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## Factors associated with outcome of liver surgery and hepatocellular carcinoma

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# CHAPTER 3

## **NORMAL TO INCREASED THROMBIN GENERATION IN PATIENTS AFTER A MAJOR PARTIAL LIVER RESECTION DESPITE PROLONGED CONVENTIONAL COAGULATION TESTS**

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**ABSTRACT**

**BACKGROUND:** Conventional coagulation tests are frequently prolonged after liver surgery, suggesting a post-operative hypocoagulability. However, these tests are unreliable for assessment of the haemostatic status in these patients. In contrast, thrombin generation testing measures the true balance between pro- and anti-coagulant factors.

**AIM:** To study the perioperative coagulation status in patients undergoing hemihepatectomy using thrombin generation assays.

**METHODS:** We examined thrombin generation profiles in serial plasma samples taken from seventeen patients undergoing right hemihepatectomy. Results were compared to ten patients undergoing pancreatic resection and twenty-four healthy volunteers. In addition, we measured conventional coagulation tests and plasma levels of several haemostatic proteins.

**RESULTS:** Following liver resection, the endogenous thrombin potential (ETP) slightly decreased until post-operative day 7. However, in the presence of thrombomodulin, the ETP increased [from 542 nM\*min (417–694) at baseline to 845 nM\*min (789–1050) on post-operative day 3] to values higher than that in healthy subjects (558 nM\*min (390–680);  $P < 0.001$ ), which contrasts with substantially prolonged PT levels. Normal to decreased thrombin generation was observed following pancreatic resection. Thrombin generation was only slightly affected by thrombomodulin after hemihepatectomy, while thrombin generation in healthy subjects decreased profoundly upon addition of thrombomodulin. This hypercoagulability following liver resection may be explained by decreased levels of protein C, S, and antithrombin and by elevated levels of factor VIII.

**CONCLUSIONS:** Thrombin generation in the presence of thrombomodulin revealed hypercoagulability in patients following liver resection. These results support the recently advocated restrictive use of plasma during liver resection and the exploration of more extensive use of post-operative thrombosis prophylaxis.

## INTRODUCTION

Major abdominal surgery is associated with a post-operative hypercoagulable state and this hypercoagulability has been recognised as a factor that contributes to the occurrence of thromboembolic complications, such as deep vein thrombosis and pulmonary embolism.<sup>1</sup> The underlying mechanisms include increased activation of platelets, reduced concentrations of anti-coagulants, and impaired fibrinolysis,<sup>2</sup> as well as more general risk factors for thrombosis, such as immobilisation, tissue damage and the presence of cancer.

Conventional coagulation tests, such as the activated partial thromboplastin time (APTT), and the prothrombin time (PT) and related international normalised ratio (INR), are frequently elevated after liver surgery,<sup>3-6</sup> suggesting a post-operative hypocoagulability. These tests are, however, not reliable for assessment of the overall haemostatic status in these patients, because they only evaluate narrow aspects of haemostasis. Specifically these tests are only sensitive for circulating levels of procoagulant factors, and do not test functionality of the natural anti-coagulant systems. Indeed, venous thromboembolism also occurs after liver surgery and, in fact, the risk increases with the extent of hepatectomy.<sup>7,8</sup> The thrombotic risk after liver resection may result from a hypercoagulable state induced by extensive tissue injury or reduction in anti-coagulant factors determined by increased consumption, blood loss or haemodilution.<sup>9-13</sup> Despite this, elevation of routine coagulation tests (PT, APTT) following liver resection frequently leads to transfusion of fresh frozen plasma (FFP).<sup>14-16</sup> However, several serious side effects of blood product transfusion may occur, including the risk of infection and the risk of transfusion-related acute lung injury.<sup>17</sup> In addition, elevation of routine coagulation tests often leads clinicians to delay thrombosis prophylaxis, based on the assumption that the patient is 'auto-anticoagulated'. This potentially increases the risk of deep vein thrombosis and pulmonary embolism in these patients.<sup>7,18</sup> Using thromboelastography (TEG), which analyses all components of the haemostatic system, it was shown that after living donor liver transplantation the majority of donors were hypercoagulable in spite of elevated routine coagulation tests.<sup>10</sup> Also patients undergoing partial liver resection for malignant disease demonstrated a brief hypercoagulable state, followed by normal clot formation as shown by TEG,<sup>5,19</sup> and yet another study using TEG reported normocoagulability after liver resection.<sup>6</sup>

