Thrombin generation assessed as endogenous thrombin potential in patients with hyper- or hypo-coagulability

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Background and Objectives. The endogenous thrombin potential (ETP) represents the balance between pro- and anti-coagulant forces operating in plasma and can be used to investigate hyper- and hypo-coagulability. As a preliminary step to larger clinical studies we investigated the effect on ETP of phospholipids, tissue factor (TF) and residual platelets in frozen plasma.

Design and Methods. We investigated platelet-poor and platelet-rich plasmas from healthy subjects, patients on oral anticoagulants (OA), or with hemophilia and women on oral contraceptives (OC), chosen as examples of the normal, hypo- and hyper-coagulable states in which ETP has been reported to be impaired.

Results. Phospholipids had only a slight effect on ETP in all conditions except in women on OC, in whom the best diagnostic efficacy was observed at 0.5 μM. TF had only a slight effect in all conditions except hemophilia, in which an ETP impairment was observed only at low (1 pM) concentration. Residual platelets had considerable effects on ETP in frozen plasmas, but this was abrogated by filtration before freezing. ETP in platelet-rich plasma at $150\times10^3/mm^3$ was similar to that obtained in filtered-plasma with 1.5 μM phospholipids in healthy subjects, patients on OA and patients with severe hemophilia, but not in those with mild- or moderate-hemophilia, where the ETP was higher in platelet-rich plasma.

Interpretation and Conclusions. The results suggest that the method can be used for investigations on the clinical value of ETP. Platelet-rich and platelet-poor plasma are suitable testing materials. The latter should be filtered before freezing to minimize the effect of residual platelets.

Key words: thrombin generation, thrombin potential, hypercoagulability, hypocoagulability.

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Thrombin generation tests (TGT) were variably used during the 1950s to investigate hemostasis in patients with hemorrhagic diseases.[1,2] These tests were based on the addition, to whole blood[1] or plasma,[2] of suitable triggers (either calcium chloride, thromboplastin, or cephalin) able to initiate coagulation. The titration of thrombin generated over time was accomplished by assessing the shortening of the clotting time induced by the test mixture on a fibrinogen solution.[1, 2] In 1986, TGT were reconsidered and modified by Hemker *et al.*[3] According to this modification, thrombin generated from the patient's plasma after addition of a suitable trigger was measured by sampling, at fixed time intervals, a small portion of the test mixture in a solution containing a thrombin-specific chromogenic substrate (S2238). The amount of generated thrombin was plotted against time to construct a thrombin generation curve (or thrombogram). The authors have also developed a computer program able to calculate the parameters which characterize the thrombogram i.e., the lag time, the peak height, the time to peak and the endogenous thrombin potential (ETP). This last is the area under the thrombin generation curve and represents the total amount of thrombin formed over time, after exclusion of the contribution to amidolysis of the $\alpha_2$macroglobulin-thrombin complex that is unable to convert fibrinogen into fibrin, but retains amidolytic activity towards the thrombin-specific synthetic substrates. It was surmised that the thrombogram may be more sensitive than traditional coagulation tests to investigate hypo- and hyper-coagulability. In 1993 TGT were further modified by the use of a thrombin-specific substrate with *slow* reactivity.[4] This modification allows photometric measurement of the para-nitroaniline split by thrombin without sub-sampling procedures, thus making the test fully automated on a clinical chemistry autoanalyzer.[4] Despite this considerable improvement, the defibrination of test plasma, which is required before testing, remains a drawback of the TGT. Recently, the chromogenic substrate has been replaced by a fluorogenic substrate (Z-Gly-Gly-Arg-AMC).[5] The main advantage of this modification is that the optical turbidity due to fibrin formation does not disturb the fluorescent signal. Therefore, both platelet-poor and platelet-rich plasma can be tested directly without defibrination. This modified method in combination with a microtiter-plate fluorometer can be regarded as a considerable step forward to the application of ETP in less specialized clinical laboratories. However, it is conceivable that the ETP

