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Existence of a hypercoagulability state prior to prosthetic hip or knee surgery

Sir,

we compared the values of D-D, TAT, and F1+2 before hip or knee arthroplasty in 79 elderly patients with those of 33 age-matched control subjects. The levels of D-D and TAT were significantly higher in patients than in controls. This hypercoagulability state may be attributed to the ostheoarticular disease and supports the appropriateness of starting antithrombotic prophylaxis prior to surgery.

Patients subjected to substitutive surgery of the hip or knee are a group acknowledged to be at increased risk for venous thromboembolic disease (VTED). It has been suggested that the condition of orthopedic patient is one of the most important risk factors; the papers by Francis *et al.*[1] concerning hip arthroplasty, and those of others investigators on knee prosthesis[2,3] support this affirmation. The hypothesis is that a presurgical state of hypercoagulability could be detected by appropriate tests.

The aim of this work was to evaluate the levels of several hypercoagulative markers in patients with hip or knee pathology before surgery.

We studied 79 consecutive elderly patients admitted for total hip or knee replacement. The control group consisted of 33 similarly aged healthy subjects. In both groups the levels of the following markers were studied (before surgery in the experimental group): D-D, with the ELISA VIDAS D-DIMER® kit (BioMerieux, Marcy-L'Etoile, France), with normal reference levels between 68-494 ng/mL. TAT and F 1+2 were assayed by ELISA with the kit Enzignost® micro (Behringwerke AG, Marburg, Germany); normal reference levels being 1.0-4.1 mg/L and 0.4-1.1 nmol/L for TAT and F1+2, respectively. The characteristics of all groups are summarized in the Table 1. Figure 1 shows the box-plots for the three markers in the study groups. The original values of D-D have been transformed into their "Ln"(equal to "n log") for graphics and statistical analysis because of their asymmetrical distribution and the fact that the range of values was extremely wide. The Anova test was performed on this variable for comparison between groups. The levels of D-D were significantly higher in patients with hip pathology than in controls ($F = 4.58$; $p=0.012$), but not in patients with knee pathology. The Kruskall-Wallis test was used for comparison of the variables TAT and F 1+2. Significant differences were found between the three groups for TAT levels ($\chi^2 = 9.12$; $p= 0.001$), with higher values in the patients with hip or knee disease than in the controls; F1+2 did not differ between the groups ($\chi^2 = 4.1$; $p=0.12$). We want to emphasize that TAT and D-D plasma levels in the patient groups were not only higher than the average levels specified by the manufacturers of the kits but also higher than those of the healthy subjects of similar age. However, our control group also showed levels of D-D and F1+2 superior to the average for the kit, possibly due to their advanced age.[4-6] The selection of elderly control subjects was, of course, intentional in order to have a population equivalent to the patient groups.

The predictive value for VTED of some hypercoagulative markers has been reported in abdominal[7,8] and in hip surgery,[9,10] though it was not an objective of this work, because systematic screening for VTED with confirmatory tests was not performed.

The potential induction of an hypercoagulability state by the same ostheoarticular process present in the orthopedic patient, counsels the appropriateness of starting antithrombotic prophylaxis previously to arthroplasty.

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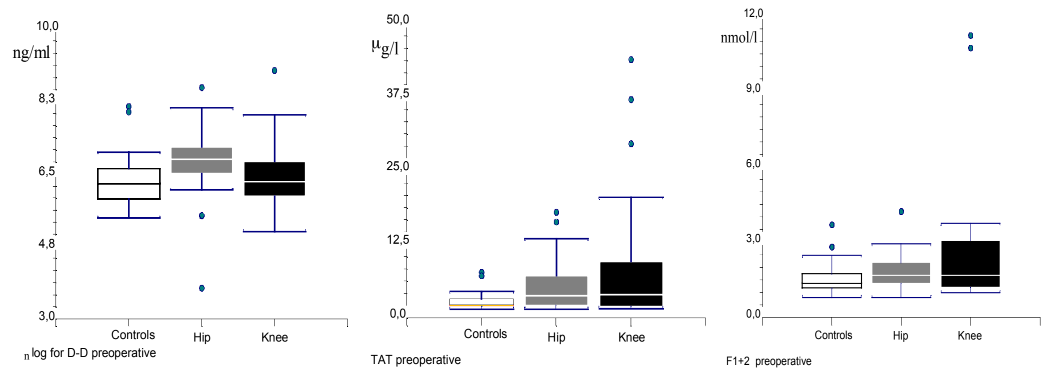

Figure 1. The central line of the box is the median (50% percentile); the lower level represents the 25% percentile; the higher limit represents the 75% percentile; the line over the box represents the value of the 75% percentile plus 1.5 times itself; the line below the box represents the 25% percentile minus 1.5 times itself; the dots represent outlying data.

