117, 2014.

[3] V. R. Deutsch and A. Tomer, "Megakaryocyte development and platelet production," *British Journal of Haematology,* vol. 134, pp. 453-466, 2006.

[4] M. Lebois and E. C. Josefsson, "Regulation of platelet lifespan by apoptosis," *Platelets,* vol. 27, no. 6, pp. 497-504, 2016.

[5] K. A. Ault and C. Knowles, "In vivo biotinylation demonstrates that reticulated platelets are the youngest platelets in circulation," *Exp Hematol,* vol. 23, no. 9, pp. 996-1001, 1995.

[6] T. Thijs, B. P. Nuyttens, H. Deckmyn and K. Broos, " Platelet physiology and antiplatelet agents," *Clin Chem Lab Med,* vol. 48, no. Suppl 1, pp. 3-13, 2010.

[7] K. Ghoshal and M. Bhattacharyya, "Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis," *The Scientific World Journal,* pp. 781-857, 2014.

[8] R. Grozovsky, S. Giannini, H. Falet and K. M. Hoffmmeister, "Regulating billions of blood platelets: glycans and beyond," *Blood,* vol. 126, pp. 1877-1884, 2015.

[9] K. R. Machlus, J. N. Thon and J. E. Italiano, "Interpreting the developmental dance of the megakaryocyte: a review of cellular and molecular processes mediating platelet formation," *British Journal of Haematology,* vol. 165, no. 2, pp. 227-36, April 2014.

[10] Y. Kurata, S. Hayashi, T. Kiyoi and S. Kosugi, "Diagnostic Value of Tests for Reticulated Platelets for Discriminating Between Hyperdestructive and Hypoplastic Thrombocytopenia," *AM J Clin Pathol,* vol. 115, pp. 656-664, 2001.

[11] G. Strauss, C. Vollert, A. Von Stackelberg, A. Weimann, G. Gaedicke and H. Schulze, "Immature platelet count: a simple parameter for distinguishing thrombocytopenia in pediatric acute lymphocytic leukemia from immune thrombocytopenia," *Pediatric Blood Cancer,* vol. 57, no. 4, pp. 641-647, 2011.

[12] Z. Van De Wyngaert, E. Fournier, E. Bera, M. Carrette, V. Soenen, J. Gauthier and T. Boyer, "Immature platelet fraction (IPF): A reliable tool to predict peripheral thrombocytopenia," *Curr Res Transl Med,* 2019.

[13] O. H. Pedersen, M. L. Larsen, E. L. Grove, P. B. Van Kooten Niekerk, S. Bonlokke, P. H. Nissen and A. M. Hvas, "Platelet characteristics in patients with essential thrombocytosis," *Cytometry B Clin Cytom,* vol. 94, no. 6, pp. 918-927, 2018.

[14] I. Pons, M. Monteagudo, G. Lucchetti, L. Munoz, G. Perea, I. Colomina and J. Obiols, " Correlation between immature platelet fraction and reticulated platelets. Usefulness in the etiology diagnosis of thrombocytopenia," *European Journal of Haemotology ,* vol. 85, no. 2, pp. 158-163, 2010.

[15] M. Sakuragi, S. Hayashi, M. Maruyama, O. Kabutomori, T. Kiyokawa, K. Nagamine and Y. Tomiyama, "Clinical Significance of the IPF% or RP% measurement in distinguishing primary immune thrombocytopenia from aplastic thrombocytopenic disorders," *Int J Hematol,* vol. 102, no. 4, pp. 369-375, 2015.

[16] A. McDonnell, K. L. Bride, D. Lim, M. Paessler, C. M. Witmer and M. P. Lambert, "Utility of the immature platelet fraction in pediatric immune thrombocytopenia: Differentiating from bone marrow failure and predicting bleeding risk," *Pediatr Blood Cancer,* vol. 65, no. 2, 2018.

[17] H. Michur, K. Maslanka, A. Szczepinski and B. Marianska, "Reticulated platelets as a marker of platelet recovery after allogeneic stem cell transplantation," *Int J Lab Hematol,* vol. 30, no. 6, pp. 519-525, 2008.

[18] T. H. Jaing, S. H. Chen, Y. C. Wen, T. Y. Chang, J. L. Huang and P. K. Tsay, "Assessment of platelet activation and immature platelet fraction as predictors of platelet engraftment after hematopoietic stem cell transplantation," *Cell Transplant,* 2016.

[19] S. H. Park, C. J. Park, M. J. Kim, M. Y. Han, B. R. Lee, Y. U. Cho and K. H. Lee, "Evaluation of parameters obtained from the Sysmex XN-2000 for predicting the recovery of the absolute neutrophil count and platelets after hematopoetic stem cell transplantation," *Int J Lab Hematol,* vol. 38, no. 2, pp. 198-208, 2016.

[20] K. Saiko, Y. Sakota, Y. Masuda, K. Matsunaga, M. Takenokuchi, K. Nishimura and S. Kumagai, "Automatic detection of immature platelets for decision making regarding platelet transfusion indications for pediatric patients," *Transfus Apher Sci,* vol. 38, no. 2, pp. 127-132, 2008.

[21] M. Ingram and A. Coopersmith, "Reticulated platelets following acute blood loss," *British Journal of Haematology,* vol. 17, no. 3, pp. 225-229, 1969.

[22] L. G. Lee, C. H. Chen and L. A. Chiu, "Thiazole orange: A new dye for reticulocyte analysis," *Cytometry,* vol. 7, pp. 508-517, 1986.

[23] J. Kienast and G. Schmitz, "Flow cytometric analysis of thiazole orange uptake by platelets: a diagnostic aid in the evaluation of thrombocytopenic disorders," *Blood,* vol. 75, pp. 116-121, 1990.

[24] B. D. Hedley, N. Llewellyn-Smith, S. Lang, C. C. Hsia, N. MacNamara, D. Rosenfeld and M. Keeney, "Combined Accurate Platelet Enumeration and Reticulated Platelet Determination by Flow Cytometry," *International Clin Cytometry Society,* vol. 88, no. 5, pp. 330-337, 2015.

[25] L. Hille, M. Cederqvist, J. Hromek, C. Stratz, D. Trenk and T. G. Nuhrenberg, "Evaluation of an Alternative Staining Method Using SYTO 13 to Determine Reticulated Platelets," *Thromb Haemost,* vol. 119, no. 5, pp. 779-785, 2019.

[26] L. Meintker, M. Haimerl, J. Ringwald and S. W. Krause, "Measurement of immature platelets with Abbott CD-Sapphire and Sysmex XE-5000 in hematology and oncology patients," *Clin Chem Lab Med,* vol. 51, no. 11, pp. 2125-2131, 2013.

[27] M. Kono, S. Matsuhiro, F. Nakazawa, M. Kaido, A. Wada and Y. Tomiyama, "Morphological and optical properties of human immature platelet-enriched population produced in immunodeficient mice," *Platelets,* vol. 30, no. 5, pp. 652-657, 2019.

[28] K. Miyazaki, Y. Koike, S. Kunishima, R. Ishii, M. Danbara, R. Horie and M. Higashihara, "Immature platelet fraction measurement is influenced by platelet size and is a useful parameter for discrimination of macrothrombocytopenia," *Hematology,* vol. 20, no. 10, pp. 587-592, 2015.

[29] N. Yoshikawa, A. Masuda, M. Jona, S. Okubo, Y. Nannya, H. Yokota and Y. Yatomi, "Evaluation of Immature Platelet Fraction Measurements Using an Automated Hematology Analyzer, Sysmex XN," *Rinsho Byori,* vol. 64, no. 11, pp. 1220-1228, 2016.

[30] J. Li, Y. Li, J. Ouyang, F. Zhang, C. Liang, Z. Ye, S. Chen and J. Cheng, "Immature platelet fraction related parameters in the differential diagnosis of thrombocytopenia," *Platelets,* 2019.

[31] A. Bimpong, "The effect of storage on the clinical utility of the immature platelet fraction," *Hematology,* vol. 14, no. 2, pp. 18-121, 2009.

[32] M. Nishiyama, S. Hayashi, O. Kabutomori, H. Yamanishi, E. Suehisa, Y. Kurata and Y. Tomiyama, "Effects of anticoagulants and storage temperature on immature platelet fraction % (IPF%) values in stored samples measured by the

automated hematology analyzer, XE-5000- utility of CTAD-anticoagulation and room temperature storage," *Rinsho Byori,* vol. 59, no. 5, pp. 452-458, 2011.

[33] M. Buttarello, G. Mezzapelle and M. Plebani, "Effect of preanalytical and analytical variables on the clinical utility of mean platelet volume," *Clin Chem Lab Med,* pp. 718-725, 2018.

[34] Y. Abe, H. Wada, H. Tomatsu, A. Sakaguchi, J. Nishioka, Y. Yabu and T. Nobori, "A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF)," *Thromb Res,* vol. 118, no. 4, pp. 463-469, 2006.

[35] O. Unay Demirel, S. Ignak and M. C. Buyukuysal, "Immature Platelet Count Levels as a Novel Quality Marker in Plateletpheresis," *Indian J Hematol Blood Transfus,* vol. 34, no. 4, pp. 684-690, 2018.

[36] C. E. Imperiali, A. Arbiol-Roca, L. Sanchez-Navarro, M. Dastis-Arias, J. C. Lopez-Delgado and D. Dot-Bach, "Reference interval for immature platelet franction on XN haemotology analyser in adult population," *Biochem Med (Zagreb),* vol. 28, no. 1, 2018.

[37] M. Joergensen and L. Bathum, "Reference intervals for mean platelet volume and immature platelet fraction determined on a Sysmex XE5000 hematology analyzer," *Scand J Clin Lab Invest,* vol. 76, no. 2, pp. 172-176, 2016.

[38] J. J. Hoffman, N. M. Van Den Broek and J. Curvers, "Reference intervals of reticulated platelets and other platelet parameters and their associations," *Arch Pathol Lab Med,* vol. 137, no. 11, pp. 1635-1640, 2013.

[39] M. O'Leary, P. Horn, B. Ward, P. Steele and D. Wright, *Pediatric Reference Intervals for New Reticulocyte and Platelet Parameters,* 2014.

[40] L. Meintker, J. Fritsch, J. Ringwald and S. Krause, "Immature platelet do not reliably predict platelet recovery in patients with intensive chemotherapy or stem cell transplantation," *Vox Sanguinis,* vol. 112, no. Feb, pp. 132-139, 2017.

[41] E. Hennel, K. Kentouche, J. Beck, M. Kiehntopf and K. Boeer, "Immature platelet fraction as marker for platelet recovery after stem cell transplantation in children," *Clin Biochem,* vol. 45, no. 10-11, pp. 749-752, 2012.

[42] J. Grabek, N. D'Elia and G. Kelsey, "Immature platelet fraction as a predictor of platelet count recovery following allogeneic bone marrow transplantation," *Pathology,* vol. 53, no. 4, pp. 493-497, 2021.

[43] F. L. Ferreira, M. P. Colella, S. S. Medina, C. Costa-Lima, M. M. Fiusa, L. N. Costa and E. V. DePaula, "Evaluation of the immature platelet fraction contribute to the

differential diagnosis of hereditary, immune and other acquired thrombocytopenias," *Sci Rep,* vol. 7, no. 1, p. 3355, 2017.

[44] B. Roemer, C. T. Nebe and C. S. Scott, *Preliminary Feasibility Design of a CELL-DYN Sapphire Method for Reticulated Platelet Analysis,* Washington DC, 2008.

Laboratory Section: _____ Test: _____

New Method/ Manufacturer _____ Current/Reference Method: _____

VALIDATION PLAN and SUMMARY

Evaluation Criteria	Experiment Required?	Data Summary	Comments
Accuracy: (e.g. linearity)	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Precision: Within run Between run Between Techs	☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Split sample Correlation	☐ Yes ☐ No # of samples ____ Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Analytic and Functional Sensitivity	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
AMR	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Carryover	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Maximum Dilution/Concentration (Clinical Reportable Range)	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Reference Range	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Interfering Substances	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Specimen Type Bias	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	
Sample Stability	☐ Yes ☐ No Date: _____ Initials: _____	☐ N/A ☐ More data required ☐ Acceptable	

The attached validation testing data and the statistical analysis thereof warrant the approval of this method and manufacturer for patient testing, as of _____

Figure 11: CCHMC Validation Plan and Summary for a Laboratory Developed Test or Assay.

Assay Verification Checklist

Department: _____ Test: _____

TASK	DATE	TECH
1. Create plan for verification with dept Leadership team		
a. Design study with approval of director and manager		
b. Complete "Experiment Required?" column on Method Validation form		
c. Study design reviewed and approved by director and manager		
2. Electrical check & KN # issued for new equipment/instrument by CE		
3. Create data collection sheet		
4. Perform verification testing including repeat on discrepant samples		
5. Write up QA of verification including statistical analysis of data:		
a. Precision		
b. Accuracy		
c. Reportable range		
d. Sensitivity & Interferences		
e. Other (list)		
6. Submit QA of verification for review by manager and director		
7. Write procedures		
a. Testing		
b. Preventive Maintenance (PM)		
8. Create following logs		
a. QC sheet		
b. Calibration log, if applicable		
c. PM log		
d. Inventory sheet		
e. Training checklist		
f. Verified Results Review sheet, if applicable		
9. Complete cost analysis with manager		
10. LIS: interface with instrumentation, if applicable (LIS.DBA.241)		
11. Cerner Millenium: CERT and PROD (follow LIS.DBA.130 procedure and LIS.DBA.130A documentation form)		
a. Build test procedure, results and interpretive data if applicable		
b. Labels		
c. Reflexive orders, if applicable		
d. Testing with a "test patient"		
12. Order CAP proficiency or establish alternative if not available		
13. Notify CAP of new activity to menu if appropriate		
14. Determine communication needs with lab leadership:		
a. Communicate changes to clinicians (internal & external) if applicable		
b. Communicate changes to other lab departments, if applicable		
15. Complete Chemical Inventory Product Form for new or deleted products		
16. Submit billing information (CPT code and cost) for compendium		
17. Obtain final approvals and determine timeline for go-live		
18. Submit information on Issue Tracking to LIS for Cerner Millenium & EPIC		
19. Clinical Lab Index: submit modifications on Issue Tracking		
20. Go-Live with testing		
21. Observe first orders for correct workflow, result posting, charge capture, etc.		

Figure 12: CCHMC Assay Verification Checklist.

CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER
3333 BURNET AVE.
CINCINNATI, OHIO 45229
513-636-7344
Software Version: v5 Analyzer S/N: 43137AZ

CELL-DYN Sapphire QC File 18 PRECISION 03/20/17 14:27 Page: 1
Lot #: Expiration Date: Status: 10/120

	WBC	RBC	RBCo	HGB	MCV	PLTi	PLTo	RETC	MPV	%rP	%R	IRF
Upper Limits:	999.	999.	999.	999.	999.	999.	999.	999.	999.	999.	999.	999.
Lower Limits:	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Target Means:	500.	500.	500.	500.	500.	500.	500.	500.	500.	500.	500.	500.

Acc Seq#	WBC	RBC	RBCo	HGB	MCV	PLTi	PLTo	RETC	MPV	%rP	%R	IRF	Date	Time	OpID
8575	7.94	4.42	4.49	12.2	83.3	238.	216.	50.9	11.6	1.36	1.15	.294	03/20/17	14:11	SSH
8576	7.76	4.41	4.49	12.4	82.9	224.	217.	57.0	11.5	.875	1.29	.268	03/20/17	14:12	SSH
8577	7.69	4.42	4.54	12.2	82.5	228.	222.	57.6	11.7	1.85	1.30	.242	03/20/17	14:13	SSH
8578	7.67	4.39	4.53	12.3	82.5	243.	226.	53.3	11.6	1.10	1.21	.272	03/20/17	14:15	SSH
8579	7.81	4.36	4.43	12.2	82.3	235.	213.	49.7	11.5	1.07	1.14	.298	03/20/17	14:16	SSH
8580	8.08	4.38	4.45	12.2	82.0	236.	217.	55.2	11.7	1.45	1.26	.263	03/20/17	14:17	SSH
8581	7.75	4.27	4.40	12.2	81.8	231.	212.	50.2	11.8	1.24	1.18	.249	03/20/17	14:19	SSH
8582	7.86	4.30	4.43	12.2	81.5	238.	214.	49.4	11.8	1.49	1.15	.272	03/20/17	14:20	SSH
8583	7.67	4.34	4.41	12.1	81.2	229.	212.	48.0	11.9	1.54	1.11	.262	03/20/17	14:21	SSH
8584	7.79	4.37	4.44	12.2	81.0	226.	206.	52.9	11.7	1.38	1.21	.296	03/20/17	14:22	SSH

Acc Seq#	WBC	RBC	RBCo	HGB	MCV	PLTi	PLTo	RETC	MPV	%rP	%R	IRF	Date	Time	OpID
N:	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0			
File Mean:	7.80	4.37	4.46	12.2	82.1	233.	216.	52.4	11.7	1.34	1.20	.272			
Std Dev:	.129	.051	.049	.074	.735	5.97	5.54	3.34	.118	.277	.068	.019			
CV (%):	1.66	1.18	1.09	.603	.895	2.56	2.57	6.38	1.01	20.7	5.64	7.16			

Figure 13: Accuracy and Precision Statistics via Onboard Sapphire QC File.

Sapphire 43137AZ QC Lot 52859

- QC Level 1 Mean=5.10 CV=79.3%
- QC Level 2 Mean=5.57 CV=52.6%
- QC Level 3 Mean=1.52 CV=43.9%

Sapphire 42992AZ QC Lot 52859

- QC Level 1 Mean= 4.23 CV= 58.8%
- QC Level 2 Mean= 5.04 CV= 47.9%
- QC Level 3 Mean= 1.48 CV= 47.4%

Figure 14: Summary of %rP means and CV%'s determined with Abbott QC.