Thromboelastography is routinely used for guiding transfusion of blood products during massive bleeding and liver transplantation by many centres.<sup>20,21</sup> However, it is still uncertain if thromboelastography-guided transfusion strategies improve outcome in patients with massive bleeding,<sup>22</sup> or decrease blood loss during liver transplantation.<sup>23</sup> Furthermore, although thromboelastography assesses the haemostatic status in a whole blood environment, the technique has three major drawbacks. (1) There are multiple ways to

perform a thromboelastographic analysis. Specifically, tests with non-anticoagulated blood as well as with citrated blood are widely used, and the results from these various methods poorly correlate.<sup>24</sup> When using citrated blood, various triggers are added to initiate coagulation (tissue factor/kaolin). Furthermore, two devices (TEG and ROTEM) are used clinically, and results from these methods are also not always in accordance.<sup>25,26</sup> (2) Quantification of the contribution of individual components of the haemostatic system to abnormalities in the thromboelastographic tracing is not possible, although it has been demonstrated that alterations in some components of the traces are dominated by platelets, coagulation or fibrinogen levels.<sup>25,26</sup> (3) Thromboelastography suffers from a unique set of pre-analytic and analytic variables that impact test reliability and reproducibility.<sup>27</sup>

The thrombin generation assay, which measures the total amount of thrombin generated during *in vitro* coagulation, has been successfully used to reassess the haemostatic status of patients with liver disease.<sup>28-31</sup> This global test, which takes plasma concentrations of both pro- and anti-coagulants into account, offers a valid alternative to the conventional coagulation tests which only test functionality of some of the procoagulant factors. Thrombin generation testing has demonstrated normal or even superior thrombin generation in patients with cirrhosis,<sup>28-31</sup> despite a prolonged PT or APTT. It has been well established that plasma levels of haemostatic proteins decrease following a partial liver resection, which has been attributed in part to a reduced synthetic capacity of the liver remnant.<sup>6,9,10</sup> However, the net results of these changes in plasmatic coagulation have not been established. We therefore studied the perioperative coagulation status in patients undergoing right hemihepatectomy using both conventional coagulation tests and thrombin generation assays. Furthermore, we compared results to those of patients undergoing a pancreatic resection which is a surgical procedure of a similar extent, but without a decrease in post-operative synthetic capacity of the liver.

## **PATIENTS AND METHODS**

### **PATIENTS**

Seventeen adult patients, who underwent a right (n=15) or extended right (n=2) hemihepatectomy were included in the study. The control group consisted of patients (n=10) who underwent a pylorus preserving pancreaticoduodenectomy (PPPD). Twenty-four adult healthy volunteers were included to establish reference values for the various tests performed. All patients were included in the University Medical Center Groningen, the Netherlands. Exclusion criteria were age younger than 18 years, pre-existing coagulation disorders, pre-operative anti-coagulation, and use of non-steroidal anti-inflammatory drugs or aspirin 1 week before surgery. Routine surgical and anaesthetic procedures were

adopted. The study protocol was approved by the local medical ethical committee and informed consent was obtained from each subject before inclusion in the study.

#### **PLASMA SAMPLES**

Plasma samples for analyses were, for both groups, drawn at the following time points: after induction of anaesthesia (baseline), at the end of surgery, and on the post-operative days 1, 3, 5, 7 and 30. Following surgery, all patients received standard thromboprophylaxis with (once-daily) low molecular weight heparin (LMWH) and at each post-operative day blood was drawn just prior to the administration of the LMWH. Blood samples from each subject were drawn by venipuncture and collected into vacuum tubes containing 3.8% trisodium citrate as an anti-coagulant, at a blood to anti-coagulant ratio of 9:1. Platelet-poor plasma was prepared by double centrifugation at 2000g and 10000g, respectively, for 10 min. at 18 °C. Plasma was aliquoted, snap-frozen and stored at -80 °C until use.

#### **THROMBIN GENERATION**

Thrombin generation testing was performed using platelet-poor plasma (PPP) with the fluorimetric method described by Hemker, Calibrated Automated Thrombography® (CAT).<sup>32</sup> Coagulation was activated by using a commercial trigger composed of recombinant tissue factor (TF, final concentration 5 pM) and phospholipids (final concentration 4 IM), in the presence or absence of soluble thrombomodulin (TM). These reagents were purchased from Thrombinoscope BV, Maastricht, The Netherlands. To calibrate the thrombin generation curves, thrombin Calibrator (Thrombinoscope BV) was added. A fluorogenic substrate with CaCl<sub>2</sub> (FluCa-kit, Thrombinoscope BV, Maastricht, the Netherlands) was dispensed in each well to permit a continuous registration of thrombin generation. Fluorescence was read in time by the fluorometer Fluoroskan Ascent (ThermoFisher Scientific, Helsinki, Finland). All procedures were followed according to the protocol suggested by Thrombinoscope B.V. Thrombin generation variables analysed were endogenous thrombin potential (ETP), peak thrombin generation, lag-time (time needed for thrombin concentration to reach 1/6<sup>th</sup> of the peak concentration) and velocity index (slope between the end of lag-time and peak thrombin generation). Furthermore, a normalised thrombomodulin sensitivity ratio (TM-SR) was determined by dividing the ETP in the presence of TM divided by the ETP in the absence of TM of an individual, by the ETP in the presence of TM divided by the ETP in the absence of TM of pooled normal plasma. A TM-SR > 1 reflects a decreased anti-coagulant response to TM in comparison to pooled normal plasma.

