

# THE ROLE OF IL-6 AND ERYTHROPOIETIN IN CHILDREN WITH SECONDARY THROMBOCYTOSIS IN INFECTION AND ANEMIA

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**Abstract:** The main regulation factor for thrombopoiesis is thrombopoietin. A relationship between the degree of thrombocytosis and the level of IL6 has not been established yet. The mechanism of secondary thrombocytosis due to iron deficiency anemia is not fully known. A cross-reaction, due to structural similarity of EPO and TPO, involving the c-Mpl receptor is suspected but not yet confirmed. Method: The study is prospective, made up of 39 children with secondary thrombocytosis, anemia and upper respiratory infection. We determined full blood count, platelet and erythrocyte parameters, CRP, ESR, IL6, EPO. Conclusions: 66,66% patients had levels of IL6 above normal. The mean value was 10,210 (std. dev. 12,7530, std. error 2,04). 15 of these patients had CRP above normal, 18 had ESR above normal, 23 had leukocytosis, suggesting secondary thrombocytosis due to infection. All patients with iron level below normal values had increased levels of either EPO or IL6, most of them with increased IL6, suggesting intra-infection cause for anemia and regulation mechanism of erythropoiesis that do not involve EPO.

**Keywords:** thrombocytosis, IL6, EPO, infection, anemia

**Rezumat:** Factorul de reglare principal al producției trombocitare este trombopoietina (TPO). Rezultatele unor studii nu au demonstrat o relație de proporționalitate indirectă între gradul de trombocitoză și nivelul seric al TPO. Mecanismul trombocitozei secundare în contextul anemiei feriprive nu este complet elucidat. Se presupune o reacție încrucișată a eritropoietinei (EPO) la nivelul receptorilor trombocitari c-Mpl datorată asemănării structurale a lanțurilor de aminoacizi. Material și metodă. Studiul este prospectiv, un lot de 39 de pacienți, cu trombocitoză secundară și infecție de căi respiratorii superioare și/sau anemie feriprivă, la care s-au determinat hemoleucogramă completă, IL6, EPO, sideremie, CRP, VSH. Concluzii. 66.66% dintre pacienți înregistrează nivel seric IL6 la valori normale. Valoarea medie a fost de 10,210 (std. dev. 12,7530, std. error 2,04). 15 pacienți dintre cei cu nivel crescut de IL6 (57,69%) aveau CRP pozitivă, 18 pacienți cu VSH peste valorile normale (69.23%), 23 de pacienți din 26 aveau leucocitoză, sugerând trombocitoză secundară unui proces inflamator. Toți pacienții cu sideremie sub pragul minim acceptat au avut IL6 sau EPO peste valoarea maximă acceptată, majoritatea cu IL6 crescut, fapt ce sugerează

etiologia intra-infecțioasă a anemiei și mecanisme ce nu depind de EPO de reglare a eritropoiezei în acest context.

**Cuvinte cheie:** trombocitoză secundară, IL6, EPO, infecție, anemie

## INTRODUCTION

A normal platelet count is necessary because of their role in vascular repairing and initiation of thromb formation. Recent studies show the implication of platelets in wounds healing, immune response and tumour molecular biology. With normal values between 150.000-400.000/dl, the term thrombocytosis is used for a number higher than 440.000/dl. Persons who have a number around the maximum considered normal have a double incidence of cardiac diseases, and, on studies conducted on lab animals and humans with metastatic cancer or tumours, it has been shown that thrombocytosis is an indicator for poor outcome. With a lifespan of 10 days, a volume around 5 liters and one third retained in the spleen, the human body produces  $1 \times 10^{11}$  thrombocytes daily, a number that can increase tenfold in circumstances of accelerated production. Main regulation factor of thrombopoiesis is thrombopoietin (TPO). Studies failed to show a correlation between level of TPO and degree of thrombocytosis, suggesting additional mechanisms. Other known factor involved in different degrees in thrombopoiesis are IL-6, IL-3, IL-11, SDF-1, IFN- $\alpha$ , TGF- $\beta$  (7,8). Studies have shown and indirect involvement of IL-6 through stimulation of TPO production (8,9). The mechanism in secondary thrombocytosis due to iron deficiency anemia is not fully known. A cross-reactivity is assumed, between erythropoietin (EPO) and TPO at the level of c-MPL receptor, due to similarities in amino acid chains. This theory is controversial, results from different studies being contradictory, but high levels of EPO have been noticed in patients with secondary thrombocytosis, suggesting its involvement in different stages of megakariopoiesis.(10,11,12,13)