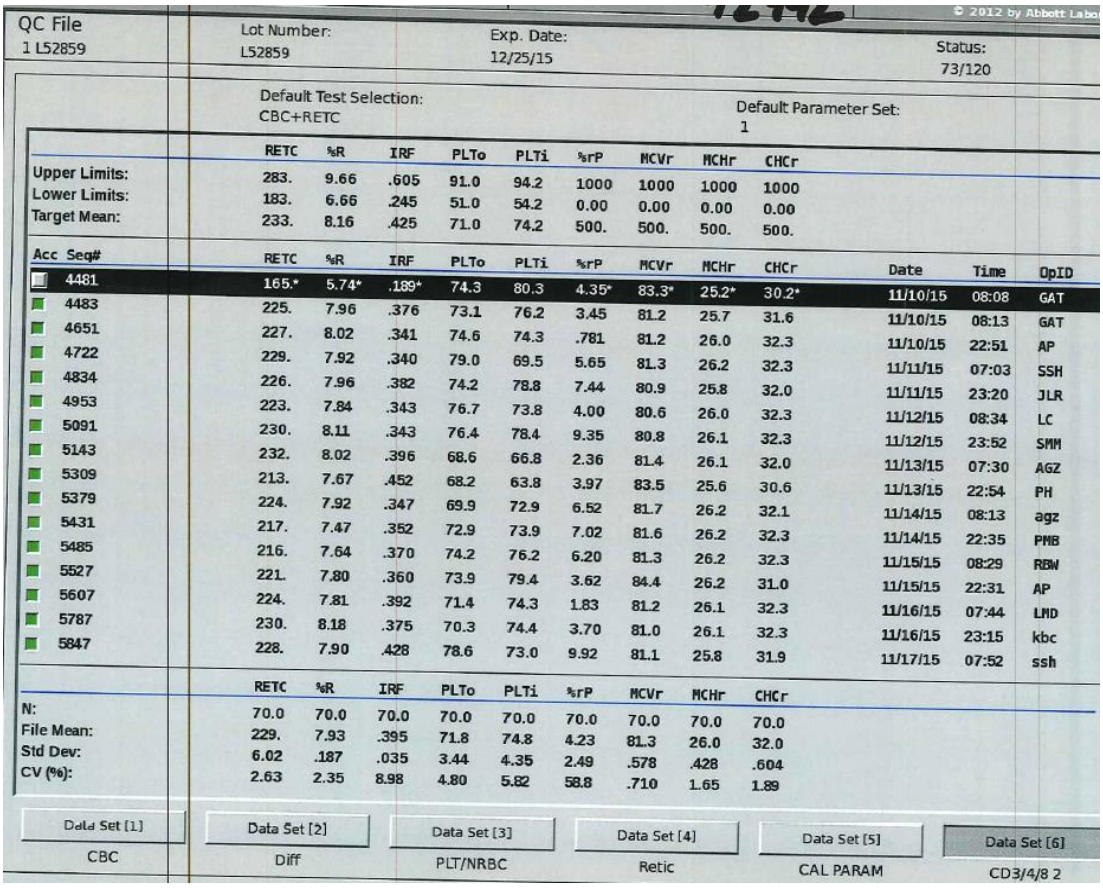


Figure 15: QC File for Lot 52859 on Sapphire SN# 42992AZ.

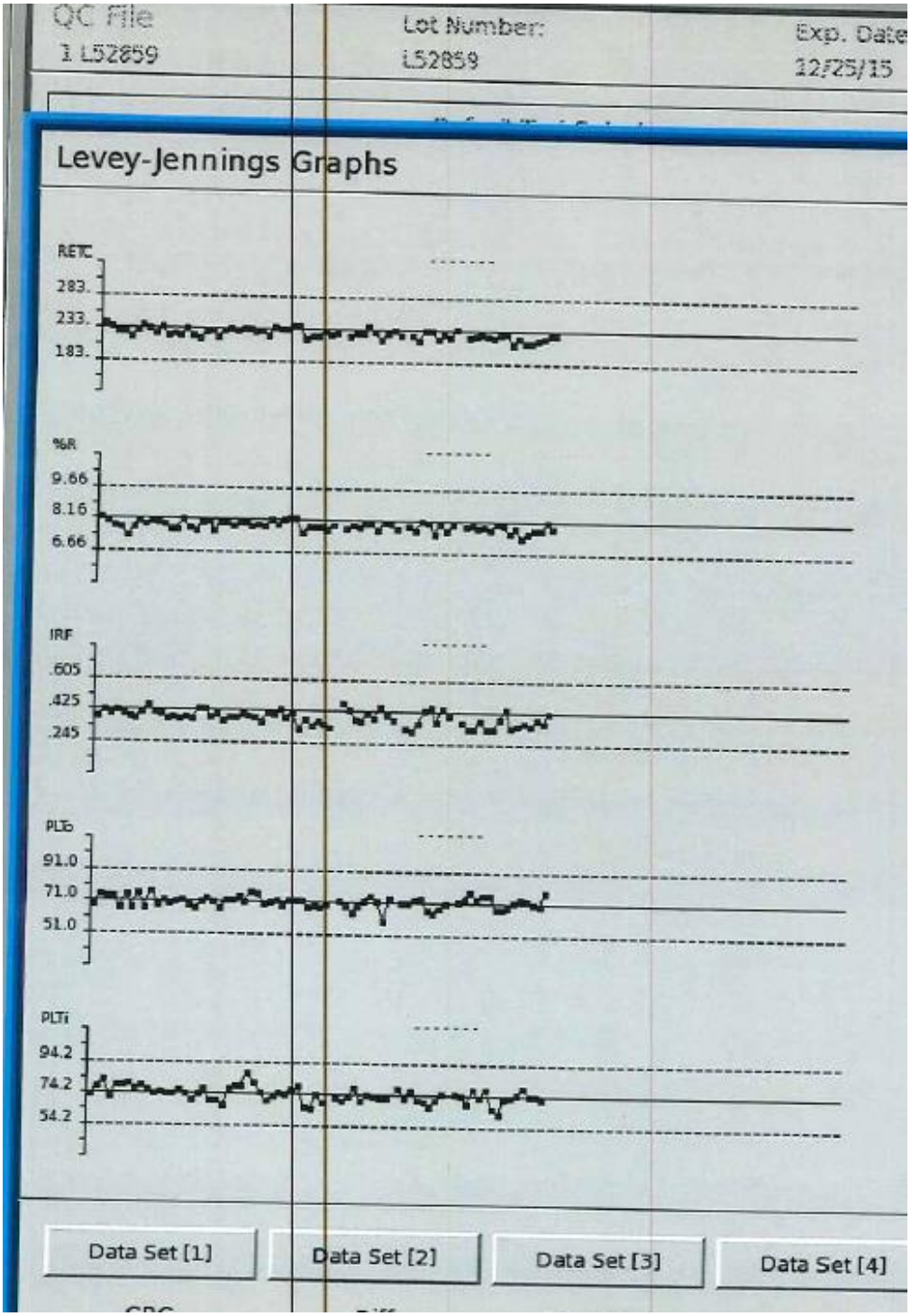


Figure 16: Levy Jennings File for Lot 52859 on Sapphire SN# 42992AZ.

APPENDIX C: ADDITIONAL STATISTICAL DATA AND DATA FILES

Statistical Analysis of 34 Subjects included in the Study+A1:D25A54A1:D23A1:DA1:D30			
Study ID	**#days from transplant to high %rP**	**#days from high %rP to Normal plt**	**#days from high %rP to last transfusion**
1	11	15	2
2	6	28	4
3	9	8	-4
4	11	12	-4
5	4	21	5
6	18		
7	8		1
8	5		8
9	11	5	-4
11	16	1	8
13	9	26	7
14a	10		
14b	3		
15	4		19
17	12	7	
18	10	18	17
19a	17		
19b	16		
19c	12		
20	10	9	-5
21	9	2	-1
22	5		0
23	9		5
24	9		8
25	3		22
26	8	7	
28	12		-2
30	13		4
31a	14	7	-14
31b	16	9	-16
31c	18	2	-5
32	5	22	4
34	10	4	23
35	4		9
36	9	8	-4
37	10		
39	2		
40	8	6	0
41	4	0	
	Average # days to see spike:	**Average # days to normal platelet:**	**Average # days until transfusions end:**
	9.49	10.33	3.11
	8.50	4.67	
Total no. included:	39	21	28

For subjects whose transfusions were discontinued after the spike:	
Average # days:	8.111111111
Total included:	18
For subjects whose transfusions were discontinued prior to spike:	
Average # days:	-5.9
Total included:	10

**14/34 subjects never returned to normal platelet post transplant and 19/34 did return to normal with spike observed

**4 subjects did not receive any transfusions during treatment

**5 subjects were transfusion dependent and platelet count never reached the normal range post transplant

**9 subjects had their transfusions discontinued but platelet count never reached the normal range post transplant

****43 subjects identified for the study but 9 samples removed due to either lack of sufficient data or no transplant was received****

Table 4: Sample Data Statistically Analyzed in Excel.

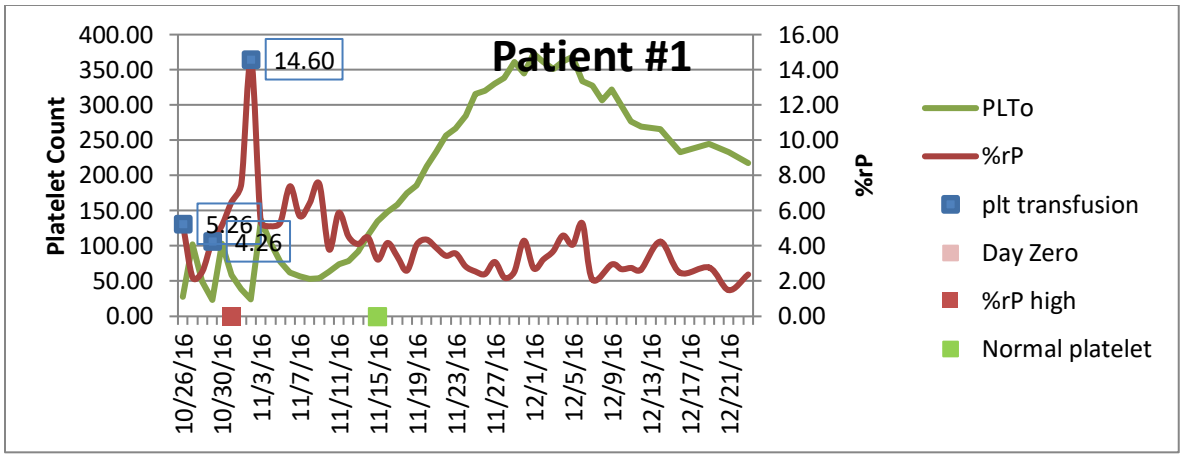
ID	Diagnosis	DOB	Age in Years	Age in Days	Gender	Transplant Date	Day %rP high	Day normal PLT	Day of last transfusion	Number of Days from TX to high %rP	Number of Days from high %rP to normal PLT	Number of Days from high %rP to last plt transfusion	High %rP value	Normal PLT value
1	dyskeratosis congenita	07/12/04	12.28	4483	Female	10/20/16	10/31/16	11/15/16	11/02/16	11	15	2	6.50	135.00
2	HLH	06/28/16	0.35	126	Female	11/01/16	11/07/16	12/05/16	11/11/16	6	28	4	11.90	179.00
3	ALL	01/30/09	7.74	2824	Female	10/24/16	11/02/16	11/10/16	10/29/16	9	8	-4	10.10	159.00
4	SCID	04/19/16	0.56	204	Male	11/09/16	11/20/16	12/02/16	11/16/16	11	12	-4	13.60	161.00
5	Fanconi Anemia	10/03/11	5.08	1856	Male	11/01/16	11/05/16	11/26/16	11/10/16	4	21	5	12.80	132.00
6	Immune dysregulation	03/16/16	0.57	208	Male	12/16/16	01/03/17	NA	02/02/17	18	NA	30	8.11	NA
7	neuroblastoma	11/07/12	3.93	1434	Male	12/06/16	12/14/16	NA	12/15/16	8	NA	1	6.76	NA
8	Ewing Sarcoma	06/16/04	12.41	4530	Male	11/10/16	11/15/16	NA	11/23/16	5	NA	8	6.25	NA
9	Congenital porphyria	12/11/11	4.91	1793	Male	11/07/16	11/18/16	11/23/16	11/14/16	11	5	-4	9.92	136.00
10	Ovarian PNET	05/25/03	13.47	4915	Female	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	pineoblastoma	10/14/10	6.02	2196	Female	10/14/16	10/30/16	10/31/16	11/07/16	16	1	8	6.60	141.00
12	Ewing Sarcoma	01/13/97	19.85	7245	Female	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	Fanconi Anemia	07/22/03	13.38	4885	Male	12/05/16	12/14/22	01/09/17	12/21/16	9	26	7	5.80	134.00
14a	AML	04/11/10	6.70	2447	Female	12/22/16	01/01/17	NA	01/29/17	10	NA	NA	10.80	NA
14b	AML	04/11/10	6.77	2471	Female	01/15/17	01/18/17	NA	01/29/17	3	NA	NA	9.64	NA
15	ALL	06/30/15	1.48	539	Female	12/20/16	12/24/16	NA	01/12/17	4	NA	19	9.46	NA
16	Wiskott Aldrich	12/05/15	1.04	381	Male	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	CD40 ligand def.	03/11/16	0.86	314	Male	01/19/17	01/31/17	02/07/17	NA	12	7	NA	11.80	149.00
18	SCID	08/19/16	0.40	146	Male	01/12/17	01/22/17	02/09/17	02/08/17	10	18	17	16.40	164.00
19a	Meduloblastoma	11/03/14	2.20	802	Female	01/23/16	02/09/17	NA	NA	17	NA	NA	8.51	NA
19b	Meduloblastoma	11/03/14	2.34	854	Female	03/06/17	03/22/17	NA	NA	16	NA	NA	8.89	NA
19c	Meduloblastoma	11/03/14	2.42	882	Female	04/03/17	04/15/17	NA	NA	12	NA	NA	11.30	NA
20	Fanconi Anemia	09/02/10	6.41	2339	Female	01/27/17	02/06/17	02/15/17	02/01/17	10	9	-5	11.40	136.00
21	Fanconi Anemia	08/28/10	6.43	2348	Female	01/31/17	02/09/17	02/11/17	02/08/17	9	2	-1	18.30	136.00
22	Aplastic Anemia	07/14/03	13.56	4949	Female	01/30/17	02/04/17	NA	02/04/17	5	NA	0	12.60	NA
23	Ewing Sarcoma	07/07/09	7.55	2754	Male	01/20/17	01/29/17	NA	02/03/17	9	NA	5	8.70	NA
24	Fanconi Anemia	12/05/07	9.16	3344	Female	01/30/17	02/08/17	NA	02/16/17	9	NA	8	8.82	NA
25	ALL	11/16/10	6.31	2302	Female	03/06/17	03/09/17	NA	03/31/17	3	NA	22	11.00	NA
26	AML	10/02/98	18.37	6704	Male	02/08/17	02/16/17	02/23/17	NA	8	7	NA	6.67	161.00
27	B cell Lymphoma	06/18/91	25.70	9382	Male	NA	NA	NA	NA	NA	NA	NA	NA	NA
28	Idiopathic aplastic anem	09/13/12	4.47	1630	Female	03/01/17	03/13/17	NA	03/11/17	12	NA	-2	11.20	NA
29	Osteopetrosis	04/12/16	0.81	295	Female	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	AML	11/21/06	10.22	3732	Female	02/08/17	02/21/17	NA	02/25/17	13	NA	4	10.00	NA
31a	ATRT	12/14/15	1.16	424	Female	02/10/17	02/24/17	03/03/17	02/10/17	14	7	-14	8.21	142.00
31b	ATRT	12/14/15	1.16	424	Female	03/20/17	04/05/17	04/14/17	03/20/17	16	9	-16	11.40	171.00
31c	ATRT	12/14/15	1.16	424	Female	04/17/17	05/05/17	05/07/17	04/30/17	18	2	-5	12.00	160.00
32	HLH	09/04/15	1.57	572	Male	03/29/17	04/03/17	04/25/17	04/07/17	5	22	4	10.50	155.00
33	CD40 ligand deficiency	08/18/04	12.58	4593	Male	NA	NA	NA	NA	NA	NA	NA	NA	NA
34	ALL T cell	04/28/03	13.83	5049	Male	02/22/17	03/04/17	03/08/17	03/27/17	10	4	23	10.90	155.00
35	B cell lymphoma	06/08/91	25.88	9447	Male	04/19/17	04/23/17	NA	05/02/17	4	NA	9	11.30	NA
36	HLH	11/29/15	1.32	481	Male	03/24/17	04/02/17	04/10/17	03/29/17	9	8	-4	13.80	147.00
37	X-linked IPEX	10/13/16	0.54	196	Female	04/27/17	05/07/17	NA	NA	10	NA	NA	10.50	NA
38	Fanconi Anemia	05/16/11	5.98	2184	Female	NA	NA	NA	NA	NA	NA	NA	NA	NA
39	CVID	05/16/01	15.88	5795	Male	03/28/17	03/30/17	NA	04/28/17	2	NA	NA	7.69	NA
40	Fanconi Anemia	01/12/10	7.23	2640	Female	04/05/17	04/13/17	04/19/17	04/13/17	8	6	0	11.30	137.00
41	CD40 ligand deficiency	07/29/16	0.76	279	Male	04/27/17	05/01/17	05/01/17	NA	4	0	NA	9.66	209.00
42	Beta Thalessemia Major	01/01/12	5.34	1949	Female	NA	NA	NA	NA	NA	NA	NA	NA	NA
43	Pre- B cell ALL	10/05/09	7.60	2773	Female	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 5: CSV File Imported into R Studio Software for Statistical Analysis and Graphing.

