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TECHNICAL SERIES

Monitoring oral anticoagulant therapy

Concepts & Practice

TULIP DIAGNOSTICS (P) LTD.

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Introduction

Since the introduction of the one stage prothrombin time test by Dr. Armand Quick in 1935, there have been various attempts to standardize the methodology and reporting of results using the Prothrombin Time reagents.

Oral anticoagulant therapy with 4-hydroxy coumarin vitamin K antagonists, such as Warfarin are commonly used for the treatment and prevention of thromboembolic episodes. Most physicians also prefer to monitor the oral anticoagulant therapy using the Prothrombin time test.

Since the individual thromboplastin reagent preparations differ significantly in their sensitivities to the deficiencies of the vitamin K dependent coagulation factors, the use of 'seconds' or 'PTR' (Ratio) as a reporting format has led to confusion amongst clinicians. In order to correctly dose deserving patients with oral anticoagulant drugs so as to achieve the dual goal of adequate anti-coagulation and reduce the risk of bleeding simultaneously, the WHO introduced a method for monitoring patients stabilized on oral anticoagulant therapy using International Normalized Ratio (INR).

The INR normalizes the PT ratio to an International reference preparation of thromboplastin. The therapeutic ranges expressed in terms of INR values for common clinical conditions requiring oral anticoagulant treatment and monitoring have been determined by double blind clinical trials.

The INR method of reporting PT results has helped to ensure that a uniform intensity of oral anticoagulant therapy is used world wide, independent of the influences of varying sensitivity of the thromboplastins used for obtaining the test result using PT reagents.

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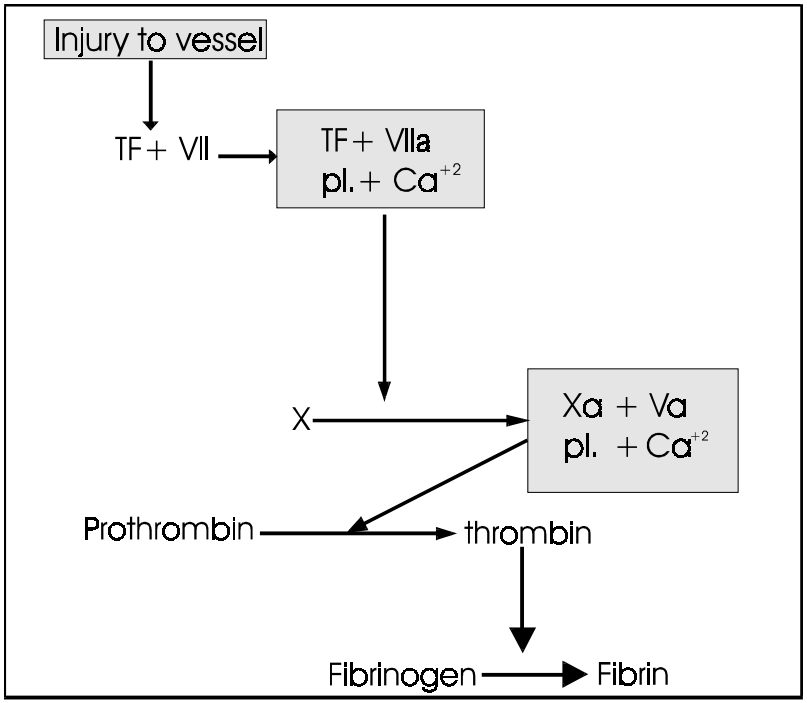
Relevance and use of the Prothrombin time test

The prothrombin time test is used to detect coagulation disorders and specifically the activity of the factors of the extrinsic pathway namely II, V, VII,X and Fibrinogen. The classical extrinsic pathway is initiated by the release of tissue thromboplastin (Tissue Factor) which is exposed to the blood when there is a damage to the blood vessel. Circulating factor VII forms a complex with the tissue thromboplastin and calcium in the presence of phospholipids. This complex activates factor X to Xa. Factor Xa catalyses prothrombin (Factor II) to thrombin (IIa). Thrombin converts fibrinogen (Factor I) to Fibrin.



Classical Extrinsic Pathway.