value resulting from TGT depends on many variables. Among such variables the concentrations of tissue factor and phospholipids added as reactants to the test system are particularly important.[6] To our knowledge no data are available on the influence of the above reactants on ETP measured with the latest generation test. This study was, therefore, aimed at investigating the influence of the concentrations of tissue factor and phospholipids as well as the influence of residual platelets on the ETP measured in platelet-poor and platelet-rich plasma from healthy subjects, patients on oral anticoagulant therapy, patients with hemophilia A and women on oral contraceptives. These groups were chosen as examples of the normal, hypo- and hyper-coagulable states in which ETP has been reported to be impaired.[6-11]

Design and Methods

Patients

We investigated plasmas from healthy subjects, patients on oral anticoagulants, patients with hemophilia A and pre-menopausal women without a history of thrombosis. Some of these women were on oral contraceptives at the time of blood sampling. Patients on oral anticoagulants and patients with hemophilia were randomly selected from among those who attended the clinic for regular monitoring. Healthy subjects, including pre-menopausal women, were randomly selected from among those who attended the clinic. They served as volunteers in the case-control studies on thrombophilia.

Plasma preparation

After informed consent, blood was collected by venipuncture into 0.105 M trisodium citrate (ratio 9:1). Platelet-rich plasma (PRP) was prepared by centrifugation at 1,000g for 10 minutes at room temperature. Platelet-poor plasma (PPP) was prepared by centrifugation at 2,000g for 12 minutes. PPP was then divided into two portions. One portion (herein called PPPF) was filtered through a 0.22μm filter to remove residual platelets. Aliquots of PPP and PPPF were snap-frozen with liquid nitrogen and kept at –80°C until testing. Filtered pooled normal plasma (herein called PNPF) was prepared by pooling equal amounts of PPPF from 10 healthy subjects. Suitable aliquots were snap frozen and kept at –80°C until testing.

Reagents for ETP measurement

Lyophilized powder blends of synthetic phospholipids (DOPS, DOPE and DOPC in the proportion of 20/20/60 (M/M), were obtained from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA). The relipidated recombinant tissue factor was recombiplastin, a calcium-free human recombinant thromboplastin (IL, Orangeburg, N.Y., USA), reconstituted with distilled water. The fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl) was obtained from Bachem, Switzerland.

ETP measurement

The ETP was measured as described elsewhere.[5] Briefly, 80 μL of plasma were pipetted into the well of a blue microtiter plate (Greiner Labortechnik, Frickenhausen, Germany) together with 20 μL of a mixture of tissue factor and phospholipids in buffer solution (20 mM HEPES, 140 mM NaCl and BSA 5mg/mL, pH 7.35). If not otherwise stated, the final concentrations of tissue factor and phospholipids were 1 pM and 0.5 μM. Phospholipids were omitted when the test was performed on PRP. The microtiter plate was then put into the pre-warmed measuring chamber of a Fluoroskan Ascent, (ThermoLabsystem, Helsinki, Finland), which automatically dispenses 20 μL of 2.5 mM fluorogenic substrate in 2.5 % dimethyl-sulfoxide with $CaCl_2$ 0.1 M into each well. The fluorescence signal was then recorded for 50 minutes at 15-second time intervals. ETP values were calculated and corrected for $\alpha_2$macroglobulin-thrombin complex activity by using the thrombogram software, made available by Synapse b.v. (Maastricht, Netherlands). The results were expressed both as relative fluoro-units (RFU)/minute and as a percentage relative to the ETP value of the pooled normal plasmas tested along with the patient's plasma (ETP %).

Other coagulation tests

Prothrombin time (PT) and factor VIII were measured on an ACL or MLA 1600 instrument (IL, Lexington, MA, USA). Reagents were Recombiplastin (IL) and Synthasil (IL).

Statistical analysis

Differences of mean values were tested for statistical significance by paired or unpaired Student's t-test or by the analysis of variance for repeated measurements. The χ^2 test was used to compare categorical data. Statistical significance was considered at $p < 0.05$.

