The determination of INR in stored whole blood

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Abstract

Aims—To examine the reliability of international normalised ratio (INR) determination on samples stored as whole blood for up to two days at room temperature.

Methods—The INR of 40 patients receiving oral anticoagulants was determined on fresh blood and on samples stored for 24 and 48 hours, using five locally calibrated prothrombin time systems. These incorporated Manchester reagent, Recombiplastin, IL PT Fibrinogen HS Plus, Manchester combined capillary prothrombin time reagent, and a freeze dried in-house reference rabbit brain thromboplastin, RBT 1010. In addition, factors II, V, VII, and X were determined on samples obtained from 18 of these patients before and after incubation at room temperature.

Results—The INR of the samples changed by differing amounts during storage, depending on which system was employed. Although the mean change after 24 hours storage was relatively small, there were individual samples that changed by > 0.5 INR with all systems. These changes would lead to adjustment in dosage of certain patients. After 48 hours these effects were greater with all systems except that employing Recombiplastin. There were only small reductions in the measured factors by 48 hours.

Conclusions—After storage of samples for only 24 hours, some patients' INR changed sufficiently to affect dosage. In view of these observations, the practice of storing whole blood samples for INR determination cannot be recommended.

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Keywords: warfarin; prothrombin time; international normalised ratio

The demand for laboratory monitoring of oral anticoagulant treatment is increasing as more patients are treated. One important reason for the increase is that the value of warfarin in reducing the risk of ischaemic stroke in high risk patients with atrial fibrillation is now widely accepted.¹ Age is considered one such high risk factor.² The prevalence of atrial fibril-

lation increases with age, from 0.5% at 59 years of age to more than 5% in patients over 70 years of age. More elderly patients are now therefore being prescribed anticoagulants. This has increased the pressure on those responsible for anticoagulant management to consider monitoring systems which are suitable for use in the community or in primary care, thus avoiding transporting non-ambulant patients to hospital outpatient anticoagulant clinics.

Expansion of anticoagulant clinics and the extension of access through "drop in" clinics have been employed with some success to cope with increased demand. Some attempts have also been made to shift the service into primary care. This has not been widespread, however. Taylor *et al* reported that general practitioners were concerned about resources and lack of expertise.³ Fitzmaurice *et al* believed that computerised dosing was of benefit in guiding the management of long term anticoagulation in primary care.⁴

Baglin *et al* have considered an alternative approach.⁵ They have shown that a system based on taking blood samples from patients and transporting these to the laboratory for testing resulted in dose control at least equal to that in a completely hospital based clinic. An advantage of this technique is that the normal laboratory quality assurance may be maintained. Such systems, however, depend on the reliability of international normalised ratio (INR) determination on samples that may be many hours old.

In this study we examined the changes in INR and in factors II, V, VII, and X in samples stored as whole blood for up to 48 hours.

Methods

Blood obtained by venepuncture from 40 patients receiving oral anticoagulant treatment

Table 2 Mean international normalised ration (INR) obtained with each of the reagents with samples at baseline and those stored for 24 and 48 hours at room temperature. The value in parenthesis is the mean change in INR

Reagent	Mean INR baseline	Mean INR sample stored for 24 hours	Mean INR sample stored for 48 hours
RBT 1010	2.31	2.45 (0.14) p<0.0001	2.70 (0.39) p<0.000
CCR	2.29	2.41 (0.12) p<0.0001	2.59 (0.30) p<0.0001
MR	2.27	2.48 (0.21) p<0.0001	2.64 (0.37) p<0.0001
RP	2.31	2.38 (0.07) p<0.02	2.42 (0.11) p<0.005
IL	2.28	2.36 (0.08) p<0.001	2.50 (0.22) p<0.0001

CCR, combined capillary reagent; IL, ILPT Fibrinogen HS Plus; MR, Manchester reagent; RBT, rabbit brain thromboplastin; RP, Recombiplastin.

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Table 1 Systems employed, international sensitivity index (ISI), and mean normal prothrombin time (MNPT)

Reagent	Endpoint detection method	Type of sample	System ISI	MNPT
RBT 1010	Manual	Citrated plasma	1.241	16.72
CCR	Thrombotrack	Citrated whole blood	1.007	19.32
MR	KC10	Citrated plasma	1.077	14.72
RP	KC10	Citrated plasma	0.874	10.31
IL	ACL	Citrated plasma	1.120	13.81

Figure 1 Bland and Altman plot of mean international normalised ratio (INR) against difference in INR at 24 hours and 48 hours. The left hand panels show the effect of storage for 24 hours; the right hand panels give the results for 48 hours. MR, Manchester reagent.

was collected into Vacutainer tubes (Becton and Dickinson, HM&S Ltd, Northampton, UK) containing 0.105 M trisodium citrate. Three tubes were taken from each patient.