## PURPOSE OF THE STUDY

This study observes the values of IL-6 and EPO in patients with secondary thrombocytosis due to infection with or without iron deficiency anemia, the correlations among level of IL-6, EPO, clinical evolution,

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inflammatory proteins and complete blood count.

### MATERIALS AND METHOD

A prospective study that involves 39 patients with secondary thrombocytosis and upper respiratory tract infection, with or without iron deficiency anemia at the age of 6-12 months, 1-2 years, 2-5 years. Exclusion criteria: chronic treatment with iron, folic acid, therapy with vinca alkaloid, cortisone, adrenaline, miconazol, clozapine, chemotherapy, low molecular weight heparin, enzyme replacement therapy, disease known as cause of thrombocytosis. Investigations: platelet count, complete blood count, ESR, CRP, haptoglobin, procalcitonin, hemoglobin, VEM, CHEM, RDW, iron, ferritin, platelet index: PDW, P-LCR, MPV, IL-6, EPO in the dynamics - day 1-7-14-21.

Laboratory: SYSMEX XS-1000, Hitachi 912 (Pediatric Clinic of Sibiu), IL6 and EPO IMMULITE System (Clinical Emergency Hospital of Sibiu).

### RESULTS AND DISCUSSIONS

66.6% patients had IL-6 levels above normal limit. Mean value was 10,210 (std. dev. 12,7530, std. error 2,04). A study conducted on patients with essential thrombocythemia and secondary thrombocytosis hasn't shown any correlation between IL-6 levels and degree of thrombocytosis, suggesting additional mechanism involved in secondary thrombocytosis due to infection (14, 15). In specialized publication is described a *in vitro* growth of megakariocytic colonies after administration of IL-6, and that the primary role of IL-6 in thrombopoiesis is in the differentiating process. *In vivo* administration of IL-6 results in modest thrombocytosis (8). Other authors suggest that the secondary thrombocytosis in infection/inflammation is due to hepatic stimulation of TPO (7). The results of our study sustain the hypothesis of IL-6 involvement in secondary thrombocytosis in infection, not being able to determine TPO, but we cannot suggest a mechanism. Out of 26 patients with high level of IL-6 only 6 had high levels of EPO, suggesting the main mechanism of secondary thrombocytosis in infection is mainly due to TPO (through IL-6, and other cytokines). 15 patients had positive CRP, 18 with ESR above normal limit, 23 had leukocytosis, pleading for an inflammatory cause of thrombocytosis. Considering the dynamics of every inflammatory index observed in our study we can plausibly assume that the thrombocytosis a result of IL-6 stimulating TPO production, associating mechanisms with different importance, not yet known. 12 (under 50%) patients out of 26 had low levels of hemoglobin and only 3 with levels below 8g/dl.

A patient with bacterial pneumonia had severe degree of anemia (hemoglobin 4.1g/dl), significant degree of thrombocytosis (680,000/mm<sup>3</sup>), high degree leukocytosis, and increased levels of IL-6 and EPO, very low levels of ferritin and iron. A future study can be focused on this kind of patients, in order to observe the mechanism of secondary thrombocytosis in patients that associate high degree of iron deficiency anemia, the

mechanism being unknown, and current data being contradictory (cross-reaction between EPO and TPO is not confirmed in *in vivo* tests). This patient had prolonged evolution, suggesting that high degree thrombocytosis could be an indicator for poor outcome (study project). Another patient, observed in a PICU Clinic, diagnosed with bacterial thrombocytosis, had platelet count over 1.000.000/mm<sup>3</sup> since admittance and for 2 weeks more, even though the clinical evolution was better and inflammatory indicators were normal. In a future study the parameters' dynamics will be studied in order to determine the role of thrombocytosis as an indicator of poor outcome.