#### **CONVENTIONAL COAGULATION TESTS**

The PT was assessed on an automated coagulation analyzer (ACL 500 TOP) with reagents (Recombiplastin 2G) and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands). Levels of factor (F) VIII and II, antithrombin (AT),

and protein C were measured on an automated coagulation analyzer (ACL 500 TOP) with reagents and protocols from the manufacturer (Recombiplastin 2G for FII, Hemosil (R) SynthASil for FVIII, Liquid Antithrombin reagent for AT, and IL reagent for protein C) (Instrumentation Laboratory).

**Table 1:** Patient characteristics

Characteristics	Hemi-hepatectomy (n=17)	PPPD (n=10)	Controls (n=24)	P
Sex (male)	6 (35)	7 (70)	13 (54)	0.201
Age (years)	62 [9.8]	67 [7.9]	27 [4.5]	<0.001
Surgical indications				
Colon cancer metastasis	9 (53)	1 (10)		
Hepatocellular carcinoma (HCC)	2 (12)	0		
Pancreatic cancer	0	7 (70)		
Cholangiocarcinoma	2 (12)	1 (10)		
Neuroendocrine tumor	1 (5.9)	1 (10)		
Leiomyosarcoma	1 (5.9)	0		
Adrenocortical Carcinoma metastasis	1 (5.9)	0		
Benign lesion	1 (5.9)	0		
Length of surgery (min)	616 (480- 650)	631 (551-739)		0.414
Estimated blood loss (ml)	700 (200-1000)	400 (300-1125)		0.980
Amount of fluids administered (ml)	4441 [1580]	5225 [1239]		0.192
RBC transfusion (units)	0	0		0.902
FFP transfusion (units)	0	0		1.000
Length of hospital stay (days)	14 (9-21)	15 (11-21)		0.863
Hemoglobin before surgery (mmol/L) *	8.6 [1.2]	8.2 [1.1]		0.407
Hemoglobin after surgery (mmol/L) *	6.7 [1.3]	6.8 [1.2]		0.841

Data are expressed as number (%), mean [s.d.], or median (interquartile range). PPPD: pylorus preserving pancreaticoduodenectomy. \* To convert values for haemoglobin to g/dL, multiply by 1.650.

### STATISTICAL ANALYSES

Values are expressed as means (with s.d.), medians (with interquartile ranges), or numbers (with percentages) as appropriate. Differences between values of pre-operative coagulation tests and thrombin generation tests, and follow-up values were evaluated by mixed linear models. To calculate differences between continuous data among independent groups, the t-test for independent samples or the Mann-Whitney U-test, as appropriate, was used. Differences between patient values and levels measured in healthy controls were compared using one-way ANOVA (with the Bonferroni post-test) or Kruskal-Wallis H test (with Dunn's post-test) as appropriate. P-values of 0.05 or less were considered statistically significant. GraphPad Prism (San Diego, CA, USA) and IBM SPSS Statistics 20 (New York, NY, USA) were used for analyses.

