Chapter 8

The Role of Thrombopoietin in Post-Operative Thrombocytosis

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The Role of Thrombopoietin (Tpo) in Post-Operative Thrombocytosis

Claudia C Folman¹,², Marike Ooms¹, Bart Kuenen¹,³, Shreyas M de Jong¹, Raymond JWM Vet¹, Masja de Haas², Albert EGKr von dem Borne¹,²

¹Division of Internal Medicine and Dept. of Haematology, Academic Medical Centre, Amsterdam the Netherlands
²Dept. of Experimental Immunohaematology, CLB and the Laboratory of Experimental and Clinical Immunology, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands
³Dept. of Medical Oncology, University Hospital "Vrije Universiteit", Amsterdam, the Netherlands

ABSTRACT

Thrombopoietin, the main regulator of thrombocytopoiesis, is a likely candidate to play a role in the increase in platelet counts that is frequently seen after surgery. In the current study, serial blood samples of patients that underwent major surgery were analysed with respect to Tpo kinetics, platelet turnover and inflammatory cytokines. Platelet Tpo content and plasma Tpo levels rose before platelet counts increased, suggesting that Tpo was indeed responsible for the elevation in platelet counts. In addition, an increase in IL-6 levels, but not in IL-11 and TNFα levels, was seen before the rise in Tpo concentration. In vitro, IL-6 was shown to enhance Tpo production by the HepG2 liver-cell line. Thus, increased Tpo levels after surgery, possibly resulting from enhanced Tpo production under the influence of IL-6 or other inflammatory cytokines, are involved in an enhanced thrombocytopoiesis.
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INTRODUCTION

In response to major surgery reactive thrombocytosis may occur [1-3]. Especially after coronary bypass surgery, reactive thrombocytosis represents a risk factor for thrombotic complications [4,5]. A maximal rise in platelet count has been observed from the seventh to the twentieth day post surgery and may encompass a platelet increase of more than 100% [1-6]. The exact mechanism leading to the rise in platelet counts is unknown. Thrombopoietin (Tpo) is the main regulator of platelet production [7] and therefore is a likely candidate to play a role in the platelet rise. Tpo alone can stimulate both proliferation and differentiation of megakaryocytic cells which eventually results in the release of platelets in the circulation [8-14]. A potential increase in circulating Tpo after major surgery may arise via different mechanisms. Tpo production might be enhanced or Tpo clearance might be decreased. Alternatively, as we reported previously [15], Tpo might be released from activated platelets, which will also result in increased levels of circulating Tpo. Under normal conditions, Tpo is produced constitutively by the liver and to a lesser extent by the kidneys and is removed from the circulation by binding to the Tpo receptor, Mpl, which is present on platelets and megakaryocytic progenitors [16-19]. In case of thrombocytopenia, bone-marrow stromal cells have also been reported to produce Tpo [20,21].

In the current study, the kinetics of circulating and platelet-associated Tpo and the potential mechanisms underlying Tpo regulation in patients undergoing major surgery were addressed in detail. Serial samples of patients undergoing orthopaedic or cardiac surgery were analysed with respect to platelet count, plasma Tpo concentration and platelet Tpo content. Plasma glycosylglycocalcin concentrations were determined as a measure of platelet turnover. Inflammatory cytokines might play a role in thrombocytopoiesis, either directly, or indirectly via enhancement of Tpo production. Plasma interleukin-6 (IL-6), interleukin-11 (IL-11) and tumor necrosis factor-α (TNFα) were measured as representatives of these cytokines. To investigate whether inflammatory cytokines or other factors could influence Tpo production, a liver-cell line, a bone-marrow endothelial-cell line and primary bone-marrow stromal cells were cultured with different cytokines and Tpo production was analysed.

