Plasma Interleukin-11 Levels in Thrombocytopenic and Non-Thrombocytopenic Neonates

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Abstract:

Thrombocytopenia is common in sick neonates, and affected neonates have adverse outcomes compared with those without thrombocytopenia. As impaired platelet production underlies many neonatal thrombocytopenias, affected neonates are potential candidates for hemopoietic growth factor therapy. The thrombopoietic cytokine recombinant human IL-11, which stimulates megakaryocytopoiesis and increases platelet counts after chemotherapy, is already licensed for clinical use. However, little is known about IL-11 in neonates. We therefore measured plasma IL-11 by ELISA in healthy term neonates, stable preterm neonates with or without thrombocytopenia, and preterm neonates with sepsis or necrotizing enterocolitis (NEC) with or without thrombocytopenia. At birth IL-11 was undetectable (<10 pg/ml) in healthy term neonates (n = 15) and 21 of 25 (84%) stable preterm neonates. Three stable preterm neonates had detectable plasma IL-11 (mean, 11.23±0.64 pg/ml), all three were born after pregnancies complicated by prolonged rupture of membranes or chorioamnionitis, the remaining neonate (IL-11, 15 pg/ml) being one of seven with early onset thrombocytopenia (presented by <72 h of age). IL-11 was also measured in 50 preterm neonates with suspected sepsis or NEC. In 20 of 50, sepsis or NEC was unconfirmed and IL-11 was undetectable, in contrast, 14 of 30 with proven sepsis or NEC had elevated IL-11 (mean, 25.41±21.3; range, 11.3–93 pg/ml)(p = 0.0003). Of the 30 neonates with proven sepsis or NEC, 19 developed thrombocytopenia: nine of 19 (47.4%) had detectable IL-11 and 10 of 19 (52.6%) did not (p = 0.746).

Conclusion: Although the role of IL-11 in platelet production in neonates remains unclear, these data suggest that IL-11 is involved in the endogenous cytokine response to sepsis or NEC in preterm neonates. Further studies of IL-11 in neonates are warranted to assess its role both in platelet production and in mediation of the endogenous inflammatory response.

Abbreviations:

Introduction:
Thrombocytopenia (platelets <150 x 10^3/mm^3) occurs in nearly 1% of all live births, and in nearly 1 of 4 newborns admitted to NICU. Although many conditions may be associated with neonatal thrombocytopenia, low platelet counts in the first few days of life are often caused by fetomaternal conditions complicated by placental insufficiency and / or fetal hypoxia, e.g. maternal PET and fetal IUGR, whereas thrombocytopoiesis developing after the third day is usually secondary to sepsis or NEC. Affected neonates are reported to be at increased risk of intracranial hemorrhage, mortality, and long-term morbidity. As a result, many thrombocytopenic neonates receive repeated platelet transfusions—although there is little evidence that this improves outcome. However, it does increase their risk of transfusion-related complications, particularly infection. Impaired megakaryocytopoiesis and platelet production is increasingly recognized as a major contributory cause to many neonatal thrombocytopenias. This suggests that HGFs such as Tpo and IL-11 which stimulate megakaryocytopoiesis, may offer an alternative approach to platelet transfusion. Tpo is the main HGF regulator of platelet production in neonates, and endogenous Tpo levels during neonatal thrombocytopoiesis may be suboptimal, making Tpo an attractive HGF option for thrombocytopenic neonates. However, the clinical application of rhTpo seems likely to be limited by the development of neutralising antibodies and by the delay of six to seven days after administration before the platelet
count rises,\textsuperscript{24} a lag time during which many neonatal thrombocytopenias will resolve. IL-11 stimulates platelet production from megakaryocytes and is now commercially available for this purpose.\textsuperscript{25} Trials show that rhIL-11 stimulates megakaryocytosis both in adults and children thereby increasing platelet counts in thrombocytopenic patients.\textsuperscript{26-28} However, little is known about endogenous IL-11 in neonates,\textsuperscript{29,30} and rhIL-11 has not been used for thrombocytopenic neonates.