Results

Precision study

The between-run precision was assessed by repeated measurements (n=14) for two plasma preparations: PNPF and filtered plasma pooled from patients on oral anticoagulants (POAT). The results, expressed as coefficients of variation (CV%), are shown in Table 1. As expected, when the test was run without phospholipids the variation was high (CV=33.4% for PNPF and 38.5% for POAT). Improved precision was obtained when phospholipids were present at 0.5 μM (CV=6.9% and 10.3%

Table 1. Between-run precision. Mean (CV%) values of 14 replicate measurements for filtered pooled normal plasma (PNPF) and pooled plasma from patients on oral anticoagulants (POAT).

Phospholipid concentration (μM)	*PNPF (RFU/min)*	*POAT (RFU/min)*
0	84 (33.8%)	26 (38.5%)
0.5	493 (6.9%)	157 (10.3%)
1.5	713 (3.2%)	241 (5.6%)

for PNPF and POAT, respectively), or at 1.5 μM (CV=3.2%. and 5.6% for PNPF and POAT, respectively).

Influence of residual platelets in frozen plasma

ETP values (Table 2) were considerably higher in frozen PPP from healthy subjects than in fresh PPP. Differences were greater when phospholipids were omitted or their concentration was low (0-1 μM, $p<0.001$). The differences became less evident at higher phospholipid concentrations (1.5 μM, $p=0.005$). The number of residual platelets in individual PPP from healthy subjects ranged from 0 to $19\times10^3/mm^3$. When PPP was filtered the differences in ETP in fresh and frozen samples were abolished even when no phospholipids were added to the test system, ($p>0.22$).

The effect of residual platelets in frozen plasma was further investigated by measuring ETP in PPP with increasing platelet counts. PRP from 4 healthy subjects was diluted with the subjects' own PPPF to obtain platelet counts in the range of 0-$50\times10^3/mm^3$. The plasma samples were then frozen and thawed to lyse platelets before testing. The tissue factor concentration in the test system was 1 pM and the phospholipid concentrations were 0, 0.5, 1 and 1.5 μM. As shown in Figure 1, when the phospholipids were omitted, there was a linear relationship between the ETP value and the number of residual platelets below $5\times10^3/mm^3$. The ETP remained unchanged at higher platelet counts. The above correlation became less evident when phospholipids were added to the system at concentrations ranging from 0.5-1 μM. No influence at all was observed at phospholipid concentration of 1.5 μM (Figure 1).

Table 2. Influence of residual platelets on ETP. Mean ETP ± SD (RFU/min) values and statistical significance (paired t-test) assessed for fresh and frozen non-filtered PPP and filtered PPP (PPPF) from 18 healthy subjects. Tissue factor concentration was 1 pM.

Phospholipid (μM)	*Non-filtered PPP*			*PPPF*		
	Fresh	*Frozen*	*p values*	*Fresh*	*Frozen*	*p values*
0	368±168	688±131	< 0.001	190±170	192±165	0.759
0.5	650± 118	732±134	< 0.001	544±115	534±121	0.223
1	715±121	748±126	<0.001	649±117	642±128	0.443
1.5	726±121	748±118	0.005	687±118	690±123	0.653

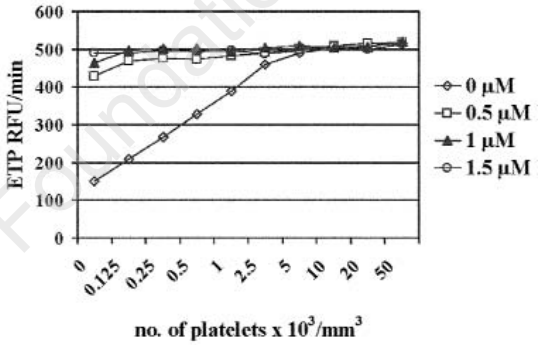

Figure 1. Mean ETP of 4 healthy subjects measured in filtered platelet-poor plasma with residual (lysed) platelets ranging from 0 to $50\times10^3/mm^3$. Tissue factor and phospholipid concentrations in the test system were 1 pM and 0-1.5 μM, respectively.

