quent in Hungary, Turkey and Lebanon[3] where it represents between 3 to 7% of the β-thalassemias. However it is very scarce in the island of Sardinia. This mutation was also reported in inland countries of Europe and the Middle East such as Czechoslovakia and Azerbaijan,[3] where it represents 14-16% of the molecular abnormalities detected in β-thalassemias. On the Iberian peninsula, to our knowledge, only a single case of IVS II-1 among 88 thalassemics has been reported in a study carried out in the north of Portugal.[4] There have been no reports until now of this molecular abnormality in Spain. This abnormality, according to our experience in molecular diagnosis of β-thalassemia, represents 1 out of 40 β thalassemics.[5]

Although this molecular abnormality is a β^0, its rarity lessens its clinical importance. However, awareness of its existence increases the spectrum of known mutations in Spain and, consequently, in the Iberian Peninsula.

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Azoospermia in a patient with sickle cell disease treated with hydroxyurea

This report describes a case of reversible azoospermia in a patient treated with hydroxyurea. This occurrence has not been previously documented in patients with sickle cell disease. We suggest that patients treated with this therapy should be informed of this potential side effect and that they should be given the necessary clinical follow-up investigations.

Sir,

Sickle cell disease (SCD) is a hemoglobinopathy characterized by an amino acid substitution in the beta chain of hemoglobin (hemoglobin S, HbS), which determines, under conditions of hypoxia, the polymerization and precipitation of hemoglobin within the erythrocytes and their subsequent deformation (sickling). The clinical picture of SCD is characterized by recurrent painful crises, hematologic crises (aplasia, splenic sequestration, hemolysis) and infections.The use of hydroxyurea (HU) causes an increase of HbF[1] which interferes with the process of polymerization of HbS; in fact at the dose of 20 mg/kg/day HU increases the value of HbF which causes an improvement in the general state of health of the patients and a reduction in the frequency and severity of painful crises (especially during the first months of therapy).[2] In our center we treat 120 patients of whom 79 are affected by thalassemia major, 24 patients by thalassemia intermedia, 15 patients by double heterozygosis for HbS and beta thalassemia (thalassodrepanocytosis) and 2 patients by SCD. One of the two SCD patients is a male who was born in 1971 and both his parents are HbS carriers with normal values of HbA_2. Our patient has required periodic blood transfusions because of recurrent severe painful crises (first crisis in 1972 at the age of 8 months); he was splenectomized in 1977. This patient was offered HU therapy at the above stated dosage. Before starting the therapy we performed: spirometry, chest X-rays, liver, biliary ducts, kidney and urinary tract ultrasound, echocardiogram, hemochrome with erythroblast count and reticulocyte assessment, Hb phoresis by HPLC, LDH, bilirubin, ALT and AST, creatinine, LH, FSH, prolactin, FT3, FT4, TSH, testosterone, and spermatic fluid analysis. All the clinical tests gave normal values except the liver ultrasound which showed a slightly enlarged liver and a non-homogeneous echostructure (the patient is $HCVAb^+$, $HCV\ RNA^+$).

Table 1. Seven months of follow-up: hematologic tests carried out and their results (NT=not tested).

Parameter	*14.04.98*	*21.05.98*	*29.10.98*	*11.11.98*
Hb (g/dL)	8.3	8.4	10.8	10.8
MCV (fL)	82	100	117	120
Erythroblasts (%)	2	12	8	NT
Reticulocytes (%)	6.5	7.5	2.5	NT
WBC(x10e^9/L)	9.7	8.7	6.4	7.6
HbS (%)	90	89	78	77
HbF (%)	2	3.3	15	16
HbA_2 (%)	3.4	3.5	3.2	3.2
HbA_1 (%)	4.5	4.2	3.8	3.8
LDH (IU/L)	1100	872	633	725
Bil.Tot. (mg/dL)	4.98	3.83	2.03	2.09
Bil.Dir. (mg/dL)	0.73	0.62	0.51	0.48
ALT (IU/L)	36	27	28	43
AST (IU/L)	19	19	29	47
Spermatazoa (x10e^6/mL)	88	92	0 (azoospermia)	0 (azoospermia)

Table 2. Follow-up after discontinuation of hydroxyurea therapy (NT=not tested).

Parameter	*01.99*	*02.99*	*06.99*	*09.99*	*12.99*
Hb (g/dL)	9.6	H	H	9.4	H
MCV (fL)	97.3			92	
Erythroblasts (%)	2			NT	
Reticulocytes (%)	10			NT	
WBC(x10g^9/L)	16.6			14.6	
HbS (%)	83.4			90	
HbF (%)	9.1			2	
HbA_2 (%)	3.1			3.6	
HbA_1 (%)	4.4			4.4	
LDH (UI/L)	1190			1320	
Bil.Tot. (mg/DL)	3.26			6.35	
Bil.Dir. (mg/dL)	1.02			1.1	
GGT (IU/L)	71			65	
ALT (IU/L)	35			44	
AST (IU/L)	26			28	
Spermatozoa (x10g^6/mL)	NT			35	

H: Hospitalization because of painful and haemolytic crisis: blood transfusion and analgesic therapy.

