The diagnostic value of plasma thrombopoietin levels and platelet autoantibodies

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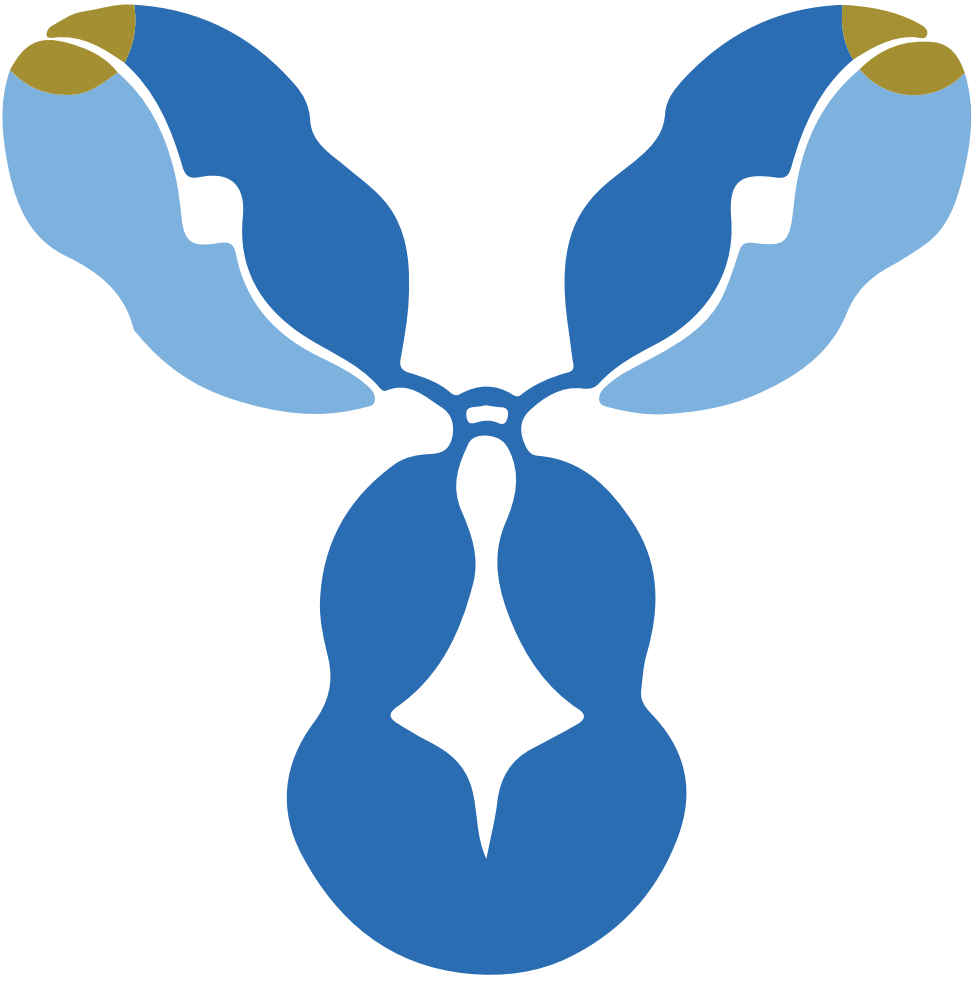

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CHAPTER 3

Fetal and neonatal thrombopoietin levels in alloimmune thrombocytopenia.

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Fetal and Neonatal Thrombopoietin Levels in Alloimmune Thrombocytopenia

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Abbreviations

EDTA	Ethylenediaminetetra-acetate
HDN	Haemolytic disease of the newborn
HPA	Human platelet antigens
ITP	Idiopathic thrombocytopenic purpura
IVIg	Intravenous immunoglobulins
NAIT	Neonatal alloimmune thrombocytopenia
Tpo	Thrombopoietin

Summary

Thrombopoietin (Tpo) is the main hematopoietic growth factor for platelet production. Plasma Tpo levels in autoimmune thrombocytopenic (ITP) patients are normal or slightly elevated. Although thrombocytopenia exists, Tpo levels are not increased because the produced megakaryocytes and platelets can bind circulating Tpo, thereby normalizing Tpo levels. In this report plasma samples from fetuses and neonates with neonatal alloimmune thrombocytopenia (NAIT), a different form of immune thrombocytopenia, were measured. Umbilical cord samples of 50 fetuses before treatment because of severe thrombocytopenia, 51 fetuses after treatment and peripheral blood samples of 21 untreated newborns with NAIT were analyzed. As controls, plasma Tpo levels were determined in 21 umbilical cord samples of 14 non-thrombocytopenic fetuses with hemolytic disease (HDN) due to red blood cell alloimmunization and in umbilical cord samples of 51 healthy newborns. The values were also compared with the plasma Tpo levels in 193 healthy adults.