Influence of phospholipid concentration

To investigate the effect of phospholipid concentration, the ETP was measured in PPPF from 19 healthy subjects, 15 patients on oral anticoagulants and 18 patients with hemophilia A. The final concentration of tissue factor in this experiment was 1 pM, whereas the final concentration of phospholipids ranged from 0.5 to 20 μM.

Healthy subjects. As shown in Table 3 and Figure 2A, ETP increased (up to 22%) with increasing concentrations of phospholipids. The values ranged from 384 RFU/min in the presence of 0.5 μM phospholipids to 455 RFU/min in the presence of 20 μM phospholipids. A plateau was reached when the phospholipid concentration exceeded 5 μM. However, when the ETP values were expressed as percentages of the values obtained with filtered pooled normal plasma (ETP%) tested in the same run, the

Table 3. Influence of phospholipid concentration. Mean ETP and standard deviation (SD) measured in filtered platelet-poor plasma (PPPF) from 19 healthy subjects expressed as RFU/min or as percentage (ETP%) relative to the filtered pooled normal plasma. Phospholipid concentration ranged from 0.5 to 20 μM. Tissue factor concentration was 1 pM. *p* values refer to the significance versus the previous value as assessed by the analysis of variance for repeated measurements.

Phospholipids	*RFU/min*			*ETP%*		
μM	*Mean*	*SD*	*p values*	*Mean*	*SD*	*p values*
0.5	384	75	–	93	15	–
1.5	441	69	<0.001	95	12	0.34
5	462	71	0.001	95	13	0.91
20	455	67	0.25	92	11	0.11

Table 4. Influence of tissue factor concentration. Mean ETP and standard deviation (SD) measured in filtered platelet-poor plasma (PPPF) from 19 healthy subjects expressed as RFU/min or percentage (ETP%) relative to the filtered pooled normal plasma. Tissue factor concentrations ranged from 1 to 40 pM. Phospholipid concentration was 0.5 μM. *p* values refer to the significance versus the previous value as assessed by the analysis of variance for repeated measurements.

Tissue factor	*RFU/min*			*ETP%*		
(pM)	*Mean*	*SD*	*p values*	*Mean*	*SD*	*p values*
1	385	80	–	88	16	–
2	415	83	<0.001	99	16	<0.001
10	491	83	<0.001	99	12	0.87
40	488	74	0.57	101	13	0.31