MATERIALS AND METHODS

Patients

Upon informed consent, four patients (3 female, 1 male; age: 70±11) undergoing hip replacement surgery, three patients undergoing a knee replacement operation (2 males, 1 female; age: 65±20) and nine patients (7 males, 2 females; age: 65±12) undergoing coronary artery by-pass graft surgery (CABG) were enrolled in the study. No complications occurred during the operations. Before surgery, patients undergoing CABG were treated with Dexamethasone (1mg/kg b.w.) and the total blood volume was adjusted by infusion of crystalloids. During surgery, CABG patients were connected to extra-corporal circulation (ECC).
Blood collection and processing
Venous blood anti-coagulated with EDTA was obtained from all surgical patients at different time points before and after surgery. For the CABG patients, blood was drawn on the day before surgery, at the day of surgery before connection to ECC, directly after disconnection and six hours after disconnection. Thereafter, blood was drawn on every subsequent day for a period of 10 days. The same blood collection strategy was applied for patients undergoing hip or knee replacement surgery, except that on the day of surgery, blood was drawn before the operation and, one, three and six hours, respectively, after the operation. Platelets were routinely counted with the Technicon H3 RTX™ System (Bayer, Tarrytown, NY, USA). Blood was processed within 4 hours after blood collection. Blood was spun at 850g for 10 min. and platelet-rich plasma was collected. After centrifugation for 7 min at 1700g, plasma was collected and stored at -20°C until use. Platelets were washed once with phosphate-buffered saline (PBS) containing 2% (w/v) BSA and 5 mM EDTA. Platelets were taken up in 1 ml of PBS and disrupted by freezing at -20°C. Before freezing, the platelet number was counted.

Tpo and GC ELISA
Thrombopoietin levels and plasma GC levels were determined with a sandwich ELISA as previously described [22,23]. In addition, Tpo was measured in supernatants of platelets disrupted by freezing.

IL-6, IL-11 and TNFα ELISA
For measurement of the concentration of IL-6 and TNFα in plasma, the commercially available IL-6 and TNFα ELISA kits (Pelikine kit, CLB, the Netherlands) were used. The Quantikine IL-11 ELISA kit was purchased from R&D systems Inc. (Minneapolis, MN, USA).

Albumin concentration
As a parameter for the change in plasma water content as a result of the infusion of colloids, and thus of the change in plasma volume, plasma albumin concentration was routinely measured in a colorimetric assay with a Hitachi 747 (Boehringer Mannheim, Mannheim, Germany). In the CABG group, the measurements of platelet counts and plasma Tpo, GC and IL-6 concentrations at the different time points were corrected for haemodilution by multiplication with the following ratio: concentration albumin before ECC divided by concentration albumin at time-point of interest.

Cell culture
The HepG2 cell line was grown in Iscove's modified Dulbecco's medium (IMDM) supplemented with 5% (v/v) heat-inactivated fetal-calf serum (FCS; Gibco BRL, Breda, the Netherlands), penicillin (100 U/ml), streptomycin (100 μg/ml) and glutamine (300 μg/ml). Before testing, cells were transferred to 12-well tissue culture plates (Costar, Cambridge, MA, USA) (400,000 cells/well) and left to adhere. The previously described human bone-marrow endothelial cell line, HBMEC-60 [24], was cultured until confluency in fibronectin (CLB)-coated 12-well tissue culture plates (Costar) and was maintained in Medium-199 with 25 mM Hepes.
buffer (Gibco BRL) supplemented with 10% (v/v) pooled heat-inactivated human serum (CLB), 10% (v/v) heat-inactivated fetal-calf serum (Gibco BRL), 1 ng/ml bFGF (Boehringer Mannheim), 5 U/ml heparin (Leo Pharmaceutical Products, Weesp, The Netherlands), 0.3 mg/ml glutamine (Sigma Chemical Co., St. Louis, MO, USA), penicillin (100 U/ml), streptomycin (100 U/ml) and Geneticin (G418) (Gibco, BRL). BM stromal cells were obtained upon informed consent from the sternal aspirate of patients undergoing cardiac surgery. The mononuclear cell fraction was isolated by density centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden; specific gravity 1.077 g/cm$^3$). Cells were plated in a 12-well tissue culture plates (Costar) and grown in medium consisting of 45% IMDM, 45% RPMI supplemented with 0.3 mg/ml glutamine and 10% FCS. Upon adherence, the non-adherent cells were removed and the medium was replaced. Thereafter, 50% of the medium was replaced once weekly until a confluent monolayer was obtained. All cells were grown in a humidified incubator at 37°C with 5% CO$_2$.

The influence on Tpo production of the following factors diluted in IMDM supplemented with 0.1% bovine serum albumin was assessed: IL-1β (10 ng/ml), IL-6 (25 ng/ml) TNFα (10 ng/ml), TGFβ (2 ng/ml). Before incubation with the various cytokines, the cells were washed free of culture medium. All cultures were performed in triplicate in a final volume of 750 µl. IL-1β was purchased from Preprotech (Rocky Hill, NJ, USA), IL-6 from the CLB, TNFα was from Calbiochem$^®$ (Bad Soden, Germany) and TGFβ from Strathmann Biotech Gmbh. Supernatants of BM stromal cells and HBMEC-60 were concentrated 15-fold by centrifugation for 30 min. at 5000g with a Centricon$^®$ centrifugal filter device YM30 (Millipore Corporation, Bedford, MA, USA).

