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# The Immature Platelet Fraction (IPF) in Neonates

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Young (immature) platelets containing residual RNA, so-called reticulated thrombocytes, appear in the peripheral blood after the release from bone marrow. It has been shown that the count of the reticulated platelet correlates with the megakaryocyte mass. As of late, the immature platelet fraction (IPF) may be reliably quantified with the fully automated blood analyzer Sysmex XE-2100. IPF may be expressed as percentage (IPF%) of the platelet count or as absolute number (IPF#)\*. Since thrombocytopenia occurs in up to 30% of all neonates admitted to intensive care, we evaluated the usefulness of IPF to estimate the platelet production rate in more than 1000 neonates. Our main findings are 1) the reference range for IPF in neonates (IPF% =  $4.1 \pm 1.8$  %; IPF# =  $9.5 \pm 3.8 \times 10^9/L$ ) is higher compared to published results for adults and children, 2) a rise in IPF% anticipates increasing platelet counts, 3) IPF% inversely correlates with the platelet count, indicating a similar mechanism of neonatal thrombocytopenia that is mainly caused by platelet consumption, 4) IPF# is decreased in neonates with severe thrombocytopenia by approximately 50% (IPF#  $4.7 \times 10^9/L$ ), indicating that a decreased megakaryopoietic activity contributes to the development of severe thrombocytopenia. We conclude that IPF can support decision-making when to monitor platelet counts in the setting of neonatal intensive care. Furthermore, the absolute IPF# should be taken into account for exact estimation of the platelet production rate.

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## INTRODUCTION

Recently released platelets from the bone marrow are reticulated and contain high amounts of RNA. In 1969, the first report on reticulated platelets was published by Ingram and Coppersmith<sup>1)</sup>. After the induction thrombocytopenia caused by acute blood loss reticulated platelets were released into the peripheral blood of dogs. These reticulated platelets stained with methylene blue occurred in increased numbers after acute blood loss. Further development of intracellular RNA-staining using thiazole-orange and measurement by flow cytometry has been established by Kienast et al.<sup>2)</sup> These authors showed that reticulated platelets reflect the platelet production rate in comparison to bone marrow biopsy in thrombocytopenic patients. Unfortunately, previous studies on reticulated platelets in neonates were performed using different methods, gating, dyes and

terminology such as thiazole-orange positive platelets, reticulated platelets or high fluorescent platelet fraction making the comparison of standardized results almost impossible<sup>3-5)</sup>. Today, a fully-automated reliable quantification of reticulated platelets, now referred to as immature platelet fraction (IPF), has been developed. Using the Sysmex XE-2100, platelets and reticulocytes are stained with two different dyes (polymethine and oxazine) and quantified in the reticulocytes/platelet channel<sup>6)</sup>. IPF represents the immature platelet fraction and thus the production rate analogous to the reticulocytes for erythropoiesis. IPF can be expressed as percentage (IPF%) of the platelet counts or as absolute number (IPF#), e.g. per nanoliter. It has been shown that IPF distinguishes between increased and decreased platelet production<sup>6)</sup>. With this new technology we achieved the evaluation of the diagnostic relevance of IPF in different settings in neonatal intensive care.

