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No Indication for APTT Screening in Patients on Oral Anticoagulant Therapy

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Summary

Patients on oral anticoagulant therapy (OAT) may bleed due to a very low factor IX level caused by a mutation at Ala-10 in the propeptide region of the factor IX gene. We evaluated screening of patients on OAT with an APTT to detect patients with this abnormality.

In 734 patients an APTT was assessed. Twenty-three patients had a disproportionately prolonged APTT. In these patients the factor IX level, the mutation at Ala-10 and the frequency of bleeding complications were assessed. No severely lowered factor IX levels were found (1 patient with 5% factor IX). No mutations at Ala-10 were found and bleeding complications were not more frequent in these patients. Conclusion: Routine APTT screening of patients on OAT is not useful to detect patients with increased bleeding or with the Ala-10 mutation in the factor IX gene.

Introduction

Oral anticoagulant therapy (OAT) with coumarin derivatives results in defective synthesis of the vitamin K-dependent proteins. These proteins include the procoagulant factors II, VII, IX and X and the anticoagulant proteins C and S. In patients, oral anticoagulant therapy is regulated on the results of the INR (international normalized ratio) which is derived from the prothrombin time. The prothrombin time measures the classical extrinsic pathway of blood coagulation which includes the vitamin K-dependent factors II, VII and X and excludes factor IX. Factor IX is involved in the intrinsic pathway of coagulation which is measured with the APTT. This implies that very low factor IX levels due to whatever cause, will be easily missed in patients on OAT. Low factor IX levels obviously contribute to bleeding, which is the most frequent complication of OAT.

Recently, two mutations at Ala-10 in the region of the gene coding for the propeptide of factor IX were found which resulted in a severely decreased factor IX level of <1% during OAT (1, 2). After discontinuation of OAT the factor IX restored to normal levels, excluding hemophilia B in these patients. The low factor IX level was believed to contribute to the severe bleeding tendency in the four patients described. The frequency of these mutations in the general population is not known at this moment. If prevalent, these mutations could be a highly relevant cause of bleeding in patients on OAT. Screening of patients candidate for OAT for the presence of the mutation could then result in prevention of bleeding.

To evaluate the importance of a prolonged APTT due to low factor IX levels in patients on OAT and the contribution of this abnormality to the problem of bleeding, we analyzed a large number of patients routinely treated at the Leiden Thrombosis Service. First, we measured the APTT as a screening test and assessed the frequency of a disproportionate prolongation of the APTT. Subsequently, the factor IX levels and the presence of the mutations at Ala-10 were assessed in the patients with a prolonged APTT.

To be informed about the relation of the factor IX level and the APTT in patients with a proportionate prolongation of the APTT we assessed the factor IX level in 15 randomly selected patients.

Our results indicate that in the Dutch situation it is not necessary to screen the APTT to exclude low factor IX levels and the presence of these factor IX mutations.

Methods

Patients routinely treated at the Leiden Thrombosis Service were evaluated. The Leiden Thrombosis Service is regionally organized like all Dutch thrombosis services and takes care of the control of the treatment with coumarin derivatives for all the outpatients in the region of Leiden, The Netherlands. The area has about 460,000 inhabitants and three hospitals. All outpatients in the covered area are referred to our service for anticoagulant therapy. Annually, about 6500 patients are under treatment and 130,000 INRs are assessed. Since 1973 all data, including INR results, dosages and bleeding complications are stored in a computerized database.

The working scheme was as follows. At three days, after routine INR measurement, the APTT was assessed of as many patients as achievable. No selection was made, except that patients using heparin were excluded. As a control the factor IX level was assessed in 15 patients randomly selected from these patients. Subsequently, patients with a disproportionate prolongation of the APTT were selected (see below). At the next regular visit at the Thrombosis Service a sample was taken from these outlier patients for assessment of the factor IX level and the presence of the mutations.

Laboratory Methods

The INR and APTT were assessed with Recombiplastin (Ortho-Clinical Diagnostics, Beerse, Belgium) and Cephotest (Nyegaard, Oslo, Norway) reagent on an Electra 1000C coagulometer (MLA, Pleasantville, New York, USA). Factor IX level was assessed on an Electra 1000C in a one stage assay using Automated APTT (Organon Teknika, Durham, NC, USA) as reagent and commercial factor IX-deficient plasma (Organon Teknika, Durham, NC, USA).