Prothrombin times were determined fresh (tube 1) and on samples stored at room temperature for 24 hours (tube 2) and for 48 hours (tube 3).

Various system combinations of thromboplastin reagent were used with manual or coagulometer technique. These were:

- (1) Freeze dried rabbit brain thromboplastin (RBT 1010, Thrombosis Reference Centre, Manchester, UK) and manual method.
- (2) Manchester combined capillary prothrombin time reagent (CCR10, Thrombosis Reference Centre, Manchester, UK) and Thrombotrack (Nycomed, Birmingham, UK)
- (3) Manchester reagent (Helena Laboratories, Sunderland, UK) and KC 10 (Amelung, Brownes (UK) Ltd, Reading, UK)

Table 3 Numbers of patients with international normalised ratio (INR) values in each therapeutic interval at baseline, 24, and 48 hours

System	INR range	Baseline	24 hours	48 hours
RBT1010/manual	<2	17	13	8
	2.0–2.5	11	13	13
	2.0–3.0	16	19	22
	3.0–4.5	6	7	8
	>4.5	0	1	2
CCR/Thrombotrack	<2	19	15	9
	2.0–2.5	9	13	16
	2.0–3.0	14	16	21
	3.0–4.5	7	8	9
	>4.5	1	1	1
MR/KC 10	<2	20	9	8
	2.0–2.5	9	17	14
	2.0–3.0	14	23	23
	3.0–4.5	6	6	5
	>4.5	0	2	4
Recombiplastin/KC 10	<2	17	16	14
	2.0–2.5	10	12	14
	2.0–3.0	17	17	19
	3.0–4.5	6	6	6
	>4.5	0	1	1
PT Fibrinogen HS Plus/ACL	<2	20	16	14
	2.0–2.5	8	12	14
	2.0–3.0	14	17	19
	3.0–4.5	6	6	6
	>4.5	0	1	1

- (4) Recombiplastin (Ortho-Clinical Diagnostics, Amersham, UK) and KC 10
- (5) PT Fibrinogen HS Plus reagent performed on the ACL 300R coagulometer (both from Instrumentation Laboratories (IL), Warrington, UK)

System 2 used whole blood in the prothrombin time test. For the other combinations plasma was obtained by centrifuging the stored whole blood at 2000 *g* for 15 minutes. International sensitivity indices (ISI) were assigned to each of the systems using fresh plasma obtained from the 40 anticoagulated patients and 20 freshly collected plasmas from 20 normal volunteers. RBT 1010 was used as the reference. This preparation was calibrated previously in a 20 centre international exercise against the existing WHO international reference preparations.⁶ INR values were calculated using these system ISI and mean normal prothrombin time (MNPT) values (see table 1). Each system was controlled by testing normal and abnormal control plasmas (Thrombosis Reference Centre, Manchester, UK) at each time of testing.

Assays of factors II, V, VII, and X were performed by standard one stage methods on fresh plasma samples and on those obtained by centrifugation of the whole blood stored for 24 and 48 hours.

STATISTICS

The results from stored samples were compared with baseline values using paired *t* tests

Table 4 Mean factor levels of samples at baseline and those stored at room temperature for 24 and 48 hours. The mean percentage change is shown in parentheses

Factor	Mean IU/dl baseline	Mean IU/dl sample stored for 24 hours	Mean IU/dl sample stored for 48 hours
Factor II	36.5	34.7 (4.9) p<0.05	32.8 (10.1) p<0.001
Factor V	110.1	108.9 (1.8) NS	101.0 (8.4) p<0.001
Factor VII	43.7	42.0 (3.9) p<0.02	39.6 (9.4) p<0.0001
Factor X	27.7	26.0 (6.1) p<0.005	25.0 (9.7) p<0.001

and Bland and Altman plots.⁷ The data were examined further to determine the distribution of patients within specific INR ranges and to observe whether there were any changes in this distribution following storage for 24 or 48 hours.

Results

Control plasma results were within specified limits for each system. Table 1 gives the system ISI and MNPT values for all the combinations employed. Figure 1 shows Bland and Altman plots comparing stored sample INR to baseline values. After 24 hours the majority of plasmas showed little change with any of the systems, although some plasmas had altered by more than 0.5 INR. These were: one with RBT 1010, two with CCR 10, three with Manchester reagent, and two with Recombiplastin. None of the plasmas altered by this amount using the IL system.

By 48 hours, however, more plasmas showed significant change from baseline: 12 with RBT 1010, eight with CCR 10, eight with Manchester reagent, two with recombiplastin, and four with the IL system. Table 2 shows the mean INR obtained with each system at baseline and after 24 and 48 hours. All give small but statistically significant differences after storage even for 24 hours. The differences increased markedly by 48 hours especially with the systems incorporating RBT 1010, CCR 10, Manchester reagent, and PT Fibrinogen HS Plus. The system incorporating Recombiplastin showed only a small mean change (0.11 INR) after 48 hours.