**RNA isolation and cDNA synthesis**

Total RNA was isolated with RNAzol (Tel Test Inc., Friendswood, Texas, USA) according to the manufacturer's instructions. Approximately 1 µg of RNA was reverse-transcribed with 5 units of M-MLV reverse transcriptase (Gibco BRL) in 1x RT reaction buffer (Gibco BRL) to which 1.5 µg of random primers (Gibco BRL), 10 mM DTT (Gibco BRL), 20 units RNAsin (Promega, Madison, WI, USA) and 0.5 mM DNTP (Pharmacia-Biotech, Uppsala, Sweden) were added. The cDNA reaction was performed for 1 hour at 37°C in a total volume of 20 µl. After an inactivation step of 10 minutes at 85 °C, the mixture was supplemented with 30 µl distilled water. Of this mixture, a 100-fold dilution was prepared, of which 5 µl was used for PCR amplification. For the standard curve, cDNA from a HepG2 culture was prepared. Five ten-fold dilutions were prepared from the (undiluted) reaction mixture.

**TaqMan RT-PCR**

Tpo primers and a Tpo probe were designed with the Primer Express version 1.0 software (PE Biosystems, Foster City, CT, USA) and the OLIGO 6.1 software (Wojciech Rychlik, Ph.D., Molecular Biology Insights, Inc., Cascade, CO, USA). The following oligonucleotides in 5' to 3' orientation were selected: Tpo forward primer: GCTGCCTGCTGTGGACTTTAG; Tpo reverse primer: GGTCACTGCTCCCAGAATGTC and Tpo TaqMan probe TGCCTTGGTCTCCTCCCATCTGGGT. The probe was chosen at the exon 4 / exon 5 transition and carried a
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FAM reporter dye at the 5’-end and a TAMRA quencher dye at the 3’-end. The housekeeping gene porphobilinogen deaminase (PBGD) was used as an input control [25]. For amplification, the TaqMan 1000 Reactions Gold with Buffer A kit (PE Biosystems) was used. The reaction was carried out in a closed tube in a total volume of 50 µl, containing 1x TaqMan buffer A, 5 mM MgCl2, 200 µM of each DNTP, 300 nM of either the Tpo or the PBGD primers, 100 nM probe, 1.25 of U AmpliTaq Gold (PE Biosystems) and 5 µl cDNA. The reaction procedure was as follows: 10 min 95°C followed by 50 cycles of 15 sec 95°C and 1 min 60°C. Fluorescence data were collected during the annealing/extension phase of every cycle by means of the ABI PRISM 7700 Sequence Detection System, containing a thermal cycler (PE Biosystems). In each experiment, a HepG2 standard curve and a negative control (no cDNA input) was included. All experiments were performed in duplicate. A standard curve was calculated in which the increase in fluorescence (ΔRn) was proportional to the concentration of template in the control. From this standard curve, the amount of amplicon in the samples was calculated.

Statistical analysis

The software package SPSS for windows, release 7.5 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A p-value <0.05 was considered significant. The paired-samples T-Test was used to compare serial sample values with baseline values. An independent sample T-Test was used to assess differences in Tpo secretion in cell cultures.