## RESULTS

### PATIENT CHARACTERISTICS

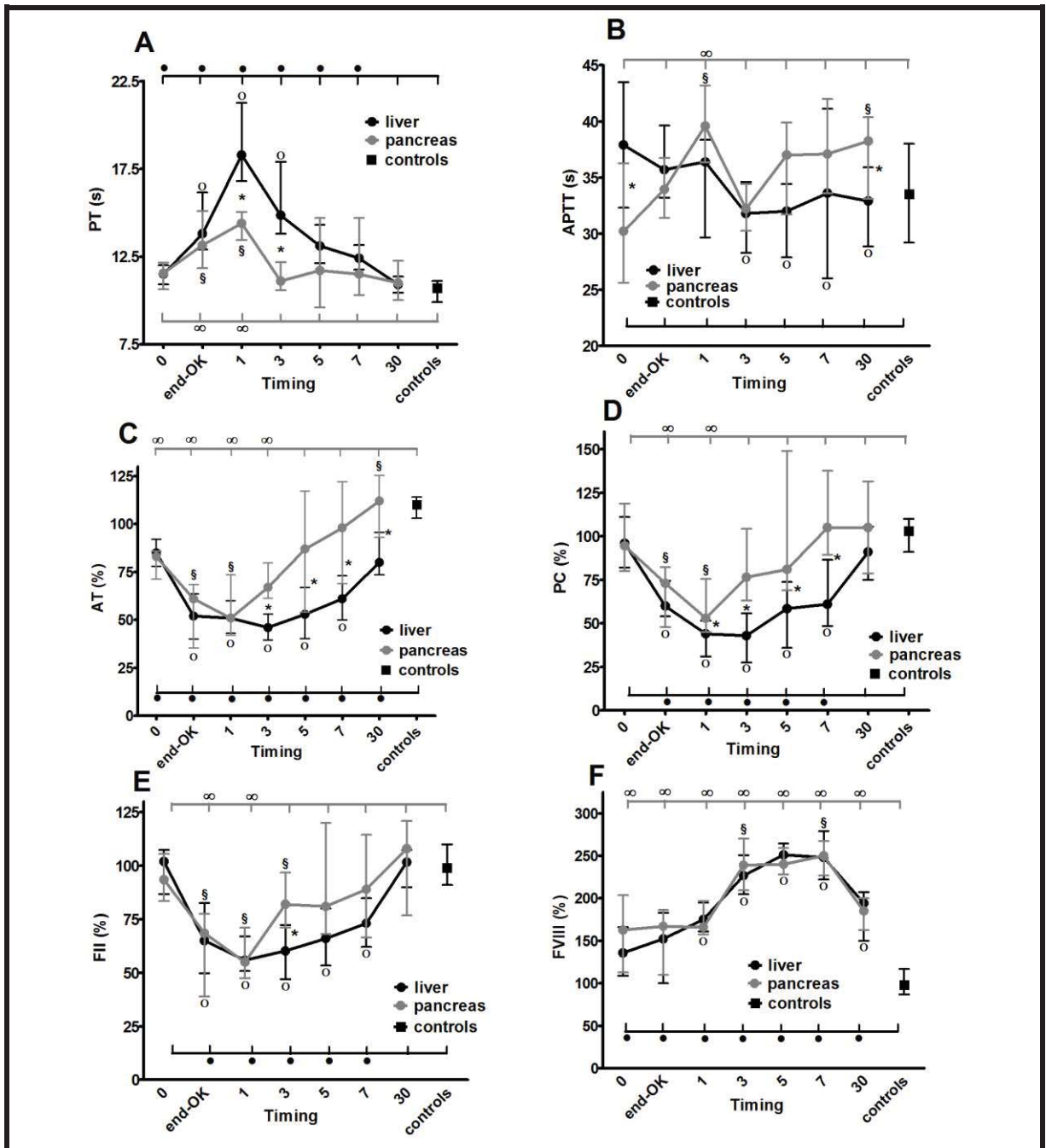
Seventeen patients who underwent a right ( $n = 15$ ) or extended right ( $n = 2$ ) hemihepatectomy, ten patients who underwent a PPPD, and twenty-four controls were included in the study. The main characteristics of the study population are reported in Table 1. The most common indication for liver resection was liver metastases from colorectal cancer and the most common indication for PPPD was pancreatic cancer. The median estimated blood loss was 700 mL in the patients who underwent a hemihepatectomy and 400 mL in the patients who underwent a PPPD. None of the patients suffered from venous thrombosis within 30 days after surgery.

### CONVENTIONAL COAGULATION TESTS

Baseline PT was 10.8 s (9.9–11.2) [median (interquartile range)] in the controls, 11.5 s (10.9–12.0) in the patients undergoing hepatectomy ( $P < 0.05$  compared to controls), and 11.6 s (10.6–12.2) in the patients undergoing PPPD ( $P > 0.05$  compared to controls). The PT progressively increased after both liver and pancreatic surgery (Figure 1), with a peak PT of 18.3 s (16.8–21.3) in the patients undergoing hepatectomy ( $P < 0.001$  compared to baseline PT) and 14.4 s (13.5–15.1) in the patients undergoing PPPD ( $P = 0.002$  compared to baseline PT) on post-operative day 1. After post-operative day 1 the PT decreased to baseline levels on post-operative day 3 in the patients undergoing PPPD and on post-operative day 7 in the patients undergoing hepatectomy. The APTT was 34.2 s (31.1–38.5) in the controls, 37.9 s (32.3–43.5) in the patients undergoing hepatectomy ( $P > 0.05$  compared to controls), and 30.2 s (25.6–36.3) in the patients undergoing PPPD ( $P > 0.05$  compared to controls) at baseline. The APTT slightly decreased following hepatectomy [reaching 31.8 s (28.8–34.6) on post-operative day 3;  $P = 0.009$ ], but increased following PPPD [reaching 39.6 s (36.4–43.2) on post-operative day 1;  $P < 0.001$ ; Figure 1].

