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INTRAOPERATIVE CLOTTING FACTOR DILUTION AND ACTIVATED HEMOSTASIS IN CHILDREN WITH EWING'S SARCOMA OR OSTEOSARCOMA: A PROSPECTIVE LONGITUDINAL STUDY

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ABSTRACT

Background. **The study was designed to evaluate prospectively intraoperative changes in coagulation and fibrinolysis in young patients with Ewing's sarcoma (n=12) or osteosarcoma (n=12) who underwent major surgery, and to relate them to hematocrit (HCT) readings.**

Materials and Methods. **Blood samples (von Willebrand factor, fibrinogen, antithrombin III, protein C, plasminogen, t-PA ag, PAI 1 activity, F1+2, D-dimer, PAP) were obtained immediately prior to starting anesthesia, two and four hours later, immediately after surgery and on the first postoperative day. Intra- and postoperative hemostatic parameters were adjusted to preoperative HCT readings.**

Results. **Major surgery induced dilution coagulopathy due to blood product transfusion to support the patient's vascular volume. Postoperatively, VWF (0.01) and fibrinogen (0.007) were found to be significantly enhanced, whereas antithrombin III levels were significantly (0.007) decreased. D-dimer formation showed a clear, significant (0.0019) rise two hours after skin incision and remained elevated through the first postoperative day. F1+2 and PAP showed only minor deviations. T-PA (0.012) and PAI 1 (0.001) rose during the operation and normalized on the first postoperative day. Within 36 hours of the initial operation, six of the 24 patients (25%) returned to surgery to stop severe hemorrhage.**

Conclusions. **These findings indicate that hemostatic parameters may be useful when monitoring surgery- and transfusion-induced hemostatic imbalance. Furthermore, the significant differences between the HCT-uncorrected concentration of the various plasma proteins clearly demonstrated the need to use HCT correction factors which may influence the necessity for and/or the frequency of substitution therapy with protein concentrates.**

Key words: coagulation, fibrinolysis, limb salvage, hematocrit

The present study was designed to evaluate prospectively intraoperative changes in coagulation and fibrinolysis in young patients with Ewing's sarcoma or osteosarcoma who underwent major orthopedic surgery, and to relate them to hematocrit readings.[1-3] Furthermore, the authors attempt to evaluate the relationship between clinical findings including complications after major surgery and parameters of coagulation and fibrinolysis.

Materials and Methods

The study comprised 24 patients aged 8 to 18 years with Ewing's sarcoma (n=12) or osteosarcoma (n=12) admitted to the Department of Orthopedics, University Hospital, Münster, Germany, for local tumor therapy. Prior to surgery, the patients with Ewing's sarcoma were treated according to the *European Intergroup Cooperative Ewing's Sarcoma Study* (EICESS 92),[4] and the patients with osteosarcoma recei-

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ved chemotherapy according to the *Cooperative Osteosarcoma Study* (COSS 86 C or COSS 91).[5] In the EICESS study most patients were designated to receive vincristine (1.5 mg/m^2), adriamycin (20 mg/m^2), ifosfamide (2 g/m^2), actinomycin D (0.5 mg/m^2) and etoposide (150 mg/m^2) every 3 weeks for 40 weeks. In most of these patients surgery was performed two to three weeks after the last course of polychemotherapy, weeks 12-14, respectively. Patients treated according to the COSS protocols received high-dose methotrexate (12 g/m^2) with leucovorin rescue and doxorubicin (30 mg/m^2), in different combinations with either cisplatinum (40 mg/m^2) or ifosfamide (3 g/m^2) for 24 to 29 weeks. Similarly to the EICESS protocol, two to three weeks after the last course of polychemotherapy, weeks 10-12, tumor resection was performed in patients with osteosarcoma. No patient had an individual or family history of bleeding or thrombophilia.

Blood samples for coagulation studies were obtained immediately prior to starting anesthesia, two and four hours later, immediately after surgery and on the first postoperative day. The blood samples were drawn into premarked 3 mL plastic tubes (citrate 3.8%/blood: 1+9; Saarstedt®), placed in ice water and centrifuged at 4°C and 3000 g for 20 minutes.

