HHS Public Access

Author manuscript

J Perinatol. Author manuscript; available in PMC 2018 October 17.

Published in final edited form as:

J Perinatol. 2017 July ; 37(7): 834–838. doi:10.1038/jp.2017.48.

The immature platelet fraction: creating neonatal reference intervals and using these to categorize neonatal thrombocytopenias

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Abstract

OBJECTIVE: The immature platelet fraction (IPF) is a laboratory measurement analogous to the reticulocyte count, but reflecting the thrombopoietic state. Similar to a reticulocyte count, it can be expressed as a percent (IPF% = percent of platelets that are immature) or as an absolute number per μl blood;the immature platelet count (IPC = IPF% × platelets per μl of blood).

STUDY DESIGN: Using a retrospective analysis of de-identified data from non-thrombocytopenic neonates, we created reference intervals for IPF% and IPC. We then tested the value of these measurements for categorizing thrombocytopenic neonates.

RESULTS: New charts display reference intervals for IPF% and IPC on the day of birth according to gestational age, and during the first 90 days after birth. Neonates with hyporegenerative varieties of thrombocytopenias (syndromes, small for gestational age, birth asphyxia) had lower IPF% and IPC than did neonates with consumptive thrombocytopenias

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CONFLICT OF INTEREST

MCS-V has a Sysmex Veterinary Analyzer as a loan from Sysmex America. She does not receive any financial support from Sysmex. The remaining authors declare no conflicts of interest.

(immune-mediated, infection, disseminated intravascular coagulation, necrotizing enterocolitis;both $P < 0.0001$).

CONCLUSION: The new reference interval charts can be used to recognize abnormal IPFs. The IPF parameters can help clarify the kinetic mechanism responsible for thrombocytopenias in neonates.

INTRODUCTION

The immature platelet fraction (IPF) is a laboratory quantification of immature platelets in the circulating blood. Thus, the IPF reflects the state of thrombopoiesis in the way a reticulocyte count reflects the state of erythropoiesis. When thrombocytopenia is due to accelerated platelet destruction, the marrow compensates by releasing younger platelets into the blood, which is recognized by an increase in the IPF. In addition, similar to the reticulocyte count, the IPF can be reported as either a 'percent', meaning the percent of platelets that are immature, or as an 'absolute number' of immature platelets per μl (also known as the immature platelet count or IPC, calculated by multiplying platelet count × IPF %). The highly fluorescent IPF (H-IPF%) represents the most immature platelet percentage. The H-IPF% is currently a research-only parameter on Sysmex hematology analyzers and is not Food and Drug Administration-approved for clinical use.

Ko *et al.*,[1] from South Korea, recently reported IPF reference intervals using Sysmex XN and XE-2100 hematology analyzers. They observed similar IPF ranges for healthy adults and for umbilical cord blood obtained at full-term deliveries ($n = 120$). IPF % ranged from 1.0 to 7.3% in adults and from 1.0 to 4.4% in cord blood. The IPC in healthy adults was 2460–15 640 μl^{-1}, and in cord blood was 2940–12 820 μl^{-1}. Yuko *et al.* reported that the IPF% from umbilical cord blood of term neonates ($n = 45$) was 2.8 ± 1.3% (mean ± s.d.). Other than values from umbilical cord blood, little data exist about the reference interval for IPF in neonates. No data have been published regarding reference intervals for H-IPF% in neonates. In general, reference intervals in neonatal hematology are highly dependent on the gestational and postnatal age of the neonate.

The purpose of our present study was to use a large multihospital database to add to our understanding of three aspects of the IPF in neonates: (1) we sought to establish reference intervals for the IPF parameters (IPF% and IPC) created specifically for gestational age at birth and for postnatal age up to 90 days after birth. (2) We aimed to determine whether the research parameter H-IPF% added unique information to that of the two other IPF parameters. (3) Among thrombocytopenic neonates, we sought to test the utility of using the IPF parameters to categorize thrombocytopenia as the kinetic result of either reduced platelet production (low or normal IPF) or accelerated platelet consumption (high IPF).