To investigate the role of IL-11 in neonatal thrombopoiesis, we measured plasma IL-11 in healthy term and stable preterm neonates and in preterm neonates at risk of developing two different presentations of neonatal thrombocytopenia\textsuperscript{20}: 1) early-onset thrombocytopenia (<72 h) associated with maternal PET or fetal IUGR; and 2) late-onset thrombocytopenia (>72 h) associated with late-onset sepsis and NEC.

**Subjects and Methods:**

This study was carried out at the Pediatric Department of Al-Minya University Hospital in the period from May 2003 to June 2004. The study included the following neonates:

**Term neonates studied at birth:**
Cord blood was obtained at birth from 15 term neonates (GA range, 38–42 wk; 8 boys, 7 girls). All of these neonates were born after uncomplicated pregnancies (i.e., no history of maternal PET, diabetes, thrombocytopenia, pyrexia during labor, or drug therapy), and none of the neonates developed significant neonatal complications.

**Preterm neonates studied at birth:**
Cord blood was obtained at birth in a total of 25 preterm neonates (GA range, 28–32 wk; 13 boys, 12 girls). Twelve of the 25 neonates were exposed to maternal PET or suffered fetal IUGR (birth weight below the 10th percentile),\textsuperscript{31} of which seven (58.3\%) developed early onset thrombocytopenia (platelets <150 x 10\(^3\)/mm\(^3\) by 72 h). The remaining neonates maintained normal platelet counts during the first 72 h. There were no significant differences in GA, severity of initial neonatal complications, and maternal drug exposure among these stable preterm neonates whether or not they had been exposed to maternal PET or suffered IUGR or early thrombocytopenia. Platelet counts were measured as part of the routine full blood count using coulter counter (Coulter Instruments, Model T660, Fullerton, California, USA), and confirmed by daily examination of blood films on all NICU patients.

**Preterm neonates studied at >72 h of age:**
Fifty preterm neonates (GA range, 28–36 wk; 26 boys, 24 girls) were studied after 72 h of age. Peripheral blood samples were obtained at the time of clinical sampling after clinical deterioration suggesting episodes of sepsis or NEC. A diagnosis of sepsis or NEC was confirmed in 30 of 50 neonates; the remaining 20 neonates whose eventual clinical course and laboratory findings did not support a diagnosis of sepsis or NEC formed the late healthy controls (all 20 maintained platelet counts >150 x 10\(^3\)/mm\(^3\)). Sepsis occurred in 20 of 30 and NEC in 10 of 30 neonates of whom 14 (70\%) and 5 (50\%) developed thrombocytopenia, respectively.

Sepsis was defined as a positive culture from blood, cerebrospinal fluid, urine, or endotracheal secretions plus one further laboratory indicator of infection: elevated CRP, chest radiograph suggesting consolidation, left shift or toxic granulation of neutrophils, or a new episode of thrombocytopenia; or in the absence of a positive culture, a clinical deterioration suggestive of sepsis in combination with two of the above indicators.\textsuperscript{32} NEC was defined as pneumatosis intestinalis on abdominal x-ray in combination with clinical symptoms and signs of the disease.\textsuperscript{33} If neonates had positive evidence of both sepsis and NEC they were assigned to the NEC group.

**Statistical Methods:**

Data were statistically represented in terms of range, mean ± SEM, and percentages. Student t test was used to compare between two groups as regards parametric data. Chi-square test was used to compare between two groups as regards non-parametric data. Pearson correlation was used to correlate two quantitative variables. For all tests, a probability (p) of less than 0.05 was considered significant.

**Results:**

**Healthy term neonates at birth:**
Plasma IL-11 was undetectable (<10 pg/ml) at birth in all 15 term neonates studied.
Preterm neonates studied at birth:
Plasma IL-11 at birth was <10 pg/ml in the majority of preterm neonates studied (21 of 25; 84%). Of the 13 neonates in this group who were not exposed to either maternal PET or suffered IUGR, three had detectable plasma IL-11 (mean, 11.23 ± 0.64 pg/ml), all of them at a low level (10.5, 11.5, and 11.7 pg/ml). All three were born after pregnancies complicated by PROM or chorioamnionitis, although none had positive blood cultures at birth or developed early onset thrombocytopenia. Of the remaining 12 neonates who were born after pregnancies complicated by PROM or chorioamnionitis, only one had detectable plasma IL-11 at birth (15 pg/ml). This neonate had IUGR and was also significantly thrombocytopenic (platelet count, 35 x 10^3/mm^3).