Mean Tpo levels from the groups of fetuses and neonates including both NAIT and control plasma were slightly but significantly elevated compared to levels in healthy adults. Tpo levels in NAIT samples were not significantly different from the levels in HDN samples or in samples from healthy newborns. Thus, like in ITP, in NAIT patients normal Tpo levels are present.

Keywords: thrombopoietin, Neonatal alloimmune thrombocytopenia, NAIT

Introduction

Thrombopoietin is the main haematopoietic growth factor for the megakaryocytic lineage and therefore for platelet production. In adults Tpo is mainly produced by the liver and kidney.1 The concentration of Tpo in blood has been found to largely depend on the number of megakaryocytes in the bone marrow and platelets in the peripheral blood. The plasma level of Tpo is regulated by binding of Tpo to its receptor (mpl) expressed by platelets and megakaryocytes. 2-6 After binding, Tpo is eventually destroyed together with the platelets. We and others have shown the diagnostic value of measurement of plasma Tpo levels for discrimination between thrombocytopenia caused by megakaryocyte and platelet production failure (highly elevated Tpo levels) and thrombocytopenia caused by elevated platelet destruction observed in autoimmune thrombocytopenia (normal or only slightly elevated Tpo levels).7-11 In the latter disorder, megakaryocyte numbers in the marrow are normal or increased as is platelet production. Because sufficient numbers of platelets are produced, even though their destruction may be rapid, Tpo levels are comparable to that in healthy individuals.

NAIT occurs in about 1 in 1000 births and results from maternal alloimmunization against platelet antigens present on fetal platelets but absent on maternal platelets. Human Platelet Antigen 1a (HPA-1a) is the most frequently offending antigen, accounting for approximately 85% of cases of severe thrombocytopenia. Because of severe thrombocytopenia in utero, in up to 10% of cases intracerebral haemorrhage occurs, often leading to severe neurological sequelae or death.12-14 As in autoimmune thrombocytopenia, in NAIT, fetal platelets are destroyed as a result of antibody binding. Furthermore, it is suggested that reduced megakaryocyte platelet production, or even megakaryocyte destruction, resulting from anti-HPA-1a antibody binding might also contribute to the thrombocytopenia in NAIT patients.15-17 In the current study, Tpo levels were measured in 129 fetal and neonatal NAIT plasma samples and 72 fetal and neonatal control plasma samples. We found Tpo levels in fetuses and neonates with NAIT to be not significantly different from the levels in fetal and neonatal controls.

Materials and methods

Patients

The study was performed with approval of all the involved institutes and all measurements were performed after informed consent.

Plasma Tpo levels were measured in samples from fetuses with NAIT (n=113), full term newborns with NAIT (n=16) and in nonthrombocytopenic fetuses and neonates, i.e. 21 first or subsequent umbilical cord samples of 14 fetuses with HDN and 51 umbilical cord plasma samples of healthy, full term newborns (Table 1). The samples were collected in New York (Weill Medical College), Leiden

(Leiden University Medical Center) and Oxford (John Radcliffe Hospital). Tpo measurement was performed in the Central Laboratory of the Blood Transfusion Service, Sanquin Diagnostics, Amsterdam. All NAIT cases included in this study involved HPA-1a alloantibodies. Sixteen neonatal samples and 50 umbilical cord samples of 50 fetuses were from NAIT patients who were not treated, Sixty-three umbilical cord samples of 51 fetuses were from NAIT patients who received treatment in utero via administration to their mothers; 40 of these 63 samples were from 34 fetuses who were also included in the untreated group. Treatment consisted of IVIg (1 gram/Kg/wk) with or without corticosteroids (prednisone 1 mg/Kg/day or dexamethason 1.5 mg/d) administered to the mother during pregnancy and/or intra-uterine platelet transfusions administered to the fetus, after umbilical cord sampling. Samples were drawn, at 24±3 (mean±std.) wk of pregnancy for the untreated fetal group, 33±4 wk of pregnancy for the treated fetal group 28±4 wk of pregnancy for the HDN group, at d 1-7 post partum for the neonatal NAIT group and directly post partum for the neonatal control group. If treatment was necessary and consisted of intrauterine platelet transfusions, samples were drawn before transfusions were given (this was also true for the patients included in the untreated fetal NAIT group). Fetal and neonatal blood samples were aspirated in syringes with EDTA or heparin. Platelet counts were performed with a coulter counter (Beckman Coulter, Inc, Fullerton, CA, U.S.A.). Methods used for aspiration of samples and for intra-uterine platelet transfusions were comparable in the different institutes and are described earlier.18,19 No problems occurred that could be related to the bloodsampling.