MATERIALS AND METHODS

The study protocol was approved by the Privacy Board of the Intermountain Healthcare Institutional Review Board. The Privacy Board granted a waiver from individual parental consent because this was a deidentified data-only study with appropriate privacy protection. The data set for this study included complete blood count (CBC) results of neonates with

dates of birth from 1 February 2013 through 31 January 2016 from 10 Intermountain Healthcare Hospital Laboratories. Only inpatients in neonatal intensive care units (NICUs) were included. Data were obtained only by Intermountain Healthcare data analysts (directed by EH). Intermountain Healthcare is a not-for-profit health-care system operating 18 hospitals with labor and delivery units in Utah and Idaho.

All blood cell counts were determined using Sysmex Hematology XE-5000 or XT-4000i analyzers (Sysmex America, Lincolnshire, IL, USA) run in the reticulocyte mode. The IPF was determined using a fluorescent polymethine dye that identifies those platelets with residual RNA, thought to represent the most immature platelets.[9] The highly fluorescent IPF is expressed as percent of the IPF. All blood tests were performed in accordance with Intermountain Healthcare Laboratory Services standard operating procedures and manufacturer's instructions. The Sysmex quality-control procedures were performed daily as recommended by the manufacturer.

Reference intervals for IPF% and IPC were calculated using only data from neonates who had platelet counts above 120 000 μl^{-1} (ref. 10) and had not received a platelet transfusion within the preceding 3 days. Reference interval charts were created by displaying the 5th percentile and 95th percentile values for the day of birth, according to gestational age. Other charts were created to display reference interval values each day for the first 90 days of life.

For the next part of the study we defined thrombocytopenia as two or more consecutive platelet counts < 100 000 μl^{-1}. Neonates with thrombocytopenia were categorized into the following three groups, based on the presumed mechanism of the thrombocytopenia: (1) hyporegenerative, (2) consumptive or (3) indeterminate. Hyporegenerative thrombocytopenias were either congenital genetically based syndromes known to have hyporegenerative thrombocytopenia, Trisomy 21, 18 and 13, the hyporegenerative thrombocytopenia of small for gestational age (SGA) or the hyporegenerative thrombocytopenia of birth asphyxia. Consumptive thrombocytopenias were either immune-mediated (allo-immune or maternal autoimmune), or accompanying infection, necrotizing enterocolitis (NEC) or disseminated intravascular coagulation (DIC).[–] Thrombocytopenias of indeterminate kinetic cause were those where none of the above associations were recognized.

The program used for data collection was a modified subsystem of Clinical Workstation. The 3M Company (Minneapolis, MN, USA) approved the structure and definitions of all data points for use within the program. The gestational age field is populated by information in the infant's medical record (not the antenatal maternal record). The means and s.d.'s were used to express values in groups that were normally distributed, and the medians and interquartile ranges to express values in groups that were not. Differences in categorical variables were assessed using the Fisher exact test or X^2 for normally distributed data and Tukey's bi-weight estimator for groups that were not. Statistical analysis used the Statit (Midas, Tucson, AZ, USA) or the R Foundation package (Statistical Computing, Vienna, Austria). The mixed effects model used the NIME program, version 3.1–105, also from the R package (Statistical Computing, Vienna, Austria). Statistical significance was set as $P < 0.05$.

RESULTS

During the study period, 24 372 CBCs with platelet counts and IPF values were available from 9172 neonates 0–90 days old. Approximately 15% of the CBCs (n = 3758) were removed from the reference interval database (Figure 1) because the platelet count was < 120 000 μl^{-1} (n = 2766), the patient had received a platelet transfusion within the previous 3 days (n = 482) or both (n = 510). This left 20 614 CBCs from 8967 neonates with normal platelet counts (> 120 000 μl^{-1}) in the database from which the reference intervals were created. Table 1 shows the race and ethnic groups of the neonates in the IPF database, as recorded in their clinical histories. The gestational age of the 8967 neonates whose CBCs were included was 35.8 ± 4.3 (mean ± s.d.) weeks, with the distribution shown in the table. The gender distribution was 59% males and 41% females.