To detect both described changes at position –10 in the Factor IX propeptide (Ala→Thr and the Ala→Val) as well as all other changes affecting this amino acid, we devised a mutagenic primer that creates a BglI ($GCCN_5GGC$)-site when a PCR is performed on the Ala-encoding wild-type allele. This primer was used as the downstream primer in the PCR. To have a positive internal

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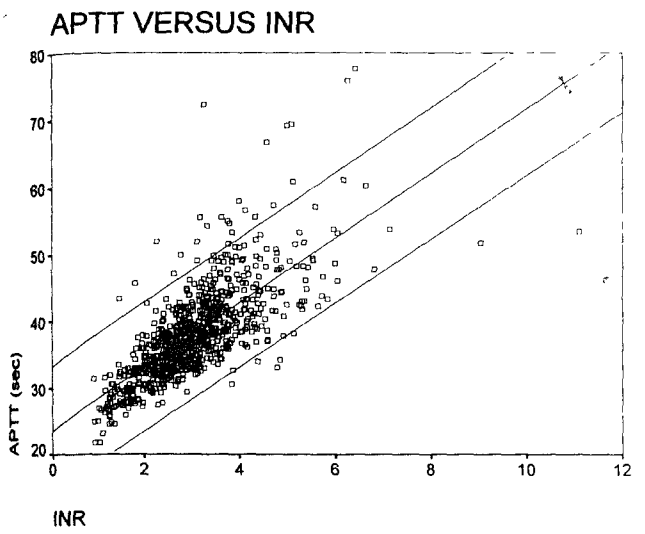

Fig. 1 APTT values in relation to INR in 733 patients treated at the Leiden Thrombosis Service. The lines indicate the upper and lower 95% confidence limits of the linear regression between the APTT and INR

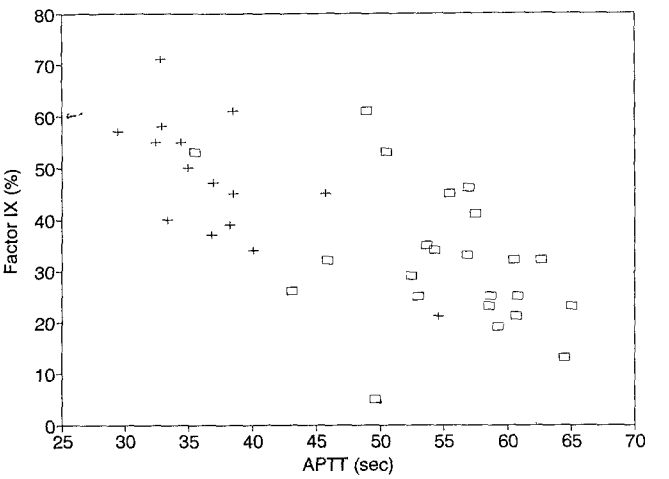

Fig. 2 Factor IX levels in relation to APTT in outlier patients (□) and in controls (+)

control for restriction enzyme activity, we changed some nucleotides in the upstream primer as well, to create a BglI site that would always be present. The sequences of the primers were (altered nucleotides are shown in lower case, and the complete or partial BglI sites are underlined):

upstream 5' ATGCCCTAAAGccAAATTGGCTTTCAGAT 3'

downstream 5' CCTCTTTGGCCGATTCAGAgccTTGTTG 3'.

The Ala-codon at -10 (GCC) is complementary to the last three nucleotides (GGC) of the BglI-site. Such a site is therefore only created when the downstream primer is followed by the GC of the Ala-codon encoded on the opposite strand.

PCR conditions were: 4 min 94° C, followed by 33 cycles of 1 min 94° C, 1 min 60° C and 1 min 71° C, followed by a 5 min extension time at 71° C in a buffer consisting of 67 mM Tris-HCl (pH = 8.8), 16.6 mM ammonium sulphate, 6.7 μM EDTA, 6.7 mM magnesium chloride, 10 mM 2-mercaptoethanol, 50 μg/ml bovine serum albumin, 10%(v/v) DMSO, 1.5 mM dNTPs and 20 U/ml Taq polymerase.

Statistical Analysis

The relation between the APTT and the INR was assessed by linear regression analysis. Patients with disproportionate APTT prolongation ("outliers") were identified as those with an APTT outside the upper 95% confidence limit of the linear regression. Standard SPSS software was used.

Table 1 Results of INR, APTT and factor IX in all patients, outliers and controls

		All patients n = 733	outliers* n=23	controls n=15
INR	mean	3.1	3.8	3.1
	range	1 0-11.1	1.7-10.0	2.3-3 9
	s.d.	1.1	1.6	0.5
APTT	mean	38.3	55.0	37.3
	range	21.8- 77.7	35.5-65.1	29.4-54.6
	s.d	7.2	6.9	6.0
Factor IX				
	mean	-	32	47.6
	range	-	5-61	21-71
	s.d.	-	13.0	12.1

*Results are from the second sample. One of factor IX control patients was found to be an outlier and is included in both groups in this table.

Bleeding Complications

The frequency of bleeding complications was assessed as previously described (3). From all patients the records were analyzed from start of treatment on. All bleeding complications were taken into account. Major bleeding was defined as intracranial bleeding, bleeding leading to admission to hospital for treatment and muscle and joint bleeds. All other bleeding complications were categorized as minor.

Results

The APTT was measured in 734 patients. In one patient the APTT was unmeasurably prolonged (>150 s, INR 2.3), due a severe factor XII deficiency (factor XII <1%, factor IX 36%, factor VIII 1.20 IU/ml, factor XI 103%). The data of this patient were excluded from further analysis.

