462

APPLICATIONS OF INTERNATIONAL RECOMMENDATIONS ON THE STANDARDIZATION OF PROTHROMBIN TIME IN ORAL ANTICOAGULANT CONTROL.
F. Dati, U. Becker, N. Heimburger.
Research Laboratories of Behringwerke AG, Marburg, FRG.

The determination of prothrombin time (PT) in oral anticoagulant control is affected by a broad variation. The responsible factors are: type of thromboplastin incorporated in the PT reagents, procedure for use, clotting factors or heparin inhibitors added to the reagent, method of expression of PT results. Recently, joint recommendations have been issued by International Committees (ICSH/ICTH) taking into account the system of International Thromboplastins and the statistical model for thromboplastin calibration established by WHO. The aim is a standardization of commercial thromboplastins for PT tests in order to allow the use of the international scale of oral anticoagulant intensity (INR: Intern. Normalized Ratio).
Following such recommendations we have standardized two new PT tests, based on coagulometric and photometric methods which rely on the same sensitive human placental thromboplastin. The coagulometric PT test (Thromborel®S) is performed with conventional coagulometers. The photometric PT assay (Chromoquick®) uses a new chromogenic substrate specific for thrombin. This method is based on the measurement of the time necessary to reach a fixed increase of absorbance (0.1 A) using a special microprocessor-controlled photometer.
The two PT reagents were calibrated either directly against a reference preparation (BCT) or via an intermediate standard thromboplastin in two multicentric studies. The calibration procedure by the WHO method allows to assign the corresponding ISI (Intern. Sensitivity Index) to the PT reagent used and the transformation of the obtained prothrombin ratio (PR) into INR by the equation $INR = PR^{ISI}$. The calculated ISI values were 1.08 for the coagulometric PT reagent (n = 330) and 1.07 for the photometric reagent (n = 365), respectively.
The reproducibility of the ISI value for the new human placental thromboplastin for 64 different batches amounts to 3.6 %, the mean ISI value being 1.12.
Comparison with the reference thromboplastins in PR values gave a good correlation.
A) Coagul. PT assay (x): r = 0.964; y = 1.03x - 0.1;
B) Photom. PT assay (x): r = 0.940; y = 1.02x - 0.1.

463

PROTHROMBIN TIME MONITORING OF ORAL ANTICOAGULANT TREATMENT: COMPARISON OF INSTRUMENTS AND THROMBOPLASTINS.
M.P. Seveso (1), A. Macagni (1), S. Viganò D'Angelo (1), C. Manotti (2), P.A. Bonini (1) and A. D'Angelo (1). Istituto Scientifico S. Raffaele Milano, Italy (1) § Divisione Medica Ospedale Regionale, Parma, Italy (2).

The prothrombin time (PT) is the most widely used assay to monitor oral anticoagulation (OA). Although it has been established that both thromboplastin and instrumentation significantly affect the results, major standardization attempts have been devoted to the calibration of reagents rather than of instruments. To provide safe laboratory monitoring of OA, an International Sensitivity Index (ISI) for thromboplastin has been introduced. We have compared two automatic coagulometers, the ACL (Instrumentation Laboratory), a laser-nephelometer centrifugal analyzer which measures the intensity of the light scattered by a plasma sample before, during and after clot formation and the KOAGULAB 40A (Ortho Diagnostics), an optical automatic coagulometer, with the tilt tube technique for the performance of PT. Five calibrated commercial thromboplastins have been used for replicate determinations in 30 normals, 30 liver disease patients and 30 patients on stabilized OA. The overall observed imprecision (C.V.) was 1.1% for ACL, 2.9% for the KOAGULAB 40A and 3.0% for the tilt tube technique. The F test for the two-way interaction of ratios was statistically significant ($p < 0.001$) for the large majority of reagent/technique combinations in normals and in liver disease patients. International normalized ratios were also significantly different ($p < 0.001$) in most instances in patients on OA. Inadequate anticoagulation (INR < 2.0) was observed in 18% of patients with the ACL, in 31% with the KOAGULAB 40A and in 33% with the tilt tube technique. Excessive anticoagulation (INR > 4.5) was observed in 19% of the patients with the ACL, in 7% with KOAGULAB 40A and in 3% with the tilt tube technique. These data highlight the need for standardization of both instrumentations and reagents to improve monitoring of OA.