Figure 2 shows IPF reference intervals on the day of birth according to gestational age. Each of the panels shows the 5th percentile, median and 95th percentile values for IPF% and IPC. A second y axis shows the mean platelet count on the day of birth according to gestational age as previously published by Henry *et al.* Figure 3 shows the IPF% and IPC reference intervals over the first 90 days after birth. A second y axis shows the mean platelet count over the first 90 days after birth. There were no significant differences in IPF parameters between males and females on the day of birth or in the first 90 days after birth in preterm or term neonates.

Simultaneous IPF% and H-IPF% measurements were highly correlated (Figure 4; R^2 = 0.936). The correlation between IPF% and IPC measurements was less (R^2 = 0.214). The IPF% correlated inversely with platelet count (Figure 5; R^2 = 0.174).

Of the 2766 CBCs in the original database with a platelet count < 120 000 μl^{-1}, 280 were from neonates who had ≥2 platelet counts < 100 000 μl^{-1}. The causes of the thrombocytopenia in these 280 were assessed by individual review of the medical records (by BCM and RDC; Table 2). The gestational age of these neonates was 3 4 ± 6 (mean ± s.d.) weeks. IPF% and IPC values were higher in neonates with consumptive thrombocytopenias than in those with hypoproliferative varieties. Neonates who had both kinetic elements (that is, the patient was SGA (hypoproliferative) and had NEC (consumptive)), or who had no recognized cause of their thrombocytopenia (indeterminate) had IPF values intermediate between the hypoproliferative and consumptive groups.

DISCUSSION

Thrombocytopenia is a common problem in NICUs. We previously reported that up to 73% of extremely low-birth-weight infants in the Intermountain Healthcare NICUs had thrombocytopenia diagnosed at least once during their hospitalization. The underlying causes of thrombocytopenia in the NICU are not always obvious. Causes can be categorized kinetically, as the result of either platelet production failure (hyporegenerative) or excessive platelet usage/consumption (consumptive). The IPF is a measurement of the youngest platelets in the circulation and, therefore, reflects the thrombopoietic activity. Thus, measuring the IPF might assist clinicians to identify the kinetic mechanism responsible for

the thrombocytopenia, as a step toward diagnosing the exact underlying cause. However, to use the IPF in clinical neonatology, rigorously created specific IPF reference intervals are needed.

In the present study, we generated reference interval charts for the IPF% and IPC measurements in neonates and infants < 90 days old, based on gestational age and postnatal age. We found, at the time of birth, higher IPF% and IPC in the most premature infants, with a progressive decrease until ~ 32 weeks. This likely reflects higher thrombopoietic activity during fetal life. Our results for IPF parameters are also consistent with previously published values using smaller sample sizes.[9] Cremer *et al.* reported that the IPF% from non-thrombocytopenic neonates admitted to the NICU ($n = 682$) was 4.1 ± 1.8% and the IPC was 9500 ± 3600 μl^{-1} (mean ± s.d.) on day of life one. The mean IPF% and IPC values on day of life 1 in our study were 4% and 8500 μl^{-1}, respectively. We observed a progressive increase in IPF values over the first 2 weeks of life, with a return toward baseline by the end of the first month. This observation is consistent with the previously reported physiological increase in platelet counts over the first 2 weeks of postnatal life, and is likely secondary to a surge in thrombopoietin production immediately after birth.

We observed a close linear relationship between the IPF% and the H-IPF% measurements. The two correlated so well that we judge that reporting both is not needed because one predicts the other. H-IPF% is currently a research-only value not approved by the Food and Drug Administration for clinical use, and our data suggest that there is no substantial clinical information gained from it in addition to the IPF%. In contrast, we found a less perfect correlation between IPF% and IPC. This is not surprising as the IPC is affected by the platelet count. This imperfect correlation suggests to us that the IPF% and IPC each measure a somewhat different aspect of thrombopoiesis, and each measurement might have unique meanings and uses.