APPENDIX D: INDIVIDUAL PATIENT DATA

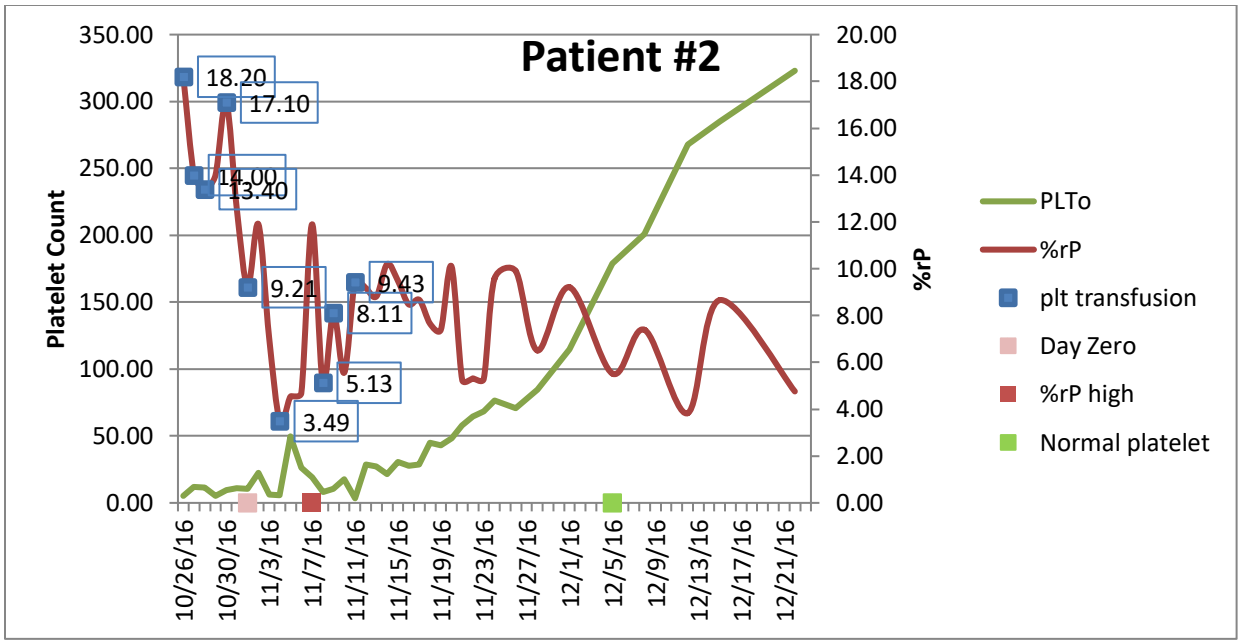
Study ID	Diagnosis	DOB	Transplant Date	Transplant Type	BM/SC/CB	Type
1	dyskeratosis congenita	07/12/04- 12 y F	10/20/2016	Allogeneic	BM	Partially matched
2	HLH	06/28/16- 3 m F	11/1/2016	Allogeneic	BM	Matched related
3	ALL	01/30/09- 7 y F	10/24/2016	Allogeneic	BM	Matched sibling
4	SCID-Cartilage Hair Hypoplasia	04/19/16- 6 m M	11/9/2016	Allogeneic	BM	Matched unrelated
5	Fanconi Anemia	10/03/11- 5 y M	11/1/2016	Allogeneic	PBSC	Matched unrelated
6	Immune dysregulation	03/16/16- 7 m M	10/10/2016 & 12/16/2016	Allo X 3	CD34 selected	Graft
7	neuroblastoma	11/07/12- 4 y F	10/11/2016 & 12/6/2016	Autologous	PBSC	
8	Ewing Sarcoma	06/16/04- 12 y M	11/10/2016	Autologous	PBSC	
9	Congenital erythropoetic porphyria	12/11/11- 4 y M	11/7/2016	Allogeneic	BM	Matched sibling
10	Ovarian PNET	05/25/03- 13 y F		Autologous	PBSC	Stem cell harvest CD34+
11	pineoblastoma	10/14/10- 6 y F	10/14/2016	Autologous	PBSC	
12	Ewing Sarcoma	01/13/97- 19 y F	12/22/2016	Autologous	HSCT	Stem cell harvest CD34+
13	Fanconi Anemia	7/22/03- 13 y M	12/5/2016	Allogeneic	BM	Matched sibling
14	AML	4/11/10- 6 y F	12/22 & 1/15	2 X CD34	Haplografts	Matched from dad
15	ALL	6/30/15- 17 m F	12/20/2016	Allogeneic	CB	Matched unrelated
16	Wiskott Aldrich	12/5/15- 12 m M	4/21/2017	Allogeneic	BM	Matched unrelated
17	CD40 ligand def.	3/11/16- 9 m M	1/19/2017	Allogeneic	BM	Matched unrelated
18	SCID-Cartilage Hair Hypoplasia	8/19/16- 4 m M	1/12/2017	Allogeneic	BM	Matched unrelated
19	Meduloblastoma	11/3/14- 2 y F	1/23 3/6 4/3	Autologous	PBSC	Stem cell harvest CD34+
20	Fanconi Anemia	9/2/10- 6 y F	1/27/2017	Allogeneic	BM	Matched sibling
21	Fanconi Anemia	8/28/10- 6 y F	1/31/2017	Allogeneic	PBSC	Matched unrelated
22	Idiopathic Aplastic Anemia	7/14/03- 13 y F	1/30/2017	Allogeneic	BM	Matched sibling
23	Ewing Sarcoma	7/7/09- 7 y M	1/20/2017	Autologous	PBSC	Stem cell harvest CD34+
24	Fanconi Anemia	12/5/07- 9 y F	1/30/2017	Allogeneic	PBSC	Matched unrelated
25	ALL	11/16/10- 6 y F	3/6/2017	Allogeneic	CB	Matched unrelated
26	AML	10/2/98- 18 y M	2/8/2017	Allogeneic	PBSC	Partially matched unrelated
27	High grade B cell Lymphoma	6/18/91- 25 y M	2/23/2017	Allogeneic	BM	Matched unrelated
28	Idiopathic aplastic anemia	9/13/12- 4 y F	3/1/2017	Allogeneic	BM	Matched unrelated
29	Osteopetrosis	4/12/16- 9 m F	2/1/2017	Allogeneic	BM	Matched unrelated
30	AML- t(8;21)	11/21/2006- 10 y F	2/8/2017	Allogeneic	BM	Matched unrelated
31	ATRT-brain tumor	12/14/2015- 18 m F	2/10 3/20 4/17	Autologous	PBSC	Stem cell harvest CD34+
32	HLH	9/4/2015- 22 m M	3/29/2017	Allogeneic	PBSC	Matched unrelated
33	CD40 ligand deficiency	8/18/2004- 12 y M	3/16/2017	Allogeneic	PBSC	Matched unrelated
34	ALL T cell	4/28/2003- 13 y M	2/22/2017	Allogeneic	BM	Matched unrelated
35	B cell lymphoma	6/08/1991- 25 y M	4/19/2017	Allogeneic	BM	Matched unrelated
36	HLH	11/29/2015- 15 m M	3/24/2017	Allogeneic	BM	Matched unrelated
37	X-linked IPEX	10/13/2016- 5 m M	4/27/2017	Allogeneic	BM	Matched unrelated
38	Fanconi Anemia	5/16/2011- 5 y F	5/8/2017	Allogeneic	PBSC	Matched unrelated
39	CVID- ADA2 deficiency	5/16/2001- 15 y M	3/28/2017	Allogeneic	PBSC	Matched unrelated
40	Fanconi Anemia	1/12/10 7 y F	4/5/2017	Allogeneic	BM	Matched unrelated
41	CD40 ligand deficiency	7/29/16- 9 m M	5/4/2017	Allogeneic	BM	Matched sibling
42	Beta Thalessemia Major	1/1/12- 5 y F	5/3/2017	Allogeneic	BM & CB	Matched sibling
43	Pre- B cell ALL	10/5/09- 7 y F	5/9/2017	Allogeneic	CB	Matched unrelated

Table 6: List of Study Subjects, Transplant Date, and Transplant Type.



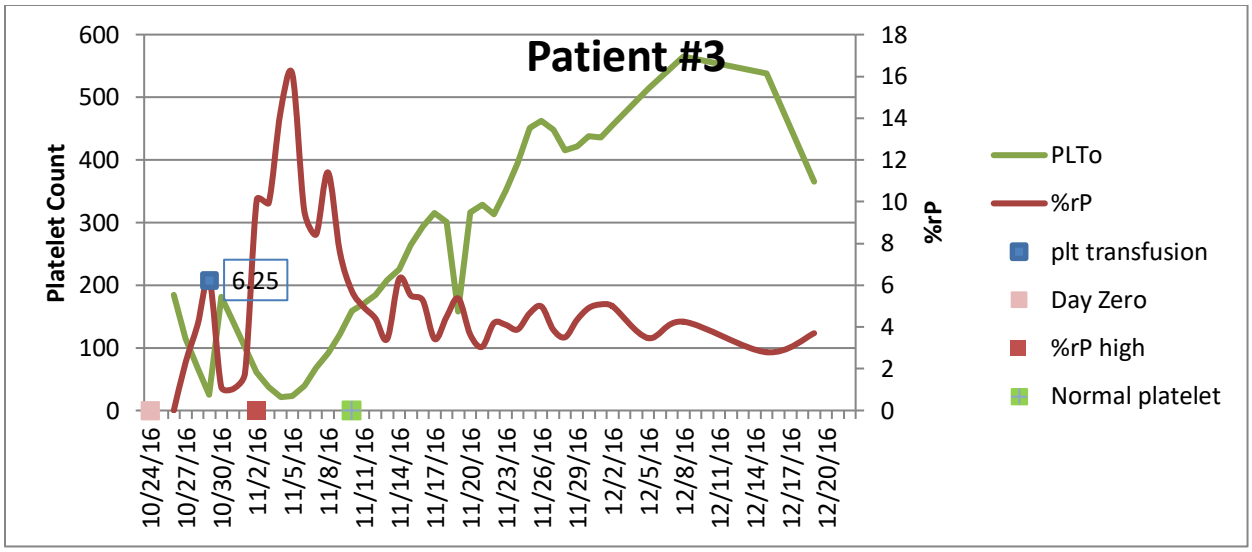
Day zero				10/20/2016
Day %rP high				10/31/2016
Day normal platelet count				11/15/2016
Day of last plt transfusion				11/2/2016
# Days from TX to high %rP				11
# Days from high %rP to normal platelet				15
# Days from high %rP to last platelet transfusion				2

Figure 17: Patient #1 age 12 years. Day Zero indicates day of transplant (TX). Spike in %rP post-transplant value of 6.5% (0.33-5.22%). %rP is identified post-transplant when %rP value exceeds the normal limit of patient's reference range. All accompanying data can be viewed in Appendix C. Please see Table 2 for CCHMC reference ranges.



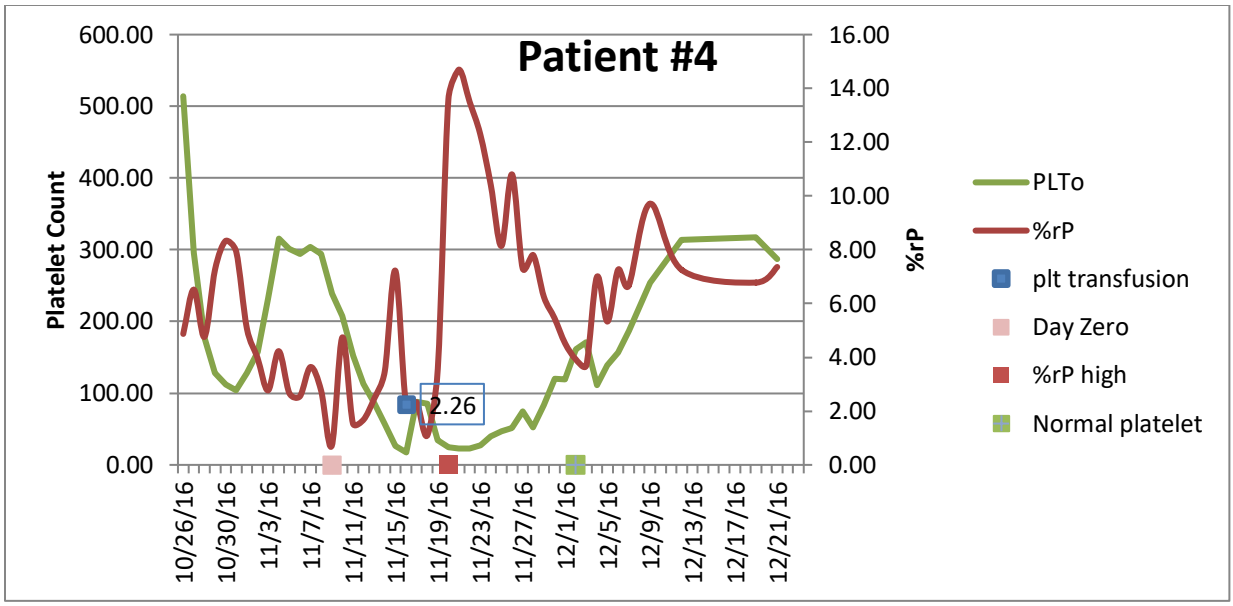
Day zero				11/1/2016
Day %rP high				11/7/2016
Day normal platelet count				12/5/2016
Day of last plt transfusion				11/11/2016
# Days from TX to high %rP				6
# Days from high %rP to normal platelet				28
# Days from high %rP to last platelet transfusion				4

Figure 18: Patient #2 age 4 months. Spike in %rP value of 11.9% (1.31-8.10%).



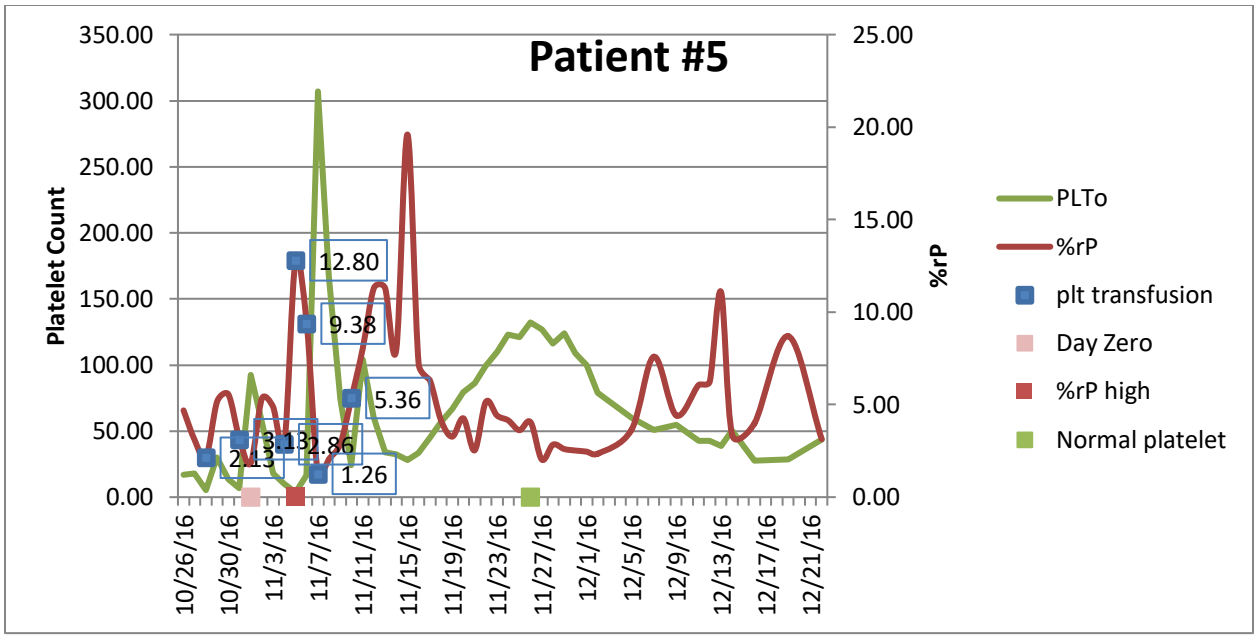
Day zero				10/24/2016
Day %rP high				11/2/2016
Day normal platelet count				11/10/2016
Day of last plt transfusion				10/29/2016
# Days from TX to high %rP				9
# Days from high %rP to normal platelet				8
# Days from high %rP to last platelet transfusion				-4

Figure 19: Patient #3 age 7 years. Spike in %rP value of 10.10% (0.26-7.33%).



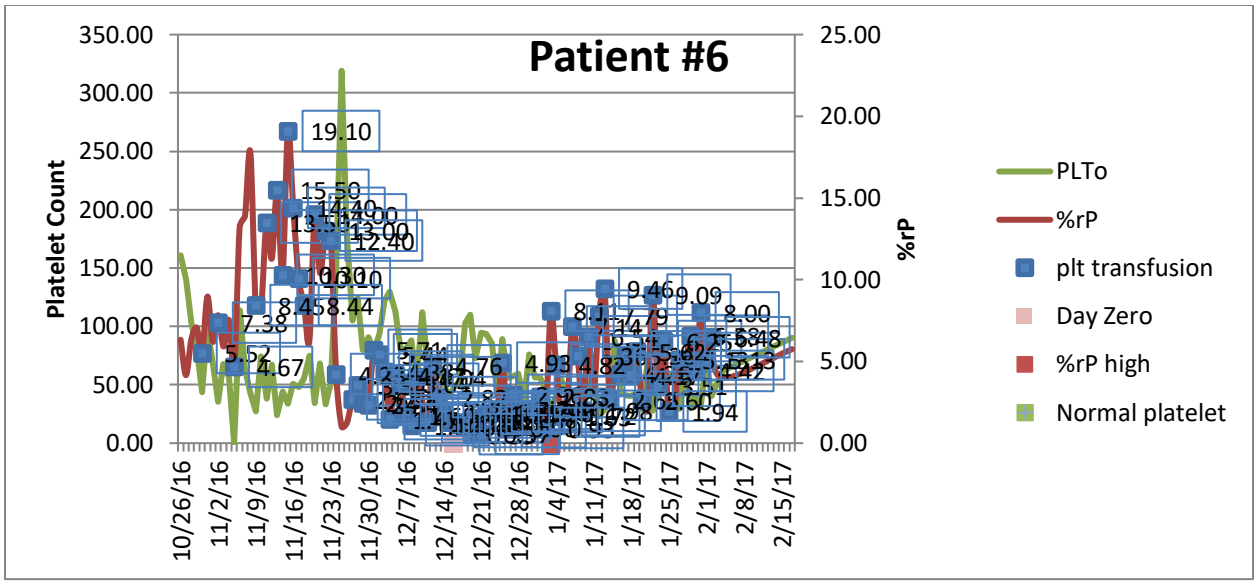
Day zero				11/9/2016
Day %rP high				11/20/2016
Day normal platelet count				12/2/2016
Day of last plt transfusion				11/16/2016
# Days from TX to high %rP				11
# Days from high %rP to normal platelet				12
# Days from high %rP to last platelet transfusion				-4

Figure 20: Patient #4 age 6 months. Spike in %rP value of 13.60% (1.31-8.10%).



