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A Comparison of Different APTT-Reagents, Heparin-Sensitivity and Detection of mild Coagulopathies

Vergleich verschiedener APTT-Reagenzien, Heparin-Sensitivität und Empfindlichkeit gegenüber milden Koagulopathien

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Summary:

The activated partial thromboplastin time (aPTT) is widely used to detect coagulation abnormalities or to monitor heparin treatment.

Many commercial aPTT-reagents are available which contain different phospholipid reagents and activators. In the present study 3 aPTT-reagents (aPTT-D, Instrumentation Laboratory, Neothromtin, Behring, PTTa, Boehringer) were compared using a computerized centrifugal analyzer. One aPTT-reagent (Pathromtin, Behring) was tested on a semiautomated coagulometer. Instrument precision was evaluated using aPTT-D as reagent.

Comparative tests were performed on plasma samples of 40 healthy donors, 3 patients with mild von Willebrand's disease (vWd), 10 patients with heaemophilia or subhaemophilia A, 1 patient with subhaemophilia A and vWd, 8 patients treated with subcutaneous injection of unfractionated heparin (UFH) and 14 patients treated with subcutaneous injection of a low molecular weight heparin (LMWH).

aPTT-D was the most sensitive reagent to detect mild vWd while Pathromtin detected none of these defects. In patients with heamophilia A and subhaemophilia A aPTT-D, Neothromtin and PTTa detected the abnormality in nearly all tested samples while Pathromtin was less sensitive.

Patients treated with subcutaneously applied UFH or LMWH often had a prolonged aPTT, especially when aPTT-D and Neothromtin were used as reagents.

Keywords:

Activated partial thromboplastin time – heparin sensitivity – von Willebrand's disease – haemophilia A – microcentrifugal analyzer

Zusammenfassung:

Die aktivierte partielle Thromboplastinzeit (aPTT) wird zur Erkennung von Gerinnungsstörungen oder zum Monitoren einer Heparintherapie verwendet. Viele aPTT-Reagenzien stehen heutzutage zur Verfügung, die unterschiedliche Phospholipide und Aktivatoren enthalten.

In dieser Studie wurden 3 aPTT-Reagenzien (aPTT-D, Instrumentation Laboratory; Neothromtin, Behringwerke; PTTa, Boehringer Mannheim) unter Verwendung eines Zentrifugalanalysators verglichen. Ein aPTT-Reagenz (Pathromtin, Behringwerke) wurde an einem halbautomatischen Kugelkoagulometer getestet. Die Präzision der Instrumente wurde mit Hilfe des aPTT-Reagenz bestimmt.

Vergleichende Teste wurden mit Plasmaproben von 40 gesunden Spendern, 3 Patienten mit mildem von-Willebrand-Syndrom (vWS), 10 Patienten mit Hämophilie oder Subhämophilie A, 1 Patient mit Subhämophilie A und vWS, 8 Patienten nach subkutaner Gabe von unfraktioniertem Heparin (UFH) und 14 Patienten nach subkutaner Gabe von niedermolekularem Heparin (NMH) durchgeführt.

aPTT-D war das sensitivste Reagenz in bezug auf das milde von-Willebrand-Syndrom, während Pathromtin am unempfindlichsten war. Bei Patienten mit Hämophilie A oder Subhämophilie A ließ sich mit Hilfe von aPTT-D, Neothromtin und PTTa die Gerinnungsstörung in nahezu allen Plasmaproben nachweisen, während Pathromtin wiederum weniger empfindlich war.

Patienten, die mit subcutanen Gaben von UFH oder NMH behandelt worden waren, zeigten oft eine verlängerte aPTT, besonders, wenn aPTT-D und Neothromtin als Reagenzien verwendet wurden.

Schlüsselwörter:

Aktivierte partielle Thromboplastinzeit – Heparinsensitivität – von-Willebrand-Syndrom – Haemophilie A – Zentrifugalanalysator

Introduction

Many commercial aPTT reagents are available which contain different phospholipid reagents and activators.

Their sensitivity to clotting factor defects and heparin effects differs widely (1–6). This is not surprising in view of the variations in performing the test and the lack of standardization of the composition of partial thromboplastin reagents.