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Thrombocytopenia in healthy newborn is rare; only 2% of healthy neonates have a platelet count  $< 150 \times 10^9/L$ <sup>7)</sup>. However, thrombocytopenia is one of the most common hematologic problems in pre-term and term neonates admitted to neonatal intensive care units, because it affects 18 to 35% of all admitted patients<sup>8,9)</sup>. It could be demonstrated, that in premature infants with a birth weight below 1000g the incidence of thrombocytopenia is nearly 60%<sup>10)</sup>. Among thrombocytopenic extremely low birth weight infants, almost 40% suffer from severe thrombocytopenia as defined by platelet counts  $< 50 \times 10^9/L$ . Neonatal thrombocytopenia occurring within 72h after birth is defined as 'early-onset' thrombocytopenia. Early-onset thrombocytopenia is often caused by congenital bacterial infection or by chronic intrauterine hypoxia (associated with placental insufficiency, intrauterine growth restriction etc.) and often resulting in 'small for gestational age' infants. In thrombocytopenic neonates platelet counts typically decrease over the first days of life and starts to increase at the end of the first week<sup>11)</sup>. This phenomenon is poorly understood, however a consumption of platelets seems likely. As a consequence, repeated blood sampling in order to determine the platelet count is a very common procedure during the first days of life. Monitoring of the platelet count is also important, since thrombocytes contribute to the postnatal occlusion of the ductus arteriosus<sup>12)</sup>. Another point is that the risk for intracranial hemorrhage, which may cause acute life-threatening complications and/or life-long disability in more than 15%, is difficult to assess, because it is closely related to the gestational and postnatal age of the neonate as well as the cause of the thrombocytopenia and the severity of concurrent conditions. Currently, only measurement of circulating platelet counts as well as determination of activated partial thromboplastin time, prothrombin time ratio, and fibrinogen are standard parameters for estimating the risk for hemorrhage as well as for decision making on platelet transfusion or, respectively, the application of fresh frozen plasma. Thus, a routine test available at a 24 /7 basis is required allowing the evaluation of the amount of platelet production as well as the distinction between inappropriate platelet production and increased platelet consumption. Furthermore, we aimed to clarify the exact pathomechanism of neonatal early-onset thrombocytopenia caused by intrauterine growth restriction or bacterial infection.

## SUBJECTS

In this review the results of different prospective studies on neonates with thrombocytopenia are briefly presented and described. The respective number of patients is given in the results section. An unselected population of all neonates admitted to our neonatal intensive care unit was included. Over a 12-month period, each infant admitted had a full blood count. Serial repetition of blood sampling was performed in sick neonates. The patients were divided into two groups according to their lowest platelet count during the first week of life. If the patient

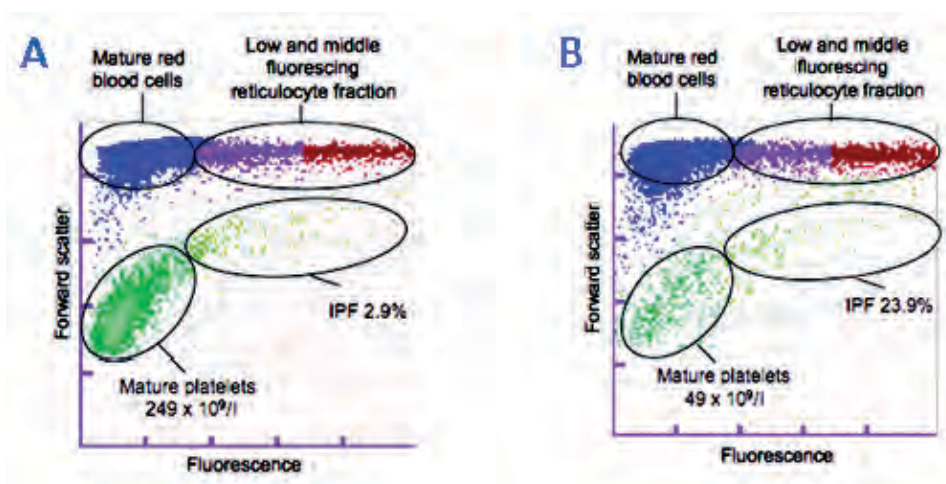
permanently had platelet counts  $> 150 \times 10^9/L$ , he was assigned to the control group. Neonates who displayed at least once a platelet count  $< 150 \times 10^9/L$  were included into the thrombocytopenia group. At admission, concentrations of interleukin-6 and/or C-reactive protein were determined to approve or rule out bacterial infection by immunoassay (IMMULITE 2000, Siemens, Erlangen, Germany; COBAS, Roche, Mannheim, Germany). Bacterial infection was diagnosed and treated if either the interleukin-6 or C-reactive protein concentration exceeded 100 pg/mL or 1mg/dL, respectively. Infants were classified as 'small for gestational age' (SGA) according to their birth weight below the 10th percentile or 'appropriate for gestational age' if the birth weight was between the 10th and 90th percentile. A control group was defined consisting of 'appropriate for gestational age' infants without infection. Written parental informed consent and organisational approval were obtained.