Oftentimes, the etiology of thrombocytopenia in neonates is not immediately clear; however, this may be important for treatment and prognosis. We found that IPF values in neonates who had consumptive etiologies were significantly higher than those in neonates with thrombocytopenia due to decreased platelet production. Thus, the IPF may help clinicians caring for thrombocytopenic neonates categorize and subsequently identify the cause of the thrombocytopenia.

Younger platelets are generally larger than mature platelets. The mean platelet volume (MPV) assesses size of platelets;thus, a higher MPV is suggestive of a more immature platelet population. Most studies in adults have shown higher MPVs in patients with consumptive thrombocytopenias compared to etiologies of decreased platelet production.[9] However, the IPF is a more direct measure of immature platelets and is likely to correlate better with increased platelet production than the MPV. When the platelet count is low, the Sysmex analyzer typically does not provide an MPV value, but the IPF is still provided. This is because when the count is low the analyzer switches from the ‘impedance mode’, where the MPV is assessed, to the ‘optical mode’ where the MPV is not assessed. Thus, for the thrombocytopenic neonates in this study we had no MPV measurements to compare with simultaneous IPF measurements.

Another potential use of the IPF in neonates was suggested by Cremer et *al*. They found that thrombocytopenic neonates who had lower IPF% values (< 8%) were more likely to have a subsequent substantial downward trend in platelet count, compared with those who had a higher IPF (> 8 %). They suggested that the IPF is of some value in predicting the trajectory of the platelet count. This predictive aspect of the IPF seems reasonable to us, as we found that thrombocytopenic neonates with a low IPF likely have a limitation in platelet production and therefore an impaired capacity to increase the platelet count. We did not assess this predictive aspect of the IPF in our data sets.

We realize limitations in our study. First, the large data set used to create the IPF reference intervals was obtained from an electronic data warehouse, and the individual neonates' charts were not manually reviewed. Data were only excluded from the reference interval data set if thrombocytopenia was present, or if the patient received a platelet transfusion in the previous 3 days. Factors that might affect the IPF but did not cause thrombocytopenia were not excluded. Second, we manually reviewed the medical records of neonates with ⩾2 platelet counts < 100 000 μl^{-1}. However, we did not analyze the IPF values based on platelet count categories (mild, moderate, severe thrombocytopenia) because that analysis would have resulted in small subsets. Thus, we cannot comment on whether the severity of thrombocytopenia affects IPF parameters, although this has been suggested in a prior study. Third, our study population is fairly homogenous with almost 80% identifying as white, and it is unknown how race or ethnicity might affect these measurements. Fourth, all results in this study were generated on a similar platform (Sysmex) because that is the instrumentation in each of our Intermountain Healthcare hospitals with a NICU. However, other instruments might generate somewhat different values.

In summary, we created neonatal reference intervals for the IPF that can be used to recognize abnormal values in the NICU. In thrombocytopenic neonates, IPF parameters may help identify the underlying kinetic mechanism causing the thrombocytopenia. Specifically, high IPF values suggest consumptive varieties of thrombocytopenia, and normal values suggest hypoproliferative thrombocytopenias.

ACKNOWLEDGEMENTS

We thank laboratory staff members Robert Childs, Dereck Daniels, Rachael Ethington, Dave Garner, Laurie Jeffs, Jennessa Jessop, Heather Kuusela, Tyson Olson, Chris Partington, Tim Patten, Brooke Qi, Lauralyn Ragin, Tara Regginello, Aba Saunders, Alicia Simon, Jennifer Sorenson, Lia Souza, Tom Taylor and Ashley Windsor for extracting data from the hematology analyzers. This study was supported in part by grant P01 HL046925 from the US Public Health Service (MCS-V).

