Changes in Haemostasis after Laparoscopic Surgery in Gynaecology: Contribution of the Thrombin Generation Test

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Key Words

Laparoscopic surgery · Gynaecology · Haemostasis · Fibrinolysis · Thrombin generation test

Abstract

Surgery induces immediate hypercoagulability by direct alteration of the vascular bed, release of procoagulant substances from the extravascular spaces and blood flow decrease, and delayed hypercoagulation in response to tissue damage which triggers inflammatory responses. Thus, the postoperative period represents a high-risk time for thrombosis. Recognition of high-risk individuals would make it possible to improve thromboembolism prevention. We studied in women undergoing laparoscopic surgery a series of markers known to be related to the thrombotic risk and confronted their results with those of a global test, the thrombin generation test (TGT) described by Hemker's

group. Our results show that two groups of patients can be distinguished according to usual risk markers (PAI-1, TAT, body mass index): the higher risk group demonstrates higher initial TGT values, but also a postoperative decrease of the TGT values whose mechanisms remain to be defined.

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Introduction

Postoperative thrombosis (deep venous thrombosis (DVT) and/or pulmonary embolism (PE)) is a relatively rare event. Yet despite efficient preventive procedures, it is a significantly frequent one in absolute terms, due to the high number of surgical procedures carried out every day. Therefore, it is too rare to be studied easily in small series of patients, but too frequent to be neglected as a medical problem.

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Recognizing patients likely to develop a thrombotic event is thus of prime importance. Clinical risk factors associated with the clinical profile and the surgical procedures have been recognized for a long time. They are based on a collective rationale and condition the preventive attitude to be adopted for an individual according to the risk group to which he belongs. However, these clinical risk factors fail to predict individual risk with high accuracy. For this reason, attempts to discern biological markers of risk are always made, in order to obtain a better understanding of the mechanisms preceding thrombosis and to adopt a preventive attitude suited to each patient. If the risk can be partially predicted from the clinical data, it is implicitly supposed that it is based on biological alterations that precede the perioperative stress. These are referred to as 'hypercoagulability' parameters. Unfortunately, no routine screening laboratory test has so far made it possible to predict the risk in the same way as activated partial thromboplastin time or prothrombin time can predict haemorrhagic risk. The thrombin generation test (TGT) described by Hemker et al. [1] in 1986, represents such an attempt to quantitate the endogenous thrombin potential.

Only very large prospective studies associating a precise clinical follow-up and an extensive biological examination are able to evaluate the predictive value of significant parameters. However, as the knowledge concerning haemostasis mechanisms becomes more intricate, preliminary studies are necessary to unravel the multitude of altered parameters which may be hypothesized to have a predictive value for thrombotic events. It would therefore be helpful to be able to recognize specific alterations related to specific surgical circumstances (such as abdominal, orthopaedic or gynaecologic surgery).

This paper reports the haemostatic alterations induced by laparoscopic surgery in gy-

naecology and attempts to shed light on the parameters related to the clinical profile (based on a simple questionnaire regarding risk factors for thrombosis), to the gynaecologic localization and to the laparoscopic procedure. The TGT is evaluated in this context to determine its potential predictive value for hypercoagulability.

Patients and Methods

The study concerned 45 women undergoing gynaecological laparoscopy for pelvic pain ($n = 4$), sterility examination ($n = 14$), endometriosis ($n = 5$), hysterectomy ($n = 6$), dermoid cyst ($n = 9$) or tubal sterility ($n = 7$). According to the indication of surgery, two groups were defined: type 1 represents the two first indications, while type 2 concerns the four latter ones. The average age was 37 ± 11 years (23–77 years). The mean operation time was 41 ± 23 min (5–90 min). The patients included in the study were brought into the operation room between 8 and 10 o'clock in the morning. The patients were in 20° Trendelenburg position in 43/45 cases and in gynaecological position in 2 cases, with 8–10 mm Hg pneumoperitoneum. Oral contraceptive use, cigarette smoking and body mass index were taken into account in the clinical questionnaire. Informed written consent was obtained for each patient before inclusion in the study. No patient had clinical manifestations of thromboembolic disease immediately after surgery or during a follow-up period of 6–8 months.