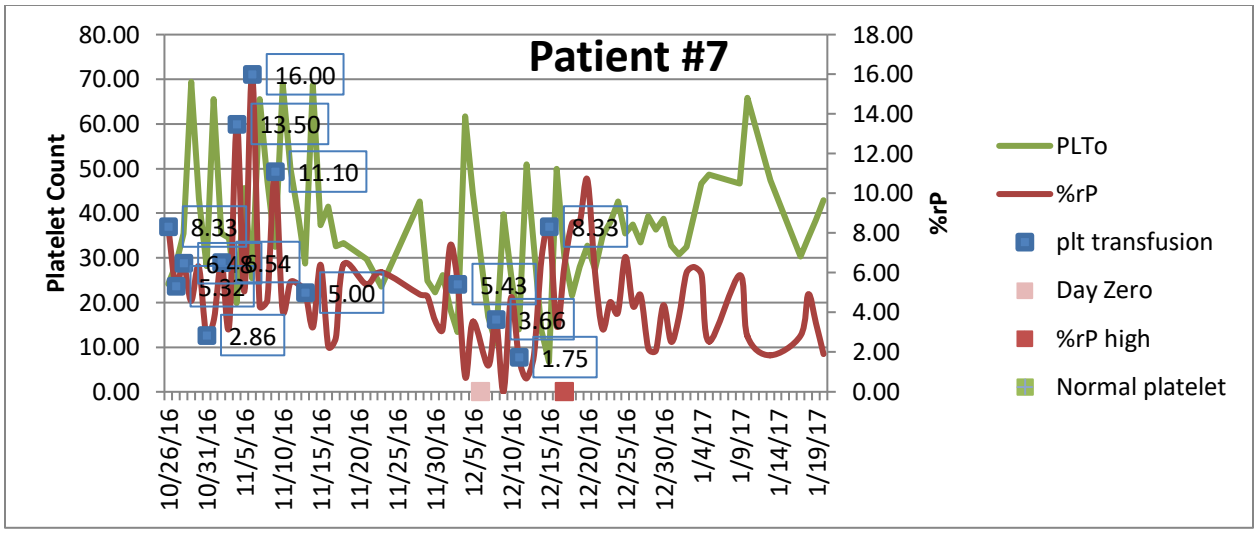
Day zero				11/1/2016
Day %rP high				11/5/2016
Day normal platelet count				11/26/2016
Day of last plt transfusion				11/10/2016
# Days from TX to high %rP				4
# Days from high %rP to normal platelet				21
# Days from high %rP to last platelet transfusion				5

Figure 21: Patient #5 age 5 years. Spike in %rP value of 12.80% (0.35-6.01%).



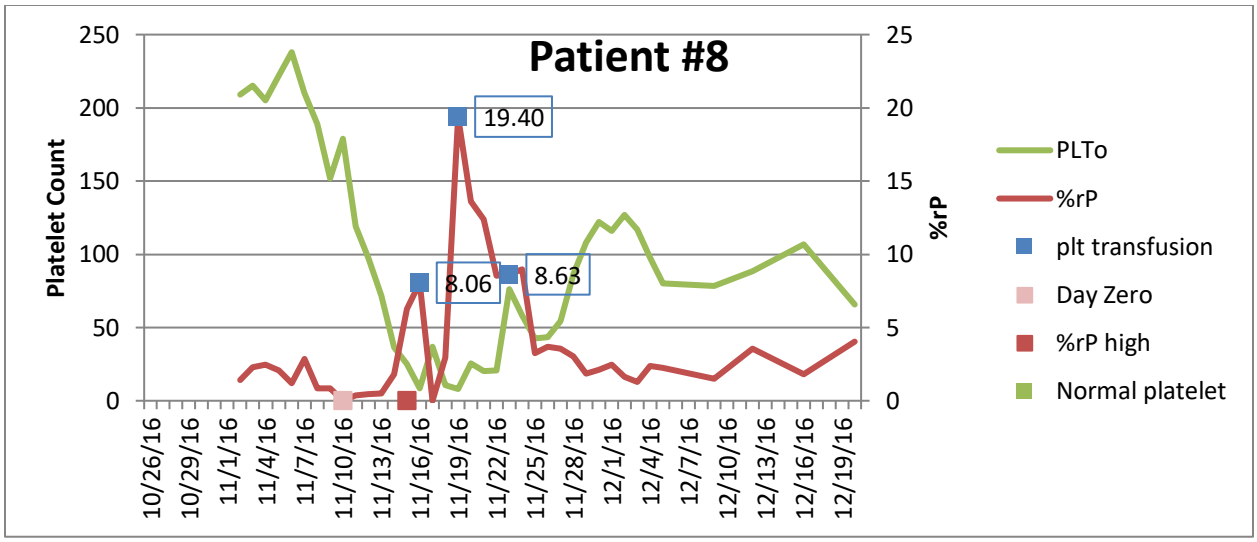
Day zero				12/16/2016
Day %rP high				1/3/2017
Day normal platelet count				NEVER
Day of last plt transfusion				NA
# Days from TX to high %rP				18
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				NA

Figure 22: Patient #6 age 6 months. Spike in %rP value of 8.11% (1.31-8.10%), however they were transfusion dependent pre- and post-transplant. The platelet count never returned to normal. This subject's data was excluded from the statistical analysis.



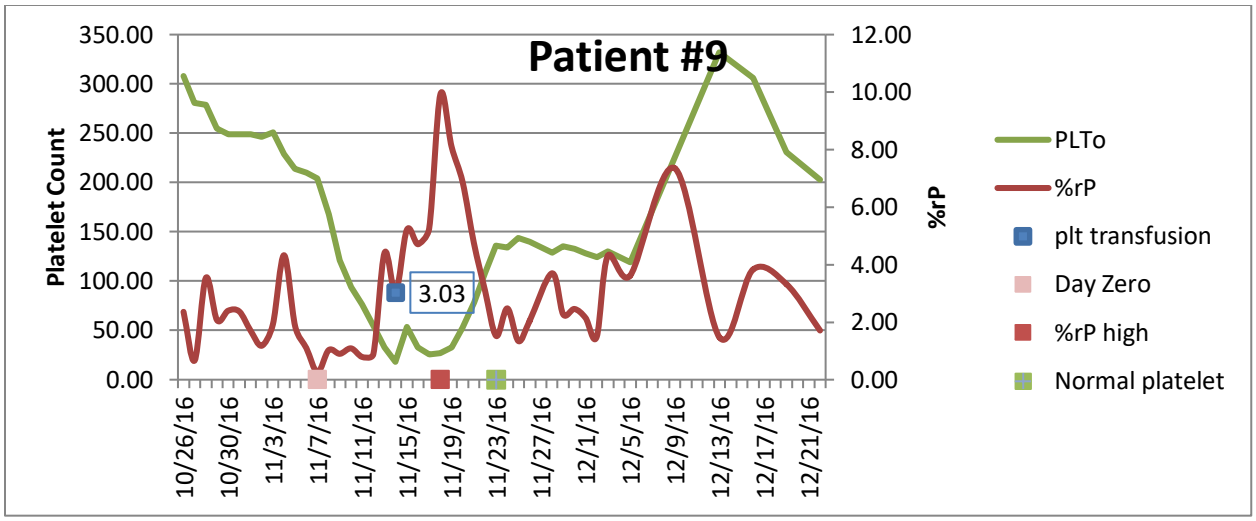
Day zero				12/6/2016
Day %rP high				12/14/2016
Day normal platelet count				NEVER
Day of last plt transfusion				12/15/2016
# Days from TX to high %rP				8
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				1

Figure 23: Patient #7 age 3 years. Spike in %rP value of 6.76% (0.35-6.01%). Platelet count never returned to normal, but patient was not transfusion dependent.



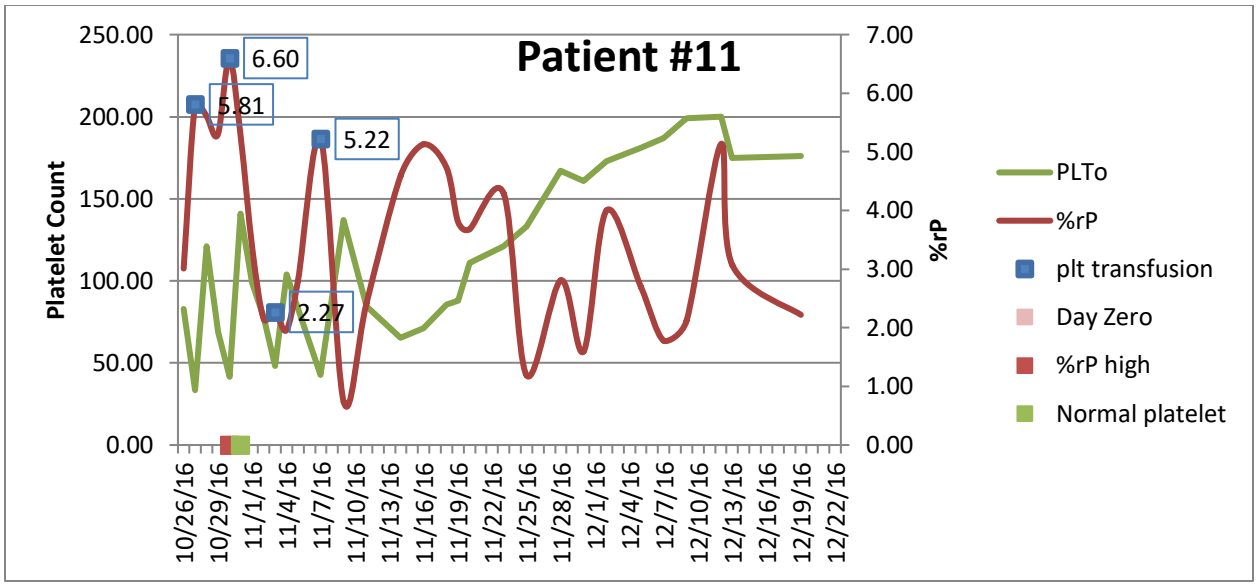
Day zero				11/10/2016
Day %rP high				11/15/2016
Day normal platelet count				NEVER
Day of last plt transfusion				11/23/2016
# Days from TX to high %rP				5
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				8

Figure 24: Patient #8 age 12 years. Spike in %rP value of 6.25% (0.33-5.22%). Platelet count did not return to normal, and patient was not transfusion dependent.



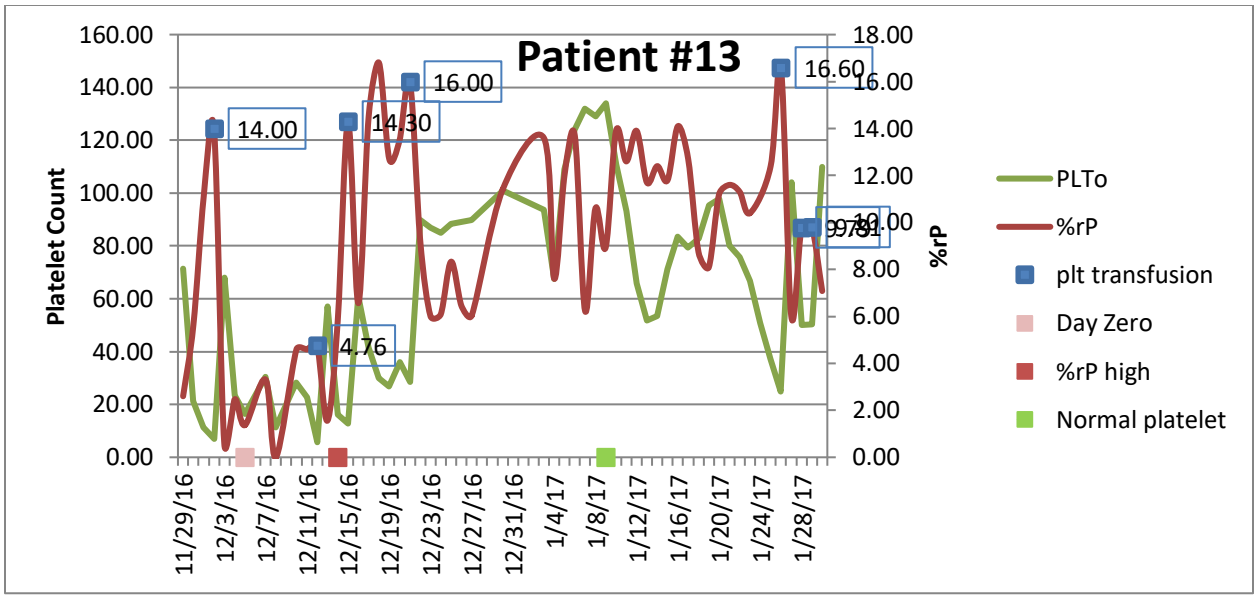
Day zero				11/7/2016
Day %rP high				11/18/2016
Day normal platelet count				11/23/2016
Day of last plt transfusion				11/14/2016
# Days from TX to high %rP				11
# Days from high %rP to normal platelet				5
# Days from high %rP to last platelet transfusion				-4

Figure 25: Patient #9 age 4 years. Spike in %rP value of 9.92% (0.35-6.01%).



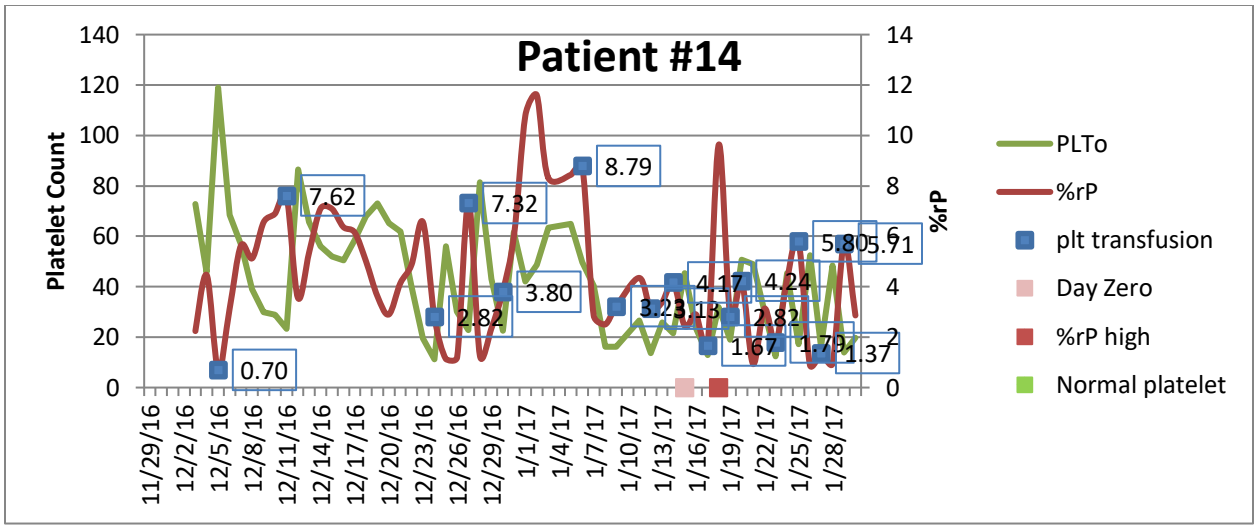
Day zero				10/14/2016
Day %rP high				10/30/2016
Day normal platelet count				10/31/2016
Day of last plt transfusion				11/7/2016
# Days from TX to high %rP				16
# Days from high %rP to normal platelet				1
# Days from high %rP to last platelet transfusion				8

Figure 26: Patient #11 age 6 years. Spike in %rP value of 6.60% (0.26-7.33%).



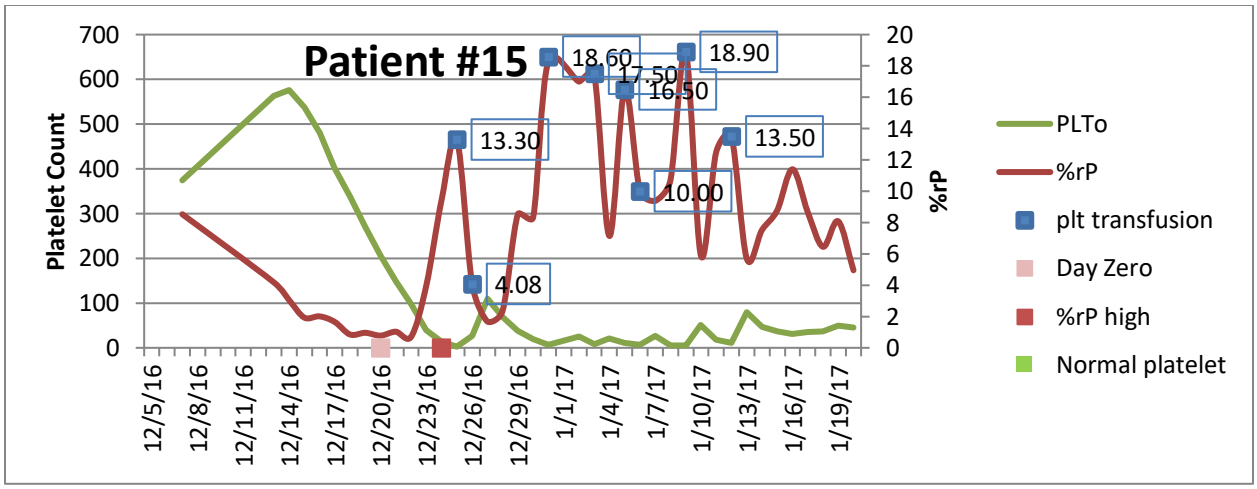
Day zero				12/5/2016
Day %rP high				12/14/2016
Day normal platelet count				1/9/2017
Day of last plt transfusion				12/21/2016
# Days from TX to high %rP				9
# Days from high %rP to normal platelet				26
# Days from high %rP to last platelet transfusion				7

Figure 27: Patient #13 age 13 years. Spike in %rP value of 5.80% (0.33-5.22%).