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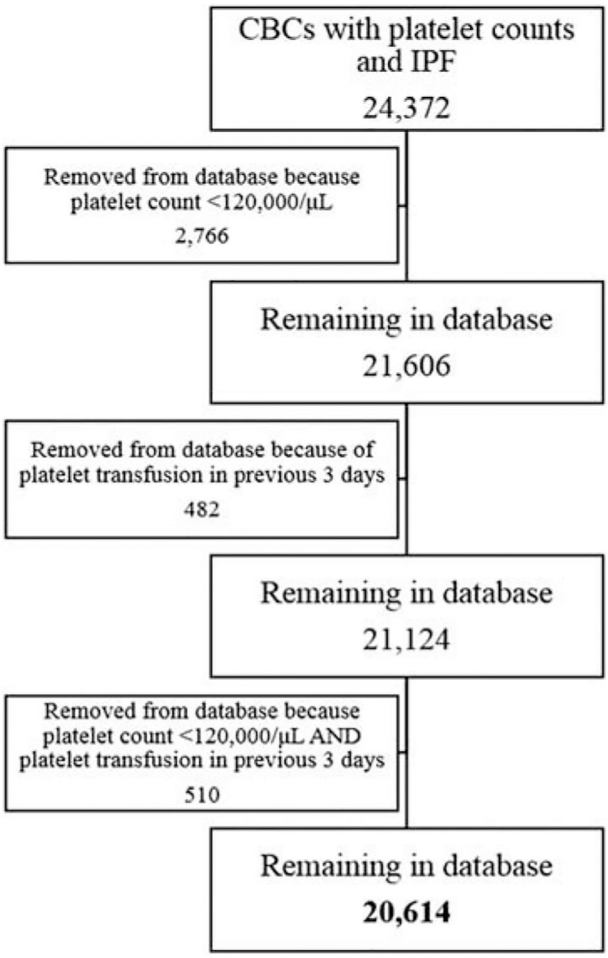

Figure 1.
Consort flow diagram. Construction of the immature platelet fraction (IPF) reference interval database

