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A rapid prenatal diagnosis of hemophilia A by DNA analysis on crude chorionic villus biopsy

Sir,

We have developed a rapid method for prenatal diagnosis of hemophilia A with a nested PCR on DNA obtained from boiled chorionic villus biopsy (CVS).

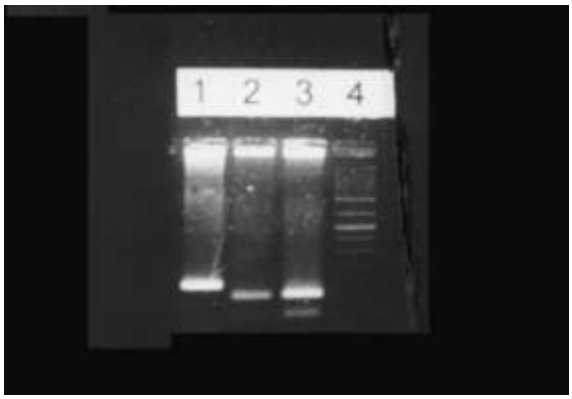

Figure 1. Prenatal diagnosis of hemophilia A by nested PCR analysis. Lane 1: undigested 142 bp fragment. Lane 2: villus, after Bcl-I enzymatic digestion, shows the (99+42) fragments. Lane 3: mother; lane 4: 100 bp ladder DNA marker.

We report on a family with severe hemophilia A due to a large deletion of factor VIII gene spanning from exon 14 to the end of the gene. The hemophiliac did not show a band for all the intragenic polymorphisms located in this deleted region, i.e. for intron (18)/Bcl I RFLP. His sister asked for genetic counselling when she was pregnant; the family study revealed a haplotype (+/deletion) for intron (18)/Bcl I polymporphism, thus she was diagnosed as a carrier.

CVS biopsy was performed at the 12th week of gestation. Karyotype analysis revealed a male fetus.

In order to obtain a rapid prenatal diagnosis of the fetal genotype, we developed a new method of DNA extraction. A single fragment of the CVS biopsy, without maternal decidua, was boiled in 5 µL of distilled water, quickly cooled on ice and utilized directly for PCR reaction.

We used two consecutive PCRs to increase the concentration and specificity of the amplified DNA.

Initially, the following external pair of primers was used: Int 18 (A) 5' ATG GCA CTG RAC-AATCTCTA 3' and Int 18 (B) 5' GGTAACATTTCCACTGTCT 3' and 35 step cycles were performed at 94° for 1 min and at 60° for 6 min.

The PCR produced a 1.8 kb fragment containing two Bcl I sites: one constant (1.4 kb) and the other polymorphic (400/300+100 bp).

Fifteen microliters of the amplified fragment were loaded onto 2% agarose gel and visualized under U.V. light with ethidium bromide staining. The villus showed a very faint 1.8 kb amplified band.

This suggested the presence of the whole FVIII gene and therefore of a healthy fetus.

To confirm the diagnosis, 10 µL of the amplified product from both mother and fetus were used as templates for the second PCR reaction with an internal pair of primers spanning the polymorphic Bcl I site (for details on primers see ref. #1).

The 142 bp fragment present in both samples was digested by the Bcl I enzyme. The results are shown in Figure 1.

The presence of a Bcl I restriction site on the amplified product confirms the diagnosis of a healthy fetus. The diagnosis was yet further confirmed by the standard procedure on DNA extracted from CVS.

In our opinion our approach is a rapid and efficient method of DNA extraction from CVS which can give a prenatal diagnosis in few hours, having used a small amount of template.

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Key words

Hemophilia, prenatal diagnosis, chorionic villus biopsy

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Burkitt's lymphomas in adults: retrospective analysis of 30 cases treated with two different schemes

Sir,

Burkitt's lymphoma represents only 5 to 10% of non Hodgkin's lymphomas (NHL) in adults and most clinical trials on this disease have been carried out in children.[1]

Using CHOP-MTX , Bernstein *et al*.[2] reported good results in localized Burkitt's lymphoma, but not in advanced disease. Magrath *et al*.[8] developed a new regimen CODOXM-IVAC and they reported good results also in advanced disease.

The aim of our retrospective study was to compare the results and toxicity observed in 30 adult patients with Burkitt's disease or L3-ALL treated in our Institution using CHOP-MTX (14 patients) from 1986 to 1991 and CODOX-IVAC (16 patients) from 1992 to 1996.

None of the patients had received previous treatment; patients with AIDS or human immunodeficiency virus or secondary lymphomas were excluded. The clinical characteristics, toxicity and response rate of patients in the two groups are reported in Table 1.

Six patients with localized disease were treated with CHOP-MTX, all achieved a CR and none relapsed; more aggressive treatment does not seem to be appropriate. Patients with advanced stage disease treated with the CODOXM-IVAC regimen had a better CR rate, and a significantly better OS than those treated with CHOP-MTX (41% vs 13% p=0.03) (Figure 1), but they suffered from more severe toxicity, a finding also reported by other authors.[3-6,10]

Seven patients received autologous stem cell transplantation as consolidation in first remission. Three are alive and well and four have died. One patient

Table 1. Clinical characteristics, toxicity and remission rate according to therapeutic scheme used.

	CHOP-M	*CODOXM-IVAC*	*P*
Age			
< 45	10	11	
> 46	4	5	n.s.
Sex			
male	9	9	
female	5	7	n.s.
LDH level			
< 500	6	8	
> 500	8	8	n.s.
Stage			
Localized	6	0	
Advanced	8	16	p=0.003
B.M.			
Neg	11	10	
Pos	3	6	
L3.ALL	2	4	
CNS			
Neg	12	13	
Pos			
Infections 3+4	3	12	p=0.02
C.R			
localized disease	6 (100%)	–	
advanced disease	2 (25%)	16 (81%)	

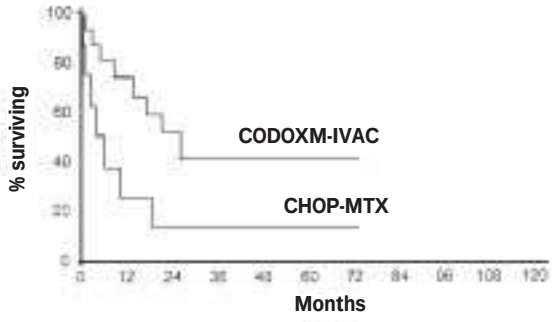

Figure 1. Overall survival of 16 patients with advanced stage treated with CODOXM-IVAC and of 8 patients with advanced stage treated with CHOP-MTX (p=0.03).