At baseline, AT levels were slightly lower in patients undergoing hepatectomy [85.0% (78.0–92.0),  $P < 0.001$ ] and patients undergoing PPPD [83.0% (71.3–85.8),  $P < 0.001$ ] compared to the controls [109.0% (99.3–117.0)]. Levels of AT substantially and significantly decreased at the end of surgery after both the hepatectomy [52.0% (40.0–63.5),  $P < 0.001$  compared to baseline value] and PPPD [61.0% (35.3–68.5),  $P < 0.001$  compared to baseline value]. AT levels further decreased in the patients undergoing hepatectomy until post-operative day 3, after which levels slowly increased towards baseline levels which were reached on post-operative day 30. In the patients undergoing PPPD, levels of AT progressively increased after post-operative day 1, reaching levels exceeding baseline levels on post-operative day 30 ( $P = 0.002$ ; Figure 1). Baseline levels of protein C were





**Figure 1:** Changes in laboratory measurements (A. prothrombin time (PT), B. activated partial thromboplastin time (APTT), C. antithrombin (AT), D. Protein C (PC), E. factor II (FII), and F. factor VIII (FVIII)) after hepatectomy and PPPD. \* $P < 0.05$  between the hepatectomy and PPPD group.  $^{\circ}P < 0.05$  vs. baseline levels in the hepatectomy group.  $^{\S}P < 0.05$  vs. baseline levels in the PPPD group.  $^{\bullet}P < 0.05$  in the hepatectomy group vs. controls.  $^{\infty}P < 0.05$  in the PPPD group vs. controls. End-OK: end of surgery, POD: postoperative day.

comparable between patients undergoing hepatectomy, patients undergoing PPPD, and controls [96.0% (82.0–111.0), 94.5% (80.0–118.8) and 104.0% (93.0–112.3), respectively]. Protein C levels decreased until post-operative day 1 in both the patients undergoing hepatectomy [44.0% (31.0–51.5),  $P < 0.001$  compared to baseline value] and the patients

undergoing PPPD [53.0% (45.0–75.5),  $P < 0.001$  compared to baseline value]. Protein C levels decreased significantly after hepatectomy compared to PPPD ( $P = 0.03$ ), and the time to reach baseline levels was substantially longer after hepatectomy (levels returned to baseline on post-operative day 30 after hepatectomy, compared to post-operative day 3 after PPPD) (Figure. 1).

Levels of FII at baseline were comparable between the groups, with 99.0% (91.0–110.0) of FII in the controls, 102.0% (86.8–107.4) in the patients undergoing hepatectomy, and 93.5% (83.5–105.5) in the patients undergoing pancreatic surgery. FII levels decreased following surgery to 55.8% (50.9–67.0) ( $P < 0.001$  compared to baseline value) in patients undergoing hepatectomy and 55.0% (47.5–71.0) ( $P < 0.001$  compared to baseline value) in patients undergoing PPPD on post-operative day 1.

Baseline levels of FII were reached again on post-operative day 4 after PPPD, but not until post-operative day 30 after hepatectomy (Figure 1). FVIII levels were, at baseline, significantly higher in both the patients undergoing hepatectomy [135.6% (109.0–165.8),  $P < 0.05$ ] and the patients undergoing PPPD [162.5% (112.5–203.5),  $P < 0.01$ ] compared to the controls [92.5% (85.5–114.8)]. Levels of FVIII increased in both groups following surgery, with the highest levels of FVIII on post-operative day 5 after hepatectomy [251.1% (227.8–264.4),  $P < 0.001$  compared to baseline value], and on post-operative day 7 after PPPD [250.0% (234.0–261.0),  $P = 0.03$  compared to baseline value]. After post-operative day 7 the levels of FVIII substantially decreased in both groups although levels remained elevated compared to the healthy controls (Figure. 1).

#### THROMBIN GENERATION

At the start of surgery, the ETP measured in the absence of TM, was comparable between the patients undergoing hepatectomy, the patients undergoing PPPD and healthy volunteers (Table 2). In the patients undergoing hepatectomy, the ETP decreased until Post-operative day 7 ( $P < 0.001$  compared to baseline value), and recovered until baseline values on day 30. In the patients undergoing PPPD, the decrease in ETP was more pronounced as compared to the decrease in the patients undergoing hepatectomy ( $P = 0.01$  compared to baseline value, and  $P = 0.045$  compared to hepatectomy on post-operative day 3).