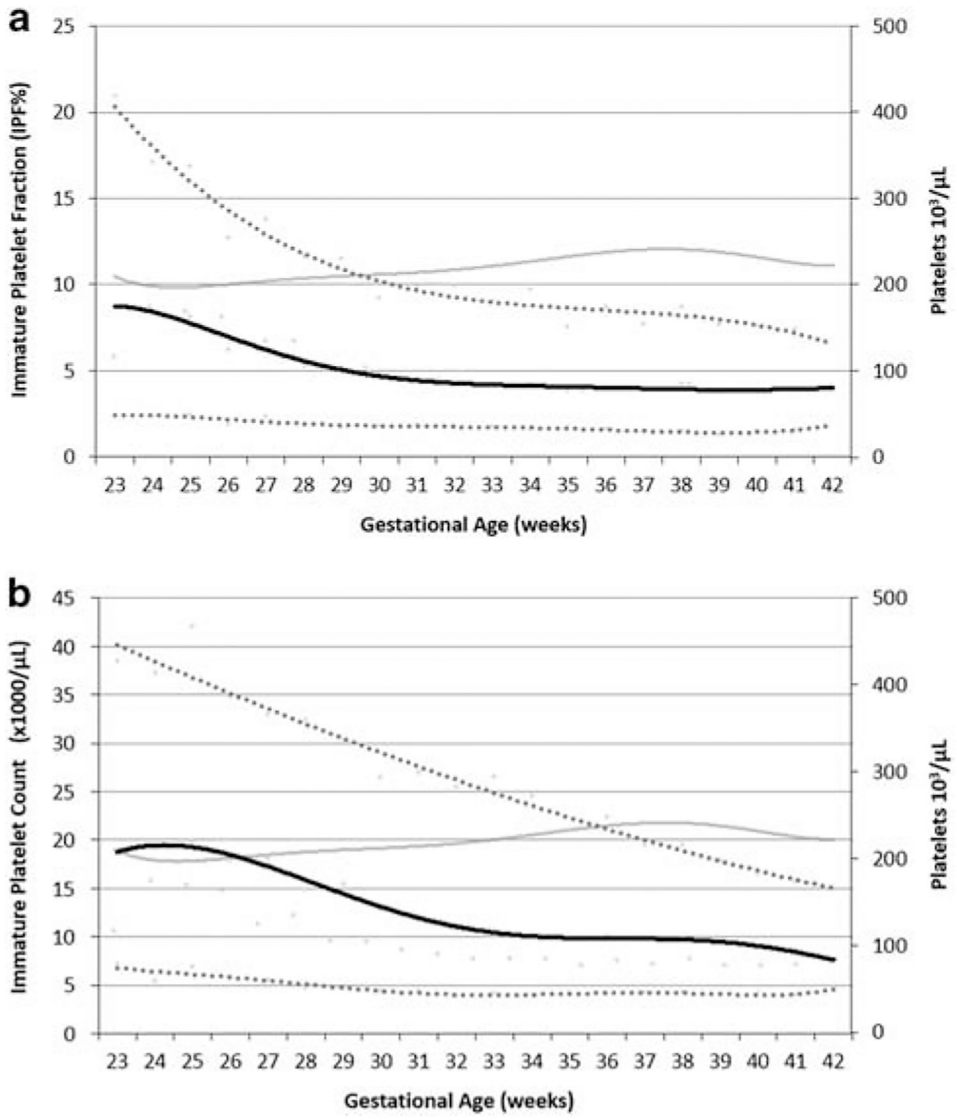

Figure 2.

Immature platelet fraction on the day of birth according to gestational age. In both panels the lower and upper dashed lines represent the 5th and 95th percentile reference intervals, and the solid black line represents the median. Circles are the actual medians for 5th, median and 95% each day. The dashed and solid lines are generated by smoothing values in the circles. The *y* axis on the right shows total platelet count, and the blue solid line represents the mean platelet count on the day of birth according to gestational age. (**a**) Immature platelet fraction. (**b**) Immature platelet count.