Blood withdrawal was carried out after anaesthetic induction (T0) and after the end of the surgical procedure (T1) and tubes were rapidly brought to the laboratory. To define the normal values of the haemostatic parameters, 10–25 healthy controls from the laboratory were also included according to the parameter (TAT, ETP, $n = 10$, fibrinolysis data, $n = 25$).

Laboratory Variables

Venous blood, anticoagulated with sodium citrate for the haemostatic variables and with EDTA for blood cell counts was withdrawn in Vacutainer® tubes. Blood samples for fibrinolysis measurement were withdrawn in Stabilyte tubes (Biopool, Umeå, Sweden). Plasma was centrifuged twice at 3,000 g for 15 min, aliquoted and rapidly frozen at -70°C , except for routine parameters which were immediately analyzed. Plasma samples thawed only once were used.

Haemostasis: Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (Fg), factors VIII:C and von Willebrand (VWF:Ag) were determined by conventional methods. Antithrombin III, Protein C (PC) and Protein S (PS) were determined by functional assays (Hemolab AT III chrom[®], bioMérieux, Marcy-L'Etoile, France; Coamatic[®], Protein C Biogenic, Maurin, France, and Proclot[®], Protein S Instrumentation Laboratory, Milano, Italy). Thrombin-antithrombin (TAT) complexes were assessed by ELISA kits (Enzygnost[®] TAT from Behringwerke, Marburg, Germany). Resistance to activated protein C (APC) was assessed by prolongation of the aPTT by APC in factor V-deficient plasma (IL Test Resistance PCA V, Instrumentation Laboratory, Italy) according to the manufacturer's instructions in an ACL 7000 apparatus (Instrumentation Laboratory). In functionally APC-resistant patients, the presence of the mutant factor V gene (1691 G → A transition) was confirmed by polymerase chain reaction and polymorphism restriction [2]. The factor II 20210 genotype was systematically determined by the technique described by Poort et al. [3].

Blood cells were counted in a Technicon H2 counter (Bayer-Technicon, Tarrytown, N.Y., USA).

Fibrinolysis: Total activity of plasminogen activators was evaluated by Euglobulin Clot Lysis Time as described by Kluft et al. [4] (ECLT, expressed in minutes).

The extrinsic system was assessed by the level of the tissue-type plasminogen activator (t-PA) and by the level and activity of its fast-acting inhibitor (PAI-1). t-PA antigen was determined by enzyme immunoassay (Imulyse[®] t-PA, Biopool) and PAI-1 activity was determined by an indirect method using a chromogenic substrate (Spectrolyse[®] PAI, Biopool).

Thrombin generation test: This was carried out in a subset of 28 patients who were analyzed both before and after surgery. Indeed, this method requires a relatively large volume of plasma due to a first step of defibrination by reptilase. Because haematocrit levels were too high, the required plasma volume was not sufficient in 10 cases (either at T0 or at T1). Moreover, as the test involves the cleavage of a chromogenic substrate whose optical density is read at 405 nm, it is hindered by an initially elevated optical density due to bilirubin or to haemoglobin and is not feasible in the case of icterical or haemolytic plasmas (7 cases). The test was carried out using a modification of the method described by Hemker and co-workers [5]. Briefly, 500 µl citrated plasma was mixed with 10 µl reptilase (Stago, Asnières, France) and incubated for 30 min at 37 °C and then kept in ice for 10 min. The fibrin clot

was wrung dry using a small plastic hook and eliminated. The remnant defibrinated plasma was proven to have normal levels of factors II, V, VII, and X and to have unaltered DD levels (as measured by the VIDAS ELISA D-dimer method from bioMérieux). The chromogenic reaction was performed on an ACL 7000 (Instrumentation Laboratory). Briefly, 100 µl of defibrinated plasma was preincubated for 10 s in the presence of calcium-free thromboplastin (Technique Biologique, Paris, France), and the reaction was triggered by a mixture of 100 mM CaCl₂ and 3 mM chromogenic substrate (SQ68 from Stago, methylmalonyl-L- α -methylalanyl-arginyl-paranitroanilide). Optical density of the reaction was continuously recorded after a lag of 10 s and for 999 s, and 1,044 digitized signals for each sample were transmitted to a regular personal computer in a format operable by the Microsoft Excel Software. Then, a programme developed locally made it possible to transform the raw data in the 'ETP' (endogenous thrombin potential) parameter, which is expressed in OD units, according to the mathematical data processing described by Hemker et al. [6]. Intra- and interassay variabilities of the method have previously been defined: their mean coefficients of variation are 3 and 7% respectively.