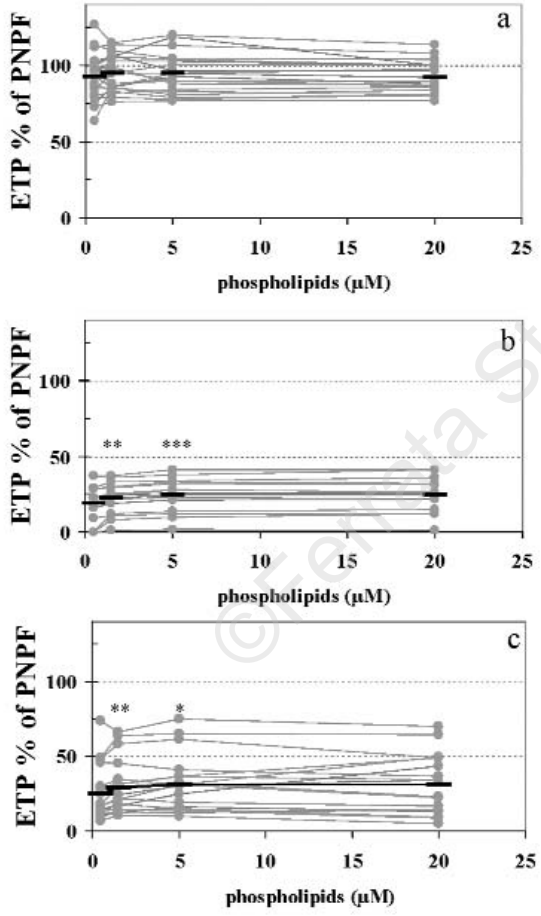

Figure 2. Influence of phospholipids on ETP% measured in filtered platelet-poor plasma of 19 healthy subjects (2a), 15 patients on oral anticoagulants (2b) and 18 patients with hemophilia A (2c). Tissue factor and phospholipid concentrations in the test system were 1 pM and 0-20 μM, respectively. Asterisks refer to the significance versus the previous value as assessed by the analysis of variance for repeated measurements (p*<0.05>0.01; ***p*<0.01>0.001; ****p*<0.001).**

phospholipid-dependent trend was abolished (*p*> 0.11), (Table 3, Figure 2A). This suggests that ETP% is probably the most appropriate way of expressing results.

Patients on oral anticoagulants and patients with hemophilia A. When the phospholipids were added to the test system at a concentration greater than 1.5 μM, small increments of ETP values were observed for patients on oral anticoagulants even when results were expressed as ETP% (Figure 2B). Mean±SD ETP % values were 19±12, 22±10, 24±11 and 25±11 at phospholipid concentrations of 0.5, 1.5, 5 and 20 μM, respectively. The same pattern was observed in patients with hemophilia A (Figure 2C). Mean±SD ETP% values were 25±18, 29±18, 31±19 and 31±20 at phospholipid concentrations of 0.5, 1.5, 5 and 20 μM, respectively.

Influence of tissue factor concentration

The influence of tissue factor concentration was assessed in both PRP and PPPF from healthy subjects, patients on oral anticoagulants and patients with hemophilia A. The final concentrations of tissue factor chosen for the investigation ranged from 1 to 40 pM. The phospholipid concentration was kept constant at 0.5 μM when the test was performed on PPPF, while no phospholipids were added when the test was performed on PRP.

Healthy subjects. Table 4 shows median ETP values for PPPF from 19 healthy subjects. As already noted for phospholipids, the ETP increased (up to 27%) with an increasing concentration of tissue factor. The mean ETP ranged from 385 RFU/min at a tissue factor concentration of 1 pM to 488 RFU/min at a tissue factor concentration of 40 pM. A plateau was reached at 10 pM (Table 4 and Figure 3a). As already noted for phospholipids, the dependency of the ETP on tissue factor concentration was much

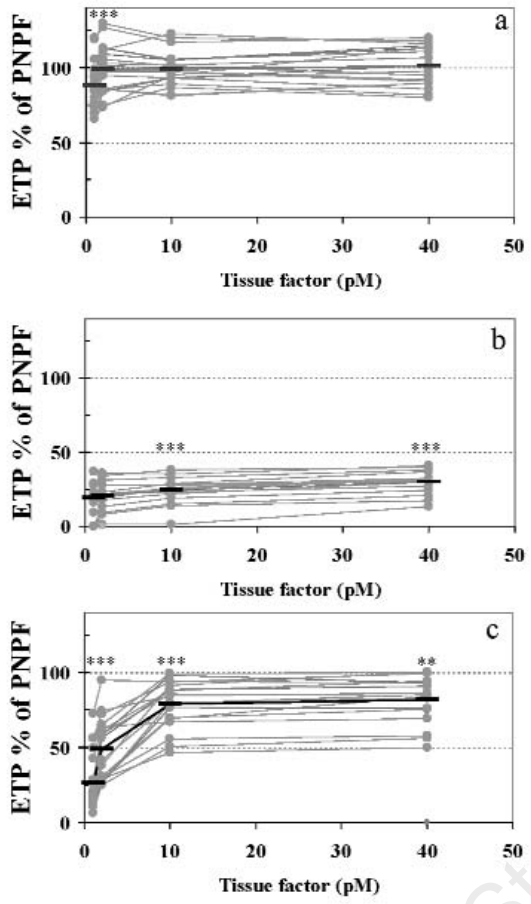

Figure 3. Influence of tissue factor on ETP% measured in filtered platelet-poor plasma of 19 healthy subjects (3a), 15 patients on oral anticoagulants (3b) and 18 patients with hemophilia A (3c). Phospholipid and tissue factor concentrations in the test system were 0.5 μM and 0-40 pM, respectively. Asterisks refer to the significance versus the previous value as assessed by the analysis of variance for repeated measurements (* p<0.05>0.01; ** p<0.01>0.001; *** p<0.001).