APTT is used to monitor treatment with heparin and LMWH and to detect coagulopathies. The test can be performed manually and on different semiautomated and automated systems.

A microcentrifugal analyzer based on clotting time as measured by light scattering (7) and a semiautomated coagulometer were used for the present investigations.

The purpose of this study was to compare 4 different aPTT-reagents in respect to their sensitivity to heparin and mild clotting factor defects. Three aPTT-tests were performed on a centrifugal analyzer, one aPTT-reagent was studied using a semiautomated coagulometer.

Materials and methods

Reagents and instruments

The 4 different aPTT-methods are shown in table 1. APTT-D, Neothromtin and PTTa were performed on a fully automated centrifugal analyzer (ACL 300, Instrumentation Laboratory, Milano, Italy). The instrument automatically delivers samples and reagents, mixes, incubates and analyzes by measuring changes of light scattering in the plasma samples. All determinations were done with the aPTT-program stored in the instrument's memory: equal quantities of plasma, reagents and $CaCl_2$; mixing time 1 min, incubation time of plasma and reagent 4 min.

PTT-determinations with Pathromtin were performed on a semiautomated coagulometer (KC 10, Amelung GmbH, Lemgo) which is routinely used in our laboratories (equal quantities of plasma, reagent + $CaCl_2$, incubation time of plasma and reagent: 2 min). Because of its content of Caolin (high turbidity) Pathromtin can not be used on the ACL 300.

Factor VIII: C was determined coagulometrically by means of factor VIII deficient plasma (Merz + Dade, Munich, FRG) and an aPTT reagent (Actin, Merz + Dade, Munich, FRG), vWF was measured by Laurell-Immunoelectrophoresis, Ristocetin cofactor was determined according to the method of Scharrer (8).

Coagulation studies

Blood was collected by venepuncture using a Syringe (Sarstedt Monovettes), containing 0.5 ml 0.109 M trisodium citrate (9 parts of blood + 1 part citrate). Platelet poor plasma was obtained by centrifugation at 800 g for 10 min at room temperature.

APTT determinations were performed on fresh plasma within 120 min after collection.

To establish the reference (normal) range 40 healthy individuals were tested (20 male, 20 female, age: 21–33 years).

APTT was also studied on

- 3 patients with mild von Willebrand's disease (vWF >30%)
- 10 patients with haemophilia or subhaemophilia A (F VIII >2%)
- 1 patient with subhaemophilia A and vWd
- 8 patients treated with subcutaneous injections of unfractionated heparin (1 x 7500 IU Calciparin s. c., Sanofi, Munich, FRG, or 1 x 10000 IU Liquemin s. c., Hoffmann La-Roche, Grenzach, FRG)
- 14 patients treated with subcutaneous injections of a low molecular weight heparin (1 x 7500 aXa s. c. LMWH Fraxiparin, Sanofi, Munich (FRG).

The blood of patients treated with UFH or LMWH was sampled 3 hours after s. c. application.

For precision calculations 9 replicate analyses on a normal control plasma (Control plasma N, Behring, FRG) and an abnormal control plasma (coagulation factors Control plasma P, Behring, FRG) were performed in the morning on both instruments. APTT-D was used as reagent for these assays.

The analyses were repeated on the same plasmas in the afternoon of the same day (about 4–6 h later) and on the 6 following days in the morning.

Results

Table 2 shows the results for each of the commercial reagents obtained in the testing of the plasma of 40 healthy donors. Since aPTT-results show a non-Gaussian distri-

Table 1: Characteristics of the 4 aPTT-methods compared in this study

aPTT-reagent	95% percentile (sec.)	normal range suggested by the manufacturer (sec.)
aPTT-D, I. L.	28–41	28–40
Neothromtin, Behringwerke	24–33	22–35
PTTa, Boehringer Mannheim	25–36	26–42
Pathromtin, Behringwerke	30–45	28–40

Table 2: 95% percentiles of the different aPTT-reagents (40 healthy donors were tested)

Reagent	Manufacturer	Activator	Source of phospholipids	Buffer + additives
aPTT/D	IL test München	micronised Silica	bovine brain cephalin	stabilisators and conservatives
Neothromtin	Behring Marburg	ellagic acid	phospholipid from vegetables	stabilized with glycin and polysaccharides
PTTa	Boehringer Mannheim	micronised Silica	lyophilized brain cephalin	stabilized with natrium-methiolat, buffer: Owrens-Veronalbuffer
Pathromtin	Behring Marburg	caolin-suspension 5 g/l	human placenta phospholipid	stabilized with lecithin, polysaccharides and antioxidants

bution in a normal population the upper limits of the reference (normal) range were calculated using the 95% percentiles.