Therapy was started in April, 1998. During the seven months of HU therapy the patient did not suffer any crises and his general health was much better. Unfortunately, after 6 months of treatment, we discovered azoospermia: so, with the patient's agreement, we discontinued the HU in November, 1998 (Table 1).

In February, June and December 1999 the patient suffered painful crises that required admission to hospital, blood transfusion and analgesic therapy. Despite our frequent requests, the patient refused to have another spermogram until September 1999: this test showed reduced number of spermatozoa (35x10^6/mL against 88 x10^6 in April 1998), reduced motility on emission (40% against 75%) and after 2 hours (30% against 65%), and reduced posterior-anterior motility (40% against 70%). Table 2 shows some tests the patient underwent after the suspension of HU and his recurrent crises.

Azoospermia and/or oligospermia is a well-known complication of SCD in untreated patients.[3] However many patients have been able to produce offspring both with the improvement of clinical care and the use of HU.[4] In previous studies different side-effects of HU have been pointed out,[5] but attention has never been focused on possible side-effects on spermatogenesis.[6] We suggest that the reversible azoospermia of our patient might be connected with HU therapy,[7] even though we cannot exclude other concomitant events or an idiosyncratic reaction to the drug. For this reason further studies are required to establish the role of HU in azoospermia and to confirm our observation. Consequently we suggest that patients treated with HU should be informed of this possibility and that they should be given the necessary clinical follow-up investigations.

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Key words

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Further evidence on the underestimation of the prevalence of TT viral DNA in blood donors

We reassessed the prevalence of TT viral DNA in Italian blood donors by using primers derived from a highly conserved non-coding region of the viral genome. Our previous underestimation of the prevalence of TTV is proved by the new figure obtained: 85% vs 5%. Again, we observed no difference with respect to a group of potential donors with elevated alanine aminotransferase levels.

Sir,

We recently reported a 5% prevalence of TTV DNA in Italian blood donors. This prevalence was not significantly different with respect to that in a group of potential donors with elevated serum alanine aminotransferase (ALT) levels.[1] At the time we started our investigation, data reported from all over the world were showing not only TTV infection to be widespread in the normal population but also to have a geographical distribution. In fact, by using primers derived from the N22 clone, a wide range of TTV prevalence had been observed,[2-8] as shown in Table 1. This region of the viral genome was being widely employed since it allowed phylogenetic analysis to be carried out. Such analysis had revealed a marked genome heterogeneity of the virus and the existence of several TTV types and subtypes. In our study we used two sets of semi-nested primers recognizing an internal region of the N22 clone and observed, by sequencing the amplified products, a comparable degree of sequence variability. On this basis, we felt that the portion of the viral genome corresponding to clone N22 was not suitable for detecting all variants of the virus and that therefore factors other than geographical differences were contributing to the wide range of TTV prevalence worldwide, especially to the discrepancies in TTV frequency observed within the same country, as in the case of Thailand[7,8] and Italy.[1,2] With respect to Japanese blood donors, Takahashi *et al.* had already pointed out the importance of using an appropriate set of conserved primers. In fact, when they selected a pair of primers (T801/T935) that specifically amplifies a portion of a non-coding region of the viral genome spanning nucleotides 6-204 of the prototype isolate TA278, they detected TTV DNA in 92% of healthy adults[9] as opposed to 12% determined earlier for blood donors in Japan.[3] More recently, Leary *et al.* confirmed, by using three novel sets of nested primers amplifying highly conserved non-coding regions, that TTV infection in the human population has so far been underestimated.[10] These findings prompted us to re-evaluate the prevalence of TTV DNA in the same 500 Italian blood donors by using the set of nested primers proved by Leary *et al.* to be the most efficient one. The region amplified is comprised between nt 3087 and nt 3392 of the TA278 sequence. The overall prevalence resulted to be 85±3% (425/500) with all the previously positive samples confirmed as positive. No statistically significant difference was observed with respect to the prevalence in the 95 potential donors with elevated ALT levels (79±8%). The specificity of the amplification was confirmed by sequencing the polymerase chain reaction (PCR) products and by comparing the sequences with those stored in databanks. Although the role of TTV in human liver disease is still unclear, our results once again point to the dubiousness of TTV having a role in unexplained hepatitis. The dramatic reversal of the previously estimated TTV prevalence in blood donors as shown here and by other groups confirms that caution should be used whenever employing PCR to study the epidemiology of viral infections, the choice of primers, in terms of specificity and sensitivity, being of crucial importance.

Table 1. Distribution and prevalence of TTV in blood donors as assessed by using primers NG059, NG063, and NG061 derived from the N22 clone.

Country	*Prevalence*	*Reference*
Italy	5%	1
Italy	22%	2
Japan	12%	3
USA	1%	4
UK	4%	5
Germany	14%	6
Thailand	36%	7
Thailand	7%	8

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