Plasma Tpo levels of 193 healthy adults to use as controls were measured previously in another study.20

Tpo ELISA

A solid phase sandwich ELISA for measurement of plasma Tpo concentrations was performed as previously described.20 Normal Tpo levels, as determined in a population of 193 healthy individuals, were 11±8 A.U./ml (range 4-32 A.U., 2.5th- 97.5th percentile). One AU equals 9 pg of recombinant Tpo (Research Diagnostics Inc. Flanders NJ USA)

Statistical analysis

Statistical analysis was performed in SPSS for Windows, release 6.1.3 (SPSS Inc., Chicago, IL, U.S.A.).

For comparison of groups the Kruskal Wallis ANOVA and the Mann-Whitney U/ Wilcoxon Rank Sum W Test was used. The correlation between two variables was calculated with Spearman correlation coefficients.

Results

The platelet count was 68±28 x 109/L (mean ± std) in the full term, untreated newborn NAIT group, 37± 34 x 109/L in the untreated fetal NAIT group, 76±61 x 109/L in the treated fetal NAIT group and 190±53 x 109/L in the nonthrombocytopenic hemolytic disease group (Table 1). Platelet counts were not determined in the healthy neonates but they had no signs or symptoms of bleeding. Further study will be necessary to analyze the influence of treatment on the fetal platelet numbers, but although the mean platelet numbers in the treated fetal group is significant higher ($p=0.003$, Table 1) than in the untreated group, the statistical analysis of platelet numbers in fetuses (n=40) included in both the untreated and treated fetal NAIT groups did not show a significant difference ($p=0.06$). Plasma Tpo levels were 21±13 A.U./ml (range 9-47) in the neonatal NAIT samples (n=16), 26±18 A.U./ml (range 4-89) in the untreated fetal NAIT samples (n=50), 26±17 A.U./ml (range 6-97) in the treated fetal NAIT samples (n=63), 27±24 (range 2-93) in the samples from healthy neonates (n=51) and 18±8 A.U./ml (range 5-34) in the fetal HDN samples(n=21).

Post hoc testing showed that plasma Tpo levels in the samples of the NAIT neonates, the untreated and the treated NAIT fetuses were comparable with Tpo levels in the samples of the non-thrombocytopenic HDN controls ($p=0.09$, 0.03 and 0.8, respectively) and the healthy neonates ($p=0.8$, 0.5 and 0.3, respectively) (Fig. 1). The plasma Tpo levels in both the fetal HDN and the healthy newborn controls were slightly, though significantly ($p<0.001$) higher than plasma Tpo levels in healthy adults. The same held true when comparing the Tpo values of the NAIT neonates and the treated and untreated NAIT fetuses with the Tpo levels in healthy adults ($p<0.001$). No difference in Tpo plasma levels were detected comparing plasma samples from fetuses with NAIT before and after treatment. No statistically significant correlation was detected between platelet numbers and Tpo levels, either in the total NAIT group (Fig. 2) or in the separate groups or between Tpo levels and age at the time of blood sampling. Furthermore, the anticoagulant used during blood collection or the institute at which blood was drawn did not influence the test results (data not shown).

Table 1: Patient characteristics

	Number of samples	Number of patients	Gestational age (weeks) (mean ± std.)	Platelet count (x 10^9/L) (mean ± std.)	Plasma Tpo levels (mean ± std.) (A.U./ml) (pg/ml)	
untreated fetal NAIT	50	50	24 ± 3	37 ± 34	26 ± 18	234±162
Treated fetal NAIT	63	51	33 ± 4	76 ± 61	26 ± 17	234±153
full term newborns NAIT	16	16	1-7 days p.p.	68 ± 28	21 ± 13	189±117
Control fetuses (HDN)	21	14	28 ± 4	190 ± 53	18 ± 8	162±72
Healthy full term newborns	51	51	directly p.p.	n.t.	27 ± 24	243±216
Healthy adults	193	193	38±11*	150-450	11 ± 8	99±72

p.p.: post partum; n.t.: not tested; *age [yrs]

Figure 1. Box plots of Tpo levels in NAIT and Controls. Box plots represent the interquartile range that contains 50% of the values. The whiskers are lines that extend ffom the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Tpo values of all separate groups were elevated compared with the adult Controls; p < 0.001.