In table 3 the distribution of patients into INR ranges is shown. The INR obtained with the different systems, on fresh samples, showed good agreement. Figure 2 illustrates the mean percentage change in INR within each INR range and confirms the smaller percentage change observed with the Recombiplastin/KC 10 and the PT Fibrinogen HS Plus/ACL systems.

These changes resulted in movement of patients between INR ranges following storage. This was particularly noticeable with the Manchester reagent/KC 10 system, where no patients with INR of more than 4.5 were found when INR was determined on fresh plasmas, while there were two after 24 hours and four after 48 hours.

There were small but statistically significant changes in factor levels between baseline and 24 hour stored samples, with the notable exception of factor V (table 4). Between baseline and 48 hour storage the differences were all statistically significant but still small—less than 10% for factor V, for example.

Discussion

At first glance, this study suggests that delay between blood collection and testing the prothrombin time is probably not of concern in anticoagulant dose adjustment. This is the likely conclusion based on comparison of the mean change in INR between freshly drawn samples and those obtained after 24 hours of storage.

Figure 2 Mean percentage change in each therapeutic interval after storage for 24 and 48 hours. The white bar represents change at 24 hours and the black bar change after 48 hours.

Unfortunately, there are some patients whose INR falls outside our arbitrary acceptable difference of 0.5 INR and who are overlooked when only the mean results are considered. This represents 2.5% of patients with RBT 1010, 5% with CCR 10, 7.5% with Manchester reagent, and 5% with recombiplastin. By 48 hours, this had risen to 30% of patients with RBT 1010, 20% with CCR 10 and Manchester reagent, and 10% with the IL system. With recombiplastin the numbers were unchanged at 48 hours.

Interestingly the thromboplastin employed seems to affect the degree by which the INR is increased on storage, Recombiplastin and PT Fibrinogen HS Plus showing smaller change than Manchester reagent, Manchester combined capillary reagent, and RBT 1010.

Baglin and Luddington⁸ concluded that there was no clinically significant change in INR when analysis was delayed for up to three days. Their conclusions, however, were based on mean INR differences and may have missed the occasional patient who behaved differently.

The Bland and Altman technique was particularly useful, since all the data were considered and shown individually. From these plots it was possible to identify the few patients whose INR changed dramatically on storage. These changes would have resulted in significant dose alteration.

Baglin and Luddington⁸ also noted that although there were some variations in stability of results with different thromboplastins, these differences over time for each thromboplastin were much less than the differences between thromboplastins. In the present study the differences between systems were reduced by performing local calibrations of the reagent and coagulometer combinations.

Another possible reason for the differences between Baglin and Luddington's study and ours is that blood was collected by different methods, Sarstedt Monovet tubes being preferred by Baglin, while we used Vacutainers. Hernandez *et al* have also reported on this subject,⁹ but, like Baglin and Luddington, they only gave mean INR values, making complete interpretation of their results difficult.

There were only small decreases in the levels of factors II, V, VII, and X and it is unlikely that these would provide the reason for the changes in INR seen with some of the patients.

In conclusion after storing samples for only 24 hours, some patients' INR values may have changed sufficiently to result in anticoagulant dose adjustment. In view of these observations, we cannot support this practice.

- Koefoed BG, Gullov AL, Petersen P. Prevention of thrombotic events in atrial fibrillation. *Thromb Haemost* 1997;78:377-81.
- Feinberg WM, Blackshear JL, Laupacis A, *et al*. Prevalence, age distribution and gender of patients with atrial fibrillation. Analysis and implications. *Arch Int Med* 1995;155:469-73.
- Taylor F, Ramsay M, Voke J, *et al*. GPs not prepared for monitoring anticoagulation. *BMJ* 1993;307:1493.
- Fitzmaurice DA, Hobbs FDR, Murray JA. Monitoring oral anticoagulation in primary care. *BMJ* 1996;312:1431-2.
- Baglin T, Lefort W, Luddington R, *et al*. Decentralised anticoagulant care: near patient sampling, dosing, or testing? [abstr] *Br J Haematol* 1996;95(suppl 1): A115.
- van den Besselaar AMHP. Multi-centre study of replacement of the International Reference Preparation for thromboplastin, rabbit, plain. *Thromb Haemost* 1993;70: 794-9.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1986;i:307-10.
- Baglin T, Luddington R. Reliability of delayed INR determination: implications for decentralised anticoagulant care with off-site blood sampling. *Br J Haematol* 1997;96: 431-4.
- Hernandez AG, de Segovia JM, Villanueva JR. Refrigerated stored specimens are not reliable for delayed INR determination. *Br J Haematol* 1997;98:781-2.