Fibrinogen, antithrombin III, protein C, plasminogen and D-dimer formation (D-dimer) were measured immediately. Platelet poor plasma was stored in plastic tubes at –80°C. Von Willebrand factor antigen (vWf), tissue type plasminogen activator antigen (t-PA), plasminogen activator inhibitor 1 activity (PAI), prothrombin fragment F1+2 (F1+2) and plasminogen/α_2-antiplasmin complex (PAP) were investigated serially in duplicate four weeks later. Controls included pool plasma from age-matched subjects, calibration plasma, normal and abnormal control plasma (IL Test™, Instrumentation Laboratory, Italy; Chromogenix, Mölndal, Sweden). Fibrinogen reagent kits purchased from Behring Werke (Marburg, Germany) were used for the Clauss method. Antithrombin III, plasminogen, protein C and PAI 1 were measured by enzymatic procedures using chromogenic substrates S2765, S2403, S2366 and S2403 from Chromogenix (Mölndal, Sweden), F1+2, D-dimer formation and PAP with Enzygnost R F1+2, D-dimer micro and PAP micro (Behring Werke, Marburg, Germany). Von Willebrand factor antigen reagent kits were purchased from Stago (Asnières, France).

Intra- and postoperative results were corrected for hemoconcentration using the published[6] correction factor $HCT_1(1\text{-}0.9\times HCT_2)$: HCT_2 $(1\text{-}0.9\times HCT_1)$. HCT_1 and HCT_2 represent the hematocrit readings prior to, and during or after the course of the operation.

Non parametric statistics[7] were performed according to Wilcoxon (Wilcoxon-rank) and Spearman (Spearman correlation coefficient) using the Apple computer (Macintosh Performa 630) Stat View 4.02 program.

Results

To achieve complete surgical tumor removal limb salvage with implantation of endoprosthetic devices, rotation plasty or hemipelvectomy was performed in 24 young patients. Median (range) surgery duration was 6.5 (4-18) hours.

During the observation period median (range) hematocrit values ranged from 36% (29-51) prior to starting anesthesia to 23% (19-42) two hours after skin incision (Wilcoxon rank: 0.0005). The majority of patients showed a hematocrit of 25% (20-44; p 0.0003) 4 hours into the operation and 28% (19-44; p 0.001) immediately after the operation. Twenty-four hours later, the hematocrit values [median 33% (24-39; p 0.001)] were still lower than prior to beginning anesthesia.

Figures 1-5 show the course of diluted and hematocrit-corrected median (range) concentrations of coagulation and fibrinolytic parameters prior to starting anesthesia, during the operation and on the first postoperative day. Surgery had a minor effect on plasma concentrations of von Willebrand factor (Figure 1); with correction for the appropriate HCT range, values were normal. Compared to median starting values, vWf was significantly enhanced to 180% (range: 36-363) 24h later. Hematocrit-corrected plasma fibrinogen levels (Figure 1) fell to the lower reference limit at the end of the operation

and like vWf showed a significant increase 24h later. While diluted values of antithrombin III (Figure 2) were found to be clearly outside the pediatric reference range, HCT-corrected values dropped during the operation, the difference was significant immediately after surgery. Postoperatively, antithrombin III levels showed clearly diminished values with a median of 54% of normal. Protein C levels (Figure 2) were more affected during surgery. A significant decrease started four hours after beginning anesthesia and lasted until the postoperative period.