Statistical analysis: Paired and unpaired Student's *t* tests were used. Potential relationships between the variables were evaluated by linear regression.

Results

Baseline Patient Characteristics at the Preoperative Withdrawals

Genotypes for the factor V and factor II mutations were systematically determined in the patients in order to define the constitutional risk of thrombosis related to Leyden mutation of factor V (APC resistance) or to the 20210 G → A mutation of prothrombin. Only 1 patient was found to be heterozygotic for both mutations, thus representing a 2.2% prevalence of the mutation in this patient group, a figure similar to the prevalence found in larger groups of controls in our geographic area. None of the samples was found to present any other preoperative haemostatic alteration suggestive of a genetic modification

Table 1. Haematological and haemostatic parameters obtained in the patients before (T0) and after (T1) surgery

		T0 (mean $\pm$ SD)	T1 (mean $\pm$ SD)	T0 vs. T1 p $\leq$		Normal range
WBC	10 ³ / μ l	6.2 $\pm$ 1.8	7 $\pm$ 2.4	<i>0.001</i>	↑	4–10
RBC	10 ⁶ / μ l	4.3 $\pm$ 0.4	4.2 $\pm$ 0.3	<i>0.001</i>	↓	4–5.5
HB	g/l	12.5 $\pm$ 1.1	12.1 $\pm$ 1	<i>0.001</i>	↓	12–16
HT	%	38.8 $\pm$ 3.5	37.3 $\pm$ 3	<i>0.001</i>	↓	37–50
PLT	10 ³ / μ l	229 $\pm$ 45	235 $\pm$ 48	0.098		140–450
TP	%	94 $\pm$ 6	91 $\pm$ 8	<i>0.002</i>	↓	70–100
aPTT	s	36 $\pm$ 4	36 $\pm$ 5	<i>0.025</i>	↑	28–40
Fg	g/l	2.8 $\pm$ 0.5	2.6 $\pm$ 0.5	<i>0.003</i>	↓	1.8–4.5
II	%	93 $\pm$ 13	113 $\pm$ 18	<i>0.001</i>	↑	70–100
VIII:C	%	115 $\pm$ 33	125 $\pm$ 56	0.052	(↑)	70–120
VIII:Ag	%	108 $\pm$ 40	105 $\pm$ 45	<i>0.047</i>	↓	70–120
AT3	%	86 $\pm$ 12	82 $\pm$ 12	<i>0.006</i>	↓	70–100
PC	%	105 $\pm$ 20	98 $\pm$ 19	<i>0.001</i>	↓	70–100
TAT	μ g/l	9 $\pm$ 17	14 $\pm$ 29	0.166		0–5
ECLT	min	181 $\pm$ 57	148 $\pm$ 52	<i>0.001</i>	↓	175 $\pm$ 35
t-PA	ng/ml	5 $\pm$ 2	6 $\pm$ 5	0.058	(↑)	5 $\pm$ 2
PAI-1	UI/ml	6 $\pm$ 7	4 $\pm$ 6	<i>0.001</i>	↓	6 $\pm$ 3
ETP	mOD	90 $\pm$ 20	93 $\pm$ 20	0.116		95 $\pm$ 30

‘p’ expresses the statistical significance of paired Student t-tests; it is italicized when significant and the arrow on the right represents the direction of the alterations (in parentheses when only tendencies were found). The abbreviations used in the first column are explained in the Methods section, apart from WBC, RBC, HB, HT, PLT, which are white blood cells, red blood cells, haemoglobin, haematocrit and platelets respectively. The last column on the right gives the normal range of the different parameters in the laboratory.

such as a deficiency of one or another of the anticoagulant proteins, as assessed by functional tests.