Table 1. Baseline demographic characteristics and mean values for the three hypercoagulative markers in the patients and in the control subjects.

Variable	*Hip*	*Knee*	*Controls*
Number of cases	53	26	33
Age (years)			
Mean	64	67	68
Percentiles 25-75%	60-73	64-70	62-73
Sex			
Male	27	3	17
Female	26	23	16
Indication for surgery *(more than one diagnosis could be present in the same patient)			
Osteoarthrosis	39	23	–
Necrosis	10	1	–
Rheumatoid arthritis	7	2	–
Miscellaneous	2	2	–
Markers			
Mean			
D-D (ng/mL)	1,135.5	847.4	727.5
TAT (µg/L)	5.3	9.0	2.8
F1+2 (nmol/L)	1.7	2.6	1.5
Percentiles 25-75%			
D-D (ng/mL)	665.14-1,339.43	403.42-992.27	365.03-812.4
TAT (µg/L)	2.1-7.6	2.05-9.8	2-2.9
F1+2 (nmol/L)	1.4-2.1	1.3-3.0	1.2-1.7

Keys words

Hypercoagulability, hip and knee arthroplasty, venous thromboembolic disease

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Cryptic insertion (15;17) in a case of acute promyelocytic leukemia detected by fluorescence *in situ* hybridization

Sir,

We report the case of a patient with acute promyelocytic leukemia (APL) with no detectable cytogenetic abnormalities. Fluorescence *in situ* hybridization (FISH) studies demonstrated an insertion of the RARα gene into one copy of chromosome 15. RT-PCR studies showed a PML/RARα transcript. The patient achieved complete remission with chemotherapy and ATRA, but relapsed during maintenance therapy with ATRA.

Acute promyelocytic leukemia (APL) is characteristically associated with the reciprocal chromosomal translocation t(15;17)(q22;q21) which is identified in up to 90% of cases by conventional cytogenetics. However, a few cases with submicroscopic rearrangements of RARα gene have been described.[1]

A 27-year-old man was admitted to our hospital because of a one-week history of weakness and fever. Blood cell count showed: Hb 79 g/L; WBC 45×10^9/L with 79% hypergranular blast cells and platelets 39×10^9/L. The bone marrow findings were consistent with classical APL (AML-M3) according to the FAB criteria. The immunophenotype showed: $CD13^+$, $CD33^+$, $HLA\text{-}DR^-$ and $CD34^-$. He was treated according to the European APL/93 protocol (ATRA in combination with cytosine arabinoside and daunorubicin) and achieved a complete remission on day 30 of treatment. The patient relapsed, 20 months after diagnosis, during maintenance therapy with ATRA. A second remission was obtained with Ara-C, mitoxantrone and etoposide. Afterwards, he received an allogeneic peripheral blood stem cell transplantation (PBSCT) from his HLA-identical sister. The patient developed a veno-occlusive disease and acute graft-versus-host dis-

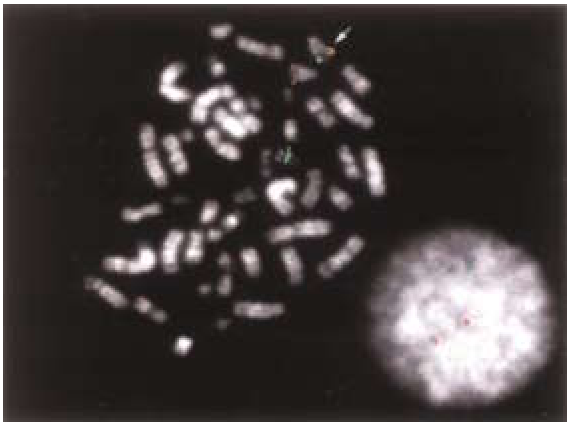

Figure 1. FISH study with specific PML (red)-RARα (green) probe. The PML-RARα fusion is the result of the interstitial insertion of RARα gene into PML gene on chromosome 15 (arrow).

ease and died on day 40 after PBSCT.

Cytogenetics: at the time of diagnosis and relapse, bone marrow samples were cultured for 48 hours according to standard procedures. A normal karyotype was observed in the 20 metaphases examined.

FISH: two-color FISH was performed using painting probes for whole chromosomes 15 (Cambio, Cambridge, UK) and 17 (Oncor, Gaithersburg, MD, USA) and revealed two intact copies of both chromosomes in the 35 metaphases analyzed. An APL t(15;17) translocation probe (Vysis, Stuttgart, Germany) demonstrated the presence of the PML/RARα fusion gene on one copy of chromosome 15 (Figure 1).