Nevertheless, all measured values were still above the lower reference limit. There was a significant rise in t-PA antigen within the reference range from 4.8 ng/mL to 8.2 ng/mL (Figure 3) at the end of the operation. Postoperatively, the level fell to 5.5 ng/mL. PAI 1 activity levels (Figure 3) followed a pattern similar to that of t-PA. Compared to starting values, there was a sharp rise above the pediatric reference range to a peak at four hours. On the first postoperative day PAI 1 activity fell significantly below the initial value. Plasma plasminogen levels (Figure 4) started at 77% of normal at the lower reference limit and were reduced to 64% by the end of the operation. Twenty-four hours later HCT-corrected plasminogen activity was still decreased in the majority of patients. PAP (Figure 4) started at 371 ug/L at the upper reference boundary and showed a small, non significant rise at two and four hours. PAP concentrations at the end of surgery and postoperatively were within the pediatric norm. Thrombin generation (Figure 5) reached a significant increase at two and four hours after beginning anesthesia. Immediately after the operation and on the first postoperative day concentrations of prothrombin fragment F1+2 did not differ from starting values. D-dimer formation (Figure 5), like thrombin generation, started above the reference boundary and was significantly elevated two hours after skin incision and at the end of surgery. HCT-corrected concentrations were significantly enhanced on the first postoperative day.

Table 1 shows a significant positive correlation between HCT and VWf, fibrinogen, antithrombin III, protein C and plasminogen,

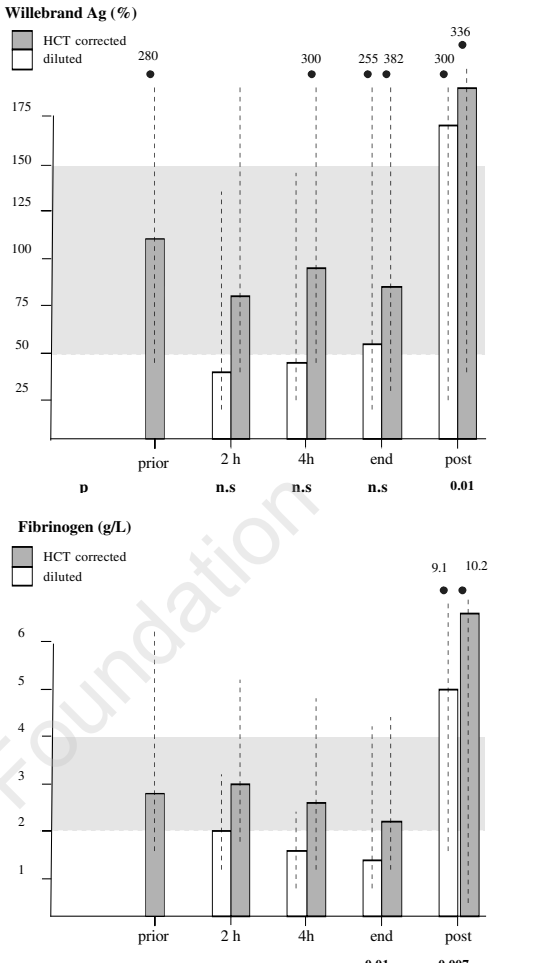
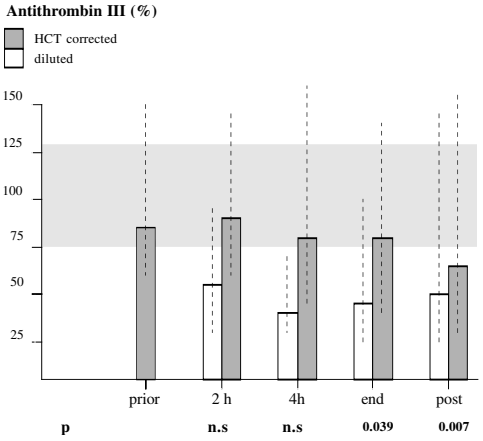


Figure 1. Hematocrit-corrected (dark column) and diluted (light column) median (range: dotted lines) plasma levels of von Willebrand factor antigen (VWf) and fibrinogen prior to, during and after surgery. HCT-corrected values vary within the pediatric reference range (shaded area). Compared to starting values, VWf and fibrinogen showed a significant (Wilcoxon rank) postoperative rise.

respectively. In contrast, no significant correlation was found between hematocrit and t-PA, PAI 1, thrombin generation, D-dimer formation or PAP. The correlation between median (range) blood loss [77 mL/kg bw (9-192)] and blood product transfusion [73 mL/kg bw (7-247)], i.e. fresh frozen plasma plus packed red blood cells, was significant and positive (r: 0.857; p= 0.0004).