The factors of the extrinsic pathway namely II, V, VII and X are synthesized in the liver parenchymal cells from precursor proteins.
These classical vitamin K dependent plasma clotting factors undergo a conformational change in the presence of Ca^{+2} ions. This is a necessary requirement for these vitamin K dependent factors (II, V, VII, X) for binding to their co-factors on phospholipid surfaces and exerting their coagulant activity.

Within the liver parenchymal cells the inactive precursor proteins of three key vitamin K dependent factors i.e. VII, IX and X are carboxylated. These carboxylated proteins then function as active factors with coagulant activity .

Oral Anticoagulant Agents

The clinical effectiveness of Oral anticoagulants has been well established based on results of numerous well designed trials for a variety of indications.
Oral anticoagulants are effective and used for:

- Prevention of primary and secondary venous thromboembolism.
- Prevention of systemic arterial embolism in patients with mechanical prosthetic heart valves or with atrial fibrillation.
- Prevention of acute myocardial infarction in patients with peripheral arterial disease.
- Prevention of stroke and recurrent infarction.

Warfarin Sodium a 4-hydroxy coumarin derivative is the most common vitamin K antagonist used for oral anticoagulant therapy.
Acenocoumarol, phenprocoumon are the other derivatives, which are also used for achieving oral anticoagulation in patient groups. The use of indandione derivatives such as phenindione has been discontinued largely due to their relative hepatic toxicity.

All the 4-hydroxy coumarin derivatives, as far as known today, have the same mode of action, but exhibit different pharmacokinetics and are metabolized differently.

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Pharmacology of Oral Anticoagulants

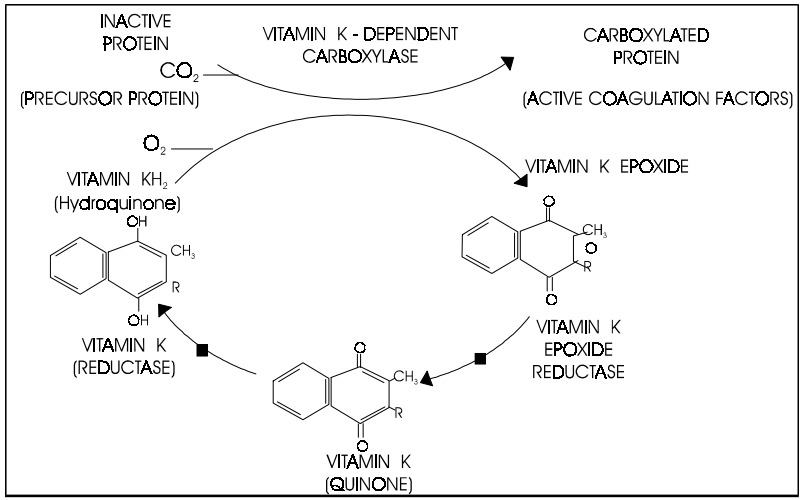
The oral anticoagulants such as Warfarin inhibit the process of carboxylation by interfering in the inter conversion of vitamin K epoxide.

This inhibition results in a decreased ability of the liver to effectively carry out posttranslational conversion of glutamyl residues (glu) on the inactive precursor proteins to γ-carboxyglutamyl (gla) residues. The gla residues impart these proteins the ability to exert their coagulant action (as factors) in a Ca^{+2} dependent fashion.

It is now known that the fully functional carboxylated proteins i.e. factors, contain a normal complement of 10 to 13 gla residues.

It has been estimated that when the number of gla residues on the carboxylated proteins reduce from 10 - 13 to 9, it results in a 30% reduction in their coagulant activity, while reduction to less than 6 residues results in a loss of more than 95% of the coagulant activity of the factors.

This inhibition in the cyclic conversion of vitamin K also results in the production and secretion of proteins that are partially carboxylated or des-carboxylated. These are dysfunctional factors.