RESULTS

Patients undergoing knee or hip surgery

In Figure 1, panels a-d, box plots are shown representing platelet counts, plasma Tpo concentration, platelet Tpo content and plasma IL-6 concentration for the patients undergoing knee or hip surgery. One day before surgery, a sample was taken to establish baseline values. One, three and six hours after surgery blood was sampled and thereafter, blood was collected daily up to day 10. The mean value of measurements performed at day 4 and 5, day 6 and 7 and day 8, 9 and 10 are shown. When compared with platelet counts at baseline (day before surgery), platelet counts initially dropped after surgery and started to rise after day 2. Four days after surgery, platelet counts significantly rose above baseline level and continued to rise until the end of the observation period (Fig. la). The increment in platelet count was preceded by a rise in plasma Tpo concentration (Fig. 1b). The Tpo level was significantly increased above baseline at day 1 after surgery, peaked around day 3 and had returned to baseline after 5 days. The amount of Tpo per platelet was significantly increased on day 1 after surgery and decreased again on day 3, despite the presence of an increased circulating Tpo concentration (Fig 1c). The rise in Tpo content per platelet preceded the increase in plasma Tpo concentration. Within hours after surgery, the plasma IL-6 concentration increased (Fig 1d). IL-6 levels were not detectable at baseline in five out of six patients. IL-6 levels were highest around 6 hrs and then started to drop again. Neither IL-11 nor TNFα could be detected at any tested
Figure 1a-d: Box plots representing the serial measurements of several variables in patients undergoing orthopedic surgery. Shown are baseline values (day -1), 1 hr, 3 hrs and 6 hrs after surgery, and 1 day, 2 days and 3 days after surgery. Mean values of day 4 and 5, day 6 and 7 and day 8, 9 and 10 are shown since the whole series could not be completed for each patient. Asterisks indicate a significant difference (*: p<0.05; **: p<0.01) compared to baseline value. Circles represent outliers (cases with values between 1.5 and 3 box lengths). Boxes represent the interquartile range containing 50% of all values. The line across the box indicates the median, whereas the whiskers extend to the highest and the lowest value. No statistical analysis was performed for IL-6, because at baseline, levels were under the detection limit of the test (10 pg). In the figure, these values were arbitrarily set at 10 pg/ml.
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follow-up of different variables during orthopedic surgery

Figure 2: Summary of the chronological profile of the different variables in patients undergoing orthopedic surgery. The relative percentage compared to baseline values are shown. In case of IL-6, levels were arbitrarily set at 10 pg/ml when results were under the limit of detection (10 pg/ml) of the assay.

time point up to day 3 after surgery. During follow-up, no significant change in plasma GC was measured. In Figure 2, the change of the different variables in time, and in relation to each other, are summarised. Within hours after surgery, IL-6 levels peaked, followed by a rise in Tpo concentration. The rise in Tpo concentration was initially reflected by an increase in platelet-associated Tpo followed by a rise in the circulating Tpo concentration. After the Tpo peak, platelet counts rose above baseline value.

Patients undergoing CABG

Figure 3 panels a-d, show the box plots for the different variables in patients undergoing CABG. Before surgery, patients received fluid infusion in order to adjust the circulating blood volume. To correct for haemodilution, platelet count, plasma Tpo, IL-6 and GC concentrations were recalculated by means of the albumin concentration. The concentration of the different variables before connection to the ECC (that is, after haemodilution) were taken as baseline value. A similar response of the different variables upon CABG surgery was seen as in the patients undergoing orthopaedic surgery. The rise in platelet count, which was significantly elevated compared to baseline at day 6/7, was preceded by a peak in plasma Tpo concentration. Platelet counts rose from baseline 163±76x10⁹/L to 320 ± 95x10⁹/L at the last time point, day 8/9/10. Similar as in the orthopaedic patients, peak Tpo levels were seen at day three after CABG surgery. Again, an increase in Tpo per platelet occurred before the peak in plasma Tpo levels. The Tpo concentration per platelet was significantly elevated compared to baseline on day 1 to 4, with peak levels found around day 2. The IL-6 concentration rose directly after surgery. For four out of eight patients, IL-6 levels were highest 6 hrs after
Figure 3a-d: Box plots representing the serial measurements of several variables in patients undergoing CABG. Except for the amount of Tpo per platelet (3c), all variables measured in plasma were corrected for haemodilution by amplification with the albumin ratio (concentration albumin before ECC divided by concentration albumin at time-point of interest). Shown are baseline values (before connection to e.c.c), mean values directly after disconnection of the e.c.c, 6 hours after disconnection and 1, 2 and 3 days after surgery. Similar as in figure 1, mean values of day 4 and 5, day 6 and 7 and day 8, 9 and 10 are shown. Triangles represent extreme values (cases with values over 3 box lengths). Further, the same legend as for figure 1 applies.
disconnection from ECC. For one patient, the peak day could not be determined since samples were only collected until 6 hrs after ECC. For two patients, an IL-6 peak was seen at day 2, and for one patient on day 4. Also in this patient group, IL-11 and TNF could not be detected and GC concentrations did not significantly change during follow-up. Figure 4 summarizes the relative changes in the different variables compared to each other. The rise in platelet count was preceded by a rise in circulating Tpo. The rise in Tpo concentration was first reflected in an increase in platelet Tpo content. In most cases, the IL-6 peak preceded the rise in Tpo.