Mean values of most of the parameters studied were found to be normal (table 1), although some of the patients were found to be outside the normal range: this percentage did not exceed 20% for any parameters except for the TAT complex which was increased in 36% of the patients, the ECLT which was increased in 20% of the patients and the factor VIII complex which was over the highest range in respectively 50 and 29% of the pa-

tients for factor VIII:C and factor VIII:Rag. No haemostatic parameter was found to be related to any of the clinical parameters noted in the questionnaire (age, underlying pathology warranting surgery, duration of surgery, oral contraceptive use, cigarette smoking and body mass index).

Haemostatic Differences between Initial and Postoperative Withdrawals

Table 1 summarizes the data of patients after surgery as compared to those before. Alterations were observed in the blood cell

counts, which were very significant although sometimes minute because all the patients vary in the same way.

Except when considering the 13% increase in WBC, the alterations never exceeded 4% of the initial value for the blood cell parameters and the global coagulation tests, while the decrease in AT3 and protein C reached respectively 5 and 8% and the increase in factors II and VIII:C were respectively as high as 21 and 9%. Protein S was not systematically assessed postoperatively due to the stability of the results. The TAT complex is often described to be increased postoperatively. Its mean value was found to be increased by 61% as compared to the initial value, although this value did not reach significance due to the very wide dispersion of the values. Two fibrinolysis parameters were also significantly altered postoperatively: the euglobulin clot lysis time, which reflects the overall potential for fibrin lysis of the plasma, was decreased 18% after surgery. This is probably related with the 32% decrease observed in the activity of the major inhibitor of the plasminogen activator, PAI-1. Interestingly, the decrease in PAI-1 was higher when surgery lasted longer than 40 min (change in PAI-1 was 0.9 ± 1.1 IU/ml when surgery lasted less than 40 min and -2.5 ± 2.2 IU/ml when surgery lasted more than 40 min, $p < 0.02$). Similarly, the decrease in PC was higher in longer surgery ($-5 \pm 6\%$ vs. $-13 \pm 19\%$, $p < 0.04$). Moreover, the heaviest type of surgery (type 2) was related to the largest increase in TAT (28 ± 50 vs. -5 ± 28 $\mu\text{g/l}$ in type 1, $p < 0.03$) and the largest stimulation of fibrinolysis (decrease in ECLT -50 ± 58 vs. 8 ± 61 min, $p < 0.05$).

When considering the patients altogether, no significant alteration in factor VIII complex nor in the endogenous thrombin potential was observed. However, a more careful examination of the data demonstrated that the latter parameter, ETP, could be consider-

ably modified in one way or another depending on the patient.

Definition of Two Groups of Patients as a Function of the ETP Test

Figure 1 shows that two groups of patients emerged from the way the ETP evolved after surgery. A first subgroup expressed a significant decrease in ETP while a second one expressed a significant increase in ETP. Interestingly, a clustering of certain parameters emerges from dividing the whole patient group in two subgroups as a function of ETP alteration. Figure 1 summarizes how the main laboratory and clinical parameters cluster with one or another of the ETP groups.

In the group with a postoperative decrease in ETP, the mean initial ETP value was higher. Moreover, higher initial levels of PAI-1 and less efficient fibrinolytic activity (ECLT) were observed at the preoperative time (T0), while higher TAT levels were found at this time when compared to the other group of patients. The patients presenting this ETP pattern were also the patients presenting the higher body mass index (23.6 ± 4.4 vs. 20.2 ± 2.1 in group B, $p < 0.006$). Conversely, in the group with a postoperative increase in ETP, lower level of ETP, PAI-1 and TAT and more efficient fibrinolysis were observed at the preoperative time (T0). However, this group of patients was also the group that reacted more dramatically to the surgical procedure, with a large increase in fibrinolytic activity postoperatively and a larger generation of TAT complexes. These patients presented limited adipose tissue storage (BMI 20.2 ± 2.1).