Table 3 demonstrates the coefficients of variation within one run, between one run and between 7 following days. APTT-D was tested on the microcentrifugal analyzer and on the semiautomated coagulometer for the comparison of the precision of both instruments.

The fully automated centrifugal analyzer was more precise than the semiautomated coagulometer.

Pathological plasma samples which were tested on the coagulometer in the afternoon showed a significantly shortened aPTT compared to the morning determinations ($p < 0.001$, results not shown). This difference between morning and afternoon run was not observed when the aPTT was determined on the centrifugal analyzer.

Table 4 shows the results obtained in plasma samples of patients with mild von Willebrand's disease or haemophilia and subhaemophilia A.

APTT-D was the most sensitive reagent detecting all patients with subhaemophilia A and 3 out of 4 patients with mild vWd. Neothromtin and PTTa were equally sensitive while pathromtin did not detect any mild vWd and only 2 patients out of 6 patients with subhaemophilia A.

The aPTT-results of patients under s. c. heparin prophylaxis are shown in Table 5.

Concerning the subcutaneous injection of LMW heparin (1 x 7500 aXa Fraxiparin s. c.) aPTT-D and Neothromtin were the most sensitive reagents showing in 30–50% of patients a prolongation of aPTT above the upper limit of the normal range. In respect to subcutaneous administration of two different UF heparins (1 x 7500 IU Calciparin s. c. or 1 x 10000 IU Liquemin s. c.) all reagents were quite sensitive with Pathromtin being the least sensitive one.

Discussion

An universal aPTT reagent should not only be sensitive to reductions of clotting factors of the intrinsic pathway, but should also detect the presence of different concentrations of heparin. Besides the results should be reproducible.

APTT-methods differ concerning the source of phospholipid, type and concentration of activator and type of buffer (see table 1).

In this study 3 aPTT-reagents have been compared on a fully automated centrifugal analyzer. Quantities of plasma, reagent and $CaCl_2$ were equal, time of mixing and activation were identical. One additional aPTT-reagent (Pathromtin) was tested on a semiautomated coagulometer since the centrifugal analyzer could not be used, because of the high turbidity of the activator caolin.

Pathromtin was the least sensitive reagent concerning the detection of mild coagulopathies and heparin.

This may be partly due to the relative short activation time (2 min) recommended by the manufacturer. According to the results of Barrowcliffe and others (1, 2) activation with caolin requires at least 10 min and shorter incubation times can lead to a reduced factor VIII sensitivity.

In respect so subcutaneously applied LMWH aPTT-D and Neothromtin were the most sensitive reagents showing

Table 3: Comparison of instrument precision, coefficient of variation within one run, between one run and between 7 following days

Instrument			coefficient of variation in %	number of determinations
KC 10	within run	normal	0,4	9
		abnormal	0,4	9
	between run	normal	0,7	18
		abnormal	1,0	18
	between day	normal	0,7	7
		abnormal	1,4	7
ACL 300	within run	normal	0,3	9
		abnormal	0,3	9
	between run	normal	0,2	18
		abnormal	0,4	18
	between day	normal	0,5	7
		abnormal	0,4	7

prolongation above the upper limit of normal range in 30–50% of patients. Concerning the s. c. application of UFH all aPTT-reagents performed well.

