was only 15% with a mean overall survival of 6.8 months.[9]

Coiffier *et al.* reported on 54 patients with relapsed or refractory NHL treated with two different doses of rituximab;[8] 31% achieved a response with the time to treatment progression (TTP) being 8 months. The CR rate was 9% and the PR rate 22%. The limitation of this study is that only half of the patients (30/54) had diffuse large B-cell lymphoma according to the REAL-classification and in 10 patients the disease could not be precisely categorized. Given the heterogeneity of the histologic subtypes, evidence on the efficacy of rituximab in the treatment of refractory or relapsed aggressive NHL could not be considered conclusive. Meanwhile, a Japanese phase-II pharmacokinetic study demonstrated an overall response rate of 37% in patients with relapsed aggressive NHL.

In accordance with the above mentioned studies, in our study 38.1% of patients with refractory or relapsed aggressive NHL responded to rituximab monotherapy. Interestingly, patients who were refractory to prior treatment by the time of inclusion, showed a higher response rate of 43.8%. Rituximab was associated with very little toxicity and symptoms were generally mild. The median EFS of 3.8 months was relatively short and the median overall survival was 8.6 months. It is an open question whether prolonging rituximab treatment up to 8 weeks, as reported by Coiffier *et al.*,[8] may increase EFS.

Our study confirmed the efficacy of rituximab as single agent therapy for aggressive NHL in patients with a poor prognosis who are ineligible for high dose chemotherapy. It is, however, important to mention the high costs of rituximab monotherapy in this setting, taking into account the relatively short EFS. The whole treatment is applicable in an outpatient setting without the toxicity commonly observed with combination chemotherapy regimens. We, therefore, consider that CD20 monoclonal antibody is a well tolerated treatment option for patients with very poor prognosis NHL in a palliative setting.

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Monoclonal Gammopathy

Malignant evolution of monoclonal gammopathy of undetermined significance: analysis of 633 consecutive cases with a long term follow-up

In 633 consecutive patients with monoclonal gammopathy of undetermined significance (MGUS) the probability of malignant evolution was 9, 17 and 51% at 5, 10 and 15 years, respectively, after diagnosis. The values of monoclonal component, Bence-Jones proteinuria and erythrocyte sedimentation rate were associated with malignant transformation.

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Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic disorder characterized by the presence in the serum of a homogeneous monoclonal immunoglobulin (M-component, MC) without any evidence of lymphoproliferative disorders.[1,2] The incidence of MGUS increases with age, from 2, 3 and 4% in patients older than 50, 70 and 80 years, respectively.[3,4] In some cases this condition is stable over the years, while in others it evolves into multiple myeloma (MM or, rarely, into another malignant lymphoproliferative disorder. In the largest study so far published the probability of malignant transformation was 12, 25 and 30% at 10, 20, and 25 years after diagnosis, respectively.[5]

From January 1974 to December 1997, 976 consecutive MGUS patients were followed at our department. Of them, 633 patients (306 males, 327 females, median age 62 years, range 27-87 years) with a minimum follow-up of 1 year were evaluated. The diagnosis of MGUS was made using standard criteria.[2] In each patient, we evaluated the following data: hemoglobin, leukocyte and platelet counts, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), lactate dehydrogenase (LDH), serum protein electrophoresis, quantification of polyclonal and monoclonal immunoglobulins (MC), presence of Bence Jones (BJ) proteinuria, β2-microglobulin, serum creatinine and calcium. In patients with ≤ 5 g/L MC, laboratory tests were monitored every year; in patients with 5-15 g/L MC, laboratory tests were monitored every six months for 3 times and thereafter every year in the case of a stable MC level. Bone marrow aspirate and

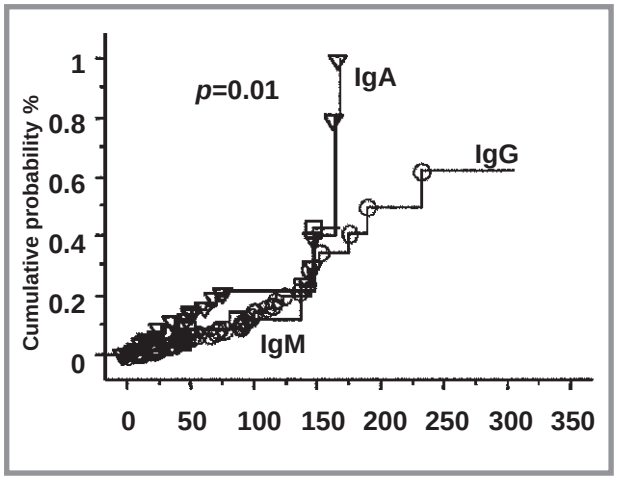

Figure 1. Correlation between the different monoclonal protein isotypes and the progression.

Table 1. Cox regression for evolution into multiple myeloma and related plasma cell disorders.