■ INHIBITION OF CARBOXYLATION BY ORAL ANTICOAGULANTS

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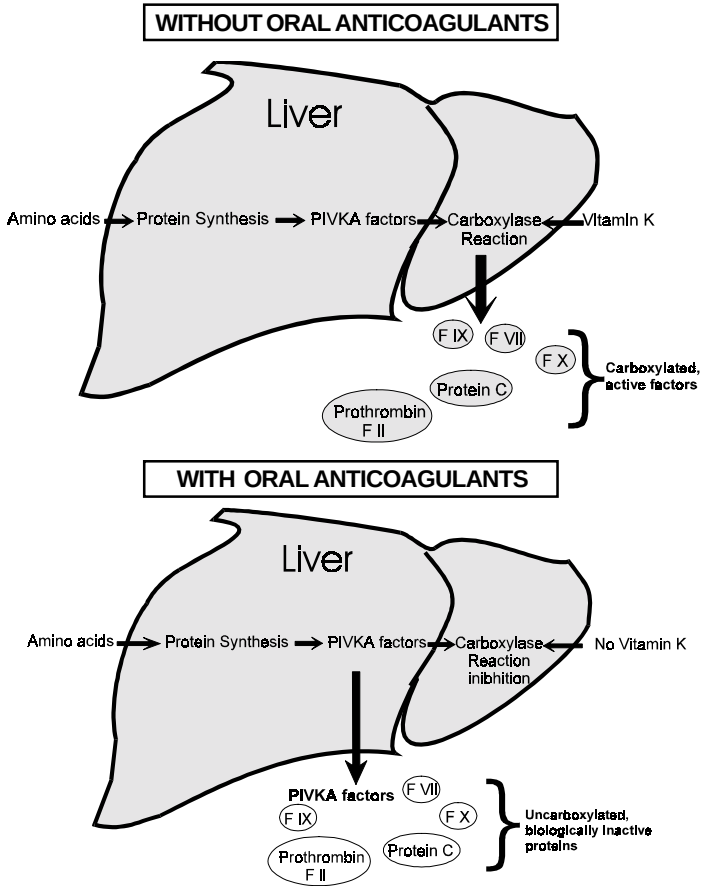
PIVKA'S

As the effect of oral anticoagulants take effect, the process of conversion of the precursor proteins to active factors through carboxylation is inhibited. The partially carboxylated or des-carboxylated, biologically inactive proteins appear in plasma. On the other hand the plasma concentration of biologically active factors VII, IX, X decreases.

This pool of partially carboxylated proteins are as a group known as PIVKA's. (Protein induced by vitamin K absence / or antagonism). Since PIVKA's are biologically inactive, they do not participate in the haemostatic control mechanism *in vivo*.



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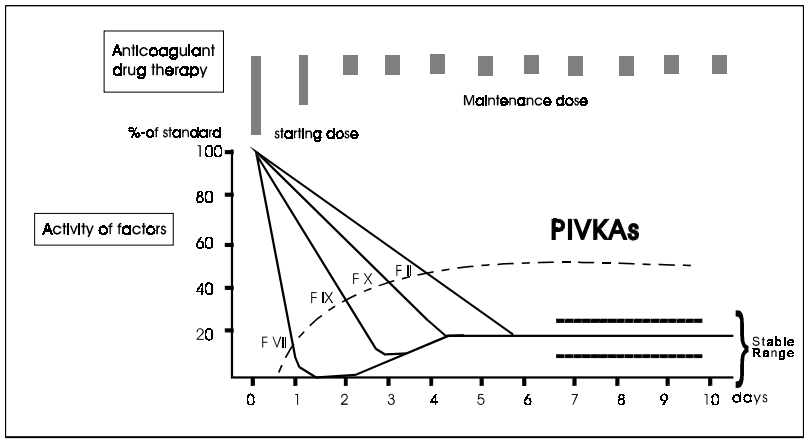
PIVKA'S also do not play a role in bleeding complications of anticoagulated patients.

However the most important practical consequence of the PIVKA effect is that it is an important confounding factor in the standardization of thromboplastins used for monitoring oral anticoagulant therapy.

PT reagents derived from rabbit or human tissues or from recombinant technology have varying sensitivity to PIVKA's.

The role of PT test in monitoring effects of oral anticoagulant therapy

The PT is the method of choice for monitoring oral anticoagulant therapy, as it is responsive to the depression of the vitamin K dependent clotting factors of the extrinsic pathway. Rate of depression of the individual clotting factors is determined by their biological half life. Factor VII falls the most rapidly followed by factors IX, X and II. As the active clotting factor levels begin to fall, the PT results start to prolong. Factor V and Fibrinogen are not affected by oral anticoagulants.



Course of Anticoagulant therapy

On the other hand upon withdrawal of the oral anticoagulant therapy (as may be required to review the effects of over anticoagulation) these factors return to normal levels in the reverse order and so do the PT results.