## PLATELET COUNT AND IMMATURE PLATELET FRACTION

Approximately 200  $\mu L$  blood were collected in EDTA-containing tubes (Becton Dickinson, Franklin Lakes, NJ, USA), incubated and measured with polymethine and oxazine dye (Retsearch (II), Sysmex) in the reticulocytes channel of the fully-automated hematological Sysmex XE-2100 analyzer (Sysmex, Kobe, Japan) equipped with the XE-IPF-Master software. Blood specimens were passed through a laser diode beam, and the resulting forward scatter light and side-ward fluorescence intensity was measured. The forward scatter light reflects the cell volume (y-axis), and the fluorescence intensity (RNA-content) is depicted on the x-axis. A pre-set gate discriminates between mature and immature platelets. IPF is expressed as absolute number (IPF#) or percentage (IPF%). An example of two original scattergrams is provided in **Fig. 1**. Platelet counts of the same specimen were measured by the electric impedance method, as the primary measurement, and optically by the scattering light method. In abnormal samples, such as those with large platelets or small red blood cells, a computer algorithm is applied and the result is switched to the result produced by the scattered light<sup>13)</sup>.

## STATISTICAL ANALYSIS

Data are given as mean and standard deviation or percentages unless stated otherwise. Statistical analysis of platelet counts and IPF was performed using ANOVA. Pearson's correlation coefficient was calculated to investigate the relationship between gestational age and platelet count, IPF% or IPF#. For statistical analysis, we used the 'PASW statistics' software (Version 18, SPSS Inc., Chicago, Illinois, USA). Statistical significance was defined by a p value  $< 0.05$ .



**Fig. 1** Original scattergrams of blood cell analysis including immature platelet fraction (IPF) in healthy (A) and thrombocytopenic neonates (B) performed with the Sysmex XE-2100 automatic analyzer equipped with the software XE-IPF-Master. Panel A: Analysis in a preterm neonate with normal platelet count ( $249 \times 10^9/L$ ) and normal IPF. Panel B: Scattergram from a blood specimen of a thrombocytopenic neonate ( $49 \times 10^9/L$ ) with high IPF value. Mature platelets appear as turquoise dots, and green dots represent immature platelets characterized by an increased cell volume and higher fluorescence intensity compared to mature platelets.

## NORMAL IPF VALUES

In total we included 1447 newborn infants who were admitted over a time period of 12 month. In total, 2856 blood samples from these patients were analyzed and the platelet count was determined. In 2562 of these samples the IPF could be measured. The median gestational age of these infants was 37.0 weeks (range: 23–42). The median birth weight was 2610g (range: 400–5125 g). Gestational age and platelet counts showed a statistically significant but low correlation ( $r = 0.13$ ,  $p < 0.001$ ). However, we found no correlation between the gestational age or the birth weight and IPF. Based on infants without thrombocytopenia we calculated the normal range for IPF at the first day of life. The mean IPF percentage was  $4.1 \pm 1.8 \%$  and absolute IPF# was  $9.5 \pm 3.8 \times 10^9/L$  (Table 1). We could demonstrate a significant negative correlation between the platelet count and the IPF% with an exponential decay ( $r = -0.62$ ,  $p < 0.001$  /Fig. 2).