RT-PCR: in vitro reverse transcription (RT) of 1 μg of total RNA to cDNA and RT-PCR amplification of PML/RARα and RARα/PML fusion transcripts were performed using standard methods (GeneAmp RNA PCR kit; Perkins Elmer-Cetus, Norwalk, CT, VSA). A PML/RARα transcript of the bcr-1 type (DNA fragment of 326 bp) was observed, however the reciprocal RARα/PML transcript failed to be amplified.

This report describes an interstitial insertion of RARα gene from chromosome 17 into the PML gene on chromosome 15 in an APL patient with an apparently normal karyotype. The cryptic PML/RARα rearrangement was detected by FISH with an APL t(15;17) probe and was confirmed by RT-PCR, which showed the presence of a hybrid PML/RARα transcript but not of the reciprocal RARα/PML transcript. A number of variant translocations associated with APL including submicroscopic translocations have been described.[2-7] However, the characterization by FISH of cases with cryptic PML/RARα rearrangements in apparently normal chromosomes 15 and 17 is unusual.[6-8] The presence of the PML/RARα fusion gene determines the sensitivity to ATRA treatment, while the cytogenetic variants of APL not leading to a PML/RARα fusion, for instance t(11;17) and t(5;17), fail to respond to ATRA.[9,10] Although the molecular consequences of this interstitial insertion are apparently identical to those observed in the classic RARα rearrangement, the molecular mechanisms should be different since another chromosome break distal to RARα has been produced to allow the insertion. Whether or not this different molecular mechanism implies a different clinical course and an unfavorable prognostic factor which could be related to the relapse of the patient during maintenance therapy with ATRA needs to be clarified.

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Key words

Acute promyelocytic leukemia, insertion (15;17), cytogenetics, FISH.

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C→T mutation at -158 $^{G}\gamma$ HPFH associated with 4 bp deletion (–225–222) in the promoter region of the $^{A}\gamma$ gene in homozygous β^0 39 nonsense thalassemia

Sir,

Two Caucasian brothers from Central Spain were found to have homozygous β^0 thalassemia with mild anemia and mild physical stigmata of thalassemia. Molecular studies revealed that both subjects were homozygotes for the nonsense mutation of codon 39 (C→T), and heterozygotes for the C→T mutation at position –158 to the $^{G}\gamma$ gene [*Xmn* I-γ (+)] and for the 4 bp deletion (–225–222) in the promoter of the $^{A}\gamma$ gene.

β-thalassemias are a heterogenous group of genetic alterations characterized by a deficient synthesis (β+) or an absence (β^0) of β globin chains. The clinical expression of this disease can range from asymptomatic cases in most heterozygote forms of β-thalassemia (thalassemia minor) to severe forms of the disease (thalassemia major) in which the patients, usually homozygotes or double heterozygotes, are transfusion dependent. However, between these two extreme clinical forms there are a wide range of clinical phenotypes.[1]

We have studied two Caucasian brothers, 26 (II_1) and 31 (II_2) years old, from Central Spain. Physical examination revealed normal body structure with a splenomegaly of 5 cm in II_1 and 6 cm in II_2, and mild signs of thalassemic facies and conjunctival jaundice in both. Their father (I_1) and mother (I_2) were not related but both had thalassemia minor. The subjects had a more severe phenotypic expression than their parents with mild anemia (Table 1).

Both subjects were homozygotes for the nonsense mutation of codon 39 (C→T) and their parents were heterozygotes for this mutation (Figure 1). This mutation produces a lack of expression of the β gene (β^0) and has been reported to be responsible for thalassemia major.[2] The existence of α-thalassemia, which would have produced a less pronounced phenotypic expression of the disease,[3] was ruled out by Southern blot analysis with *Bam* HI, *Bgl* II, *Hph* I, *Nco* I and *Eco* RI restriction enzymes and α and ζ probes.