The ETP in the patients undergoing PPPD also recovered to baseline values on post-operative day 30 (Figure 2). Despite the decrease in thrombin generation in the absence of TM, in the presence of TM thrombin generation increased following hepatectomy (Table 2; Figure 2). The ETP increased from 542 nM\*min (417–694) at baseline ( $P > 0.05$  compared to controls) to 845 nM\*min (789–1050) on post-operative day 3 ( $P < 0.001$  compared to baseline;  $P < 0.001$  compared to controls).

**Table 2:** Parameters derived from the thrombin generation test in patients following hepatectomy, patients following PPPD, and healthy controls.

	In absence of thrombomodulin				In presence of thrombomodulin			
	ETP	Peak	Lag-time	Velocity index	ETP	Peak	Lag-time	Velocity index
<b>Controls</b>	1009 [856-1154]	233 [191-245]	1.9 [1.7-2.0]	94 [58-105]	558 [390-680]	142 [99-176]	1.7 [1.7-2.0]	630 [47-87]
<b>Patients following hepatectomy</b>								
Baseline	1141 [960-1364]	201 [183-204]	1.8 [1.7-2.2]	68 [51-95]	542 [417-694]	128 [97-156]	1.7 [1.7-2.0]	57 [41-78]
End-OK	987 [915-1223]+	200 [170-220]	1.7 [1.3-1.8]	85 [75-97]+	760 [593-878]*+	161 [124-164]+	1.6 [1.3-1.7]	77 [61-87]+
POD1	993 [781-1179]+	194 [146-212]	2.0 [1.7-2.0]	85 [71-98]	820 [628-956]+	167 [135-185]	2.0 [1.7-2.0]	79 [64-93]
POD3	981 [931-1150]+	203 [186-227]	2.0 [1.7-2.0]	99 [88-106]+	845 [789-1050]*+	189 [177-203]*+	2.0 [1.7-2.0]	94 [88-102]*+
POD5	907 [823-1073]+	203 [180-218]	1.7 [1.7-2.0]	95 [88-103]+	787 [721-896]*+	181 [174-208]+	1.7 [1.7-2.0]	93 [79-104]*+
POD7	883 [745-994]+	199 [176-215]	1.7 [1.7-2.0]	89 [82-106]+	715 [654-847]	186 [162-198]+	1.7 [1.7-2.0]	93 [74-103]*+
POD30	1173 1080-1173]	243 [194-293]	1.8 [1.7-2.0]	103 [58-126]+	818 [501-1101]	179 [116-248]+	1.7 [1.7-2.0]	77 [50-115]+
<b>Patients following PPPD</b>								
Baseline	1036 [810-1189]	165 [118-205]*	1.8 [1.7-2.3]	49 [33-87]*	361 [243-680]	78 [55-168]*	1.7 [1.7-1.9]	36 [25-80]*
End-OK	980 [686-1112]+	152 [116-193]*	1.8 [1.5-2.2]	64 [36-80]*	559 [243-823]	110 [63-127]*	1.7 [1.7-1.9]	53 [32-62]
POD1	772 [656-874]*+	128 [96-162]*+	2.3 [1.6-3.3]	47 [26-72]*	388 [178-691]	91 [35-140]*	2.0 [1.6-2.7]	42 [11-63]*
POD3	868 [658-962]*+	194 [146-212]*	1.7 [1.7-2.1]	79 [65-99]+	627 [469-754]	154 [120-187]+	1.8 [1.7-2.1]	70 [59-94]+
POD5	825 [524-882]*+	182 [122-207]*	2.0 [1.9-2.2]	67 [49-104]	399 [347-701]	119 [98-185]	2.0 [1.7-2.3]	59 [47-83]
POD7	733 [549-966]*+	162 [127-212]*	2.2 [1.9-2.5]	69 [45-84]*	317 [259-601]	89 [74-165]	2.0 [1.9-2.5]	49 [32-83]
POD30	979 [901-1121]	185 [149-206]*	2.2 [1.9-2.3]	70 [43-78]*	476 [331-602]	112 [74-136]*	2.0 [1.7-2.1]	48 [32-66]*

Data are expressed as median [interquartile range]. TM: thrombomodulin. ETP: endogenous thrombin potential. End-OK: end of surgery. POD: postoperative day. PPPD: pylorus preserving pancreaticoduodenectomy. \*P<0.05 compared to controls; +P<0.05 compared to baseline.

