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Monitoring oral anticoagulant treatment from plasma stored for up to 48 hours and frozen plasma

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ABSTRACT

Background and Objective. **The number of patients being referred for lifelong anticoagulant treatment has increased markedly in the last years. The prothrombin time test is sometimes difficult to perform the same day as sample collection. The aim of this study is to determine International Normalized Ratio (INR) and vitamin-K dependent factor levels of frozen plasma and plasma stored for up 48 hours.**

Design and Methods. **The INR of 84 patients receiving acenocoumarol were determined fresh (0 hours), on samples stored between 2°C and 8°C for 24 hours and 48 hours, and on frozen samples (–40°C) using 4 different thromboplastin reagents (Thromboplastin IS; Thromborel; Simplastin; and Thromboplastin D+G). In addition, factors II, VII, IX, X were determined in 34 of these patients in all these situations. We used the interclass correlation coefficient to compare the results obtained at 0 hours and the results obtained in the subsequent measurements. Both measurement and proportional errors were also estimated by linear regression analysis.**

Results. **The correlation coefficient of the INR between fresh and frozen plasma was 0.98, 0.98, 0.92 and 0.97 for IS, Thromborel, Simplastin and D+G respectively. The correlation between 0 and 24 hours was 0.98, 0.91, 0.95 and 0.85 for IS, Thromborel, Simplastin and D+G respectively. By 48 hours although IS still had r=0.94, Thromborel, Simplastin, and D+G had r=0.55, r=0.50 and r=0.81, respectively. By 24 hours in stored plasma and in frozen plasma the activity of vitamin-K dependent factors was slightly reduced (r=0.97 at 24h / r=0.94 with frozen plasma for factor II, r=0.92 / 0.96 for factor VII, r=0.83 / 0.98 for factor IX, and r=0.98 / 0.95 for factor X). By 48 hours however, significant reductions were noted in the activity of these factors (r=0.94 for factor II, r=0.88 for factor VII, r=0.70 for factor IX, and r=0.98 for factor X).**

Interpretation and Conclusions. **The INR can be reliable determined in frozen plasma and in plasma stored at 2-8 ° C for up to 24 hours.**

Key words: oral anticoagulants, monitoring, thromboplastins, storage

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Following evidence of clinical benefit of anticoagulation in non-valvular atrial fibrillation, the number of patients being referred for lifelong treatment has increased significantly.[1,2] In addition, there is an increasing trend towards sample collection from patients receiving oral anticoagulants at sites distant from the anticoagulant services and the prothrombin test is often difficult to perform within two hours of sample collection.[3] Alternative models such as patient self-management or a nurse specialist service have been proposed in selected patients.[4,5] Another option would be to perform international normalized ratio (INR) on frozen plasma or plasma stored for at least 24 hours.

This study examines whether freezing plasma or storing it for up to 48 hours at 2ºC to 8ºC affects INR and vitamin-K dependent factors levels using different thromboplastin reagents.

Design and Methods

Study design and methods

A total of 84 outpatients on long-term acenocoumarol therapy attending laboratory monitoring and with a range of anticoagulation were recruited. All patients had an INR between 2.0 and 4.5. The venous blood was collected into evacuated siliconized tubes containing 3.2% citrate anticoagulant (Becton Dickinson, Meylan Cedex, France). The supernatant plasma after centrifugation at 2,000 x g was tested: 1) immediately (0 hours); 2) after 24 hours and 48 hours of incubation between 2ºC and 8ºC; and 3) after storage at –40ºC (less than 1 month).

Thromboplastin reagents. Rabbit brain thromboplastin IS, international sensitivity index (ISI) 1.30 (Dade International, Miami, USA); rabbit brain thromboplastin Simplastin, ISI 1.27 (Organon Teknika, Barcelona, Spain); human thromboplastin Thromborel, ISI 1.07 (Behring, Marburg, Germany); and rabbit brain thromboplastin D+G, ISI 1.20 (Diagnostic Grifols, Barcelona, Spain).

Instrumentation. Sysmex CA-6000 (Tao Medical Electronics, Kobe, Japan); Thrombolyzer Chrom (Behnk Electronics, Hamburg, Germany); and ACL 6000 (Instrumentation Laboratory, Barcelona, Spain).

Plasma samples were subjected to a PT testing and

Table 1. Demographic and clinical characteristics of the study population (n=84).

Age (years) (mean±SD)	65.4±11.1
Sex (male/female)	38/46
Indication for anticoagulation	
Atrial fibrillation	38 (45.2%)
Valvular prosthesis	22 (26.2%)
Cardiomyopathy	13 (15.5%)
Venous thromboembolism	11 (13.1%)

Table 2. Intra-class correlation coefficients of the INR measured in fresh samples with the INR measured in stored samples.