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Factors Influencing Anticoagulant Effects of Warfarin

GUT

Anticoagulant Effect Potentiated

- Low vitamin K intake
- Reduced vitamin K absorption in fat malabsorption

Anticoagulant Effect Counteracted

- Increased vitamin K intake
- Reduced absorption of warfarin by cholestyramine

PLASMA

Anticoagulant Effect Unchanged

- Displacement of warfarin from albumin binding does not influence anticoagulant effect of coumarins

LIVER

Anticoagulant Effect Potentiated

- Drugs:

Phenylbutazone
Metronidazole
Sulfinpyrazone
Trimethoprim
Sulfamethoxazole
Disulfiram
Amiodarone
Erythromycin
Anabolic steroids
Clofibrate
Cimetidine
Omeprazle
Thyroxine
Ketoconazole
Fluconazole
Isoniazid
Piroxicam
Tamoxifen
Quinidine
VitaminE (megadoses)
Phenytoin
Liver disease

Hypermetabolic States:

- Pyrexia
- Thyrotoxicosis

Anticoagulant Effect Counteracted

- Drugs:

Barbiturates
Rifampicin
Griseofulvin
Carbamazepine
Penicillin
Alcohol

HAEMOSTATIC PLUG

Impaired Haemostatic Plug Formation

Impaired Coagulation:

- Reduced vitamin K dependent coagulation factors
- Reduction in concentration of other coagulation factors
- Other anticoagulants (heparin,ancrod)

Impaired Platelet Function:

- Thrombocytopenia
- Aspirin
- Other nonsteroidal anti inflammatory drugs
- Ticlopidine
- Moxalactam
- Carbencicillin and high doses of other penicillins

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Problems in monitoring Oral Anticoagulant therapy

Historically the Prothrombin time (PT) results are reported in "seconds" or as a percentage of coagulant activity or as ratio (PTR).

Physicians use sufficient oral anticoagulant drugs to maintain the PT ratio at 1.5 to 2.0 or 1.3-2.0 times the median value of the normal range. The problem with this approach is that it fails to take into account the variation in sensitivity or "responsiveness" of different PT reagents.

PT ratio (PTR)

For example: For a given reduction in the plasma levels of the clotting factors, more responsive PT reagents (A) results in greater prolongation of the PT in 'seconds' as compared to an unresponsive PT reagent (B). Practically this translates into different PT times (seconds) or ratios for the same patient sample, when tested using reagent A or B.

For e.g.:

Lab.	Reagent	FNP Normal	Test	PTR	Therapeutic ratio
X	Reagent A	12.0	30	2.5	1.5 -2.0
Y	Reagent B	12.0	18	1.5	1.5 -2.0

This patient appears over anticoagulated when tested with reagent (A) and just about adequately anti coagulated when tested with reagent (B).

% Activity

The other method of reporting PT results is as a percentage of coagulation activity. First a dilution of normal plasma is made. Then the normal dilution curve is plotted using these dilutions. Then the patients clot time is converted to % activity of factors from the dilution curve.

Since the dilutions of normal plasma do not contain PIVKAS and Thromboplastins vary in their sensitivity to PIVKAS, the accuracy of the normal dilution curve has been rightly challenged.

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Thromboplastins based on their tissue origin and method of preparation vary markedly in their responsiveness to depression of vitamin K dependent factors and PIVKAS. Therefore PT results reported using different reagents are not interchangeable between laboratories when the older system such as seconds, ratios or percentage activity are used to report PT results.

Standardisation of Prothrombin time Reagents & Reporting System

In view of the aforesaid variations in the reagent sensitivities an attempt to standardize PT test results was initiated internationally. By mid 1970 a number of regulatory and advisory groups such as WHO (World Health Organization), ICTH (International Committee for Thrombosis and Haemostasis) and ICSH(International Committee for Standardisation in Haematology) started work towards standardisation of PT for monitoring oral anticoagulant therapy.

The first step was the preparation of IRP's (International Reference Preparations) of thromboplastins by the WHO. This was done to have an accurate reference reagent which could serve as a standard for calibrating responsiveness / sensitivity of commercial thromboplastin reagents.

The various IRP's were made from thromboplastins derived from various animal tissues in use for manufacture of commercial thromboplastin (PT) reagents. This was done in order to have more precise, like to like comparison.