**Figure 2:** Changes in the thrombin generation assay [(A) endogenous thrombin potential (ETP) without thrombomodulin (TM), (B) endogenous thrombin potential (ETP) with TM, (C) thrombomodulin sensitivity ratio (TMSR)] after hepatectomy and PPPD. \* $P < 0.05$  between the hepatectomy and PPPD group. ° $P < 0.05$  vs. baseline levels in the hepatectomy group.

§ $P < 0.05$  vs. baseline levels in the PPPD group. ● $P < 0.05$  in the hepatectomy group vs. controls. ∞  $P < 0.05$  in the PPPD group vs. controls. End-OK, end of surgery; POD, post-operative day.

In comparison, total thrombin generation only slightly increased in the patients following PPPD (Figure 2). When these data were recalculated to a normalised thrombomodulin sensitivity ratio (TM-SR), it became evident that after hepatectomy thrombomodulin was less effective at regulating thrombin generation in these patients compared to controls (Figure 2). The TM-SR increased from  $1.2 \pm 0.4$  (mean  $\pm$  s.d.) at baseline to  $2.1 \pm 0.2$  on post-operative day 3 in the patients undergoing hepatectomy ( $P < 0.001$  compared to baseline;  $P < 0.001$  compared to controls). In comparison, the TM-SR only slightly increased in the patients following PPPD.

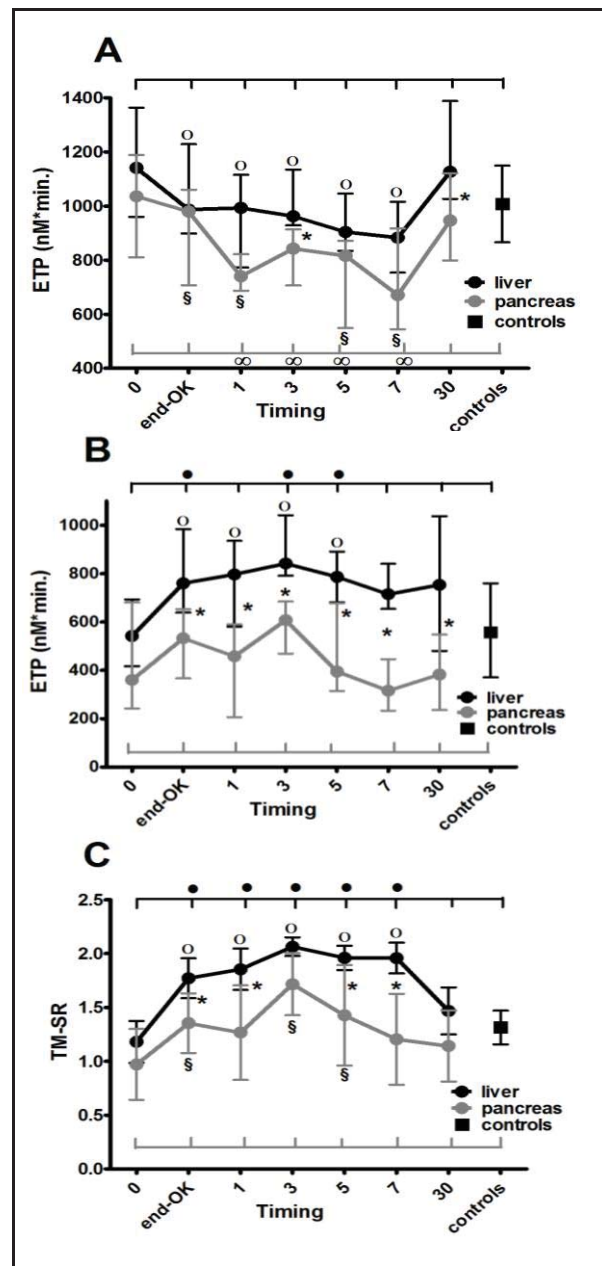


Table 2 shows the other parameters derived from the thrombin generation test. In the absence of TM, also peak thrombin generation levels remained comparable to baseline values following hepatectomy, and slightly decreased in the patients following PPPD on post-operative day 1. Following surgery, the lag-time remained comparable to baseline values for both patient groups. The velocity index increased until post-operative day 30 following hepatectomy, but only slightly increased on post-operative day 3 and declined again thereafter following PPPD. In the presence of TM, peak thrombin generation also gradually increased following hepatectomy, compared to only an increase on post-operative day 3 following PPPD. Following surgery, the lag-time remained the same in both patient groups. The velocity index, however, increased in both groups following surgery, with a maximum on post-operative day 3.