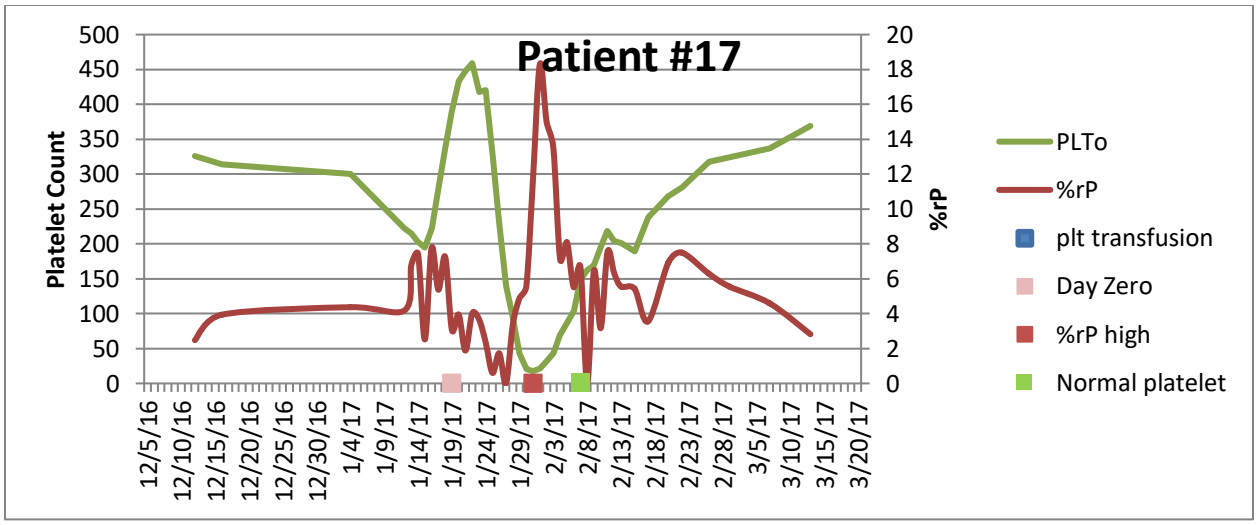
Day zero		**12/22/2016**		**1/15/2017**
Day %rP high		1/1/2017		1/18/2017
Day normal platelet count		NEVER		NEVER
Day of last plt transfusion		1/29/2017		1/29/2017
# Days from TX to high %rP		10		3
# Days from high %rP to normal platelet		NEVER		NEVER
# Days from high %rP to last platelet transfusion		NA		NA

Figure 28: Patient #14 age 6 years. This patient received two transplants and data was collected for both. Spike in %rP value of 10.80% (0.29-7.33%) for transplant #1 and spike in %rP value of 9.64% (0.29-7.33%) for transplant #2.
Patient was transfusion dependent with no return to normal platelet count.



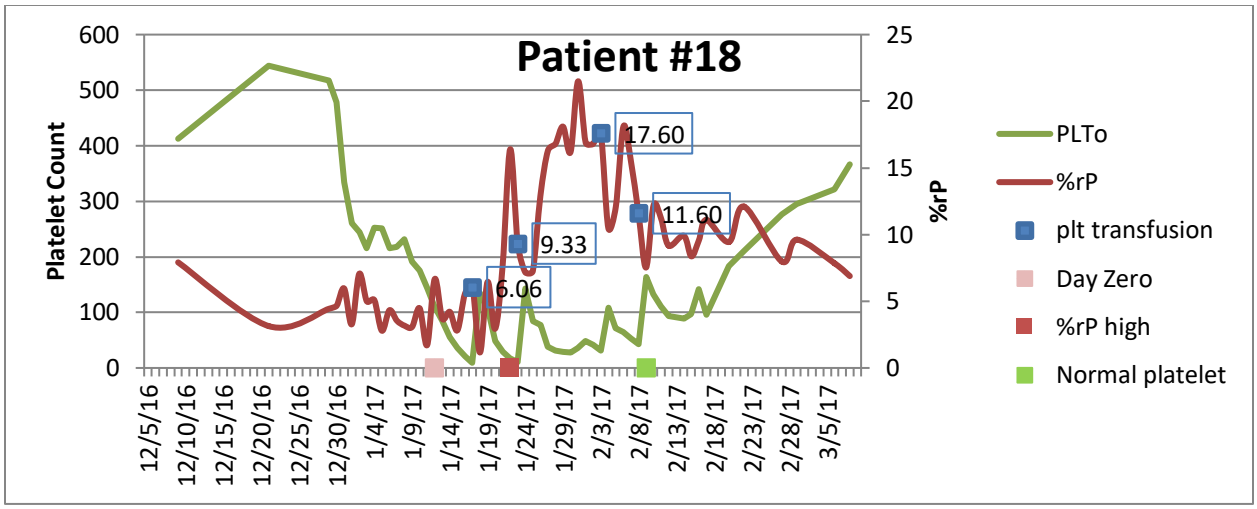
Day zero				12/20/2016
Day %rP high				12/24/2016
Day normal platelet count				NEVER
Day of last plt transfusion				1/12/2017
# Days from TX to high %rP				4
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				19

Figure 29: Patient #15 age 1 year. Spike in %rP value of 9.46% (0.95-8.93%). Patient was transfusion dependent with no return to normal platelet count.



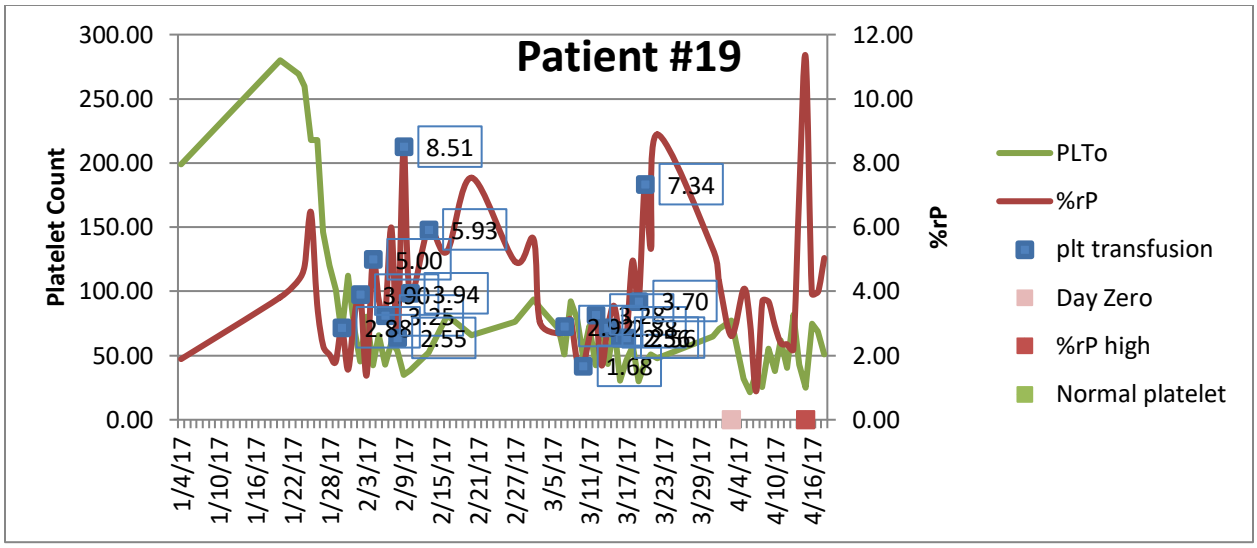
Day zero				1/19/2017
Day %rP high				1/31/2017
Day normal platelet count				2/7/2017
Day of last plt transfusion				NONE
# Days from TX to high %rP				12
# Days from high %rP to normal platelet				7
# Days from high %rP to last platelet transfusion				NA

Figure 30: Patient #17 age 10 months. Spike in %rP value of 11.80% (1.31-8.10%). No transfusions administered.



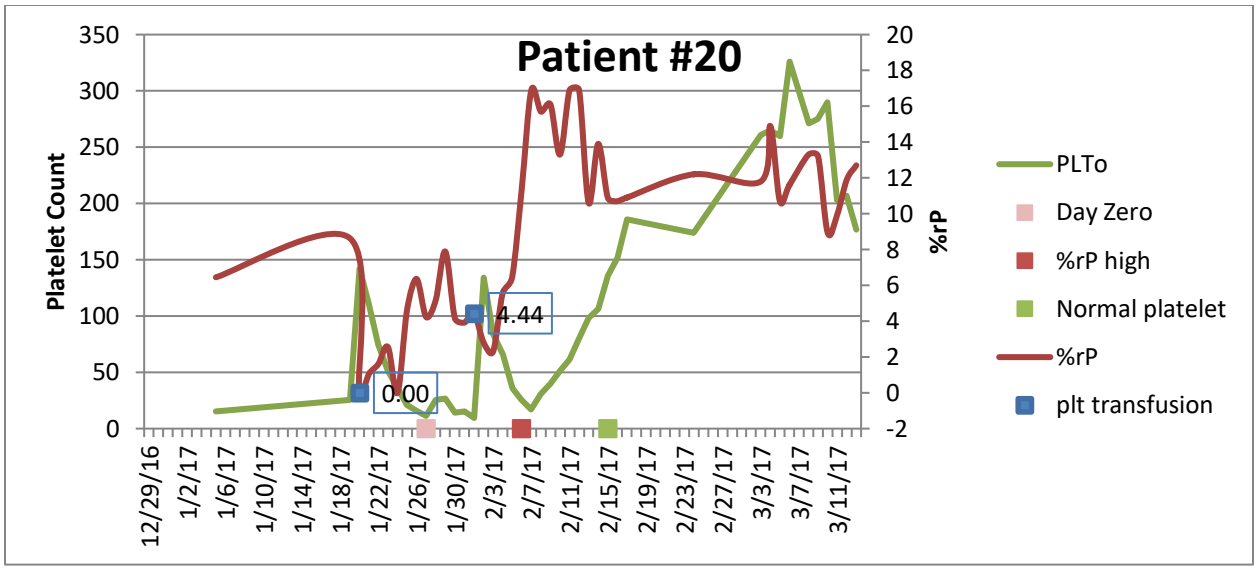
Day zero				1/12/2017
Day %rP high				1/22/2017
Day normal platelet count				2/9/2017
Day of last plt transfusion				2/8/2017
# Days from TX to high %rP				10
# Days from high %rP to normal platelet				18
# Days from high %rP to last platelet transfusion				17

Figure 31: Patient #18 age 4 months. Spike in %rP value of 16.40% (1.31-8.10%).



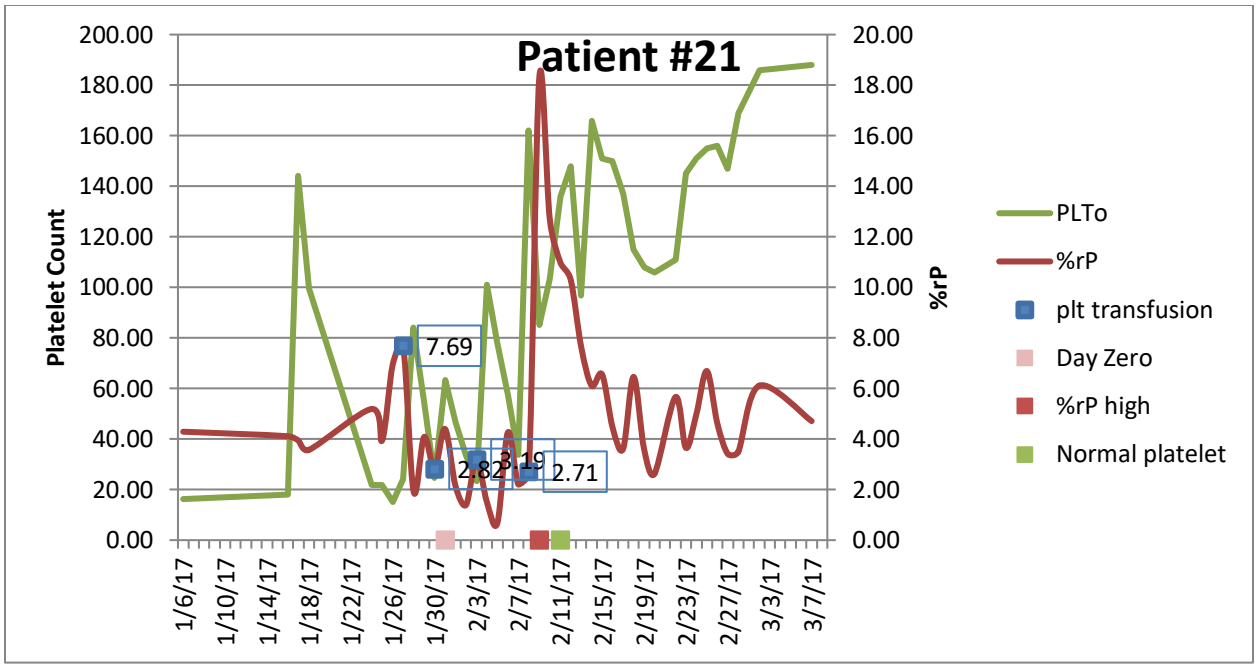
Day zero		1/23/2017	3/6/2017	4/3/2017
Day %rP high		2/9/2017	3/22/2017	4/15/2017
Day normal platelet count		NEVER	NEVER	NEVER
Day of last plt transfusion		NA	NA	3/20/2017
# Days from TX to high %rP		17	16	12
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				NA

Figure 32: Patient #19 age 2 years. This patient received three transplants and data was collected for all. Spike in %rP value of 8.51% (0.95-8.93%) for transplant #1, 8.89% (0.95-8.93%) for transplant #2, and 11.30% (0.95-8.93%) for transplant #3. Patient was not transfusion dependent but no return to normal platelet count.



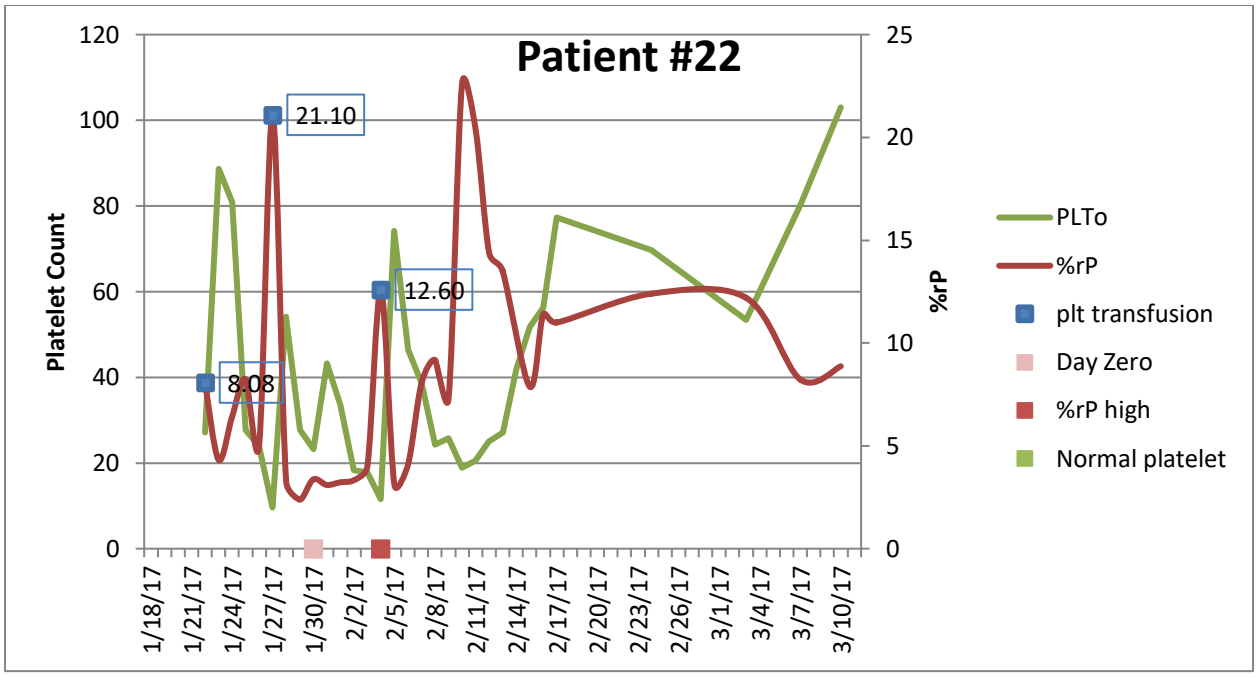
Day zero				1/27/2017
Day %rP high				2/6/2017
Day normal platelet count				2/15/2017
Day of last plt transfusion				2/1/2017
# Days from TX to high %rP				10
# Days from high %rP to normal platelet				9
# Days from high %rP to last platelet transfusion				-5

Figure 33: Patient #20 age 6 years. Spike in %rP value of 11.40% (0.26-7.33%).