Neither age, hormonal therapy, smoking habits nor surgery duration were associated with any of the haemostatic alterations observed before or after surgery.

Fig. 1. ETP results (in mOD) in patients before (T0) and after (T1) the laparoscopic procedure. Patients were distributed into two groups according to the direction of ETP modification after surgery when compared to the ETP value before surgery. ETP decreased after surgery in patients in group A and increased in

those in group B. Accompanying parameters at T0 and T1 in the two groups are compared by Student t-tests (paired between T0 and T1 in a given group and unpaired between groups); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

The patients engaged in this study were young women in good general health, submitted to rapid laparoscopic surgical procedures. Only slight pre- or postoperative alterations could then be expected. Indeed, haemostatic parameters were in the normal ranges for more than 80% of the patients apart from PAI-1, VIII:C, VIII:Rag and the TAT complex. The latter presented an overall increase when compared to normal laboratory values ($9 \pm 17 \mu\text{g/l}$ in patients, $n = 45$, vs. $0.5 \pm 0.5 \mu\text{g/l}$ in normal controls, $n = 10$, $p < 0.026$) and was above the limits of normal values in 36% of the patients. The above-mentioned alterations are related to the inflammatory response, which is closely interconnected with the haemostatic system [7], thus explaining the increase in TAT.

Type of surgery, duration or method are known to modify laboratory parameters to a variable extent due to a haemodilution whose intensity depends on the anaesthetic procedure, and due to demargination of leukocytes. The increase in WBC levels observed postoperatively in our study is usual during anaesthesia, while the modest postoperative haemodilution observed was probably involved in the slight yet significant decrease in most plasmatic factors (RBC, Hb, Ht, Fg, AT3, PC, VIII:Rag...). However, some factors or functions were increased in spite of this small haemodilution (factor II, fibrinolysis efficiency), so more complex alterations of the haemostatic equilibrium occur after surgery.

The increase in postoperative fibrinolytic activity found in our study in women undergoing gynaecologic surgery under laparoscopy is in line with recent results published in laparoscopic cholecystectomy, where the global fibrinolytic activity was found to be increased postoperatively [8, 9] and where an increase in t-PA activity was described during

intervention [10]. There appears a discrepancy in the course of fibrinolysis after laparoscopic and after open surgery [11]. Indeed, in the literature, fibrinolysis activity is usually described to decrease during and immediately after open surgery due to an increase in PAI-1 activity [12, 13]. This surgery-induced 'shut down' of fibrinolytic activity is thought to be related to a peroperative release of platelet PAI-1 [14], high levels of the pituitary hormone arginine vasopressin [15] and of inflammatory cytokines [16] in response to the surgical stress which induces a release of the endothelial protein PAI-1. A difference in fibrinolytic response according to the type of surgery has been described when comparing orthopaedic and abdominal surgery [17]. In only concerned the intensity of decrease in fibrinolytic activity (which is more pronounced in orthopaedic surgery).

Laparoscopic surgery as thus confirmed by our study induces an increase in fibrinolytic activity during the immediate postoperative period, which may mean a lower thrombotic risk for patients undergoing this procedure as compared to open surgery.

Although potentially less thrombogenic, laparoscopic surgery is a setting where individual cases of fatal or nonfatal thrombotic events are reported, either localized in the splanchnic vessels [18, 19] or in the veins of the lower limbs (DVT) and pulmonary arteries (pulmonary embolism) [20–22]. Most papers evaluating the risk of postoperative thrombotic events after laparoscopic surgery (most often after laparoscopic cholecystectomy) estimate the risk to be lower than with open surgery, although not negligible [23]. The lingering bias due to the overrepresentation of young and healthy patients early in the era of laparoscopic surgery is mentioned in some reviews [23]. In others, authors emphasize on the contrary on the 'extremely high incidence of venous thrombosis' that corre-

lates with the haemodynamic changes, which occur in the venous system during pneumoperitoneum [24]. Identifying patients with increased risk of thromboembolism thus remains of prime interest in laparoscopic surgery as well as in open surgery.