In the last decade some forms of non HPHF-deletion, which can "improve" the expression of the disease, have been described. These forms are due to point mutations of one base upstream of the $^{G}\gamma$ or $^{A}\gamma$ gene. Most of these mutations are associated with levels of HbF from 5 to 25% in heterozygotes and levels of HbF greater than 5% when associated with heterozygote β-thalassemia.[4] In the two cases reported here the parents are carriers of heterozygous β-thalassemia and the levels of HbF are lower than 3% in both (Table 1). On the other hand, the substitution C→T at position –158 of the $^{G}\gamma$ gene [*Xmn* I-γ (+)] is associated with increases in HbF in situations of severe anemia and stress erythropoiesis (homozygote SS, homozygote or double heterozygote β-thalassemia) which would result in a decrease in the clinical severity of these situations.[5-7] However, these *Xmn* I-γ (+) are not associated with a significative increase in HbF in normal individuals or heterozygote β-thalassemias.[7] The molecular studies revealed that the mother and the two brothers had the C→T mutation at position –158 to the $^{G}\gamma$ gene [*Xmn* I-γ (+)] in the heterozygote form (Figure 1). This finding could explain the clinical picture of the disease, with a mild anemia of 10.5 to 11.5 g/dL of HbF and a $^{G}\gamma/^{A}\gamma$ ratio of 2:1, higher than the expected 2:3, in the brothers, and a HbF level less than 3% in the mother who has heterozygote β-thalassemia. Other forms of non HPHF-deletion are associated with levels of HbF greater than 5% when associated with heterozygote β-thalassemia.[4] In this context, the presence of another form of non HPHF-deletion associated in this family is not probable.

At the level of the promoter of the gene $^{A}\gamma$ both the brothers and the parents had a 4 bp deletion (–225–222) (Figure 1). This deletion of 4 base pairs is

Table 1. Hematologic values and biochemical studies.

Measurement	*I_1 (father)*	*I_2 (mother)*	*II_1*	*II_2*
RBC × 10^{12}	6.9	6.3	4.5	4.6
PCV (L/L)	41.6	38.1	33.7	31.4
Hb (g/dL)	13.6	12.5	11.3	10.6
MCV (fL)	59.2	61	75	67.6
MCH (pg)	19.6	20	25.5	22.8
MCHC (g/L)	33.1	32.8	33.6	33.8
RDW (%)	15.5	14.9	26.1	26
Reticulocytes (‰)	6.2	5.9	7.4	8.3
IMR: (MFR+HFR)×100/LFR	3.5	6.9	13.4	10.7
Hb A_2 (%)	5	5.2	2.5	3.1
Hb F (%)	2.9	2.3	97.5	96.9
$^{G}\gamma/^{A}\gamma$	-	-	2/1	2/1
LDH (U/L)	-	-	226	186
Total bilirubin (mg/dL)	-	-	4.8	3.40
Serum iron (g/dL)	-	-	171	108
TIBC (g/dL)	-	-	193	184
Ferritin (ng/mL)	-	-	368.2	454.8

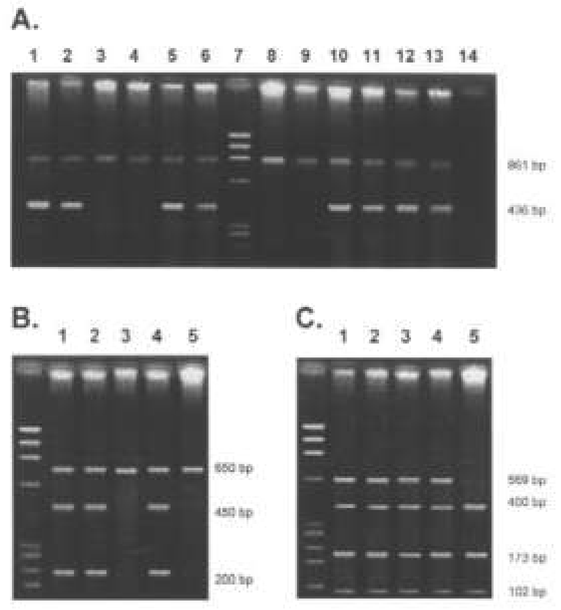

Figure 1.
A. Ethidium bromide-stained gel illustrating the products obtained after amplification using primers specific for detection by PCR-ARMS of the point mutation CD39 (C→T) (436 bp). The first six lanes correspond to the normal assay. Lane 1 and 2 are heterozygote controls, Lanes 3 and 4 represent the propositus II$_1$ and II$_2$ respectively. Lanes 5 represent the father (I$_1$) lane 6 the mother (I$_2$), respectively. Lane 7 is the DNA Marker X174 *Hae* III. Lines 8 to 13 correspond with the mutate assay; lane 8 and 9 heterozygote controls, lanes 10 and 11 represent the propositus II$_1$ and II$_2$, respectively. Lane 12 represent the father (I$_1$) and line 13 the mother (I$_2$). Lane 14 is the H_2O control. The internal control corresponds to the 861 bp fragment.