Preterm neonates with suspected sepsis or NEC:
Fifty neonates were screened for suspected sepsis or NEC at >72 h of age and had concurrent plasma IL-11 levels measured. Their clinical details are shown in Table I. In the 20 neonates in whom sepsis or NEC was unconfirmed and who maintained normal platelet counts, plasma IL-11 was undetectable. In contrast, in the 20 neonates with sepsis and 10 with NEC; plasma IL-11 was detectable in 10 (50%) of 20, and 4 (40%) of 10 (mean, 25.41 ± 21.3; range, 11.3–93 pg/ml). The difference between plasma IL-11 levels in the group of neonates with sepsis or NEC (n=30) compared with the group of neonates without these conditions (n=20) was significant (p=0.0003). However, there was no significant relationship between plasma IL-11 and clinical severity of sepsis or NEC as reflected by CRP on the day of sampling or peak CRP, or survival (Table I). Nineteen of the 30 neonates with sepsis or NEC developed thrombocytopenia. Of these, nine of 19 (47.4%) had detectable plasma IL-11, and 10 of 19 (52.6%) did not (p=0.746). In addition, there was no significant correlation between plasma IL-11 and platelet count in the preterm neonates studied as a single group (r =-0.013; p = 0.267).

Discussion:
Recent evidence suggests that hyporegenerative thrombocytopenia is common in sick neonates. Clinical trials show that rhIL-11 stimulates human megakaryocytopoiesis, making rhIL-11 a potential therapy for thrombocytopenic neonates. However, there are few data about IL-11 and its role in thrombopoiesis in neonates. To investigate this we measured plasma IL-11 levels at birth in healthy term neonates and at birth and during the neonatal period in stable preterm neonates and preterm neonates at high risk of developing thrombocytopenia.

We found that plasma IL-11 levels were undetectable at birth in healthy term neonates and the vast majority of stable preterm neonates. As the level of sensitivity of the IL-11 by ELISA was 10 pg/ml, this indicates that the normal, physiologic level of circulating IL-11 at birth is <10 pg/ml. Only four of the 25 preterm neonates tested at birth had detectable IL-11 (three suffered PROM or chorioamnionitis and one had fetal IUGR and thrombocytopenia). By contrast, almost half of the 20 neonates with sepsis or NEC had elevated plasma IL-11 (>10 pg/ml). However, despite the known effects of IL-11 on megakaryocytopoiesis in vitro in adults and in vivo in adults and children with chemotherapy-induced thrombocytopenia, we found no correlation between plasma IL-11 levels and platelet count in the neonates we studied. The majority of neonates with thrombocytopenia did not have elevated plasma IL-11, and elevated IL-11 levels were found equally in neonates with and without thrombocytopenia.

Although comparison of data among different age groups has to be interpreted with caution, our data are consistent with those of Cremer et al., who measured IL-11 levels in thrombocytopenic children with aplastic anemia and a variety of inherited platelet disorders and found detectable IL-11 in only 27% of thrombocytopenic children. However, by contrast, our data differ from those of Chang et al., who found a

![Table I: Clinical details of 50 preterm neonates presenting after the age of 72 h with suspected sepsis or NEC](image)

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<td>Gestational age (weeks)</td>
<td>Birth weight (kg)</td>
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<tr>
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<td>Mean ± SEM</td>
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<td>11 (22%)</td>
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<td>28–36</td>
<td>1.450 ± 0.06</td>
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<td>1.050–1.800</td>
<td>6(12%)</td>
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<tr>
<td>Birth weight (kg)</td>
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<td>Sex Male</td>
<td>Thrombocytopenia</td>
<td>Mortality</td>
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<td>8(16%)</td>
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<td>Placental insufficiency</td>
<td>Thrombocytopenia</td>
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<td>9 (47.4%)</td>
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<td>10 (52.6%)</td>
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close reciprocal relationship between plasma IL-11 levels and platelet count in thrombocytopenic children undergoing chemotherapy.