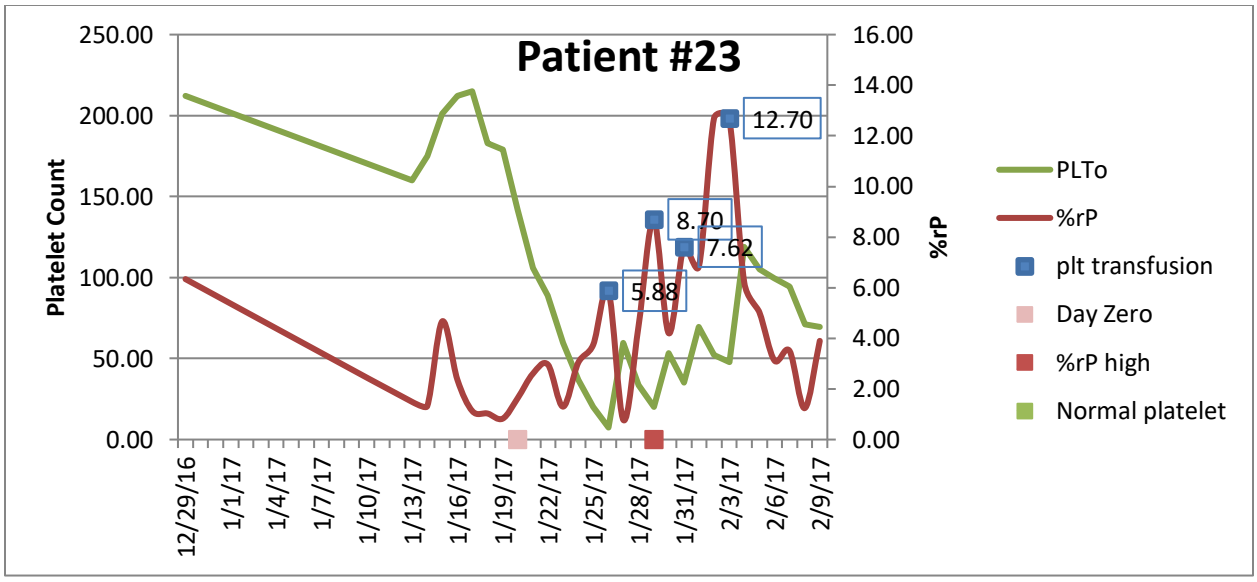
Day zero				1/31/2017
Day %rP high				2/9/2017
Day normal platelet count				2/11/2017
Day of last plt transfusion				2/8/2017
# Days from TX to high %rP				9
# Days from high %rP to normal platelet				2
# Days from high %rP to last platelet transfusion				-1

Figure 34: Patient #21 age 6 years. Spike in %rP value of 18.30% (0.26-7.33%).



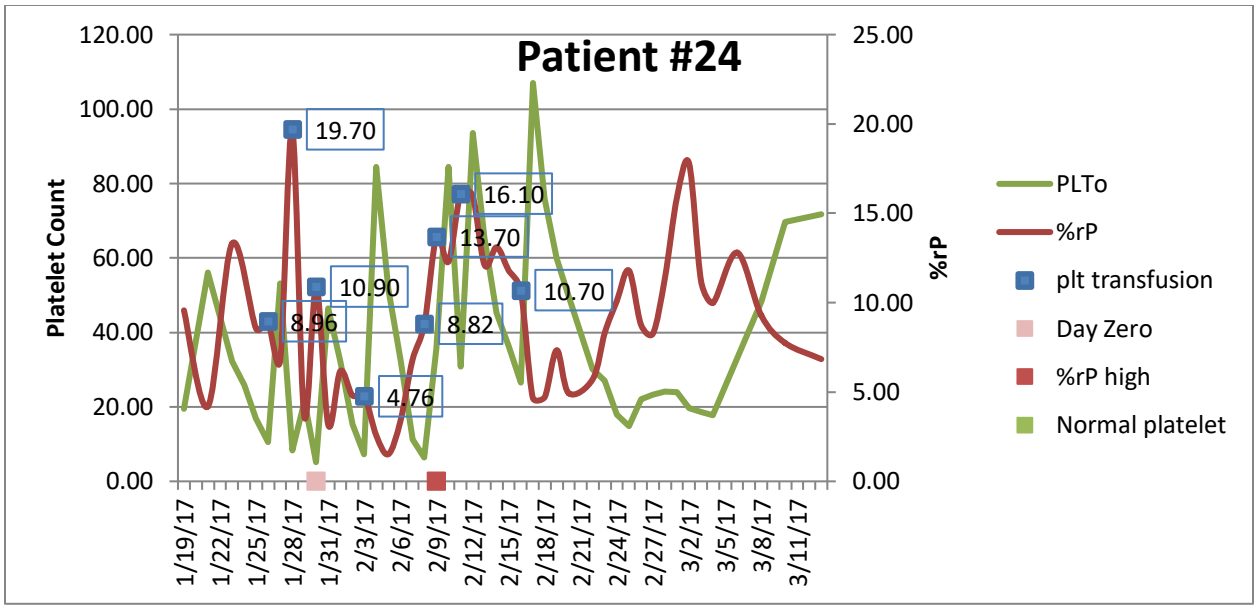
Day zero				1/30/2017
Day %rP high				2/4/2017
Day normal platelet count				NEVER
Day of last plt transfusion				2/4/2017
# Days from TX to high %rP				5
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				0

Figure 35: Patient #22 age 13 years. Spike in %rP value of 12.60% (0.33-5.22%). Patient was not transfusion dependent but no return to normal platelet count.



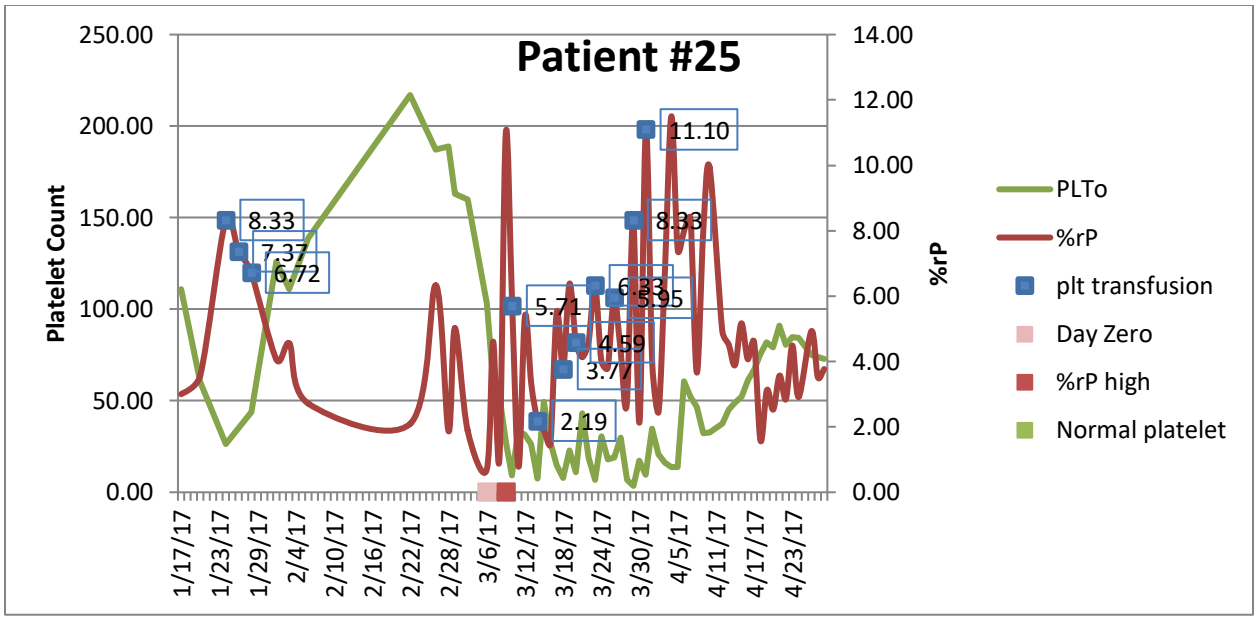
Day zero				1/20/2017
Day %rP high				1/29/2017
Day normal platelet count				NEVER
Day of last plt transfusion				2/3/2017
# Days from TX to high %rP				9
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				5

Figure 36: Patient #23 age 7 years. Spike in %rP value of 8.70% (0.26-7.33%). Patient was not transfusion dependent but no return to normal platelet count.



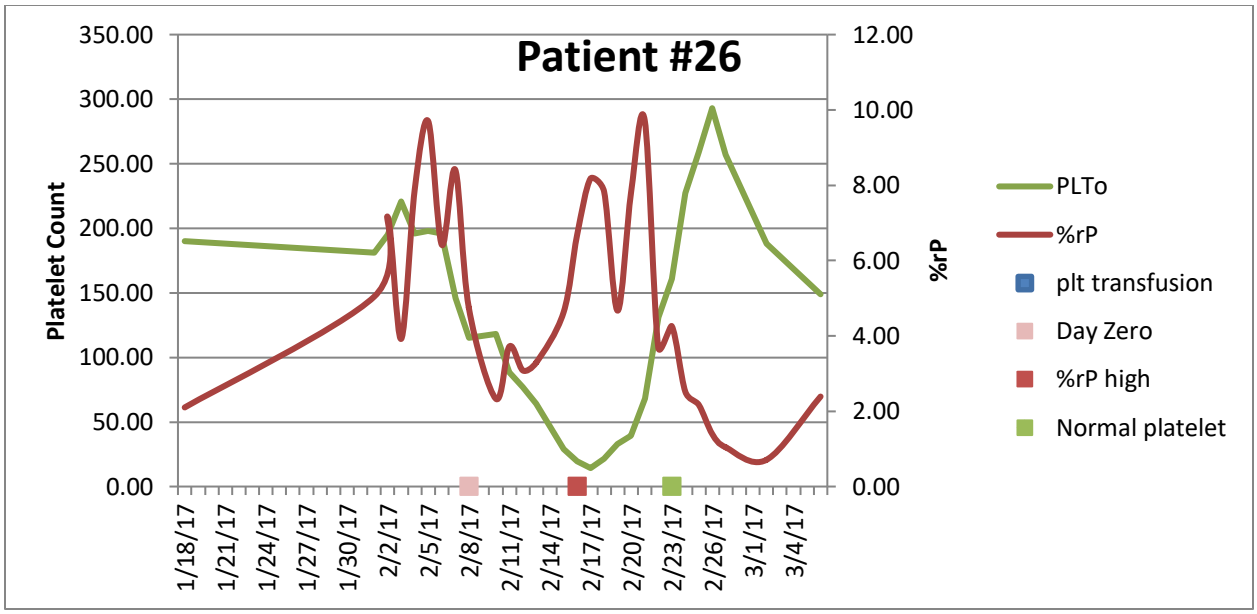
Day zero				1/30/2017
Day %rP high				2/8/2017
Day normal platelet count				NEVER
Day of last plt transfusion				2/16/2017
# Days from TX to high %rP				9
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				8

Figure 37: Patient #24 age 9 years. Spike in %rP value of 8.82% (0.26-7.33%). Patient was not transfusion dependent but no return to normal platelet count.



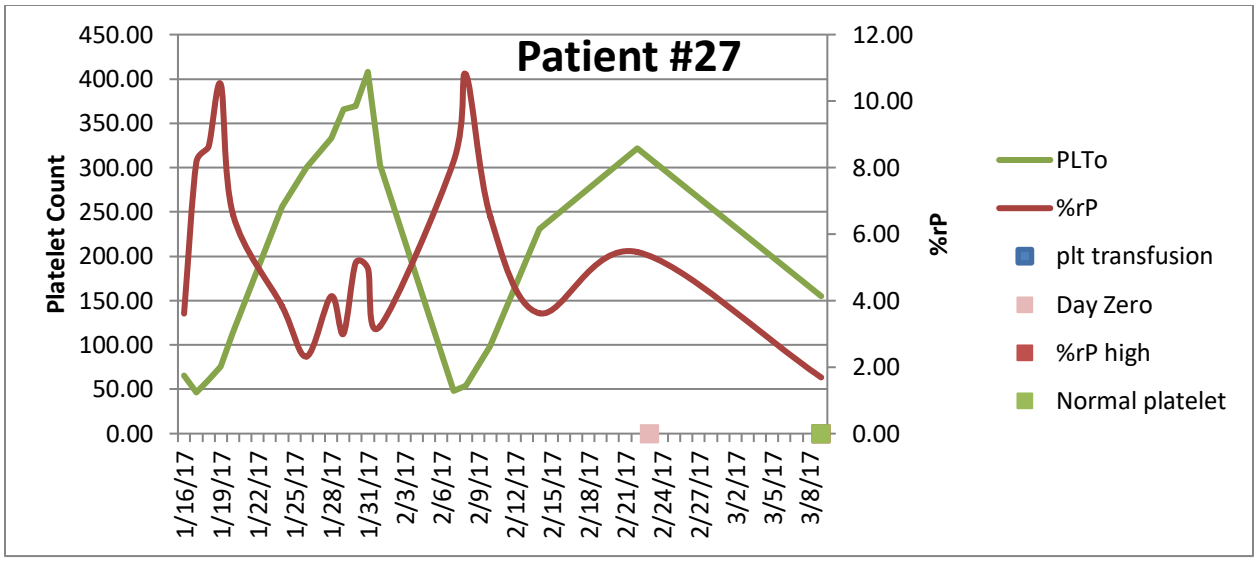
Day zero				3/6/2017
Day %rP high				3/9/2017
Day normal platelet count				NEVER
Day of last plt transfusion				3/31/2017
# Days from TX to high %rP				3
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				22

Figure 38: Patient #25 age 6 years. Spike in %rP value of 11.00% (0.26-7.33%). Patient was not transfusion dependent but no return to normal platelet count.



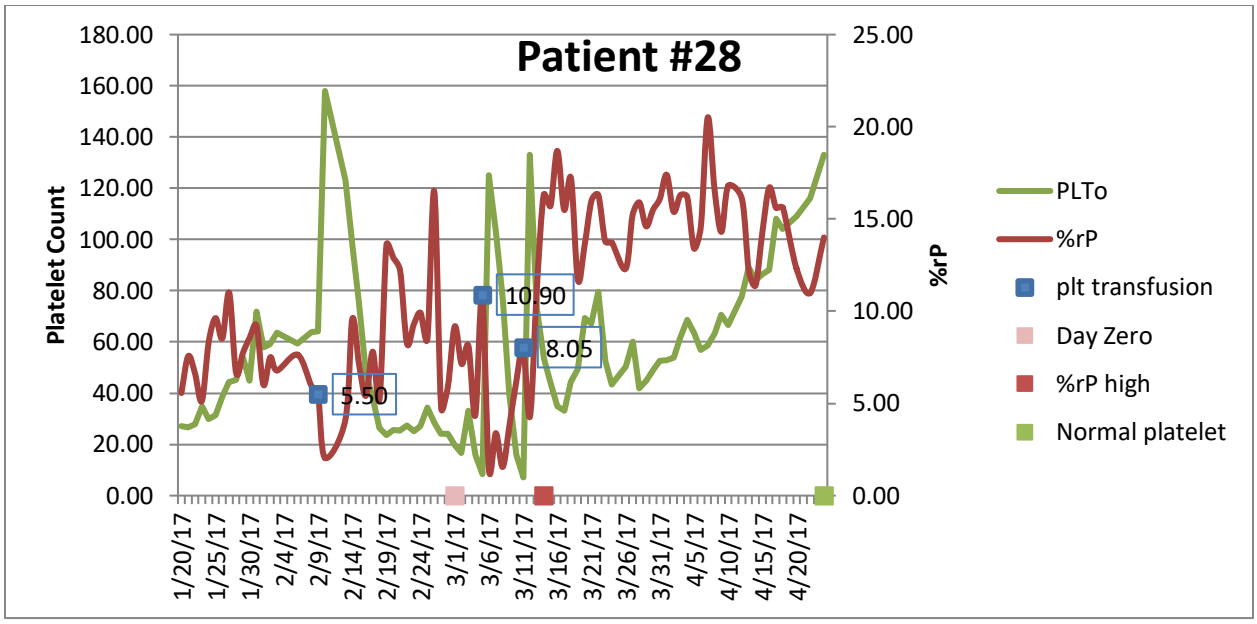
Day zero				2/8/2017
Day %rP high				2/16/2017
Day normal platelet count				2/23/2017
Day of last plt transfusion				NONE
# Days from TX to high %rP				8
# Days from high %rP to normal platelet				7
# Days from high %rP to last platelet transfusion				NA

Figure 39: Patient #26 age 18 years. Spike in %rP value of 6.67% (0.33-5.22%). No transfusions administered.



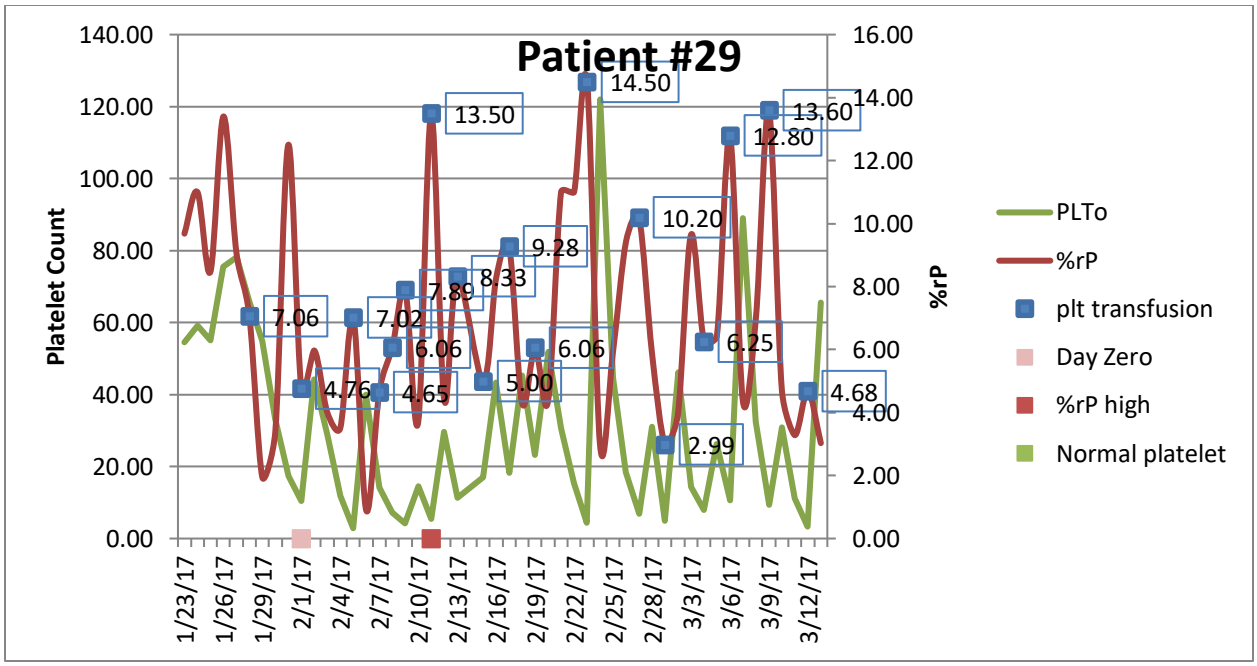
Day zero				2/23/2017
Day %rP high				NA
Day normal platelet count				3/9/2017
Day of last plt transfusion				NONE
# Days from TX to high %rP				NA
# Days from high %rP to normal platelet				NA
# Days from high %rP to last platelet transfusion				NA

Figure 40: Patient #27 age 25 years. Subject removed from study for lack of data.