We couldn't determine a correlation between degree of thrombocytosis, level of IL-6 and between levels of IL-6 and CRP. We noticed a high degree of leukocytosis in patients with significant levels of IL-6 but only in 50% of the patients, fact that doesn't allow a plausible statistical conclusion, but does suggest a correlation between level of IL-6 and degree of leukocytosis. PDW and MPV may suggest active platelet production, high PDW and normal MPV. None of the patients had PDW out of the reference range and MPV was normal in all patients. We can conclude that, when assessed, the platelet production was normal, the stimulating processes were over, even though 65% of the patient had increased levels of IL-6. The mechanism of IL-6 involvement described by different authors includes a feedback control process of TPO, partly explaining high levels of TPO and normal platelet (according to PDW) production in patients with secondary thrombocytosis.

P-LCR was in normal limits in all patients. P-LCR indicates large circulating platelets, associated with thrombotic incidents being more active. The results suggest that in secondary thrombocytosis there is an increased production of normal (aspect and function) platelets, with a low risk of thrombotic incidents. P-LCR, PDW and MPV can be indicators for chronic myeloid leukaemia (values over normal limits) (16). Although it is not a common disease in children, these indicators can be used for screening in patients with thrombocytosis. None of the patients included in this study had criteria for chronic myeloid leukaemia. Out of the 11 patients with high level of EPO, 7 had low iron levels, and four of them having also increased levels of IL-6 suggesting supplementary mechanisms involving EPO in patients with infection and anemia. 5 out of 7 patients had high EPO, low iron, and high RDW suggesting increased erythropoiesis; these patients had haemoglobin level around 8g/dl. 10 patients with high level of EPO had low VEM.

An inverse proportion relation can be established between EPO and VEM, all patients with EPO higher than 100 had VEM around 50-60. We noticed significant increases in EPO levels in patients with haemoglobin level of 8 and below, VEM under 80, data similar to other authors, suggesting a threshold for stimulating erythropoiesis.

All patients with low iron levels had IL-6 had

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high levels of either IL-6 or EPO, most of them with increased IL-6, suggesting infection as the cause of anemia, and supplementary mechanism for erythropoiesis regulation that do not involve EPO; most patients had low VEM and increased RDW. Ferritin and other indicators for iron deficiency anemia could not be determined.

We could not establish a proportion relationship between EPO and level of iron.

Mean age was 15.03 months (std dev 10,363), 66,7% under 12 months, 89,7% under 24 months. We could establish a proportion relationship between age, degree of thrombocytosis or level of IL-6 and EPO, but we noticed a high incidence under 2 years. Patients were included in the study according to the criteria in the order of hospital admission. Only 6 patients needed hospitalization more than 7 days, 4 of them with high level of IL-6, all four with platelets over 600.000/mm<sup>3</sup>. The other two patients admitted for more than 7 days also had platelets over 600.000/mm<sup>3</sup> but normal level of IL-6. We can assume that high IL-6 and platelets over 600.000/mm<sup>3</sup> could indicate poor outcome (requires large scale study).

### CONCLUSIONS

Highest incidence of secondary thrombocytosis was notice in patients below 2 years of age but with no proportion relationship between IL-6, EPO, degree of thrombocytosis and age.

We can plausibly conclude that IL-6 stimulates production of platelets through (according to literature data) hepatic stimulation of TPO production in secondary thrombocytosis due to infection. In order to determine the exact mechanism TPO dosage and dynamic study of every parameter is needed.

Secondary thrombocytosis in infections and anemia seems to be modulated by mechanism involving primarily IL-6/TPO. There are also mechanisms modulated through EPO, due to the fact that we noticed patients with normal IL-6, thrombocytosis, high EPO and low iron.

Is it possible that IL-6 is involved, either through TPO or other mechanism, in occurrence of leukocytosis because in 50% of patients we could establish a direct proportion relation between IL-6 and degree of leukocytosis.

P-LCR can be used for differential diagnosis of thrombocytosis/thrombocythemia in patients with infection and anemia, this indicator being normal in all patients included in the study. P-LCR, PDW and MPV can be used for diagnosis of chronic myeloid leukaemia.

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