Originally the following IRP's were made available:-

IRP's Tissue source	Code	Assigned ISI
Thromboplastin (Human combined)	67/40	1.0
Thromboplastin (Bovine combined)	68/434	1.0

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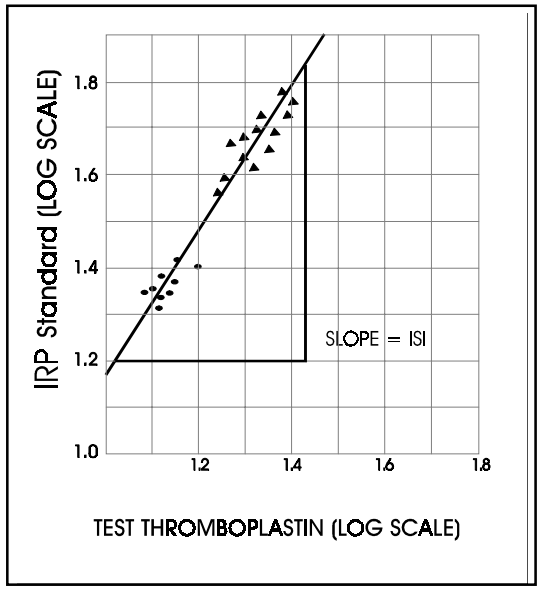
Subsequently after the stocks of primary IRP's were exhausted secondary IRP's have been made available under other code numbers. Till date however IRP's for recombinant thromboplastins are not available for primary calibration.

Basis of Calibration

This calibration system of individual commercial preparations vs. the reference preparations (IRP's) is based on the fact that when the commercial preparations of thromboplastin (test thromboplastin) and the reference thromboplastin (IRP's) of same tissue origin are tested on the same plasma samples, a linear relationship would exist between log of PT ratios obtained with reference and test thromboplastins.

Log of PT of reference thromboplastin is plotted on the Y-axis and the log of test PT are plotted on the X axis respectively on a log/ log paper.

The slope of the calibration line would then indicate the sensitivity of the test thromboplastin in comparison to the IRP.



Calibration of Test Thromboplastin vs. WHO IRP

▲ Samples from patients stabilised on oral anticoagulant for minimum 6 weeks.
• Normal patient population samples.

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The ISI (International Sensitivity Index) indicates the sensitivity of test thromboplastin in comparison to the international reference preparation. When the calibration line has a slope =1.0, the test thromboplastin equates to the IRP in sensitivity or responsiveness.

The ISI in practical terms is a measure of the responsiveness (prolongation of PT values) of a given thromboplastin to the reduction of the vitamin K dependent coagulation factors. The lower the ISI (more close it is to 1.0) the more sensitive is the thromboplastin.

The recommended calibration methodology includes normal plasma samples as well as plasma from patients on oral anticoagulant therapy containing PIVKAS. Hence the assignment of sensitivity also corrects for thromboplastin sensitivity to PIVKAS.

This method for standardization of PT reagent sensitivity based on "ISI" was the first step towards eliminating reagent based variability in expression of PT results.

Manufacturers assign ISI value for each lot of thromboplastin by the WHO recommended methods to assist laboratorians in calculation of the INR (International Normalized Ratio).

The INR method of reporting results

By definition INR represents the PT ratio which would have been obtained for a particular patient sample as if the WHO reference thromboplastin itself (ISI=1.0) had been used in the PT determination.

$$INR = [R]^{ISI}$$

$$INR = \left[\frac{\text{Patient PT in seconds}}{\text{Mean of the normal range}} \right]^{ISI}$$

A PT ratio is obtained by dividing the patient PT in seconds by the **"Mean of the normal range"**(MNPT). This ratio is then "normalized" by raising the results to the power of the ISI of the PT reagent used.

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Lower the ISI of the reagent used, closer will be the INR to the observed PT ratio.

Ideally when the ISI of the reagent is 1.0 then the INR is a simple PT ratio since $(R)^{1.0} = R$.

Currently many coagulation instruments are available that can perform this exponential calculation by entering the ISI of the reagent in use.

Alternatively a table is provided by reagent manufacturers for reading off "INR" directly for the given patient PT ratio, corresponding to the ISI value of the reagent used for e.g.