Together these data suggest that IL-11 plays a role in platelet production that is different from that of Tpo. For Tpo there is now good evidence that plasma levels consistently increase as megakaryocytopenesis and platelet production decrease.37,38 Given that IL-11 clearly stimulates megakaryocytopenesis in vitro and in vivo (at least in adults and children), the absence of a correlation between plasma IL-11 and platelet count in our neonatal patients could be explained in a number of ways. First, plasma IL-11 levels may increase only in the setting of severe thrombocytopenia. Our data would generally be consistent with this: plasma IL-11 was not increased in the neonates with early thrombocytopenia secondary to maternal PET or IUGR if the thrombocytopenia was modest (50–150 x 10^3/mm^3). However, IL-11 was increased in the neonate with severe thrombocytopenia secondary to IUGR and in slightly less than half of the neonates with significant thrombocytopenia secondary to sepsis or NEC. Second, although the contribution of plasma IL-11 to platelet production might be modest, under physiologic conditions, locally produced IL-11 (not detectable in the systemic circulation), either alone or in synergy with other megakaryocytopenic cytokines such as IL-6 and stem cell factor, may play a significant role in platelet production. Such a model would be consistent with the observed effects of IL-11 in vitro in which it seems to exert its effects predominantly on more mature cells of the megakaryocyte lineage and to have a greater effect in the presence of stem cell factor and IL-6.39 Third, we were only able to obtain a single sample for IL-11 measurement and, therefore, may have missed transiently elevated levels in some neonates. Finally, it may be that IL-11 does not play any significant role in neonatal megakaryocytopenesis and that the IL-11 levels we measured reflect a different aspect of its known pleiotropic properties.39

In our neonates, IL-11 was most commonly detectable during sepsis and NEC. Both these conditions are characterized by a local and systemic inflammatory response mediated by the major proinflammatory cytokines, tumor necrosis factor-α, IL-1B, and IL-6.40-42 IL-11 is known to play a role in the endogenous pro- and counter-inflammatory response down-regulating the proinflammatory response by modifying the release of the above cytokines.43 This action underlies the development of IL-11 as a therapeutic agent in conditions characterized by chronic inflammation "e.g. rheumatoid arthritis and Crohn’s disease".44 The elevated IL-11 levels we found in neonates with sepsis and NEC may therefore reflect their endogenous counter-inflammatory response rather than their megakaryocytopenic or thrombopoietic status. This may also explain the elevated plasma IL-11 we found at birth in the three healthy neonates with a history of PROM or chorioamnionitis. Clearly further studies of IL-11 in healthy and sick or thrombocytopenic neonates will be required to define the significance of detectable IL-11 levels in these patients.

In summary, the data presented in this research show that healthy neonates with normal platelet counts do not have detectable levels of plasma IL-11. In contrast, many preterm neonates with proven sepsis or NEC have elevated plasma levels of IL-11. However, as there was no correlation between plasma IL-11 and platelet count, the significance of these findings is unclear. Therefore, further work is indicated to delineate the role of IL-11 in neonatal platelet production in view of both the therapeutic availability of rhIL-11 and the high incidence of thrombocytopenia necessitating platelet transfusion in sick neonates. Finally, the observation that elevated IL-11 is most consistently seen during sepsis and NEC also deserves further evaluation in view of the potential role of IL-11 as an important counter-inflammatory cytokine in these potentially devastating inflammation-mediated conditions in neonates.

References:

38. Emmons RV, Reid DM, Cohen RL. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 1996; 87: 4068-71.
40. Morecroft JA, Hamilton PA, Holmes SJ. Plasma interleukin-6 and tumour necrosis factor levels as predictors of disease severity and outcome in