## THE COURSE OF PLATELET COUNTS AND IPF

Patients with severe disease and /or low platelet counts had repeated blood sampling to monitor platelet counts. If the platelet counts dropped below  $150 \times 10^9/L$  during the first week, the patient was categorized into the thrombocytopenia group ( $n = 156$ ). As shown in Figure 3, platelet counts continuously decreased in these patients until day five of life. On day six and seven, platelet counts increased and returned to a platelet count  $> 150 \times 10^9/L$ . In patients without thrombocytopenia platelet counts remained stable over the first four days of life and then increased rapidly. In the thrombocytopenia group as well as in controls increasing platelet counts were

anticipated by an increase of IPF% (Fig. 3). Based on these findings, we calculated the risk for a severe decrease of platelets on the following day and tried to predict the course of the platelet count. In only 5 out of 76 patients the platelet count dropped more than  $50 \times 10^9/L$  even though the IPF exceeded 8%<sup>14</sup>.

## NEONATAL INFECTION AND 'SMALL FOR GESTATIONAL AGE' INFANTS

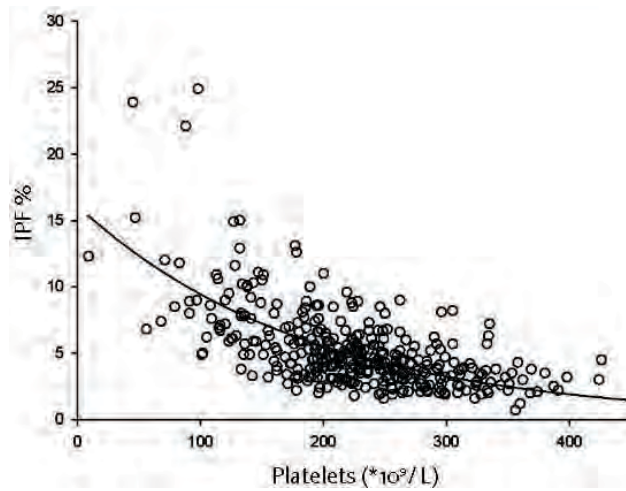
To elucidate the platelet production rate in neonates with congenital bacterial infection ( $n = 134$ ) we analyzed neonates with elevated proinflammatory cytokine concentration and compared them with controls ( $n = 656$ )<sup>11</sup>. In general, neonates with infection had significantly lower platelet counts but displayed similar IPF. The same was true for SGA infants. SGA infants suffered more frequently from thrombocytopenia. However, the platelet production rate, reflected by the IPF, was similar compared to controls.

## ABSOLUTE VERSUS RELATIVE IPF

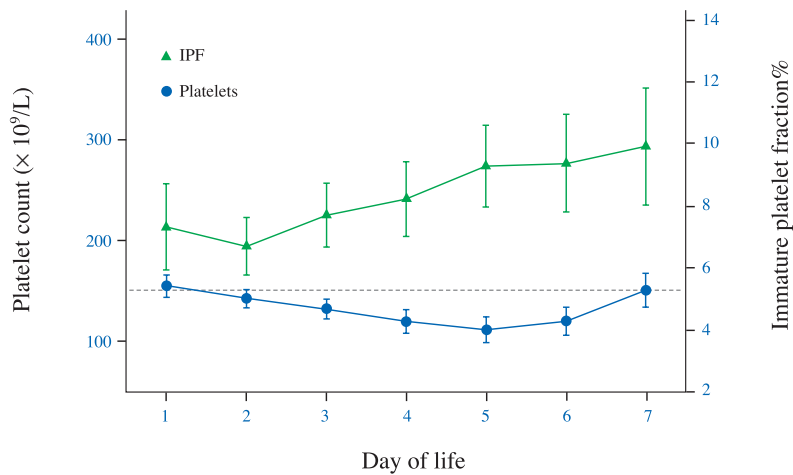
In neonates with thrombocytopenia we found a strong inverse correlation between the platelet counts and the percentage of immature platelets (IPF%). Especially in patients with severe thrombocytopenia (platelet counts  $< 50 \times 10^9/L$ ) IPF% was constantly increased. Since the percentage of IPF depends on the absolute platelet count, it may be reasonable to calculate the absolute IPF values by multiplication of the platelet count with the IPF%. The absolute IPF is independent of the platelet count. Severely thrombocytopenic neonates ( $n = 24$ ) with

**Table 1** Immature platelet fraction in non-thrombocytopenic neonates, children<sup>16)</sup> and adults<sup>6)</sup> Data are given as mean and standard deviation or range.