B. Ethidium bromide-stained gel illustrating the products obtained after amplification using specific primers and digestion with *Xmn* I for the polymorphic variation of C→T in the 158 bp 5' of the Cap site of the $^G\gamma$ gene. Lane 1 represents propositus II$_1$ [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 2 represents propositus II$_2$ [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 3 represents the father (I$_1$) (*Xmn* I– (–) haplotypes) with a 650 bp fragment, lane 4 the mother (I$_2$) [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 5 represents a negative control with a 650 bp fragment. DNA Marker X174 *Hae* III.

C. Agarose gel electrophoresis of Fnu 4HI-digested A promoter DNA amplified by PCR. Lanes 1 and 2 represent the propositus II$_1$ and II$_2$ respectively with 569, 400, 173 and 102 bp fragments (heterozygotes for 4 bp deletion). Lanes 3 and 4 represent the father (I$_1$) and mother (I$_2$) respectively with 569, 400, 173 and 102 bp fragments (heterozygotes for 4 bp deletion). Lane 5 is a normal control DNA with 400, 173 and 102 bp fragments. DNA Marker X174 *Hae* III.

closely associated in *cis* with haplotype II in cases of β-thalassemia with the mutation of codon 39, and has been reported to cause decreased expression of $^A\gamma$ when it is associated in *trans* with haplotype I and IX β^0 39.[8] It has not been well determined whether this decrease in $^A\gamma$ expression can affect expression of the gene $^G\gamma$ in *cis* or in *trans*.[9,10] This way, the presence of the deletion of 4 base pairs from 225 to 222 in the promoter region of the $^A\gamma$ gene, in our two cases (II$_1$ and II$_2$), would favor the expression of $^G\gamma$[10] which would already be augmented by the presence of *Xmn* I-γ (+).

Although the existence of other related genetic factors which produce an increase of HbF in the *Xmn* I-γ (+) cannot be ruled out, the C→T substitution at position –158 to the $^G\gamma$ gene is in a region which contains sequences which are important in regulation of γ gene expression[5] and probably, in addition to being a genetic marker, is responsible for most of the $^G\gamma$ synthesis in these *Xmn* I-γ haplotypes. It is, therefore, important to study this factor in patients with β-thalassemia because of the prognostic implications of this disease.

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Key words

–158 $^G\gamma$ HPFH, homozygous $\beta^0$39 thalassemia, $^A\gamma$ gene, thalassemia intermedia

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In vivo effect of chloroquine on platelet aggregation in anesthetized rats

Sir,

In vivo platelet aggregation was studied by a platelet count ratio (PCR) technique. Following the intravenous administration of collagen or ADP to rats the mean PCR was lower in controls than in two groups administered graded doses of chloroquine ($p<0.05$ and 0.01 respectively). Chloroquine inhibits platelet aggregation *in vivo* in rats.

Previous reports on the effect of chloroquine on platelet aggregation were based on *in vitro* and *ex vivo* studies where aggregation inducers and chloroquine were added to isolated platelets, or aggregation inducers added to platelets withdrawn from chloroquine-treated human volunteers.[1-3] Since not all the factors that affect aggregation *in vivo* may be available *in vitro* or *ex vivo*, the effect of chloroquine on platelet aggregation *in vivo* has been examined.

Rats were randomly assigned into a control or two test groups (n=6). The control group was administered 0.9% NaCl (1 mL/kg, ip). The first test group was given ADP at a dose of 8.6 mg/kg, ip[1] while the second test group was administered a higher dose of chloroquine (40 mg/kg, ip). After one hour, collagen (1 mg/kg, iv) was administered under urethane anesthesia (1.5 g/kg, ip) to all groups to induce platelet aggregation *in vivo*.

Blood (1 mL/rat) was taken by cardiac puncture for estimation of platelet aggregation.This was measured by a PCR technique[4] in which a lowering of the count ratio signifies an increase in platelet aggregation and vice versa. These experiments were repeated using another aggregation inducer, ADP (90 µg/kg, iv) and normal saline (1 mL/kg, iv). The doses of ADP and collagen were slightly higher than those reported for rabbits[5] since preliminary studies showed that lower doses were ineffective. Serum chloroquine concentration was estimated by the method of Prauty and Kuroda.[6]

Mean serum chloroquine concentrations one hour after administration were 5.06±1.29 mg/L and 10.98 ±3.75mg/L (mean±SD; $p<0.01$) in rats administered chloroquine at doses of 8.6 mg and 40 mg/kg respectively (n=5).

In the rats given i.v. collagen, the PCR were 0.283±0.165, 0.560± 0.175 and 0.694±0.193 in the control, first and second test groups respectively. The ratios for the two test groups were significantly higher ($p<0.05$ and 0.01) than that of the control group. Results after ADP were similar. Platelet count ratios following the infusion of normal saline were 0.818± 0.094; 0.830±0.073 and 0.876±0.070 for control, first and second test groups respectively. The ratios obtained with saline were not significantly different between the three groups (Figure 1).