Within 36 hours of the initial operation six of the 24 patients who required massive blood product transfusion intraoperatively returned to surgery to stop severe hemorrhaging. Although extensive hematomas were located near



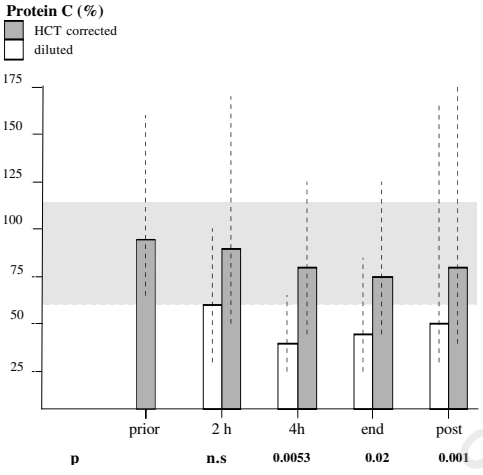
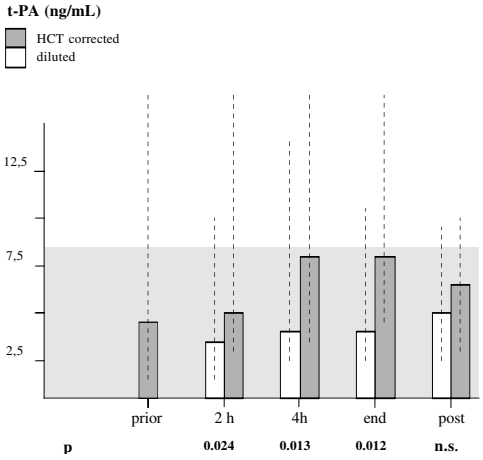


Figure 2. Hematocrit-corrected (dark column) and diluted (light colum) median (range: dotted lines) plasma concentrations of antithrombin III and protein C prior to, during and after surgery. Except for postoperative antithrombin III levels, which are significantly below the pediatric norm, HCT-corrected plasma levels vary within the pediatric range (shaded area).



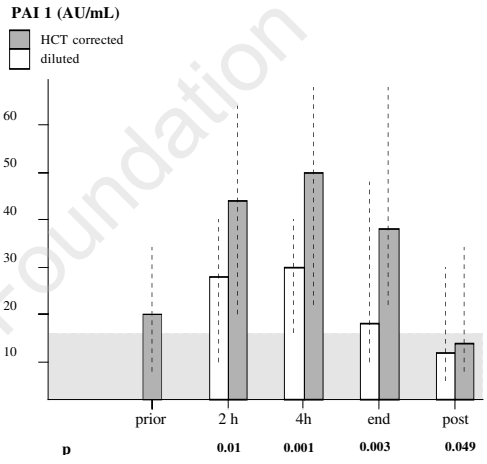


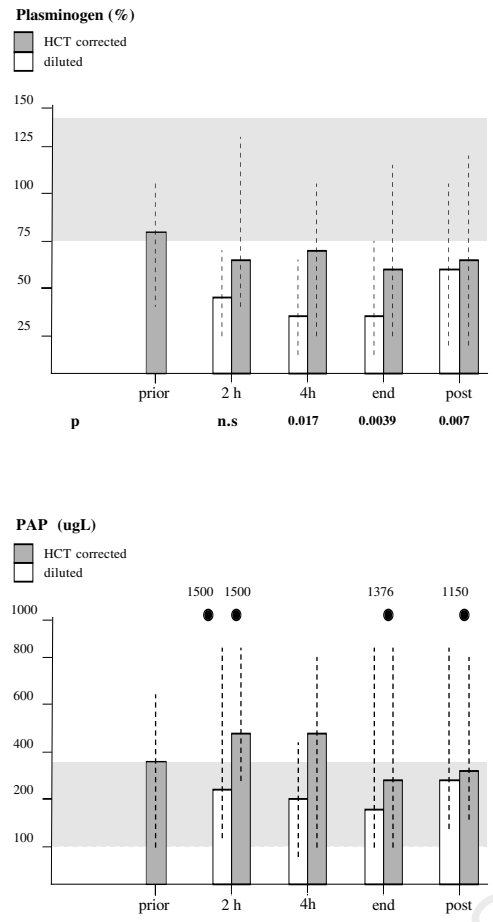
Figure 3. The course of hematocrit-corrected (dark column) and diluted (light column) median (range: dotted lines) plasma values of t-PA ag and PAI 1 activity prior to, during and after surgery. Compared to starting values, both parameters show a significant rise within the reference range (shaded area), with a return to baseline values 24 hours after surgery.