INR Conversion Table

PT RATIO	ISI 1.0	1.05	1.10	1.15	1.20	1.25	1.29
1.0	1.0	1.00	1.10	1.00	1.00	1.00	1.00
1.1	1.10	1.11	1.11	1.12	1.12	1.13	1.13
1.2	1.20	1.21	1.22	1.23	1.24	1.26	1.27
1.3	1.30	1.32	1.33	1.35	1.37	1.39	1.40
1.4	1.40	1.42	1.45	1.47	1.50	1.52	1.54
1.5	1.50	1.53	1.56	1.59	1.63	1.66	1.69
1.6	1.60	1.64	1.68	1.72	1.76	1.80	1.83
1.7	1.70	1.75	1.79	1.84	1.89	1.94	1.98
1.8	1.80	1.85	1.91	1.97	2.02	2.08	2.13
1.9	1.90	1.96	2.03	2.09	2.16	2.23	2.29
2.0	2.00	2.07	2.14	2.22	2.30	2.38	2.45
2.1	2.10	2.18	2.26	2.35	2.44	2.54	2.60
2.2	2.20	2.29	2.38	2.48	2.58	2.68	2.77
2.3	2.30	2.40	2.50	2.61	2.72	2.83	2.93
2.4	2.40	2.51	2.62	2.74	2.86	2.99	3.09
2.5	2.50	2.62	2.74	2.87	3.00	3.14	3.26

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Recommended therapeutic ranges for oral anticoagulant therapy

Indications	INR	Intensity
• Prophylaxis of venous thrombosis (high risk surgery) • Treatment of venous thrombosis • Treatment of pulmonary embolism • Prevention of systemic embolism - Tissue heart valves - Acute myocardial infarction (to prevent systemic embolism) - Valvular heart disease • Atrial fibrillation	2.0 -3.0	LOW
• Mechanical prosthetic valves (high risk) • Prevention of recurrent myocardial infarction	2.5 -3.5	HIGH

Other Factors influencing the INR

The variability in the responsiveness of the PT reagents, is corrected through the "ISI" calibration, however three additional technical factors influence the INR:

- Derivation of MNPT
- Magnitude of difference in the ISI value of test thromboplastin and IRP (ISI=1.0)
- Method of clot detection employed during PT test.

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MNPT

MNPT is a critical requirement in the derivation of INR. Ideally each laboratory must derive its own MNPT from 20 or more normal patients for a given PT reagent and Lot under use. This corrects within laboratory test variables that influence PT results. If "normal control plasmas" are used in place of patient plasma for arriving at the MNPT it can effect the evaluation of the patients level of anticoagulation. For eg:

Reagent ISI=2.5	Test Day 1	Test Day 2	Test Day 3
Patient PT (sec)	16.0	16.0	16.0
Normal Control (10.4 – 12.3 sec)	11.5	10.4	12.3
INR Formula $[R]^{ISI}$	$\left[\frac{16.0}{11.5}\right]^{2.5}$	$\left[\frac{16.0}{10.4}\right]^{2.5}$	$\left[\frac{16.0}{12.3}\right]^{2.5}$
Resulting INR	2.27	2.89	1.92

If the control time is greater than the mean normal range (MNPT), the PT ratio for any patient PT will be smaller, potentially leading to over coagulation. If the control time is lesser than MNPT the ratio for any patient PT will be greater, leading to under coagulation.

On the other hand MNPT for a particular laboratory using the same combination of methodology, reagent and instrument would remain constant.

ISI value of PT used and method of clot detection

INR looses some precision when comparisons are made with thromboplastins with markedly different ISI values as against the IRP (ISI=1.0) and different methods of clot detection e.g.: manual, mechanical,optical etc.

Therefore manufacturers must provide ISI values adapted to the method used for clot detection. Also the reagent used for reporting results should be ideally as close to 1.0 as possible.

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Practical Considerations for Warfarin therapy

Oral administration of Warfarin results in a rapid absorption of the drug, however an observable anticoagulant effect is delayed. This delay is due to the time required for des-γ-carboxylated (dysfunctional) vitamin K dependent factor to replace the normal clotting factors.

Depending on the dose the delay may range from 1 to 7 days.

The early anticoagulant effect is mainly caused by the loss of fully carboxylated procoagulant factor VII which has a half life of approximately 5 hours.