N. of pts.	*Thromboplastin reagents*	*Instrumentation*	*24h*	*48h*	*Frozen plasma*
32	Thromboplastin IS	Sysmex CA-6000	0.98	0.94	0.98
19	Simplastin	Thrombolyzer Chrom	0.95	0.50	0.92
15	Thromborel	ACL 6000	0.91	0.55	0.98
18	Thromboplastin D+G	ACL 6000	0.85	0.81	0.97

INR. In addition, factors II, VII, X were determined in 32 of these patients using thromboplastin IS in fresh and frozen samples and after incubation in the refrigerator. Factor IX activity was determined in all these situations using the Automated APTT (Organon Teknika, Barcelona, Spain).

Statistical analysis

Data are expressed as mean and standard deviation. The INR results in the different situations (fresh samples, at 24 hours, at 48 hours and frozen samples) using the same thromboplastin were compared by means of ANOVA. The relationship between fresh sample results and subsequent measurements were analyzed using the intra-class correlation coefficient.[6] Both measurement and proportional errors were also estimated by linear regression analysis. A measurement error existed when the regression line did not pass through the origin (i.e. the intercept was not 0). A proportional error existed when the slope was not equal to 1 for the linear regressions. All statistical analyses were performed by means of SPSS 7.5 for Windows 95.

Results

The demographic and clinical characteristics of the study population are presented in Table 1. The number of patients studied with each thromboplastin reagent, and the instrumentation used are shown in Table 2. The correlation coefficients of the INR at 24 hours and 48 hours, and frozen plasmas with the INR obtained immediately after blood collection using four different thromboplastin reagents are shown in Table 2 and Figure 1. There was no difference in the INR when a sample was tested with the same reagent when fresh and after freezing. The INR in fresh samples was not statistically different from that at 24 hours with all

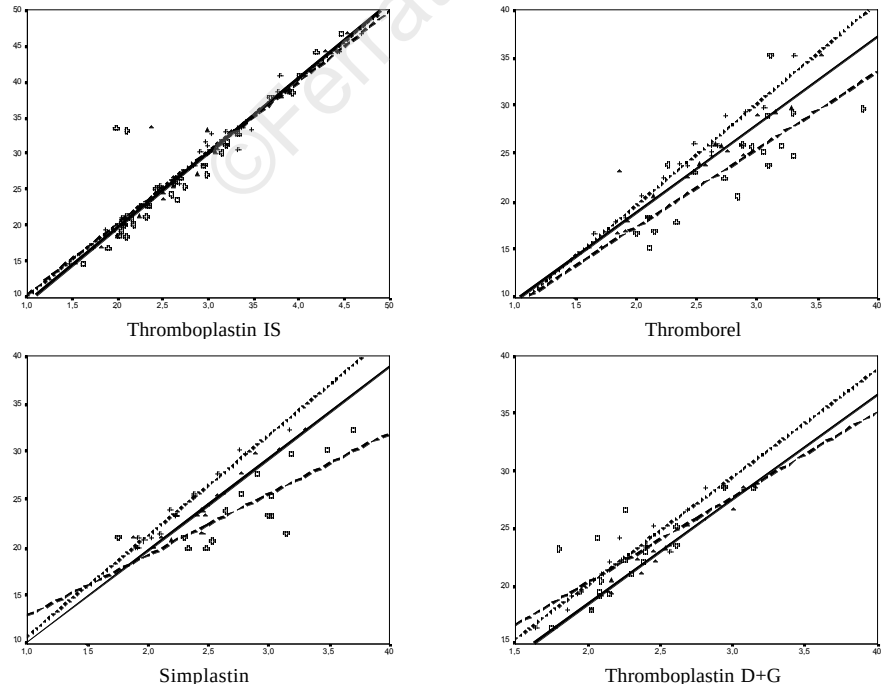

Figure 1. Regression analysis of the INR performed in fresh samples (y-axis) and the INR performed in different situations (x-axis). At 24 hours (▲---); at 48 hours (○ ---); and frozen samples (+…).

Table 3. Intra-class correlation coefficients of the activity of vitamin-K dependent factors at 0 hours with the activity of these factors in stored samples.

	24 hours	*48 hours*	*Frozen plasma*
Factor II	0.97	0.94	0.94
Factor VII	0.92	0.88	0.96
Factor IX	0.83	0.70	0.98
Factor X	0.98	0.98	0.95

four thromboplastin reagents. The lowest correlation coefficient was observed with thromboplastin D+G with a value of 0.85. By 48 hours, although thromboplastin IS still had a high correlation coefficient, the rest of the thromboplastins showed significant differences compared with the results obtained when samples were tested at 0 hours. In addition, measurement errors were detected only in samples stored 48 hours. (Table 2 and Figure 1).

In sample stored for 24 hours and in frozen plasma the activity of vitamin-K dependent factors was slightly reduced compared with that in fresh samples. By 48 hours, however, significant reductions in activity of factor VII and factor IX were noted (Table 3 and Figure 2). Measurement and proportional errors were observed in the determination of factor IX activity in samples stored 24 hours and 48 hours but not in frozen samples.