the skin, soft tissue and vascular incisions, no local vascular defects were found. All six patients (Table 2) showed systemic evidence of DIC with decreased antithrombin III and fibrinogen levels, together with platelet consumption ($< 50\times10^9$/L), enhanced thrombin generation and D-dimer formation. No thrombotic event occurred in this series of patients.

Discussion

The capacity to dissolve polymerized fibrin is an important mechanism for counteracting fibrin deposition. Due to its lytic action on intravascular fibrin, fibrinolysis may be interpreted as a defence mechanism. On the other hand, dissolution of fibrin clots in wounds may lead to devastating hemorrhage, and a balance between coagulation and fibrinolysis is therefore crucial for survival after severe trauma. Nevertheless, plasma coagulation and fibrinolytic response after trauma and surgery follow a similar pattern.

The main finding from this study was that major orthopedic surgery in young patients with Ewing's sarcoma or osteosarcoma induced dilutional coagulopathy as a result of the emergency support of vascular volume by blood pro-



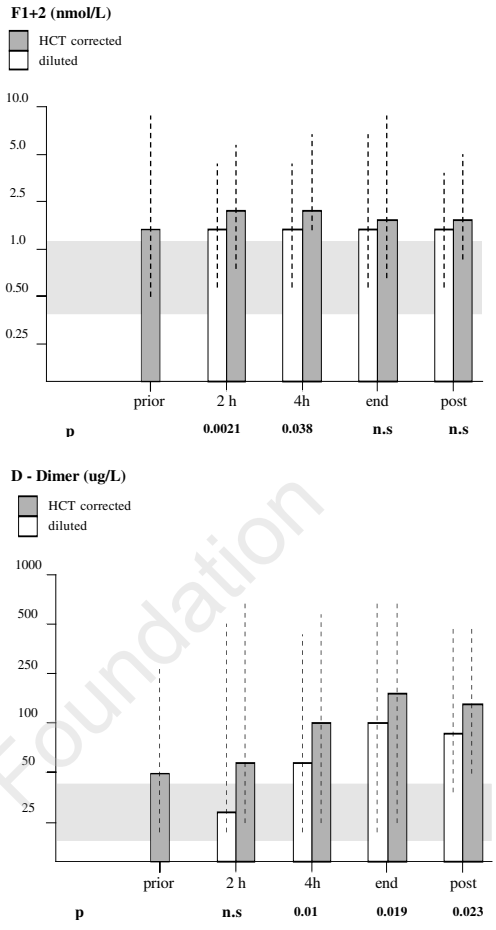


Figure 4. The course of median (range: dotted lines) hematocrit-corrected (dark column) and diluted (light column) plasminogen and PAP plasma concentrations prior to, during and after surgery. Whereas HCT-corrected and diluted plasminogen levels significantly decreased below the pediatric range (shaded area), PAP concentrations were affected to a lesser degree by surgery.