## DISCUSSION

In the present study, we observed a hypercoagulable status in samples taken from patients following a right hemi-hepatectomy, despite substantially prolonged conventional coagulation tests. Our results using thrombomodulin-modified thrombin generation testing are in line with several recent studies that found a normal to hypercoagulable state following liver surgery.<sup>5,6,10,19</sup> In contrast to previous work, our study provides a mechanistic explanation for the hypercoagulable state, i.e. a profound and sustained post-operative deficiency of the natural anti-coagulants AT and protein C.

Samples taken after liver resection were profoundly resistant to the anti-coagulant action of thrombomodulin, the physiological activator of the natural anti-coagulant protein C. While thrombin generation substantially decreased in healthy volunteers when thrombomodulin was added to the plasma, thrombin generation only slightly decreased by the addition of thrombomodulin in plasma from patients following hemi-hepatectomy. Consequently, thrombin generation in the presence of thrombomodulin was higher than that of healthy volunteers. The thrombomodulin resistance following liver surgery is in part explained by the decreased levels of protein C and elevated levels of FVIII.<sup>28</sup> Combined with the low levels of AT, the net effect is a normal or even supranormal thrombin generation when tested in the presence of thrombomodulin after hemi-hepatectomy, despite decreased levels of procoagulant proteins.

The decrease in coagulation factors following hemi-hepatectomy is in part explained by the decreased synthetic capacity of the liver remnant. However, we also observed a transient decrease in FII, AT, and protein C following pancreatic surgery. Therefore, it is likely that consumption of coagulation proteins as a result of surgical damage may also play a role. Finally, haemodilution may also partly explain the decrease in coagulation factor levels, and the decrease in haemoglobin following surgery combined with the limited blood loss indeed suggests our patients to be slightly haemodiluted.

Despite similar effects of both liver and pancreas surgery on levels of coagulation factors, we observed a difference in thrombin generation following hemi-hepatectomy and PPPD. Although thrombin generation tested in the presence of thrombomodulin was increased following liver surgery, thrombin generation under these experimental conditions was normal in patients following pancreatic surgery. Furthermore, when thrombin generation was tested in the absence of thrombomodulin, we observed a decreased in thrombin generation in both groups, but this decrease was much more pronounced following pancreatic surgery. De Pietri et al.<sup>6</sup> have also shown signs of hypocoagulability in patients undergoing pancreatic surgery using TEG. As the decrease in protein C and AT were more

extensive following hemi-hepatectomy compared to the decrease in patients following PPPD, this may in part explain the difference in thrombin generation between both groups.

As reported before,<sup>5,6,19,28</sup> we also observed a substantial prolongation of conventional coagulation tests in patients following liver surgery. However, these tests cannot reflect the true haemostatic status of patients undergoing liver surgery, as they are only sensitive for levels of procoagulant factors and do not take the reduction in anticoagulant factors, which also occur following liver surgery, into account. The thrombomodulin-modified thrombin generation test is, however, sensitive to all anticoagulant proteins in the plasma. Therefore, this test measures the true balance between the pro- and anti-coagulant factors. Thrombin generation testing in the presence of thrombomodulin has demonstrated normal or even superior thrombin generation in patients with chronic liver disease, despite prolonged conventional coagulation tests.<sup>28,29,31</sup> These findings have challenged the long held dogma of chronic liver disease being associated with a bleeding tendency due to changes in the plasmatic coagulation system.<sup>33</sup> Our present findings show comparable findings in patients that underwent liver resections, which may have important clinical consequences. First, we strongly advise not to correct routine coagulation tests by using blood product transfusion during or after hepatectomy. In fact, several serious side effects of blood product transfusion may occur, including the risk of infection and the risk of transfusion-related acute lung injury.<sup>17</sup> Furthermore, routine thrombosis prophylaxis should not be withheld in patients undergoing liver resection, as this may result in higher rates of venous thromboembolism in post-operative liver disease patients. More extensive prophylaxis may even be required as VTE rates are still substantial in those patients receiving optimal routine thromboprophylaxis.<sup>33</sup>

In conclusion, although conventional coagulation tests point to hypocoagulability in patients undergoing liver resection, thrombin generation in the presence of thrombomodulin revealed hypercoagulability following liver resection. This hypercoagulability was associated to a profound thrombomodulin resistance which was likely attributable to decreased levels of protein C and elevated levels of FVIII. Therefore, clinicians should be aware of the limitations of the use of conventional coagulation tests to guide haemostatic management during liver surgery. Furthermore, the results of our study support the exploration of more extensive use of anti-coagulant medication in the post-operative period, as was previously suggested by others.<sup>7,18,33</sup>

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