Open surgery induces a stress that has been shown to be associated with the risk of developing a DVT with an odds ratio of 1.69, comparable to that related with age or prior history of venous thromboembolism [25]. In highly thrombogenic surgery like elective hip replacement, DVT is significantly associated with age and obesity, but also with factor VIII:C activity and TAT complexes [26]. Rocha et al. [27] pioneered a preoperative identification of patients with high risk of venous thrombosis by creating an index associating the results of D-dimers, t-PA and PAI-1. This index was able to predict a DVT after hip replacement with a very high sensitivity and specificity (respectively 100 and 95%).

In the same circumstances, Cofrancesco et al. [28] demonstrated that preoperative levels of fragment F1+2 of prothrombin (an activation marker of prothrombin activation in thrombin), TAT and D-dimers were associated with the risk of development of DVT after surgery. However, these indexes are not yet commonly used and they have to be confirmed in large prospective multicentric studies.

Another approach should ideally be a global test that may predict hypercoagulability in the same way as PT and aPTT predict hypo-coagulability rapidly, efficiently and inexpensively. Thromboelastography (TEG) has been proposed to evaluate hypercoagulability after laparoscopic cholecystectomy in a clinical series [29] and in an experimental series in swine [30]: both studies confirmed the post-operative hypercoagulability. However, this method is not available in most institutions and Hemker et al. [1] have proposed a plas-

matic alternative with the TGT. This method was initially developed to unravel the physiology of thrombin generation in whole plasma independently of its breakdown by antiproteases. Thrombin 'burst' resulting from a standardized activation can be measured in partially reconstructed coagulation systems to recognize the relative influence of different components of the complex network of enzymatic reactions. It can be used in patients submitted to anticoagulant treatment to analyze the main targets of action, and in patients in different pathological circumstances [6]. First clinical results demonstrated an increase in the endogenous thrombin potential (ETP) in various clinical circumstances such as congenital antithrombin deficiencies, use of oral contraceptives, DVT and coronary artery disease [5]. Thus, the perioperative period in which hypercoagulation occurs is an interesting target for ETP investigation. Although our series is too small to allow any quantification of the prevalence of thrombotic complication, which has been evaluated to be about 0.3% in the general population undergoing laparoscopic surgery [31]; interesting descriptive results of the biological data are however possible in these patients.

The important results of this part of the study was that the ETP did not vary homogeneously among the patients. A first group of patients was shown to present a decrease in ETP after surgery while a second one had an increase in ETP. The first also had higher initial ETP values associated with altered fibrinolysis parameters, increased TAT levels and higher body mass index. Thus, this first group was found to present baseline alterations consistent with hypercoagulability, which have been demonstrated in previous studies to be related to an increased risk of thrombosis [26, 27, 31]. The ETP of these patients is not further increased after surgery (as inherently related to the definition of the patient group),

but is in fact decreased, as if the surgery-induced alterations which are regularly encountered (such as the increase in TAT complex due to the stimulation of the coagulation process at the surgical area) may no longer occur. Similarly, the laparoscopy-induced stimulation of fibrinolysis reported by different authors [8–10] was not significant in this group of patients, whereas in the other group, fibrinolysis was strongly stimulated by laparoscopy. The ETP results of the present study are in line with those obtained in our laboratory in patients with hypercoagulability due to malignant colic diseases and submitted to abdominal surgery: elevated baseline ETP values were followed by a significant decrease in ETP one day postoperatively (publication in progress).

In conclusion, we confirm in this study that gynaecologic laparoscopic surgery induces a stimulation of the fibrinolytic activity, which may in turn have a protective effect against postoperative thrombotic complications when compared to open surgery. However, some hallmarks of inflammation and coagulation activation were also present in the subgroup of patients who presented preoperatively a higher potential for thrombin generation as assessed by the TGT. Surgical stress did not enhance thrombin generation in this group but revealed a decreased potential to generate thrombin. The TGT may therefore be an interesting global test to recognize patients with enhanced risk of developing a thrombosis postoperatively, and it needs to be further evaluated in larger series of patients to strengthen the relevance of this preliminary study.

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