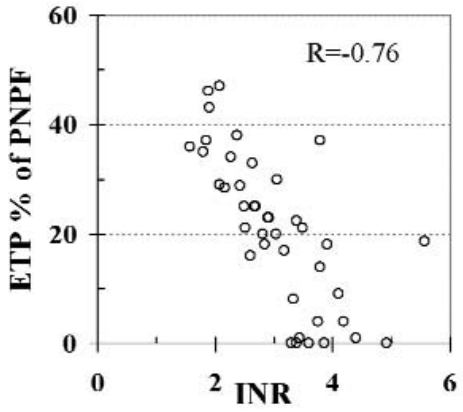

Figure 4. Correlation between ETP% and INR values in 41 patients on oral anticoagulants. Phospholipid and tissue factor concentrations in the test system were 0.5 μM and 1 pM, respectively.

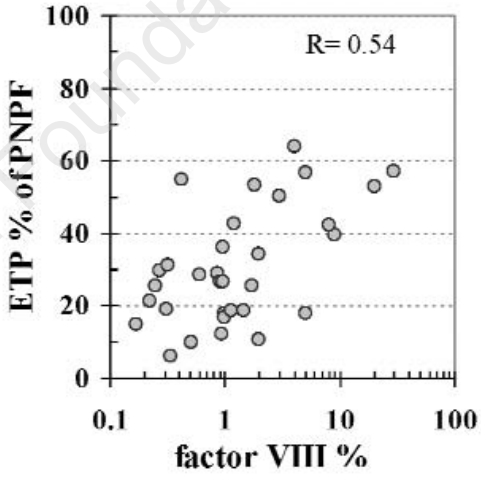

Figure 5. Correlation between ETP% and factor VIII concentration in 39 patients with hemophilia A. Phospholipid and tissue factor concentrations in the test system were 0.5 μM and 1 pM, respectively.

less when results were expressed as ETP %. No significant differences were found when the tissue factor concentration was greater than 2 pM.

Patients on oral anticoagulants. As shown in Figure 3b the ETP increased with increasing tissue factor concentration. Mean±SD ETP% values were 19±12, 21±10, 29±9 and 30±8 when tissue factor concentrations were 1, 2, 10 and 40 pM, respectively.

Patients with hemophilia A. Unlike in healthy subjects and in patients on oral anticoagulants, the ETP of patients with hemophilia A increased markedly with increasing tissue factor concentration. Mean±SD ETP% values were 26±18, 49±21, 79±16 and 82±15 at tissue factor concentrations of 1, 2, 10 and 40 pM, respectively (Figure 3c). The extent of influence of the tissue factor concentration on PRP was similar to that observed on PPPF (*data not shown*).

Correlation between ETP and clotting tests in patients on oral anticoagulants and patients with hemophilia A

A significant (hyperbolic) correlation was observed between ETP and INR in 41 patients on oral anticoagulants (r=-0.76, p<0.001; Figure 4), or between

Table 5. Distribution of ETP% values obtained with two different concentrations of phospholipids for 76 women. The 75th percentile of the distribution of results for women not on oral contraceptives was used as the cut-off value. Tissue factor concentration was 1 pM.

	ETP % phospholipids (0.5 μM)		*ETP % phospholipids (5 μM)*	
Cut-off values	<114	≥114	<103	≥103
Oral contraceptives				
No	41	14	43	12
Yes	1	20	6	15
χ^2 *p* values	<0.001		<0.001	

ETP and log-transformed factor VIII (r=0.54, $p<0.001$; Figure 5) in 39 patients with hemophilia A.