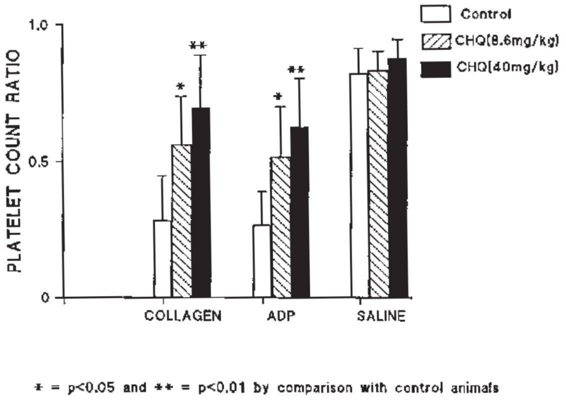

Figure 1. Platelet count ratio in rats administered collagen, adenosine diphosphate (ADP) or normal saline (iv) following ip administration of normal saline (1 mL/kg) to control rats (n=6) and chloroquine diphosphate to test rats at either 8.6 mg/kg (n=6) or 40 mg/kg (n=6). Values are represented as means±SD.

Based on *in vitro* and *ex vivo* studies some investigators have concluded that therapeutic concentrations of chloroquine have a negligible effect on platelet aggregation and are not a significant risk to patients with compromized hemostasis.[1] However, *in vitro* and *ex vivo* studies may not reflect *in vivo* events since some endogenous aggregation inducers and inhibitors from non platelet sources may be reduced or unavailable.

We have shown that a therapeutic dose of chloroquine inhibits platelet aggregation *in vivo* in rats and so, its use in patients with compromized hemostasis could be risky if the results are confirmed in humans. Conversely, chloroquine administration could be beneficial in the reduction of hyperaggregability of platelets in malaria[7,8] and in the prevention of thrombosis.

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Key words
Chloroquine, platelets, in vivo aggregation.

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Successful treatment of AA amyloidosis secondary to Hodgkin's disease with 4'-iodo-4'-deoxydoxorubicin

Sir,

A case of AA amyloidosis secondary to Hodgkin's disease is reported. After complete remission of the lymphoma, treatment with the drug 4'-iodo-4'-deoxydoxorubicin resulted in an improvement of the nephrotic syndrome and removal of amyloid from liver tissue. The drug could be a therapeutic option for secondary amyloidosis.

Secondary (AA) amyloidosis is known to be associated with a variety of diseases in which inflammation is a common feature.[1] Apart from control of underlying disease, currently there are no treatments able to remove amyloid from involved tissues. Preliminary reports on the use of the drug 4-iodo-4'-deoxydoxorubicin in primary (AL) amyloidosis seem encouraging.[2] We report here a case of AA amyloidosis secondary to Hodgkin's disease in which treatment with 4'-iodo-4-deoxydoxorubicin resulted in substantial improvement of clinical status and removal of fibrils as assessed by liver biopsy.

The patient was a 37-year-old male whose complaints were fatigue and significant maleolar edema. An abdominal ultrasound showed enlarged retroperitoneal lymph nodes and after biopsy, a diagnosis of Hodgkin's disease was made. From the blood analysis severe hypoproteinemia (4.2 g/dL), hypoalbuminemia (1.1 g/dL) and increased alkaline phosphatase (1163 U/L) were found as well as proteinuria (12 g/L). During pathologic staging, amyloid deposition was found in hepatic sinusoids (Figure 1). Immunohistochemical staining confirmed amyloid AA deposition. After complete staging the definitive diagnosis was mixed cellularity Hodgkin's disease stage II A with secondary amyloidosis. A renal biopsy was not performed due to an increased risk of bleeding; the nephrotic syndrome was attributed to amyloidosis.

After six cycles of COPP/ABV chemotherapy, a complete remission was achieved, assessed by computerized tomography. Nevertheless, proteinuria, hypoalbuminemia and edema persisted, probably due to renal deposition of amyloid. A repeat liver biopsy showed similar findings to those at diagnosis, with the same amount of amyloid deposition. Four months after complete remission, biochemical parameters and edema remained at similar levels.