	Newnates	Children	Adults
Number of patients	682	100	50
Immature platelet fraction (%)	4.1 ± 1.8	2.7 ± 1.3	3.4 (1.1 – 6.1)
Immature platelets (× 10 <sup>9</sup> /L)	9.5 ± 3.8	7.2 ± 3.4	8.6 (3.1 – 16.4)



**Fig. 2** Regression analysis between platelet count and IPF% in neonatal blood specimens at the first day of life (n = 347).



**Fig. 3** Longitudinal analysis of platelet count (mean ± standard error of mean) and IPF percentages (mean ± SEM) during first seven days of life. Patients with thrombocytopenia are presented (platelet count once < 150 × 10<sup>9</sup>/L). Triangles represent IPF%, circles represent platelet counts. The dashed line indicates the lower limit for normal platelet count (< 150 × 10<sup>9</sup>/L).

platelet counts  $< 50 \times 10^9/L$  had significantly lower IPF# ( $4.7 \times 10^9/L$ ) compared to controls ( $9.5 \times 10^9/L$ ,  $p < 0.001$ ).

## DISCUSSION

The immature platelet fraction is an innovative tool to assess the platelet production rate ad hoc. The fully automated measurement of IPF by flow cytometry has been increasingly used since it is reliable and reproducible<sup>15</sup>. Interpreting IPF in neonates involves comparing their measured values with 'normal ranges'. We established such ranges by drawing blood on large numbers of neonates admitted to intensive care (because of ethical concerns, blood samples are not taken from healthy neonates). Hence, these IPF values have to be interpreted carefully. Interestingly, we found a higher platelet production rate in neonates compared to children between the age of six months and 18 years<sup>16</sup> and adults<sup>6</sup> (**Table 1**). These findings are in line with previous studies showing that neonates have a higher proliferation rate of megakaryocytes than adults<sup>17</sup>. However, thrombocytopenia on the neonatal intensive care unit is frequent and the mechanisms leading to thrombocytopenia are not entirely clear. We were able to demonstrate, that IPF is a useful tool to estimate the platelet production rate. However, IPF measurements are not suitable to determine the level of platelet consumption. It seems that the drop of platelet count on the first days of life is due to platelet consumption which is not compensated by an adequate increase of platelet production. This fact becomes evident in the case of severe thrombocytopenia: The significantly lower IPF# value in neonates with severe thrombocytopenia compared to neonates with platelet counts  $> 50 \times 10^9/L$  reflects a decreased megakaryopoietic activity. Brown et al. evaluated reticulated platelets and thrombopoietin concentrations in twenty neonates with sepsis. They found reduced absolute counts of reticulated platelets in thrombocytopenic neonates<sup>18</sup>. Although thrombopoietin concentrations in neonates are increased in the early phase of infection and inversely correlate with the platelet count, this stimulus is obviously not sufficient enough to maintain normal platelet counts<sup>18,19</sup>. Since normalisation of platelet count can be anticipated by rising IPF values, serial measurements of IPF might be helpful for decision-making if a neonate with severe thrombocytopenia requires platelet transfusion. In the case of constant platelet degradation IPF can predict the course of the platelet count. Therefore, the determination of the moment in which to repeat blood analysis may be supported by the evaluation of the platelet production rate in the peripheral blood by means of IPF. Thus, IPF may provide guidance to improve the management of neonatal thrombocytopenia.

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