Figure 5. The course of median (range:dotted lines) hematocrit-corrected (dark column) and diluted (light column) concentrations of thrombin generation and D-dimer formation prior to, during and 24 h after surgery. In contrast to a small significant increase of prothrombin fragment F1+2 two and four hours after skin incision, D-dimer formation shows a clearly significant rise starting two hours after anesthesia was begun and extending through the postoperative period. The shaded area represents the pediatric norm.

duct transfusion. Since the amount of anticoagulant commonly used (ratio of 1+9) is based on a theoretical HCT of 40-45%, lack of an arithmetic correction for hemoconcentration or hemodilution of the actual HCT will be responsible for spurious measurements of significantly increased or diminished levels of coagulation parameters.[8,9] In order to maintain a constant plasma-to-anticoagulant ratio, the published[6] correction factor was used. Intraoperative changes in hematocrit-related parameters, i.e. VWf, fibrinogen, antithrombin III and protein C, were within the pediatric reference range.

The effect of major surgery on PAP and thrombin generation was less pronounced, although the latter showed significantly elevated levels two and four hours after anesthesia was begun. Furthermore, F1+2 showed no correlation with HCT. The observed activation is probably due to surgery-induced tissue damage and vascular damage or the underlying malignant disease.[10] In contrast, similar to findings published in the literature[11,12] D-dimer formation demonstrated a clear, significant rise two hours after skin incision that extended through the first postoperative day. This change in D-

Table 1. Correlation (Spearman rank) of hematocrit readings with parameters of coagulation and fibrinolysis during the course of major surgery in 24 children suffering from Ewing`s sarcoma (n=12) or osteosarcoma (n=12).

parameter	*count*	*Rho*	*p-value*
Fibrinogen	96	0.586	0.0001
AT III	96	0.707	0.0001
Protein C	96	0.544	0.0001
Plasminogen	96	0.530	0.0001
Willebrand	96	0.612	0.0001
t-PA	94	0.162	0.183
PAI 1	90	- 0.177	0.156
F1+2	98	0.118	0.338
PAP	96	0.204	0.107
D-dimer	90	- 0.503	0.705

Table 2. Postoperative coagulation, fibrinolysis blood loss and amount of blood product transfusion in patients who returned to surgery because of hemorrhaging (n=6).

patient	*Fib mg/dL*	*AT III %*	*D-dimer ug/L*	*blood loss mL/kg*	*transfusion mL/kg*
1	60	50	355	192	117
2	80	43	130	110	180
3	50	58	130	111	101
4	49	41	155	183	130
5	105	22	240	143	128
6	95	52	150	95	95

dimer formation reflects the capacity to dissolve insoluble cross-linked fibrin, which serves to regulate the postoperative coagulation imbalance resulting from low inhibitor levels, i.e. antithrombin III and high acute phase proteins such as fibrinogen and vWF.[13] There have been few detailed studies on the components of the fibrinolytic response during the course of surgical procedures.[11,12,14] In this series t-PA ag increased through the operation period and declined on the first postoperative day to a level close to starting values. PAI 1 activity followed a more pronounced, yet similar pattern to that of t-PA. Postoperatively, PAI 1 activity was significantly decreased compared to values prior to starting anesthesia. These findings are consistent with data in the literature.[15-17] In contrast, the observation of normal t-PA ag and PAI 1 values fails to confirm the reported fibrinolytic shutdown.[11,14-16,18,19]

In our group of patients, 6/24 (25%) experienced clinically significant postoperative hemorrhage. All of theses patients showed signs of systemic DIC with low AT III plasma levels, consumption of fibrinogen, low platelet counts and clearly enhanced D-dimer formation. Compared to the patients who did not require further surgery, these six needed massive blood transfusions, more than 90 mL/kg bw, which may be another risk factor in addition to the underlying malignancy as a possible trigger mechanism for DIC.[20] Further studies are necessary to evaluate the possible prophylactic use of AT III concentrates in patients with accelerated consumption in order to avoid low AT III levels combined with enhanced D-dimer formation.[21]

In conclusion, our data indicate that hemostatic parameters are useful when monitoring surgery- and transfusion-induced hemostatic imbalance. Furthermore, the significant differences between the HCT-uncorrected concentrations of the various plasma proteins clearly demonstrated the need to use HCT-correction factors which may influence the necessity for and/or the frequency of substitution therapy with protein concentrates.

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