At that point, we started treatment with 4-iodo-4'-deoxydoxorubicin in an attempt to improve the patien-

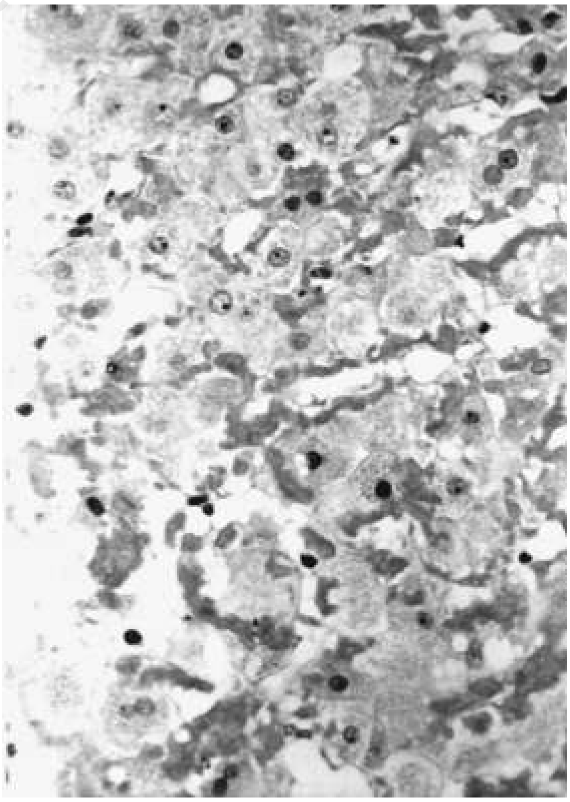

Figure 1. Liver biopsy showing extracellular amyloid deposition (Congo red, ×600).

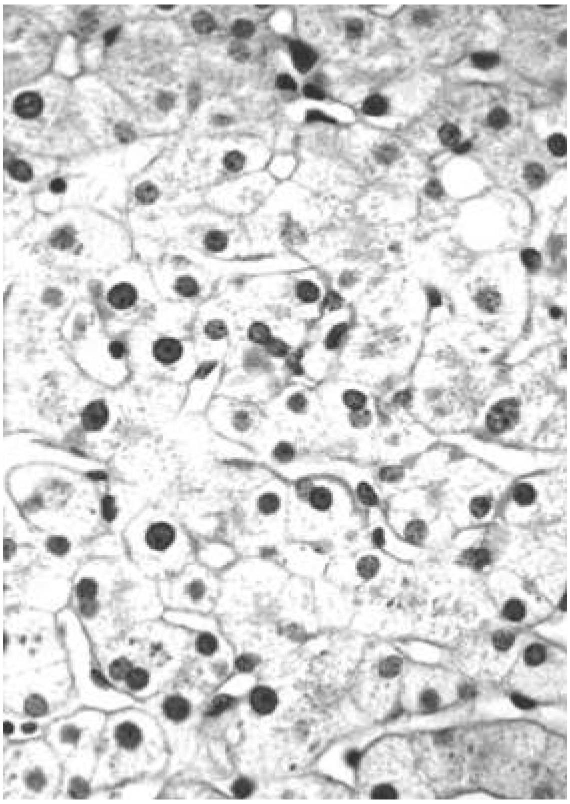

Figure 2. Liver biopsy; substantial removal of amyloid after treatment (PAS, ×600).

t's situation. Two weeks later, after four cycles of weekly administration at a dose of 30 mg/m^2, a new evaluation was performed. Increased albuminemia (2.5 g/dL) and proteinemia (4.8 g/dL), decreased alkaline phosphatase (711 U/L) and decreased proteinuria (5 g/L) were found. Fatigue and edema disappeared and a new liver biopsy showed substantial decrease in amyloid deposits (Figure 2). After one year of follow-up, the patient's status is similar, with hypoalbuminemia and proteinuria at levels comparable to those achieved at the end of therapy and no drug-related toxicity.

Initial reports of *in vitro* binding to amyloid fibrils[3] led to clinical studies[2] that suggest that 4-iodo-4'-deoxydoxorubicin might achieve not only blockage of amyloid deposition but also removal of fibrils from the extracellular matrix. The drug has been successfully used for the treatment of AL amyloidosis but to date, there are no reports of its use in AA amyloidosis.

The possibility of improvement after resolution of underlying Hodgkin's disease cannot be completely ruled out,[4] but the evolution of biological parameters was not uniform. No improvement was achieved four months after complete remission of the lymphoma, but proteinuria and edema dramatically changed after four cycles of therapy with 4-iodo-4'-deoxydoxorubicin. Thus, it is reasonable to think that the drug is responsible for partial resolution of the disease. In our opinion, use of this drug for the treatment of AA amyloidoses, as well as AL amyloidosis, should also be investigated.