Figure 2. Platelet counts w plasma Tpo levels in NAIT. Platelet counts and plasma Tpo levels are not correlated. Tpo levels are comparable in the different groups (p > 0.1), with or without the two outliers. The two outliers cannot be explained by differences in clinical data, treatment, or sampling procedures. Perhaps Tpo release ffom platelets occurred in vitro as a result of some unwanted platelet clotting in the EDTA samples.

Discussion

Screening for NAIT is not routinely performed and treatment of the initial case in a family mostly consists of post-natal treatment with intravenous gammaglobulin (IVIg) and/or platelet (negative for the antigen involved) transfusions. In subsequent pregnancies treatment of the mother with IVIg and corticosteroids and of the fetus with intrauterine platelet transfusions are possible and often employed. Trials are in progress to clarify the relative merits of each form of treatment although increasingly avoidance of fetal sampling is desired if feasible. We and others detected normal or only slightly elevated Tpo levels in ITP patients.7-10 We now find that Tpo levels in NAIT fetuses are also normal or only slightly elevated compared to levels in non-thrombocytopenic age-matched controls. Thus, as in ITP, in alloimmune mediated thrombocytopenia it appears that normal Tpo removal occurs resulting in normal levels being maintained in the circulation. This indicates that there is predominantly normal platelet production in fetuses and newborns with NAIT. It seems to contradict the hypothesis that the anti-HPA-1a antibodies would bind to glycoprotein IIb/IIIa on megakaryocytes and either inhibit thrombocytopoiesis or destroy them outright.15-17 We, like others21,22, found slightly but significantly higher Tpo levels in fetal/neonatal plasma compared to plasma from adults (Fig. 1). For the slightly elevated Tpo levels in fetuses/neonates (also with normal platelet counts) several explanations are possible - e.g. an increased production of Tpo considering that in fetuses and neonates the spleen may also contribute to Tpo production23; less expression of Tpo receptors on megakaryocytes in neonates, as described by Kuwaki et al24, resulting in slower removal; or an increased constitutive production of Tpo, inasmuch as the rate of growth of the fetus as well as the neonate is remarkably high, and, therefore, there is a continuous requirement for blood elements for neovasculature. Further study is necessary to explain the higher Tpo levels in neonates compared to adult levels.

The down regulation of Tpo plasma levels by platelets appears to prevent an increase in Tpo levels in all cases in which thrombocytopenia is due to increased platelet destruction. Thus, the fact that, as in ITP patients, Tpo levels in NAIT patients are not or only marginally increased may have important therapeutic implications. Injection of Tpo, or Tpo-mimicking peptide, could likely be applied to increase platelet production and platelet count. This approach has been shown to be effective in chimpanzees with HIV-ITP25 and in a few patients.26