However these oral anticoagulant reagents also cause suppression in the synthesis of **natural anticoagulant protein C and protein S.** Due to this, in the early phase of initiation of oral anticoagulant therapy there is a potential for initial prothrombotic effect. This event underlines the syndrome of coumadin induced skin necrosis, especially in patients with hereditary deficiencies of protein C and protein S.

Therapy can begin with an anticipated maintenance dose (e.g:5mg/day). A small loading dose of about twice the average maintenance dose may also be used initially. This dosage achieves a steady state anticoagulant effect in 5-7 days. The use of a large loading dose (e.g.20-40 mg) has little benefit. Such dosing not only produces a marked factor VII deficiency (which alone may not protect against thrombosis) but also an acquired protein C deficiency, which could produce a prothrombotic state.

If the need for antithrombotic effect is more urgent, heparin should be given as indicated. Heparin is then discontinued when INR is in the therapeutic range.

Considerations for frequency of Laboratory tests for monitoring oral anticoagulant therapy

PT monitoring of patients initially should be performed daily for the first 5 days and 2 to 3 times a week for the first 1 to 2 weeks. Depending on the stability of the PT results from the thirdweek onwards the frequency

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of monitoring may be further reduced to every 4-8 weeks.

While some patients on long term Warfarin therapy have unexpected fluctuations in dose response, some have unexplained requirement for increase in dosage.

The unexpected fluctuations in dose response could be due to: change in diet, undisclosed concomitant drug use, poor patient compliance, surreptitious self-medication, alcohol consumption, intermittent illness or unsuspected changes in the responsiveness of the PT reagent used to perform the PT test.

Management of patients with high INR values with or without bleeding complications

INR	Recommendations
INR> 3.5 but <6.0 patient not bleeding	Rapid reversal not indicated,omit Warfarin for a few doses and resume warfarin at lower dose when INR reaches therapeutic range
INR>6.0 but < 10.0 patient not bleeding	If rapid reversal required give 1-2 mg sc Vit.K • reduction of INR will occur in 8 hrs • back in therapeutic range in 24 hrs • If INR remains high at 24 hrs additional dose of 0.5mg Vit.K may be given. • Warfarin can be resumed at lower dose when INR returns to therapeutic range
INR > 10.0 patient not bleeding	Give 3 mg sc. Vit.K • INR will be reduced substantially by 6 hrs • Check INR at 6 hrs • Vit.K can be repeated if necessary
INR > 20.0 or patient bleeding	• Give 10 mg sc.Vit.K supplemented with FFP or prothrombin complex. • Check INR every 6 hrs • Administration of Vit.K inj. may be repeated every 12 hrs • In case of life threatening bleeding or serious warfarin overdose replacement with prothrombin complex concentrate is indicated, supplemented with i.v. Vit.K 10mg; repeated as necessary depending on INR

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Advantages of the INR system

- Major advantage of the INR system is that it helps alleviate confusion in the interpretation of PT results. Usually laboratory changes like change in thromboplastin and/ or equipments could go unnoticed by the attending physicians. The INR remains constant even with such changes.

- INR system affords comparison of PT results between laboratories.

- INR system provides a more accurate and convenient means of monitoring patients who travel extensively.

- INR therapeutic ranges for different clinical conditions are based
- on inter national collaborative studies. Usage of standardized dosage reduces the risk of thrombotic episodes or secondary bleeding.

Disadvantages of the INR system

- The prothrombin time test is always a part of the preoperative screening panels. It is also frequently used to evaluate other haemostatic disorders such as liver disease, DIC, LA, hereditary factor deficiencies and acquired vitamin K deficiency.

- Since these disorders have been excluded from the derivation of the ISI,INR has a diagnostic and therapeutic value mainly applicable for patients stabilized on oral anticoagulants. Therefore laboratories may prefer to report both the INR and patients time in seconds depending on clinical application.

- The INR systems effectiveness would still depend on the calibration of the coagulation instruments as well as thromboplastin reagents used.

- Derivation of the correct MNPT and use of the mean normal range in each laboratory.

- Usage of thromboplastin reagents with ISI of preferably 1.0 or as close to 1.0 as possible.

- The correct use of the formula to compute the INR.

- Uniform understanding of the INR system by clinicians as well as laboratorians.

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