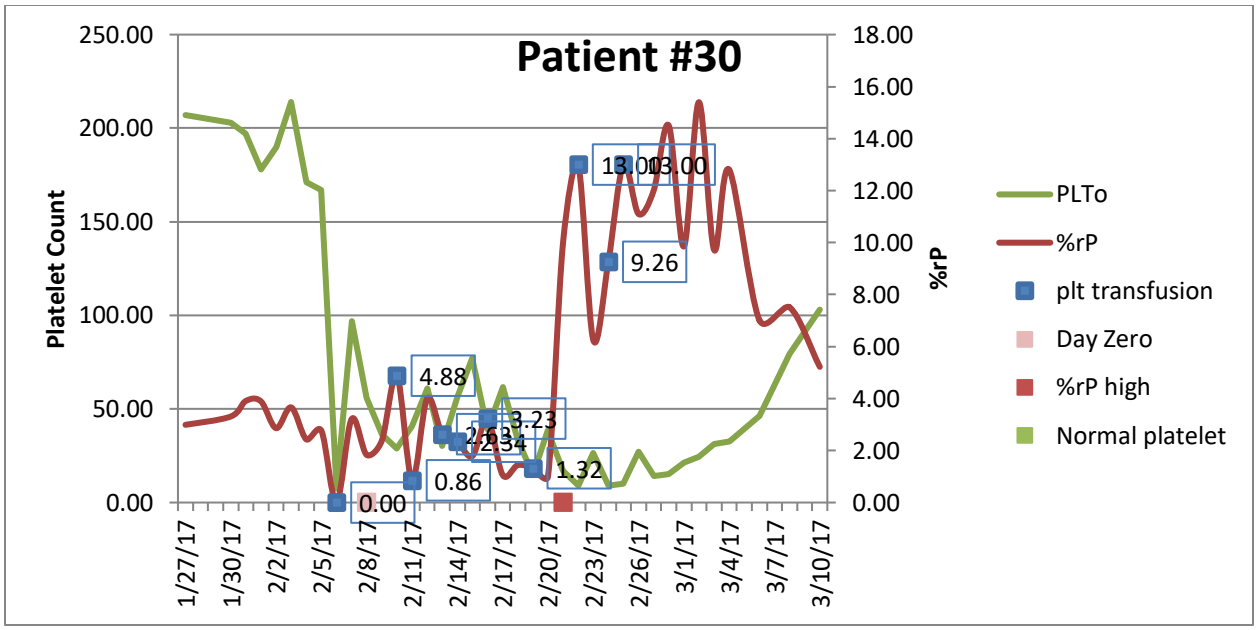
Day zero				3/1/2017
Day %rP high				3/13/2017
Day normal platelet count				NA
Day of last plt transfusion				3/11/2017
# Days from TX to high %rP				12
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				-2

Figure 41: Patient #28 age 4 years. Spike in %rP value of 11.20% (0.35-6.01%). Platelet count did not return to normal within data collection period.



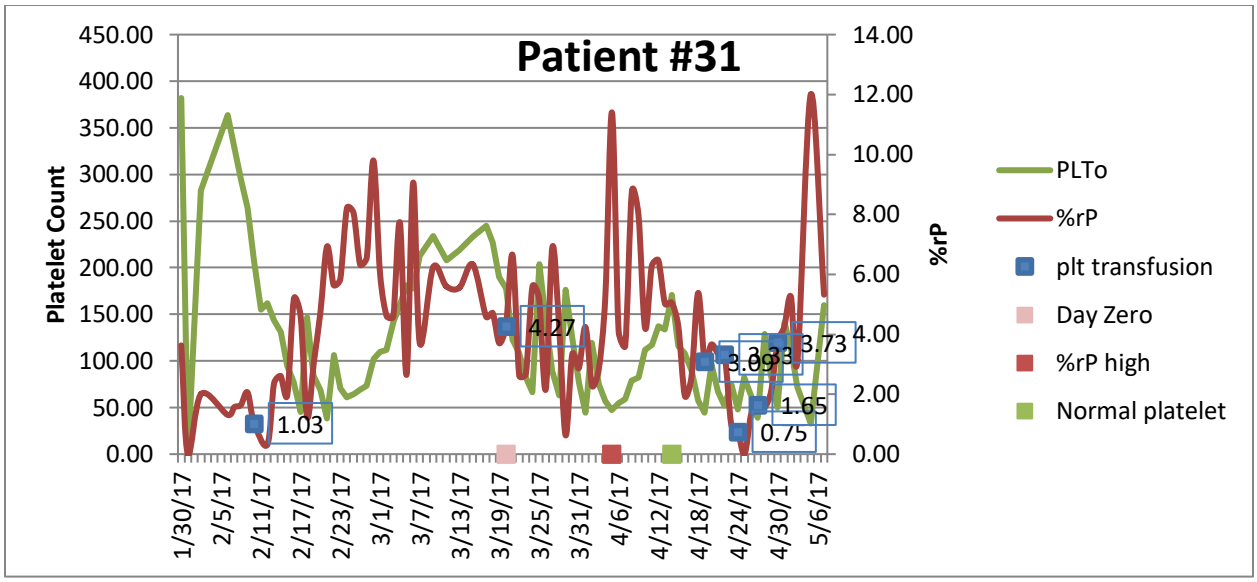
Day zero				2/1/2017
Day %rP high				2/11/2017
Day normal platelet count				NEVER
Day of last plt transfusion				NA
# Days from TX to high %rP				10
# Days from high %rP to normal platelet				NA
# Days from high %rP to last platelet transfusion				NA

Figure 42: Patient #29 age 9 months. Subject removed from study due to number of transfusions received.



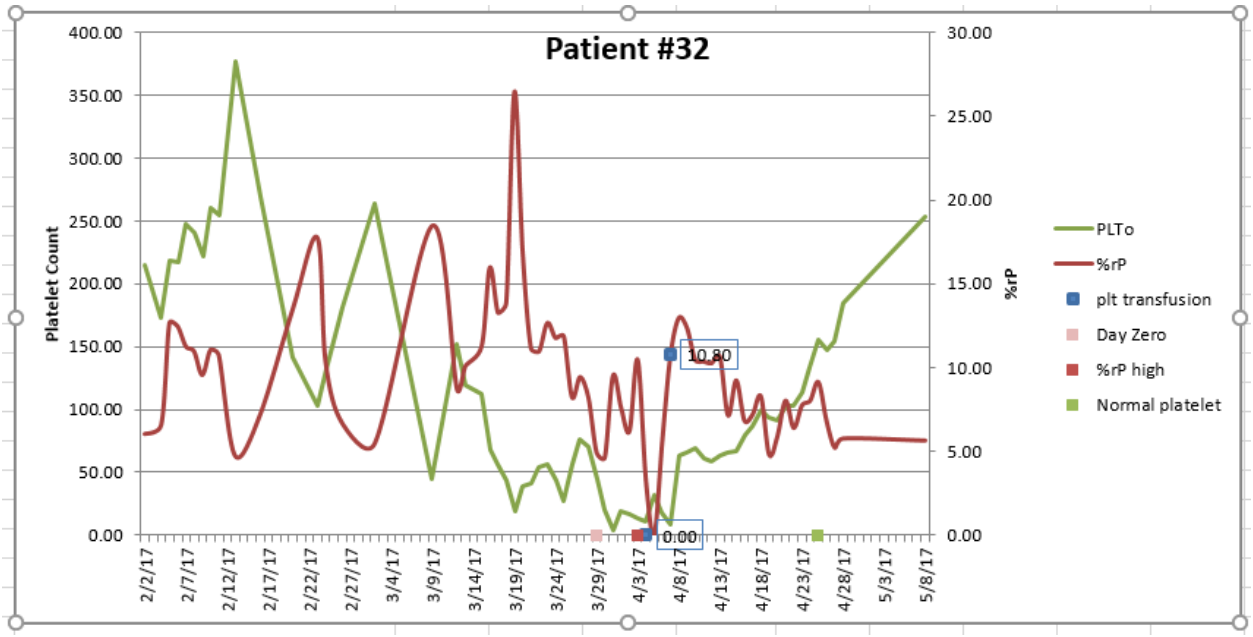
Day zero				2/8/2017
Day %rP high				2/21/2017
Day normal platelet count				NEVER
Day of last plt transfusion				2/25/2017
# Days from TX to high %rP				13
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				4

Figure 43: Patient #30 age 10 years. Spike in %rP value of 10.00% (0.26-7.33%). Patient was not transfusion dependent but no return to normal platelet count.



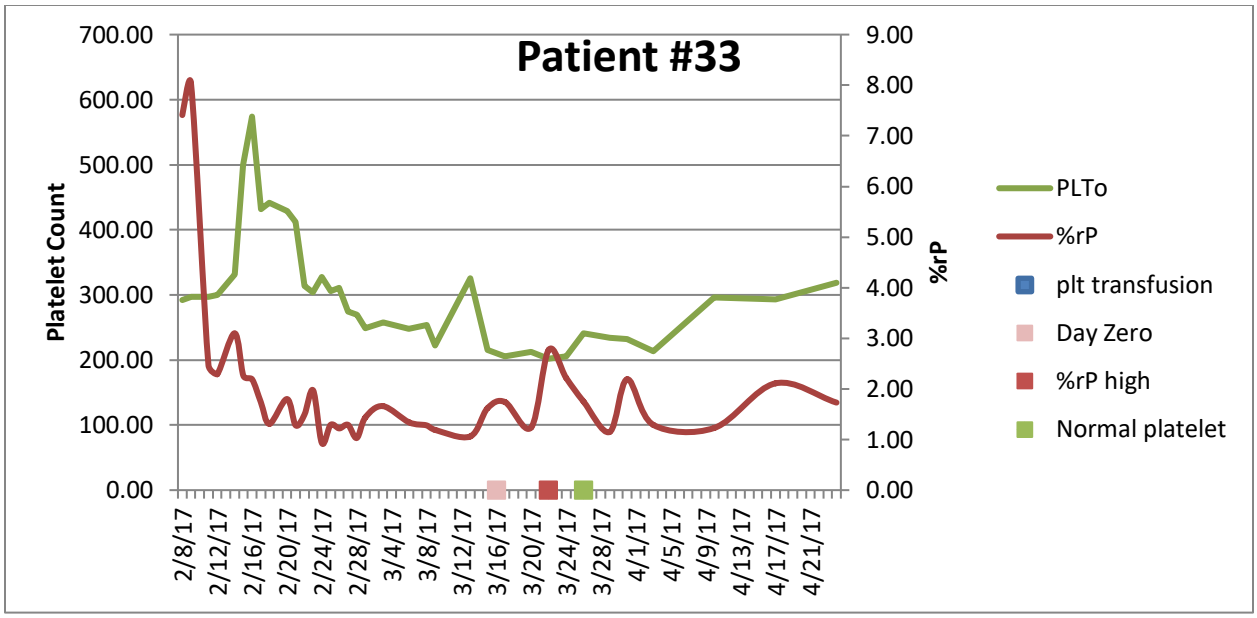
Day zero	2/10/2017	3/20/2017	4/17/2017
Day %rP high	2/24/2017	4/5/2017	5/5/2017
Day normal platelet count	3/3/2017	4/14/2017	5/7/2017
Day of last plt transfusion	2/10/2017	3/20/2017	4/30/2017
# Days from TX to high %rP	14	16	18
# Days from high %rp to normal platelet	7	9	2
# Days from high %rP to last platelet transfusion	-14	-16	-5

Figure 44: Patient #31 age 1 year. This patient received three transplants and data was collected for all. Spike in %rP value of 8.21% (0.95-8.93%) for transplant #1, 11.40% (0.95-8.93%) for transplant #2, and 12.00% (0.95-8.93%) for transplant #3.



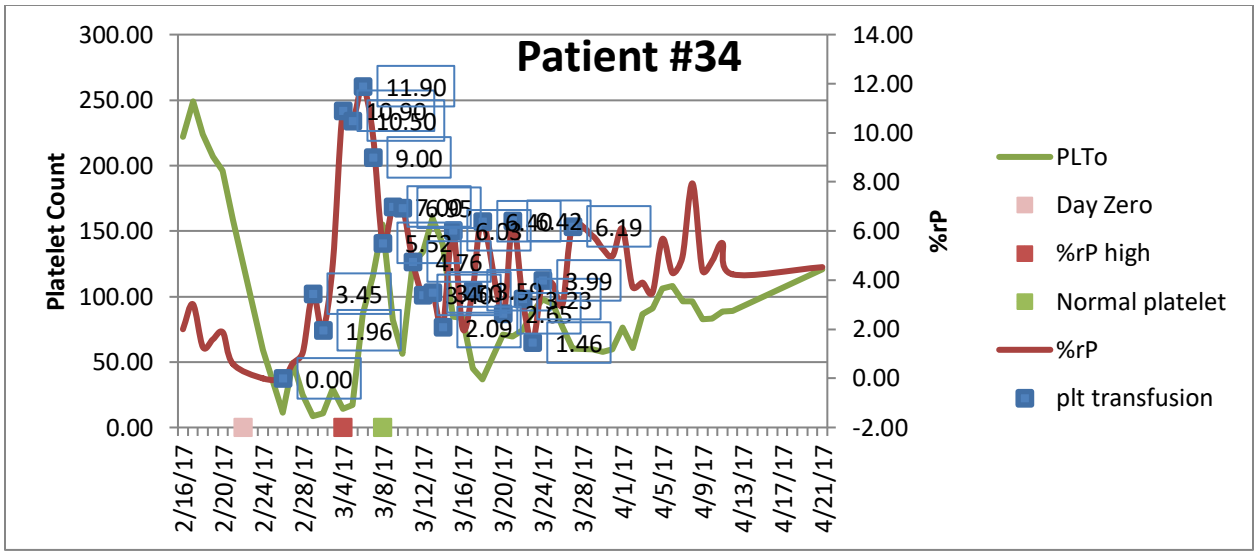
Day zero				3/29/2017
Day %rP high				4/3/2017
Day normal platelet count				4/25/2017
Day of last plt transfusion				4/7/2017
# Days from TX to high %rP				5
# Days from high %rP to normal platelet				22
# Days from high %rP to last platelet transfusion				4

Figure 45: Patient #32 age 1 year. Spike in %rP value of 10.50% (0.95-8.93%).



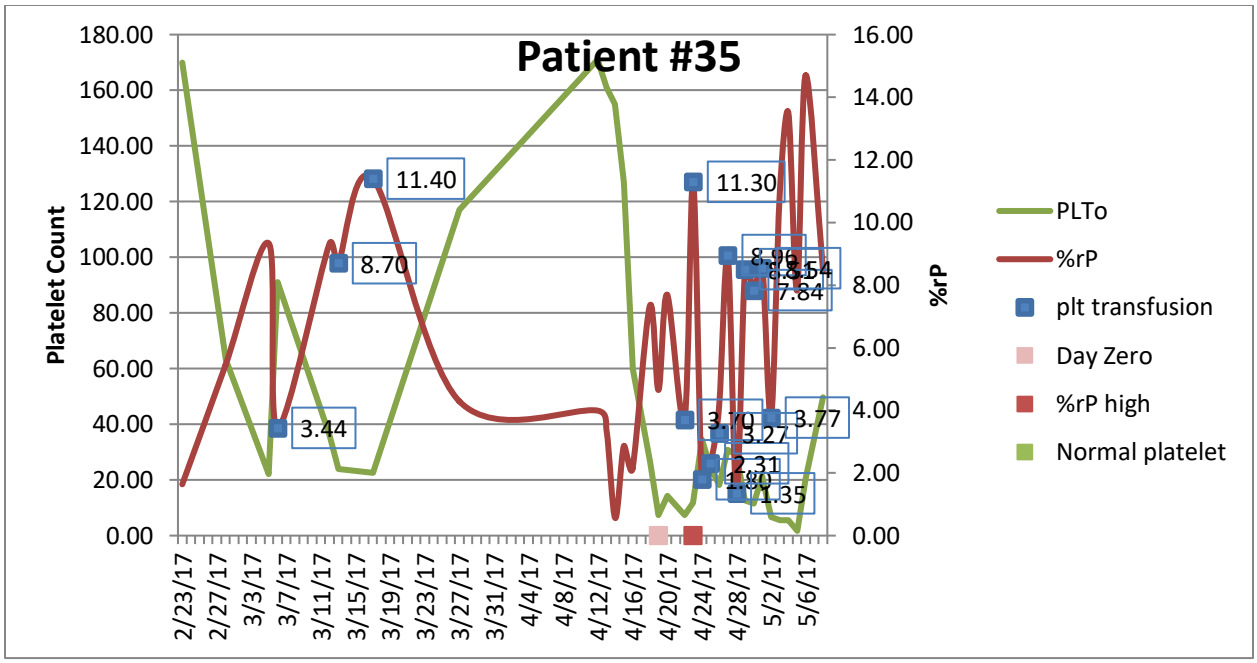
Day zero				3/16/2017
Day %rP high				3/22/2017
Day normal platelet count				3/26/2017
Day of last plt transfusion				NONE
# Days from TX to high %rP				6
# Days from high %rP to normal platelet				4
# Days from high %rP to last platelet transfusion				NA

Figure 46: Patient #33 age 12 years. No spike in %rP observed. Subject removed from study statistics.