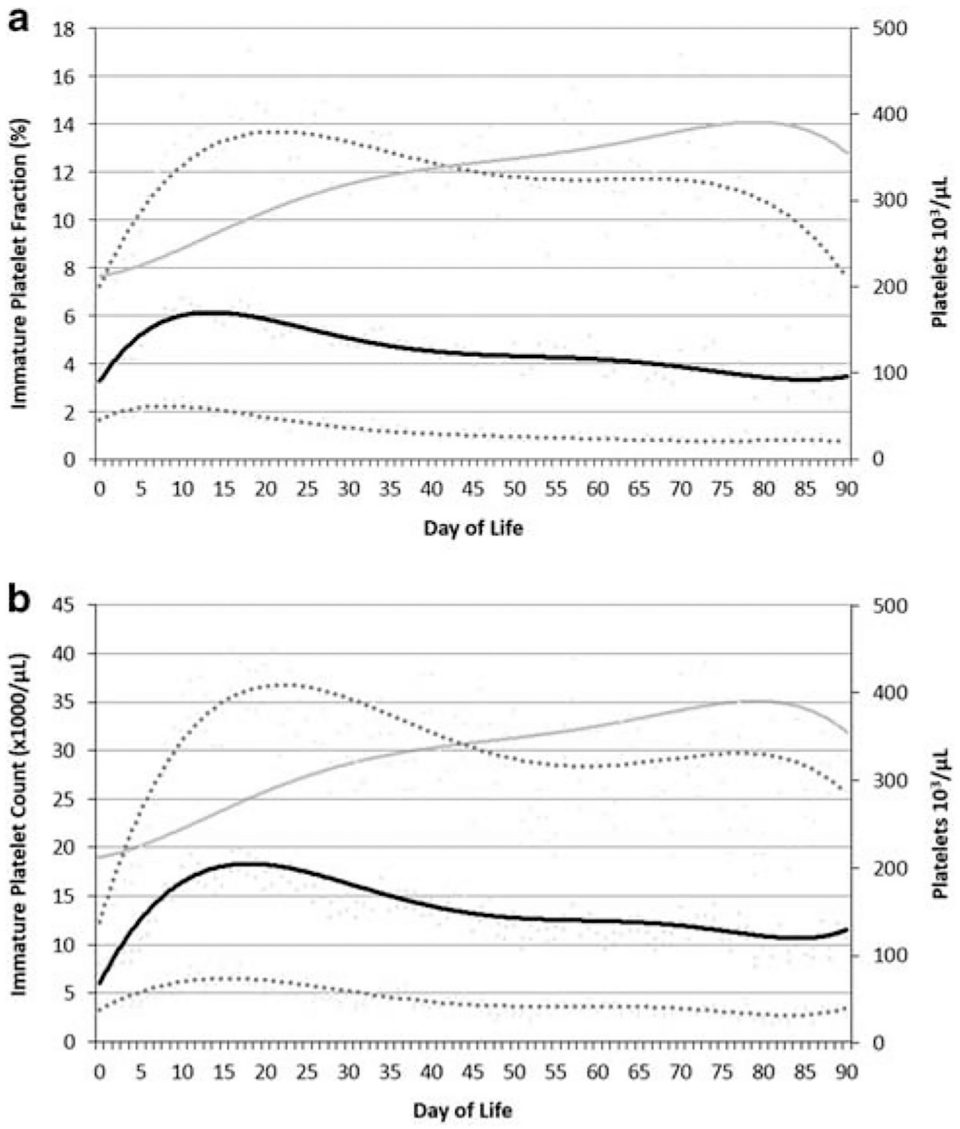

Figure 3.

Immature platelet fraction over the first 90 days. In each panel the lower and upper dashed lines represent 5th and 95th percentile reference intervals, and the solid black line represents the median. Circles are the actual medians for 5th, median and 95% each day. The dashed and solid lines are generated by smoothing values in the circles. The *y* axis on the right shows total platelet count, and the blue solid line represents the mean platelet count over the first 90 days after birth. (**a**) Immature platelet fraction. (**b**) Immature platelet count.

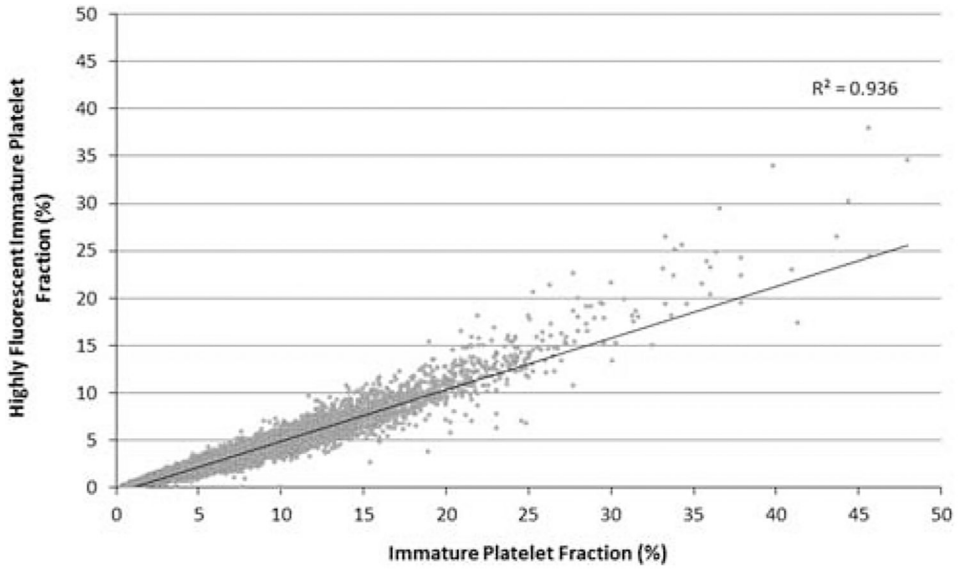

Figure 4.