Mean INR of the remaining 733 patients was 3.1 (range 1.0 to 11.1, standard deviation 1.1). Mean APTT of the 733 patients was 38.3 (normal ≤32.5) s, with a standard deviation of 7.2 and a range of 21.8-77.7 (see also Table 1). Linear regression analysis revealed the following relation between INR and APTT: APTT= 23.5 + 4.8 *INR. In Fig. 1 the INR and APTT results are plotted with the linear regression line and its 95% confidence limits. Twenty-five outliers were identified. The mean APTT of the outliers was 57.6 s, with a range of 43.4-77.7 s and a standard deviation of 9.1. Of the 25 outlier patients one died of lung cancer before follow-up was possible and another patient had stopped anticoagulant therapy. Of the remaining 23 patients the APTT and factor IX level were (re-)assessed in a second sample (see Table 1). The mean APTT in this second sample was 55.0 s with a standard deviation of 6.9. The mean factor IX level was 32% with a range of 5–61% and a standard deviation of 13. In Fig. 2 the factor IX levels are presented in relation to the APTT.

The results of the APTT and the factor IX levels in the 15 randomly selected control patients are presented in Table 1 and Fig. 2. In these patients the APTT ranged from 29.4 to 54.6 with a mean of 37.3 s and a standard deviation of 6.0. The factor IX level had a mean of 47.6% with a range from 21 to 71%.

For the detection of the mutation we used PCR on genomic DNA to amplify a 176 bp fragment. The resulting fragment will contain two

BglI-sites if an Ala-residue is encoded by both alleles, but only one if another codon would be present in this position. The assay is therefore sensitive for both the Ala → Thr and the Ala → Val mutation, that have both been described as well as for all other possible mutations at this position. The additional BglI-site in the PCR-fragment serves as a positive control for digestion by the enzyme. All patients with out-of-proportion APTT prolongation turned out to carry an Ala-codon on both alleles, excluding the presence of a mutation in this position.

Bleeding Complications

The history of bleeding complications of the 24 outlier patients were analysed. All data available from the start of treatment on were analysed. Total follow-up time was 109.5 years, with a range from 2 weeks to 14½ years, a mean of 4.2 years. A total number of 10 bleedings was observed, one of which was severe (digestive tract bleeding). So severe bleeding was found at a frequency of 0.91/100 treatment years and minor bleeding at 8.2/100 treatment years. The patient with the lowest factor IX level of 5% had been treated for 14½ years and experienced only two minor conjunctival bleeds.

Discussion

We analysed 734 patients on routine treatment with oral anticoagulants by screening with an APTT, followed by assessment of the factor IX level if the APTT was disproportionately prolonged. In Fig. 2 the relation between the factor IX level and the APTT is shown both for the controls and for the outliers, indicating that our APTT is sensitive to factor IX in this type of patients. We did not find any patient with a very low factor IX level or a mutation at Ala-10 in the propeptide region of the factor IX gene. This abnormality therefore proves not to be an important cause of bleeding in this large group of over 700 anticoagulated patients. Using a nearly identical approach Peters et al. found one patient with a factor IX level of 7.8%, without a mutation at the Ala-10 position (4). We found one patient with a factor IX level of 5%. This was a 43-year-old woman who was treated with oral anticoagulants for more than 14 years because of recurrent venous thromboembolism. She experienced only two minor conjunctival bleeds and no major bleeding complications. The PCR-assay we used is specific for mutations affecting codon -10 of the factor IX gene. The presence of another mutation affecting the factor IX level during OAT in this or other patients therefore cannot be excluded.

We analysed a random patient sample, including patients who recently started therapy. Selection bias caused by patients with the mutation who stopped OAT because of bleeding therefore cannot have majorly influenced our results. We may have missed patients if presence of the mutation does not always result in a very low factor IX level and a prolonged APTT. However, these patients will not have an increased bleeding tendency and it is not clinically relevant to find them. The method we used cannot give an exact estimate of the frequency of the mutation in the population. However, we found 0 mutations in 734 patients and from the 95% confidence interval of 0/734 it can be deduced that less than 0.4% of the population will have a mutation (when the mutations, if present, would always be detected by APTT screening as we performed). This means that screening for the mutations of all patients who are candidates for OAT is not indicated.

Another conclusion can be drawn from our data. We analysed bleeding complications in patients with an disproportionately prolonged APTT. We found a frequency of major bleeding of 0.91 per 100 treatment years and a frequency of all bleeding of 8.2 per 100 treatment years. In 1988 and 1991 we found 2.7 and 2.1 major bleeds per 100 treatment years respectively (3, 5). For all bleeding we found 16.5 and 16.6 bleeds per 100 treatment years respectively (3, 5). The results of this investigation therefore compare very favourable with the previous data. So, in general, assessment of APTT is not indicated to identify patients who will experience increased bleeding on OAT.

Presence of a mutation at Ala -10 in the factor IX gene cannot be excluded as a rare cause of bleeding in patients on OAT. The same is true for other abnormalities in the intrinsic pathway of blood coagulation. Therefore, in patients with pronounced bleeding complications during OAT assessment of an APTT is indicated. When the APTT is disproportionately prolonged, factor assays have to be performed. It may well be that the mutation, due to a higher frequency, has more relevance in other population groups.

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