APTT-D is the most sensitive reagent to detect mild von Willebrand's disease, while Neothromtin, PTTa and aPTT-D are equally sensitive in detecting subhaemophilia or haemophilia A. Since aPTT-D and Neothromtin show a si-

Table 4: aPTT-determinations of the plasma of patients with mild von Willebrand's disease or subhaemophilia and haemophilia A, vWF = von Willebrand factor

Patients	diagnosis	aPTT-D (sec)	Neothromtin (sec)	PTTa (sec)	Pathromtin (sec)	Fakt. VIII %	Fakt. VIII Ass.Ag %	Ristocetin Cofactor %
K. F.	Haem. A	69	56	73	61	2	–	–
F. G.	Haem. A	65	41	52	56	6	–	–
J. H.	Haem. A	56	42	49	51	9	–	–
Di. B.	Haem. A	52	43	46	50	14	–	–
Da. B.	Haem. A	53	43	44	54	16	–	–
R. B.	Subhaem. A	53	40	45	52	16	–	–
E. B.	Subhaem. A	48	34	35*	40*	19	–	–
P. S.	Subhaem. A	61	38	- 44	43*	23	–	–
A. B.	Subhaem. A	46	35	43	43*	28	–	–
U. G.	Subhaem. A	46	41	45	51	28	–	–
P. S.	Subhaem. A mild vWs type 1	55	34	39	42*	28	50	115
G. S.	mild vWs type 1	47	30*	36*	39*	61	42	67
I. S.	mild vWs type 1	51	33*	37	41*	54	62	60
S. W.	mild vWs type 1	41*	31*	33*	36*	73	31	75

* aPTT within the 95% percentiles (within the normal range)

" mean of double investigations

Table 5: aPTT-determinations of the plasma of patients tested with low molecular weight heparin subcutaneously (1 x 7500 aXa Fraxiparin s. c.) or unfractionated heparin subcutaneously (1 x 7500 IU Calciparin s. c. or 1 x 10000 IU Liquemin s. c.)

Patient	Therapy	aPTT-D (sec)	Neothromtin (sec)	PTTa (sec)	Pathromtin (sec)
R. L.	1 x 7500 aXa s. c. Fraxiparin	41*	32*	32*	34*
S. A.	1 x 7500 aXa s. c. Fraxiparin	35*	33*	29*	43*
N. A.	1 x 7500 aXa s. c. Fraxiparin	31*	26*	28*	34*
R. B.	1 x 7500 aXa s. c. Fraxiparin	35*	33*	33*	33*
S. H.	1 x 7500 aXa s. c. Fraxiparin	39*	30*	31*	35*
L. F.	1 x 7500 aXa s. c. Fraxiparin	31*	33*	30*	33*
N. T.	1 x 7500 aXa s. c. Fraxiparin	34*	29*	28*	28
P. T.	1 x 7500 aXa s. c. Fraxiparin	47	26*	33*	36*
E. S.	1 x 7500 aXa s. c. Fraxiparin	46	24*	29*	30*
M. K.	1 x 7500 aXa s. c. Fraxiparin	40*	37	36*	40*
H. J.	1 x 7500 aXa s. c. Fraxiparin	47	35	36*	38*
M. D.	1 x 7500 aXa s. c. Fraxiparin	42	35	36*	39*
M. G.	1 x 7500 aXa s. c. Fraxiparin	49	35	37	37*
N. E.	1 x 7500 aXa s. c. Fraxiparin	50	45	41	56
T. V.	1 x 7500 IU s. c. Calciparin	41*	68	40	73
R. M.	1 x 7500 IU s. c. Calciparin	48	38	42	34*
M. L.	1 x 7500 IU s. c. Calciparin	56	54	45	39*
L. N.	1 x 7500 IU s. c. Calciparin	105	71	87	85
R. G.	1 x 7500 IU s. c. Calciparin	76	55	55	49
F. M.	1 x 10000 IU Liquemin	56	32*	41	36*
F. S.	1 x 10000 IU Liquemin	70	36	48	34*
T. Z.	1 x 10000 IU Liquemin	80	70	>120	80

* aPTT within the 95% percentiles (within the normal range)
° mean of double investigations

milar sensitivity to subcutaneously administered LMWH and UFH this has to be considered when these reagents are used for the detection of mild coagulopathies in hospitalized patients, who are frequently heparinized.

A fully automated centrifugal analyzer can easily be used for routine coagulation tests as aPTT.

Preheating, delivery of samples and reagents, mixing, incubation and analysis of the coagulation time are performed under standardized automated conditions. Consequently differences in results concern predominantly different reagents and individual plasmas used.

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