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Key words

Amyloidosis AA, 4'-iodo-4'-deoxydoxorrubicin, Hodgkin's disease.

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Hepatitis C virus infection and mixed cryoglobulinemia in patients with lymphoproliferative diseases

Sir,

In the last few years hepatitis-C virus (HCV) has been implicated in the pathogenesis of diverse processes originating from B-clonal lymphoid proliferation, such as mixed cryoglobulinemia (MC) and B-cell non-Hodgkin's lymphomas (NHL).[1,2] However, other studies carried out in other geographic areas have not confimed these observations.[3] We, therefore, analyzed 95 patients affected by B-cell lymphoproliferative diseases (B-LPD), seen from October 1991 to December 1995 at the Hematology Department of the University Hospital of Zaragoza, Spain.

B-LPD was diagnosed on the basis of morphologic and immunologic evaluation of lymph nodes, bone marrow or peripheral blood specimens. All the processes were classified according the REAL classification.[4] Detection and characterization of cryoglobulins were performed according to previously described methods.[5] Antibodies (Ab) to HCV were

Table 1. B-LPD subgroup, prevalence of MC, Ab to HCV and HCV-RNA.

Diagnosis	*Patients (%)*	*Patients with MC and the type*	*Patients MC+/HCV+*		*Patients MC-/HCV+*	*Patients with HCV-RNA*
Chronic lymphocytic leukemia	21(22.1)	3 (1 tpe II, 2 type III)	2		–	1
Hairy cell leukemia	3 (3.1)	–	–		–	–
Prolymphocytic leukemia	1 (1.05)	–	–		–	–
Multiple myeloma	18 (18.9)	7 type I	–		4	2
Lymphoplasmacytoid lymphoma/ immunocytoma	9 (9.4)	4 (3 type II, 1 type III	2		1	3
Follicle center lymphoma	20 (21)	6 (4 type II, 2 type III)	2		2	4
Marginal zone lymphoma	5 (5.2)	1 type II	1		–	1
Mantle cell lymphoma	3 (3.1)	–	–		1	1
Diffuse large lymphoma	10 (10.5)	1 type II	1		–	1
B-lymphoblastic lymphoma	3 (3.1)	–	–		–	–
Burkitt's lymphoma	22 (2.1)	–	–		–	–
TOTAL	95	15/95 (10 type II, 5 type III) (15.7%)	8/15 (53.3)	p<0.01 (Fisher)	8/80 (10%)	13/16 (81.2%
				16/95 (16.8%)		

B-LPD: B-cell lymphoproliferative diseases; MC: mixed cryoglobulinemia. Ab: antibodies; HCV: hepatitits C virus.

detected with a third generation ELISA test; positive samples were tested in duplicate and, if reactive, confirmed by a second generation RIBA test. The HCV positive samples were tested for RNA-HCV with a RT-PCR method. Thirty healthy blood donors were analyzed as a control group using the same tests as those for the patients. We considered the prevalence of HCV infection obtained in the largest study performed in our area (1.32%).[6]

Ninety-five patients (mean age, 62±12 years) were included in the study; 62 (65.2%) were male and 33 (34.8%) female. All patients were HIV negative. All the tests performed in the control group were negative.The prevalence of MC and HCV infection was 15.7% and 16.8 % respectively. The diagnosis, the classification of patients's disease and the presence of MC and virological findings are shown in Table 1. We noted a high prevalence of MC and HCV infection in the patients diagnosed as having immunocytoma. In the patients with multiple myeloma we detected a high prevalence of HCV infection not associated with MC but a high prevalence of cryoglobulinemia type I (not HCV-induced) was observed in this group. We also found that, of the patients with MC, 8 (53.3%) were HCV-positive , whereas in the group without MC 8 patients (10%) were HCV-positive (p<0.001).

We found a high prevalence of MC associated with HCV infection in patients with immunocytoma. These data support the findings described by Silvestri and others[1,2] about the pathogenic role of HCV in the development of some B-LPD. HCV lymphotropism has been shown by Zignego *et al.*[7] in peripheral blood mononuclear cells and, recently, by Muratori *et al*.[8] using *in situ* RT-PCR and by Sansonno *et al.*[9] who found viral proteins in the cytoplasm of neoplastic lymphoid cells. Based on its lymphoproliferative characteristics, HCV has been proposed to have an etiologic role in the development of B-LPD; recently Ivanovski *et al.*[10] decribed the presence of cryoprecipitable rheumatoid factor (RF) encoded by the lymphoma-derived V sequences, indicating a role for chronic antigen stimulation by HCV-containing immune complexes in the development of clonal B-cell disorders. These hypotheses need to be validated by further studies.

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