References

1. Kaushansky K 1998 Thrombopoietin. N Engl J Med 339: 746-54.
2. Debili N, Wendling F, Cosman D, Titeux M, Florindo C, Dusanter-Fourt I, Schooley K, Methia N, Charon M, Nador R et al 1995 The Mpl receptor is expressed in the megakaryocytic lineage from late progenitors to platelets. Blood 85: 391-401.
3. Broudy VC, Lin NL, SAbath DF, Papayannopoulou T, Kaushansky K 1997 Human platelets display high affinity receptors for thrombopoietin. Blood 89: 1896-904.
4. Folman CC, de Jong SCM, de Haas M, von dem Borne AEGKr 2001 Analysis of the kinetics of thrombopoietin uptake during platelet transfusion. Transfusion 41: 517-21.
5. Fielder PJ, Hass P, Nagel M, Stefanich E, Widmer R, Bennett GL, Keller GA, de Sauvage FJ, Eaton D 1997 Human platelets as a model for the binding and degradation of thrombopoietin. Blood 89: 2782-8.
6. Li J, Xia Y, Kuter DJ 1999 Interaction of thrombopoietin with the platelet c-mpl receptor in plasma: binding, internalization, stability and pharmacokinetics. Br J Haematol 106: 345-56.
7. Tahara T, Usuki K, Sato H, Ohashi H, Morita H, Tsumura H, Matsumoto A, Miyazaki H, Urabe A, Kato T 1996 A sensitive ELISA for measuring thrombopoietin in human serum: serum thrombopoietin levels in healthy volunteers and in patients with haematopoietic disorders. Br J Haematol 93: 783-8.
8. Emmons RVB, Reid DM, Cohen RL, Meng G, Young NS, Dunbar CE, Shulman NR 1996 Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 87: 4068-71.
9. Kosugi S, Kurata Y, Tomiyama Y, Tahara T, Kato T, Tadokoro S, Shiraga M, Honda S, Kanakura Y, Matsuzawa Y 1996 Circulating thrombopoietin level in chronic immune thrombocytopenic purpura. Br J Haematol 93: 704-6.
10. Porcelijn L, Folman CC, Bossers B, Huiskes E, Overbeeke MA, vd Schoot CE, de Haas M, von dem Borne AE 1998 The diagnostic value of thrombopoietin levels measurements in thrombocytopenia. Thromb Haemost 79: 1101-5.
11. Cohen-Solal K, Vitrat N, Titeux M, Vainchenker W, Wendling F 1999 High-level expression of Mpl in platelets and megakaryocytes is independent of thrombopoietin. Blood 93: 2859-66.
12. Mueller-Eckhardt C, Kiefel V, Grubert A, Kroll H, Weisheit M, Schmidt S, Mueller-Eckhardt G, Santoso S 1989 348 cases of suspected neonatal alloimmune thrombocytopenia. Lancet 1: 363-6.
13. Williamson LM, Hackett G, Rennie J, Palmer CR, Maciver C, Hadfield R, Hughes D, Jobson S, Ouwehand WH 1998 The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening. Blood 92: 2280-7.
14. von dem Borne AEGK, van Leeuwen EF, von Riesz LE, van Boxtel CJ, Engelfriet CP 1981 Neonatal alloimmune thrombocytopenia: detection and characterization of the responsible antibodies by the platelet immunofluorescence test. Blood 57: 649-56.
15. Warwick RM, Vaughan J, Murray N, Lubenko A, Roberts I 1994 In vitro culture of colony forming unit-megakaryocyte (CFU-MK) in fetal alloimmune thrombocytopenia. Br J Haematol 88: 874-7.
16. Bizzaro N and Dianese G 1988 Neonatal alloimmune amegakaryocytosis. Case report. Vox Sang 54: 112-4.
17. Tsukada T, Ueyama J, Miyakoshi S, Morinaga S, Mutoh Y, Yamaguchi H, Matsuya S 1990 [Diagnostic significance of the number of bone marrow megakaryocytes in cases with decreased platelet production evidenced by platelet kinetic study]. Rinsho Ketsueki 31: 903-10.

18. Bussel JB, Zabusky MR, Berkowitz RL, McFarland JG: Fetal alloimmune thrombocytopenia. N.Engl.J.Med. 337:22, 1997
19. Kanhai HHH, Porcelijn L, van Zoeren D, Klumper F, Vietor H, Meerman RH, Brand A: Antenatal care in pregnancies at risk of alloimmune thrombocytopenia: report of 19 cases in 16 families. Eur J Obstet Gynecol Reprod Biol 68:67, 1996
20. Folman CC, von dem Borne AEGKr, Rensink IHJAM, Gerritsen W, van der Schoot CE, de Haas M, Aarden L 1997 Sensitive measurements of thrombopoietin by a monoclonal antibody based sandwich enzyme-linked immunosorbent assay. Thromb Haemost 78: 1262-97.
21. Sola MC, Juul SE, Meng YG, Garg S, Sims P, Calhoun DA, Dame JB, Christensen D 1999 Thrombopoietin (Tpo) in the fetus and neonate: Tpo concentrations in preterm and term neonates, and organ distribution of Tpo and its receptor (c-mpl) during human fetal development. Early Hum Dev 53: 239-50.
22. Ishiguro A, Nakahata T, Matsubara K, Hayashi Y, Kato T, Suzuki Y, Shimbo T 1999 Age-related changes in thrombopoietin in children: reference interval for serum thrombopoietin levels. Br J Haematol 106: 884-8.
23. Wolber EM, Dame C, Fahnenstich H, Hofmann D et al 1999 Expression of the thrombopoietin gene in human fetal and neonatal tissues. Blood 94: 97-105.
24. Kuwaki T, Hagiwara T, Yuki C, Kodama I, Kato T, Miyazaki H 1998 Quantitative analysis of thrombopoietin receptors on human megakaryocytes. FEBS lett 427: 46-50.
25. Harker LA, Marzec UM, Novembre F, Sundell IB, Waller EK, Karpatkin S, McClure HM, Kelly AB, Stead RB 1998 Treatment of thrombocytopenia in chimpanzees infected with human immunodeficiency virus by pegylated recombinant human megakaryocyte growth and development factor. Blood 91: 4427-33.
26. Harker LA 1999 Physiology and clinical applications of platelet growth factors. Curr Opin Hematol 6: 127-34.