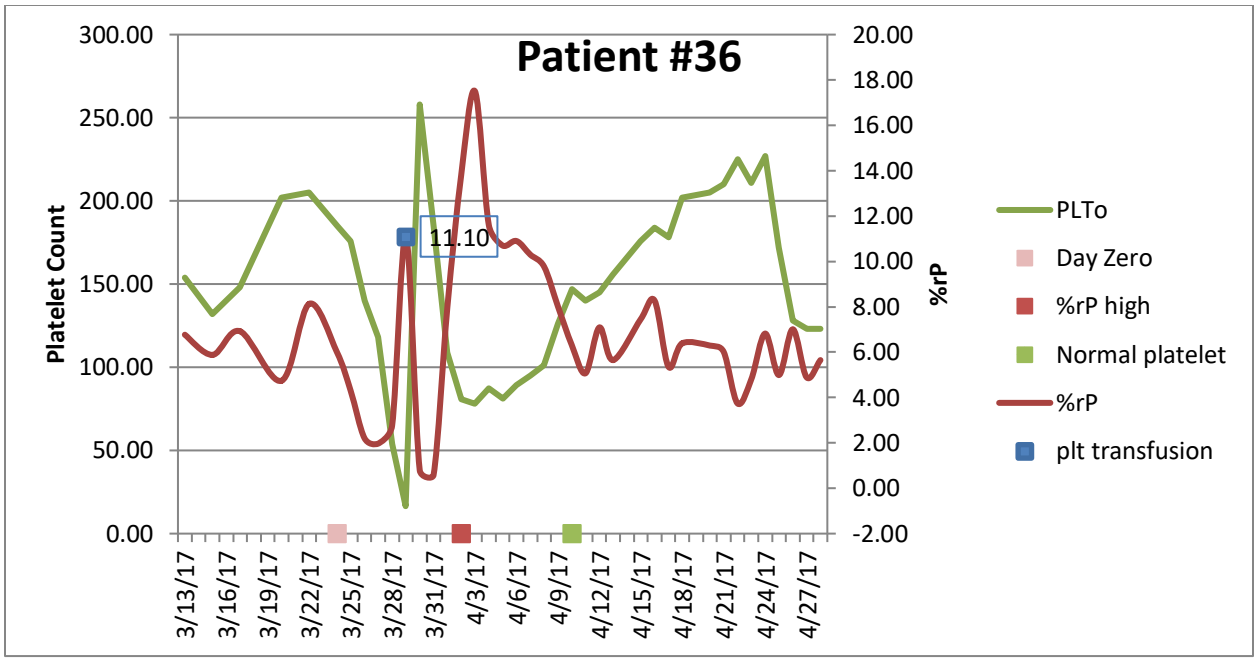
Day zero				2/22/2017
Day %rP high				3/4/2017
Day normal platelet count				3/8/2017
Day of last plt transfusion				3/27/2017
# Days from TX to high %rP				10
# Days from high %rP to normal platelet				4
# Days from high %rP to last platelet transfusion				23

Figure 47: Patient 34 age 13 years. Spike in %rP value of 10.90% (0.33-5.22%).



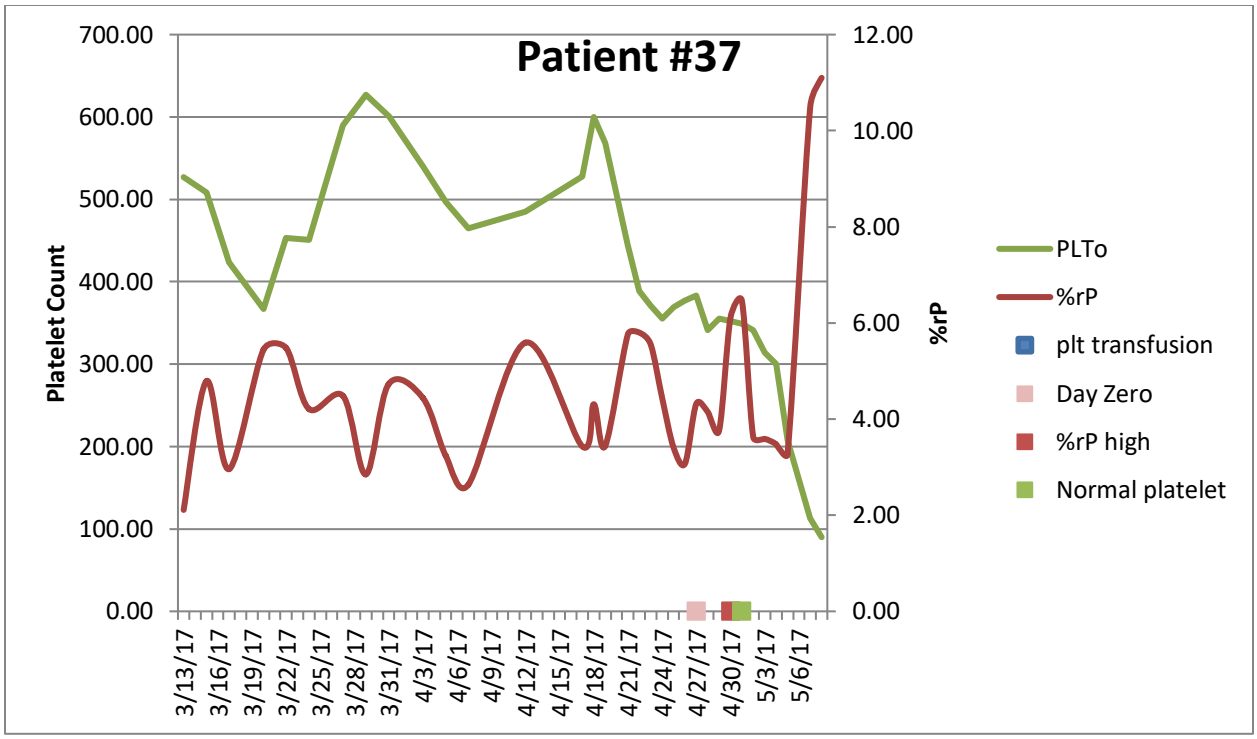
Day zero				4/19/2017
Day %rP high				4/23/2017
Day normal platelet count				NEVER
Day of last plt transfusion				5/2/2017
# Days from TX to high %rP				4
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				9

Figure 48: Patient #35 age 25 years. Spike in %rP value of 11.30% (0.33-5.22%). No return to normal platelet within data collection period.



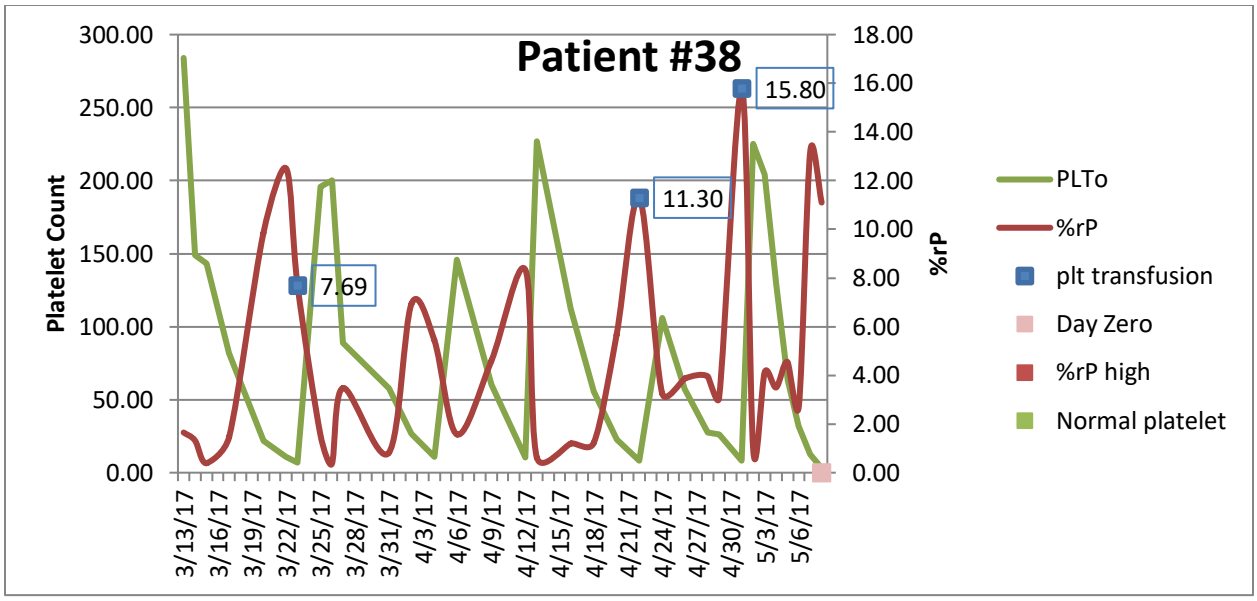
Day zero				3/24/2017
Day %rP high				4/2/2017
Day normal platelet count				4/10/2017
Day of last plt transfusion				3/29/2017
# Days from TX to high %rP				9
# Days from high %rP to normal platelet				8
# Days from high %rP to last platelet transfusion				-4

Figure 49: Patient #36 age 1 year. Spike in %rP value of 13.80% (0.95-8.93%).



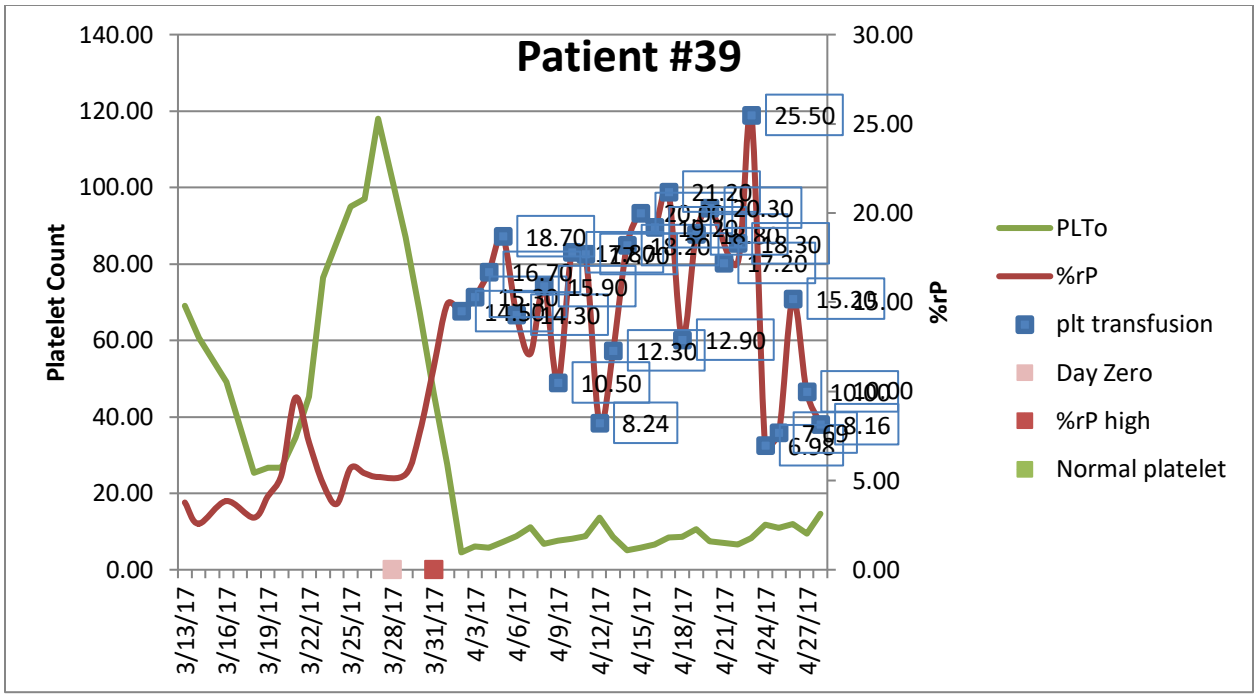
Day zero				4/27/2017
Day %rP high				5/7/2017
Day normal platelet count				NA
Day of last plt transfusion				NONE
# Days from TX to high %rP				10
# Days from high %rP to normal platelet				NA
# Days from high %rP to last platelet transfusion				NA

Figure 50: Patient #37 age 6 months. Spike in %rP value of 10.50% (1.31-8.10%). No return to normal platelet within data collection period.



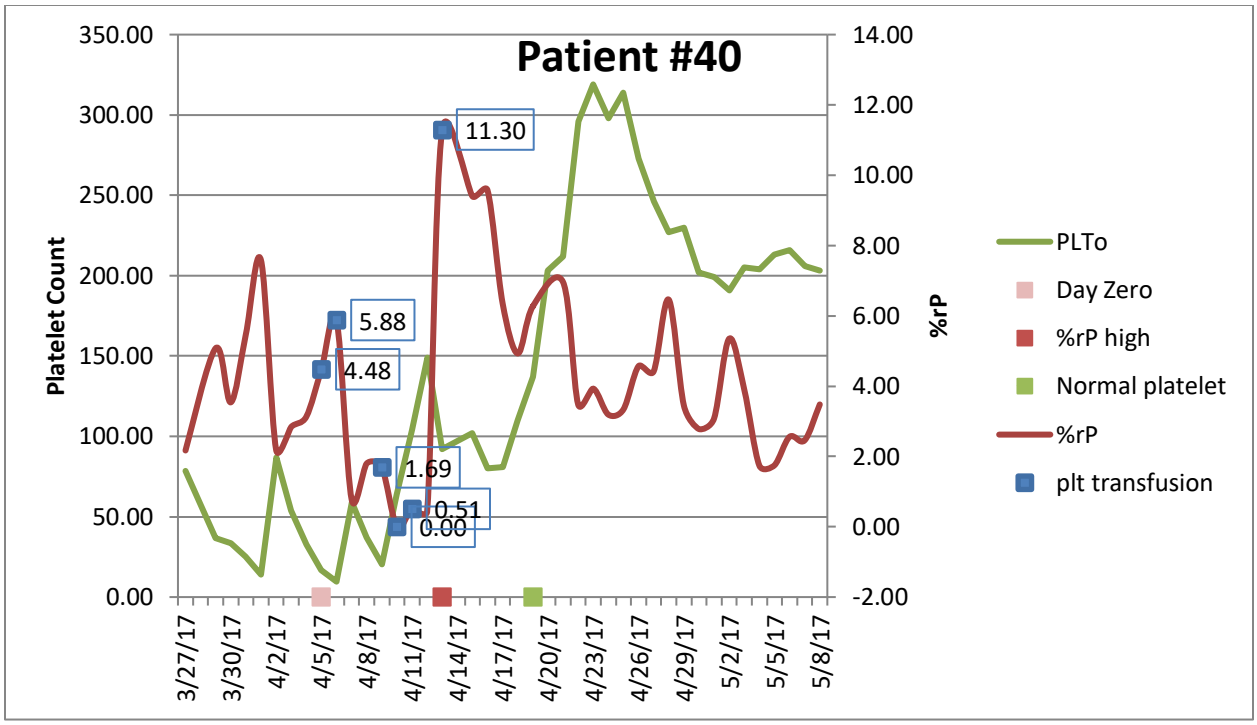
Day zero				5/8/2017
Day %rP high				NA
Day normal platelet count				NA
Day of last plt transfusion				NA
# Days from TX to high %rP				NA
# Days from high %rP to normal platelet				NA
# Days from high %rP to last platelet transfusion				NA

Figure 51: Patient 38 age 5 years. Subject removed from study for lack of sufficient data post-transplant.



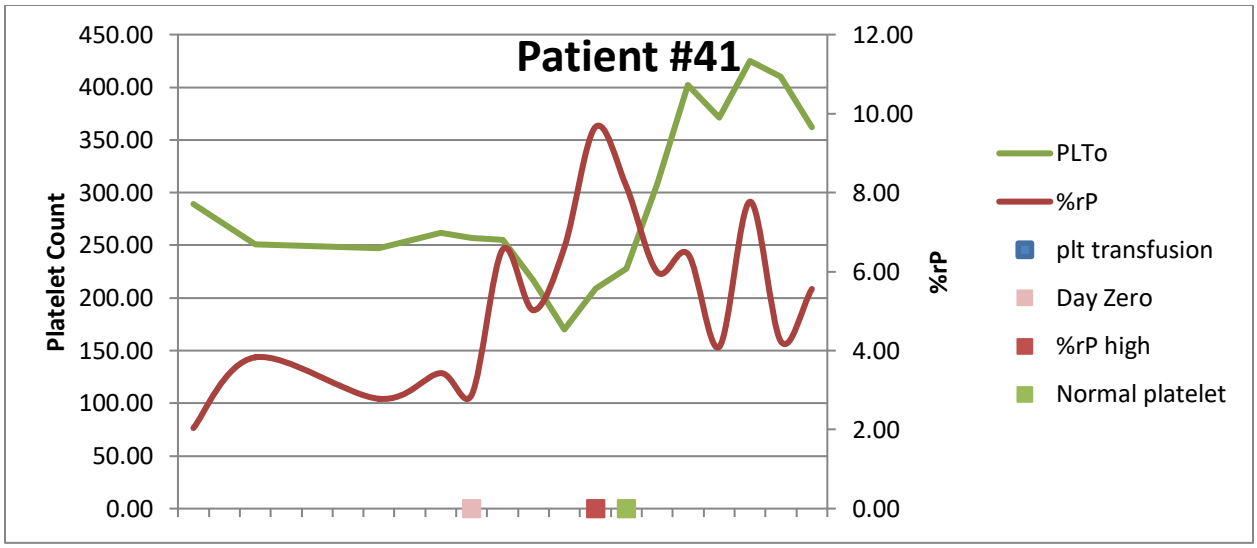
Day zero				3/28/2017
Day %rP high				3/30/2017
Day normal platelet count				NEVER
Day of last plt transfusion				4/28/2017
# Days from TX to high %rP				2
# Days from high %rP to normal platelet				NEVER
# Days from high %rP to last platelet transfusion				NA

Figure 52: Patient #39 age 15 years. Spike in %rP value of 7.69% (0.33-5.22%). Patient was transfusion dependent with no return to normal platelet count.



Day zero				4/5/2017
Day %rP high				4/13/2017
Day normal platelet count				4/19/2017
Day of last plt transfusion				4/13/2017
# Days from TX to high %rP				8
# Days from high %rP to normal platelet				6
# Days from high %rP to last platelet transfusion				0

Figure 53: Patient #40 age 7 years. Spike in %rP value of 11.30% (0.26-7.33%).



Day zero				4/27/2017
Day %rP high				5/1/2017
Day normal platelet count				5/1/2017
Day of last plt transfusion				NONE
# Days from TX to high %rP				4
# Days from high %rP to normal platelet				0
# Days from high %rP to last platelet transfusion				NA

Figure 54: Patient #41 age 9 months. Spike in %rP value of 9.66% (1.31-8.10%). No transfusions administered.