464

THE THROMBOTIC COMPLICATIONS OF TWO GROUPS OF PATIENTS WITH DIFFERENT INR THERAPEUTIC RANGES. THE NECESSITY OF INTENSE ORAL ANTICOAGULANT TREATMENT.
Ir. Kontopoulou-Griva, J. Spiliotopoulou, L. Digenopoulou, J. Georgopoulos. 1st Regional Transfusion Centre, Hippocration Hospital, Athens, Greece

One of the reasons why oral anticoagulants fell into disrepute is the absence of internationally acceptable standarised procedures for controlling the level of anticoagulation. This deplorable situation resulted in over and under coagulation and uncertainty in the therapeutic range. The International Normalised Ratio (INR) can safely be applied in patients on oral anticoagulants.

We present two Groups of patients under long term anticoagulation, mainly because of prosthetic heart valves that have recently been added to our outpatients clinic. These patients were till then attended by two cardiologists with different attitudes on the intensity of the anticoagulant treatment. The thromboplastin reagent used is that of ox origin and the results are expressed on INR.

The Group A with 32 patients had at the time that we started attending them an INR $\bar{x} = 1,80 \pm 0,48$ and a daily dose of acenocoumarol $\bar{x} = 1,65 \pm 0,51$.

The Group B with 49 patients had an INR $\bar{x} = 2,75 \pm 0,51$ and a daily dose of acenocoumarol $\bar{x} = 2,52 \pm 1,53$.

Seven patients of the Group A referred thrombotic complications, while three patients of the Group B referred transiant thrombotic complications.

The statistical analysis with the t-test of the INR between the two Groups is $p<0,001$ while that of the thrombotic complication with the x^2 is $p<0,05$.

The introduction of the INR and the acceptance by the medical people of the necessity of the intense oral anticoagulant treatment especially on high risk patients with mechanical heart valves as is the majority of the presented patients, will minimize the thromboembolic complications without high risk of bleeding.

466

ACUTE INTERRUPTION OF ORAL ANTICOAGULANT THERAPY: A THROMBOTIC RISK?.
J.Rouvier, H.Vidal, J.Gallino, M.Boccia, A.Scazziota and R.Altman. Centro de Estudios Médicos y Bioquímicos, Viamonte 2008, (1056) Buenos Aires, Argentina.

It is still on discussion how oral anticoagulant therapy must be interrupted. A progressive diminution of drug intake have been proposed in order to avoid a "rebound" of vitamin K-dependent procoagulant factors. At the present, it is well known that coumarin drugs affect not only the biologic activity of factors II, VII, IX and X but also Protein C (PC), an inhibitor of coagulation kinetics, and their cofactor Protein S. With the aim to determine the recovery level of PC in relation with the others vitamin K-dependent factors, the effect of suppression of anticoagulant therapy in patients under chronic treatment with acenocoumarin was studied.
Quick time, functional factors II, VII, X (one stage methods), functional PC (Francis method) and immunological Factor II and Protein C (Laurell) were determined before and 36 hours after suspension of acenocoumarin administration.
Results showed that: 1) Recovery levels of functional Protein C (increased from 28.55% ±2.57 to 72.64% ±5.9) were significantly higer than functional Factor II (22.09% ±2.34 to 30.73% ±8.64), Factor VII (22.55% ±2.01 to 40.73% ±4.85) and Factor X (23.27% ±2.66 to 39.18% ±3.19). Statistical analysis (Newmann-Keuls test) showed at least a $p<0.01$ between PC increase and factors II, VII or X increment.
2) No significant differences were seen between immunological levels of Factor II before and after suspension of acenocoumarin.
3) Levels of immunological PC in patients under anticoagulant therapy were higer than functional PC. After acenocoumarin suppression, not correlation was seen between immunological and functional Protein C recovery.
It is concluded that acute suppression of acenocoumarin does not induce a thrombotic tendency because the recuperation of functional Protein C is more important than factors II, VII and X recovery.