ETP in women on oral contraceptives

We investigated the influence of phospholipid concentration on the capacity of the ETP to detect hypercoagulability secondary to oral contraceptive intake, given the literature reports of such a state.[7] Overall, women on oral contraceptives had higher ETP values than those not on oral contraceptives ($p<0.001$). The mean ± SD ETP% values obtained in 55 women not taking and in 21 taking oral contraceptives were 104±16 and 134±23, respectively, at a phospholipid concentration of 0.5 μM. The corresponding values at a 5 μM concentration of phospholipids were 95±13 and 111±15. Using the 75th percentile of the distribution of results for women not on oral contraceptives, we found that a greater proportion of women on oral contraceptives had higher than normal ETP values and this held true regardless of the phospholipid concentration in the test system (Table 5). However, the above proportion was greater at 0.5 μM (20/21 patients) than at 5 μM phospholipids (15/21 patients) (Table 5). These results suggest that in this setting the optimal amount of phospholipids in the test system is 0.5μM.

ETP in platelet-rich-plasma (PRP)

ETP was also measured in the PRP from 20 healthy subjects, 14 patients on oral anticoagulants and 14 patients with hemophilia A. The concentration of tissue factor was kept constant at 1 pM. The platelet count was adjusted to $150\times10^3/mm^3$. Table 6 shows the mean ETP of PRP, compared to that of PPPF from the same subjects. The phospholipid concentration in the test system ranged from 0 to 1.5 μM. In the group of healthy subjects and patients on oral anticoagulants, the ETP values for PPPF and phospholipids at a concentration of 1.5 μM were similar to those of PRP ($p>0.1$), whereas in patients with hemophilia A the ETP values were higher in PRP than in PPPF ($p<0.05$). However, when the patients were grouped according to their factor VIII level (i.e., mild >5%; moderate 1-5% and severe <1%), the

Table 6. Mean ETP (RFU/min) and standard deviation (SD) in healthy subjects, patients on oral anticoagulants and patients with hemophilia A obtained for filtered platelet-poor plasmas (PPPF) or platelet-rich plasma (PRP). Tissue factor concentration was 1 pM. [a]not significantly different from the value obtained for PPPF with 1.5 μM phospholipids ($p>0.1$). [b]significantly different from the value obtained for PPPF ($p<0.05$).

	No.	*PPPF with phospholipids (μM)*				*PRP platelets*
		0	*0.5*	*1*	*1.5*	*$150\times10^3/mm^3$*
Healthy subjects	20	182±155	529±122	644±122	693±118	662±119[a]
Patients on oral anticoagulants	14	N.D.	84±61	108±77	113±88	130±67[a]
Hemophilia A (VIII%)	14	23±28	112±82	151±102	197±115	277±183[b]
Mild (>5 %)	5	43±33	186±70	238±107	299±138	409±126
Moderate (1-5%)	4	24±20	115±44	136±6	175±28	374±68
Severe(<1%)	5	3±4	35±34	73±50	112±34	67±58

discrepancy between PRP and PPPF remained for moderate and mild hemophilia, but was no longer present for severe hemophilia (Table 6).

Discussion

As expected, the concentration of phospholipids used in the test system influenced the imprecision of the measurement; the lower the concentration the higher the imprecision. The concentrations of phospholipids affected not only the imprecision but also the absolute ETP value (RFU/min); the higher the concentration of phospholipids, the higher the ETP values, with a plateau reached at the concentration of 5 μM (Table 3, Figure 2). However, the concentration-dependent variation induced by phospholipids can be abolished or minimized by expressing the results as a percentage relative to the pooled normal plasma tested along with the patient's plasma (Table 3, Figure 2A-C). Interestingly, we observed that residual (lysed) platelets in frozen PPP had the same degree of effect as the phospholipid concentration and this effect was related to the platelet count. ETP variation is more evident at a platelet count ranging from 0 to $10\times10^3/mm^3$, which is the platelet count in PPP usually obtained by conventional centrifugation (2,000g) in most clinical laboratories.[12] In order to minimize this unwanted effect it is recommended that platelets are removed by filtration before freezing the samples for later ETP testing. Alternatively, addition of phospholipids at a concentration greater than 1.5 μM can overcome the influence of residual platelets in plasma. However, filtration and addition of small amounts of phospholipids in the test system is preferable, as this makes the test more responsive to thrombin generation due to hypercoagulability as shown by the ETP values obtained in plasma from women on oral contraceptives (Table 5).