**Cell cultures**

To investigate whether stimulation of (potentially) Tpo-producing cells with inflammatory cytokines could influence Tpo production, HepG2 cells, BM stromal cells and HBMEC-60 were cultured in the presence or absence of several growth factors. HBMEC-60 did not produce Tpo after 48 hrs of culture with IL-1, IL-6, TNF or TGFβ. After a fifteen-fold concentration of the supernatants, still no Tpo could be detected. Similarly, no Tpo was detected in either the non-concentrated or concentrated supernatants of BM stromal cells after 48 hours of exposure to the cytokines. The HepG2 cell line did produce Tpo (Figure 5a). A significant increment in Tpo concentration was present when cells were cultured for 24 hrs with IL-6. On average, Tpo production by HepG2 cells increased 1.5-fold in the presence of IL-6. IL-1, TNF and TGFβ did not significantly influence the final Tpo concentration. To assess whether the increment in Tpo concentration in the IL-6 cultures resulted from an increased Tpo mRNA production per cell, the Tpo mRNA content was analysed with TaqMan RT-PCR.
Figure 5: Tpo production by unstimulated and stimulated HepG2 cells. Panel A: Mean and standard deviation of the Tpo concentration in the supernatants of triplicate cultures after 24 hrs are shown. The cultures stimulated with IL-6 showed a significantly higher Tpo concentration compared to the control cultures. Panel B: Tpo mRNA transcription in the different cultures relative to transcription of the house-keeping gene PBGD, which served as an input control. RNA was isolated from triplicate cultures and was processed separately. Mean and standard deviation are shown.

Figure 5b shows the Tpo mRNA content relative to the expression of PBGD mRNA. No significant difference in Tpo mRNA expression was present in either one of the tested conditions compared to the control.

DISCUSSION

The current study shows that the reactive increase in platelets that is often seen after major surgery is most likely caused by an increment in circulating Tpo, the main regulator of thrombocytopoiesis. The increase in platelet Tpo content suggested that the rise in circulating Tpo is first reflected by enhanced Tpo uptake by platelets. Previously, several in vitro studies have shown that platelets bind and internalise Tpo [17-19,26]. A change in average Tpo content per platelet in vivo, however, has to our knowledge not been reported before. It is of notice that the Tpo uptake per platelet initially increased, as evidenced by the increased platelet Tpo content, but as circulating Tpo levels increased, the platelet Tpo content decreased again. The cause of this decrease is unknown. Possibly, the total receptor expression diminishes due to internalisation of the Tpo-Mpl complex and subsequent processing. Alternatively, platelets with a high Tpo content might represent aged platelets that leave the circulation.
In accordance with our findings, an elevation in circulating Tpo upon surgery has been reported by Hobisch-Hagen et al. [27] and Cerutti et al. [6]. The former group reported significantly elevated serum Tpo levels (compared to levels before haemodilution) already occurring three hours after surgery. This early increment might be attributed to the measurement of serum instead of plasma Tpo levels. Previously, we have shown that platelets can release Tpo and that serum levels are on average 3.4 times higher than plasma Tpo levels [15,22]. In serum, both Tpo released from platelets and circulating Tpo is measured. Cerutti et al. [6] reported elevated plasma Tpo levels with a peak at day 3, which was associated with a rise in IL-6 concentrations, in patients undergoing hip replacement surgery. In our study, IL-6 levels peaked within hours after surgery and preceded the peak platelet-associated Tpo.

A similar sequence of events, an IL-6 peak followed by an increase in circulating Tpo and platelet counts, was noted in patients with Kawasaki disease and upon induction of endotoxemia in healthy individuals [28,29].

Recently it was shown that in healthy individuals who received a bolus injection of rHu-MGDF, the rise in platelet count, a reflection of enhanced thrombocytopoiesis, followed a few days after the rise in circulating Tpo [30]. In line with this observation, we observed a two- to three-day delay before platelet counts started to rise. This suggests that the increased Tpo levels in the surgery patients might indeed have caused the rise in platelet counts.

Although platelet counts decreased immediately after surgery and gradually increased in time, no change in the GC concentration was observed. Previously, GC has been shown to be a marker for platelet turnover and is thought to be enzymatically cleaved from the platelet during destruction [31-33]. It is likely, that the follow-up of the patients in this study was too short to detect an increment in GC concentration, because the newly produced platelets were still viable at the end of the follow-up. This is in agreement with results from a study in which patients were treated with MGDF [34]. In these patients, GC levels started to rise 3 days after the start of the increase in platelet count.