Discussion

Coagulation tests including prothrombin time are usually performed within the 2 hours following blood collection. When testing is not to be done immediately, plasma is usually removed from packed blood cells.[7] Although several guidelines for storage of specimens collected for coagulation testing have been published, the likelihood of interlaboratory variability in results is great.[8,9] The aim of this study was to establish the influence that time and temperature had on INR and vitamin-K dependent factors using different thromboplastin reagents.

Our study shows that the reproducibility of INR is not affected when plasma is frozen. The highest correlation coefficients of INR of fresh samples vs INR of stored samples were obtained with frozen samples. Frozen samples did not affect the activity of any vitamin-K dependent factors. The activity of factors II, VII, IX and X was similar between fresh samples and frozen samples. In addition, these data were also observed with all four thromboplastin reagents tested.

The data show that the reproducibility of the INR is not compromised in specimens stored between 2ºC and 8ºC for 24 hours. The correlation coefficients with unstored samples were higher than 0.9 with three thromboplastins. Only one thromboplastin showed a correlation coefficient of 0.85. At 24 hours the activity of factors II, VII and X was similar to the activity of these factors in fresh samples. Only the activity of factor IX decreased slightly after storage of the samples for up to 24 hours. Some authors have suggested that storage at room temperature for 24 hours even without removal of the plasma from blood cells would be better than storage for the same period at 2ºC to 8ºC.[10,11] Activation of factor VII induced by prolonged storage of the samples in the cold may produce shortening of the PT.[12] In contrast,

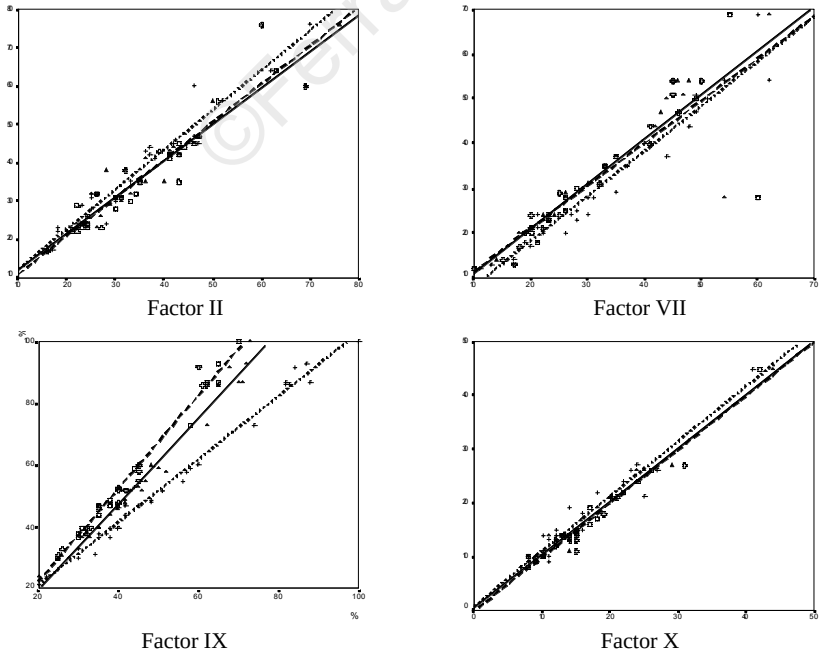

Figure 2. Regression analysis of the vitamin-K dependent factors activity performed in fresh samples (y-axis) and in different situations (x-axis).
At 24 hours (▲--); at 48 hours (○ ---); and frozen samples (+…).

our study clearly shows that samples stored at 2ºC to 8ºC for up to 24 hours are still satisfactory for clinical testing and factor VII activity is not affected after this incubation. Our results have been confirmed by other authors.[13] An explanation for discrepancies between studies could be that we used thromboplastins with an ISI between 1.0 and 1.3. It seems that INR performed with thromboplastins with an ISI near to 1.0 would give more precise results than those with thromboplastins with higher ISI in samples stored in cold for a prolonged time.[14] However, this hypothesis must be confirmed by further studies. In fact, we did not find any relationship between the reproducibility of the INR and the ISI of the different thromboplastins.

Samples kept for up to 48 hours at 2ºC to 8ºC show marked differences from those used for immediate testing. As expected, prolonging the incubation for more than 24 hours produced a decrease in the activity of vitamin-K dependent factors. Although all vitamin-K dependent factors showed reduced activity at 48 hours, factor IX had the greatest degree of variation.

In conclusion, we found that INR can be performed in frozen plasma with results similar to those that would be obtained from testing fresh samples. Plasma samples can also be maintained at 2ºC to 8ºC for up 24 hours without affecting the reproducibility of test results.

Contributions and Acknowledgments

EG: design of the study and writing the paper; JMT: statistical analyses; MAO, JF, MTJ: laboratory work and data collection; EG, EP, AP, ER: assessment of the patients and interpretation of the results; all the authors: final approval of the definitive version. The order of appearance of the names is based on the importance of each individual contribution.

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Disclosures

Conflict of interest: none.

Redundant publications: no subsantial overlapping with previous papers.

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