Correlation of IPF% with highly fluorescent immature platelet fraction (H-IPF%). H-IPF%, highly fluorescent immature platelet fraction.

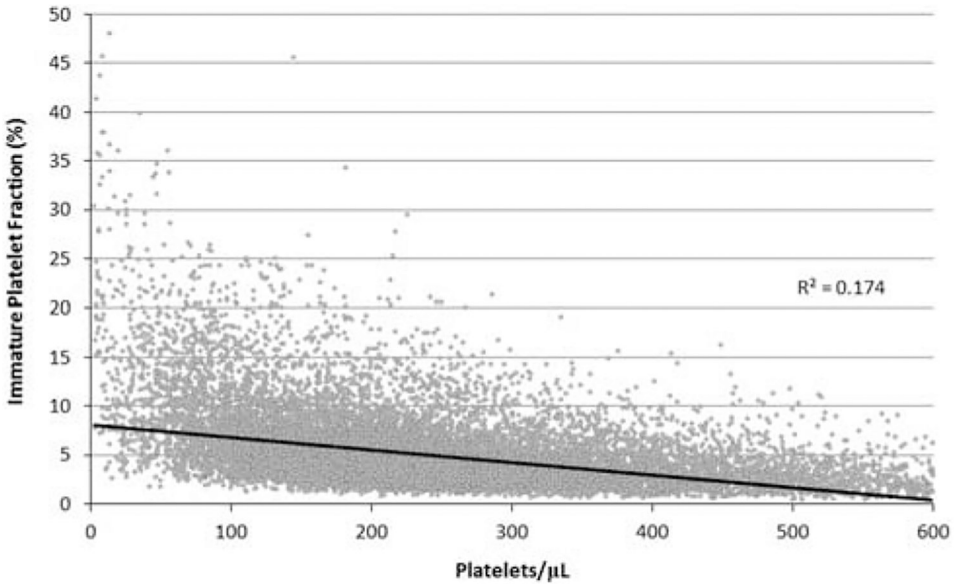

Figure 5.
Correlation of IPF% with platelet count. IPF%, immature platelet fraction.

Table 1.

Race/ethnicity/gender and gestational ages of neonates contributing data to the IPF reference interval database

Group	Number	Percent of total
American Indian or Alaska Native	52	0.6
Asian	235	2.6
Black or African American	121	1.3
Hispanic	608	6.8
Middle Eastern	25	0.3
Multiple	11	0.1
Native Hawaiian or Pacific Islander	195	2.2
Unknown or not recorded	609	6.8
White	7111	79.3
Total	8967	100
Male	5290	59
Female	3677	41
Total	8967	100
23–28 Weeks gestation at birth	238	2.7
29–34 Weeks gestation at birth	1386	15.5
≥34 Weeks gestation at birth	7343	81.9
Total	8967	100

Abbreviation: IPF, immature platelet fraction.

Table 2.

Immature platelet fraction values in thrombocytopenic neonates

	N = *280*	***IPF%***	***IPC***
Hypoproliferative *	92	10.4 ± 2.9	10.95 ± 4.99
Consumptive **	98	20.9 ± 7.9	24.63 ± 13.25
Both	76	17.9 ± 5.9	21.36 ± 9.40
Indeterminate ***	14	12.8 ± 8.1	9.36 ± 3.86
P–values (hypoproliferative versus consumptive)		< 0.0001	< 0.0001

* A syndrome associated with hypoproliferative thrombocytopenia or SGA or birth asphyxia.

** Immune-mediated, NEC, DIC, sepsis.

*** None of the above diagnoses. IPF% and IPC (mean ± s.d.) from 280 CBCs were the neonates had two or more platelet counts < 100 000 μl^{-1}. Each of the 280 were judged (independently of the IPF) to have thrombocytopenia from either (1) a hypoproliferative cause*, (2) a consumptive cause**, (3) elements of both or (4) indeterminate cause***.