The concentration of tissue factor had a more pronounced effect than phospholipids on the ETP values even when results were expressed as a percentage relative to the pooled normal plasma; this was especially so in patients with hemophilia A. In this group of patients, normal or near normal levels of ETP can be obtained with as little as 10 pM of tissue factor (Figure 3C), suggesting that this concentration is sufficient to trigger coagulation via the extrinsic pathway. Conversely, a relatively low tissue factor concentration (i.e., 1 pM) resulted in a subnormal value (Figure 3C). This is in agreement with the bleeding tendency of these patients and suggests that the ETP measurement with low levels of tissue factor may be of help to assess thrombin generation in patients with hemophilia. Another interesting observation stemming from this study is that the generation of thrombin in patients with mild or moderate hemophilia A can be enhanced by the presence of platelets even when the concentration of the trigger (tissue factor) is minimal (Table 6). Though the number of patients investigated is too limited to draw definite conclusions, this finding is in agreement with the clinical observation that these patients rarely bleed spontaneously.

The other issue of interest is the type of blood specimen to be used for ETP measurements. For a comprehensive picture of the balance between pro- and anti-coagulation, ETP should ideally be measured in whole blood. However, the method is not yet available. Therefore, the second choice is PRP or PPP. Although, ETP values obtained in PRP or PPP are not interchangeable in all instances (see Table 6), the ETP measurement in frozen plasma is more practical. Results from this study suggest that frozen plasma is suitable provided that it is filtered to remove platelets.

As expected, we found relatively good correlations between ETP and the traditional coagulation tests, such as the PT-INR for patients on oral anticoagulants (r=-0.72) and factor VIII levels for patients with hemophilia A (r=0.54). All these promising results pave the way to future investigations on the clinical value of ETP measurements. Despite the fact that previous reports have emphasized a better association between ETP and hypo- or hyper-coagulability than the traditional coagulation tests,[13] ETP has not been widely employed so far, probably because of the demanding technical procedures required by the early methods. The recent modifications, which include a fluorogenic substrate and no defibrination, make the method more practical and suitable for performance with relatively simple equipment even in general clinical laboratories. An additional advantage of the current method is that platelet turbidity does not disturb the fluorescence signal, thus making it possible to investigate the role played by platelets in thrombin generation. The method is reproducible, but it should be realized that ETP expressed as RFU/mL is an arbitrary unit, calculated directly from the fluorescence signal. Therefore, any instrument variation that causes changes in the light source or absorption (lamp, filter aging, etc.), will affect ETP values. These systematic variations can be minimized to a certain extent by reporting the results as a percentage relative to pooled normal plasma. An internal thrombin standard to compensate for the above variation would probably be more appropriate.

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Pre-publication Report & Outcomes of Peer Review

Contributions

AT, VC: design of the study; VC, PG, MC, CB: method set up; MC, CB: measurements; VC: data collection and statistical analysis; AT: coordination; VC, AT: writing; AT; primary responsibility for all Tables and Figures.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received January 29, 2002; accepted March 24, 2003.

In the following paragraphs, the Deputy Editor summarizes the peer-review process and its outcomes.

What is already known on this topic

Over many years efforts have been made to investigate the endogenous thrombin potential (ETP) in blood.

What this study adds

The authors develop a suitable and reproducible method to study ETP in PRP or PPP.