The exact origin of the increase in circulating Tpo is unknown and might involve several mechanisms. According to the model in which the total platelet and megakaryocyte mass affects the amount of circulating Tpo via receptor-mediated binding [35], a decrease in platelet mass might result in increased Tpo levels. In patients undergoing CABG, a decrease in platelet mass was not reflected by a decrease in platelet counts. However, it is plausible that also in these patients platelets are consumed. The absence of a decrease in circulating numbers might result from an enhanced platelet release by the splenic pool or other compartments, thus masking platelet turnover in the periphery.

Another mechanism that might contribute to the Tpo increment is Tpo release by activated platelets. Previously, we and others have shown that Tpo levels are elevated in patients with disseminated intravascular coagulation [15,36,37] and correlate with markers for thrombin generation [15]. In those patients, massive platelet activation occurs. Mild induction of endotoxemia in healthy individuals already leads to an elevation of plasma Tpo levels [29]. In analogy, Tpo might also be released by platelets activated upon surgery.
A third mechanism that might be involved, is enhanced production of Tpo. To date, no regulation at the mRNA level has been reported for the liver and the kidney in response to thrombocytopenia or thrombocytosis [38]. In contrast, Tpo production by BM stromal cells can be induced. In patients with thrombocytopenia as a result of bone-marrow aplasia or idiopathic thrombocytopenic purpura, an increased Tpo mRNA concentration in bone marrow was found [20,21]. It has been reported that platelet α-granular proteins such as PDGF, FGF, PF4, TSP and TGFβ could influence the production of Tpo by BM stromal cells [39,40]. Apart from these proteins, enhanced Tpo production might be induced under the influence of cytokines and/or growth factors that are involved in the inflammatory reaction upon surgery. Elevated levels of IL-6 and acute-phase reactants such as C-reactive protein in combination with elevated Tpo levels have been reported in patients with thrombocytosis secondary to inflammation or infection [41-43]. In two studies in which blood was drawn at one time-point in each patient, Tpo levels correlated with IL-6 levels and acute-phase reactants [41,43]. To assess whether the production of Tpo could be influenced by inflammatory factors, cell lines or cells potentially involved in Tpo production i.e. HepG2, BMEC and BM stromal cells, were cultured with IL-1β, IL-6, TNFα, TGFβ and compared with unstimulated cells. Tpo production was not seen for the HBMEC and the BM stromal cells. In contrast, Sakamaki et al. [39] showed that TGFβ increased the expression of Tpo mRNA. However, Sungaran et al. [40] also found no upregulation but rather a down regulation of Tpo mRNA in BM stromal cells under the influence of TGFβ. Similar to our findings, Wolber et al. [44] reported no Tpo production by BM fibroblasts either cultured without growth factors or with IL-1β, IL-6 or TNFα. HepG2 cells did produce Tpo, and a significant increase in Tpo was seen upon stimulation of these cells with IL-6. Analysis of the amount of Tpo mRNA expression showed no evidence that the IL-6 related increase in Tpo production resulted from enhanced Tpo mRNA expression. Although an increase in Tpo mRNA was noted, it did not reach statistical significance. However, Wolber et al. [44] reported a significant two-fold increase in Tpo mRNA content, in both the liver-cell lines HepG2 an Hep3B that were cultured with IL-6, using competitive PCR for quantification. They also reported a 1.5-fold increase in Tpo protein production, which is in agreement with our findings [44]. In contrast, Hino et al. [45] and Yang et al. [46] observed no enhanced protein production. In addition to Tpo, IL-6, which is a thrombopoietic factor as well as a pro-inflammatory factor, might also be directly involved in thrombopoiesis. Although to a lesser extent than Tpo, IL-6 is able to support megakaryocytopoiesis [47]. The increase in IL-6 that was seen after surgery might therefore play a direct role in enhancing thrombopoiesis. Apart from IL-6, other thrombopoietic factors such as IL-1, IL-3, and IL-12 might contribute. Further studies should be performed to investigate this. In conclusion, it is likely that the surgery-related increase in platelet counts is caused by increased platelet production under the influence of Tpo and, possibly, to some extent by IL-6 or other cytokines with some thrombopoietic activity. Upon surgery, Tpo levels increased, as was demonstrated by an increased Tpo content per platelet followed by an increased level of
circulating Tpo. The exact origin of the Tpo elevation is unknown, but possibly multiple mechanisms are involved, i.e. accumulation as a result of diminished uptake, Tpo release by activated platelets, and enhanced production under the influence of IL-6.

These findings may contribute to the clarification of the mechanisms underlying the finding that aspirin reduces the risk of pulmonary embolism and deep-vein thrombosis after surgery, as was demonstrated again recently in a large multicentre trial [48].

REFERENCES