Factor	*Cox analysis (p value)*
Sex	NS
Age (< 60 vs. > 60 years)	NS
Serum MC IgA-type	0.01
Serum MC concentration (≥ 17 g/L)	< 0.001
Bence Jones proteinuria	< 0.001
Erythrocyte sedimentation rate	< 0.001
Lactate dehydrogenase	NS
C reactive protein	< 0.03
β2-microglobulin	NS
Serum monoclonal k or l light chain	NS

NS: not significant.

skeleton X-rays were performed if a significant increase of MC (>3-5 g/L) was observed during the follow up. Age, sex, amount and type of MC, presence of BJ proteinuria, ERS, LDH, CRP and β2 microglobulin levels at diagnosis were evaluated as predictors of progression. In statistical analysis, data were expressed as median (range) when appropriate and were analyzed by the Mann-Whitney rank sum test. Percentages were compared by χ^2 test with Yates' correction and Fischer's exact text. The cumulative probability of progression was calculated with the Kaplan-Meier function; curves were compared with the log-rank test. The Cox proportional hazards model was used to evaluate the effect on progression of the different variables examined at diagnosis. A p value of < 0.05 was considered statistically significant.

Monoclonal component of IgG class was documented in 437 cases (69.0%), while in 113 cases (17.8%) it was IgA type and in 71 cases (11.2%) was IgM type. Biclonal gammopathy was found in 12 cases (1.9%). The median serum MC concentration was 7 g/L (range, 1-25) and κ light chain (57%) was prevalent over λ chain (43%). BJ proteinuria was detected in 118 patients (18.6%) at diagnosis. Erythrocyte sedimentation rate, β2-microglobulin, CRP and LDH at diagnosis were raised in 167 (26.4%), 81 (12.8%), 48 (7.6%) and 35 (5.5%) patients, respectively. After a median follow-up of 67 months (range, 12-310 months), MC remained stable in 551 patients (87.1%), whereas in 82 cases (12.9%) the MGUS evolved into MM (74 cases: IgG-type 49, IgA-type 25) or WM (8 cases: IgM-type). BJ proteinuria was present in 28/82 cases (34.1%), whereas the values of ESR, β2-microglobulin, CRP and LDH were abnormal in 43/82 (52.4%), 14/82 (17.1%), 9/82 (11.0%) and 4/82 (4.9%) cases, respectively.

The cumulative probability of MGUS transformation into MM or WM (Figure 1A) was 9, 17 and 51% at 5, 10 and 15 years, respectively [median 180 months (95% confidence interval, 156-204)]. With Cox regression analysis, we examined different factors (sex, age, type and amount of serum MC, presence of BJ proteinuria, ESR, LDH, CRP, b2-microglobulin levels and type of light chain) with respect to the risk of MGUS progression into malignant disease. Type (IgA) and concentration (≥ 17 g/L) of MC, presence of BJ proteinuria and increased ESR and CRP values were associated with a higher risk of progression to disease (Table 1). Twenty two percent of patients with IgA isotype progressed to malignant transformation, as compared with 11% of patients with IgG and 13% of those with IgM isotypes (Figure 1B). One out of 12 (8.3%) of the patients with biclonal MC progressed to MM. However, in a multivariate analysis, only the presence at diagnosis of Bence Jones proteinuria (p=0.02), serum MC concentration more than 17 g/L ($p < 0.001$) and raised ESR (p=0.01) were independent predictors of progression.

In our study, the cumulative transformation probability reached 51% at 15 years, a percentage which is higher than that reported in other previous studies.[5-7] This is probably because patients with a follow-up shorter than 1 year were excluded from teh evaluation[5,7] and/or because of a longer follow-up time.[6,7] However, even if the probability of progression increases with the duration of follow-up, malignant transformation during the first years after diagnosis is still possible. For this reason, we believe that it is necessary to evaluate, at diagnosis, those factors associated with a higher progression rate in order to follow those patients with poor prognostic indicators more closely. In our study, Bence Jones proteinuria, amount of MC and raised ESR were the most important predictors of progression, confirming the results of other previous studies.[8,9] In our opinion, the use of these simple parameters may be useful for correct managementof a great number of patients affected by MGUS.

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Thrombosis

Homozygosity of the *T* allele of the 46 C→T polymorphism in the *F12* gene is a risk factor for acute coronary artery disease in the Spanish population

Following new guidelines that contain recommendations on the desirable features of a genetic association study, we performed a case-control study to establish the risk of acute coronary artery disease (CAD) related to the polymorphism (46 C→T) in the *F12* gene. We found a 6-fold higher risk of acute CAD associated with the homozygosity of the *T* allele of the *F12*, *46C→T* polymorphism in the Spanish population.

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Acute coronary artery disease (CAD) is complex and results from interactions between environmental and genetic factors.[1,2] Association studies have shown that there is a relation between factor XII levels and the 46 C→T polymorphism in the development of CAD, but the results are controversial.[3-6]

Our association study is unique because it was designed to avoid the usual biases of association studies in regard to establishing a polymorphism as a risk factor. We followed the new guidelines containing recommendations on the desirable features of a genetic association study.[7,8] From the GAIT Project (a family-based study), we demonstrated a high heritability (h^2 = 67%) of factor XII, and we also reported that the structural *F12* gene influenced both susceptibility to thrombosis and plasma levels of factor XII.[9-10] Following these results, we conducted an age-gender-ethnic matched, case-control study of an independent sample of Spanish individuals to assess the risk of acute CAD associated with the 46 C→T polymorphism of the *F12* gene and factor XII levels.

We included 174 patients who were diagnosed as having acute CAD, and 211 control subjects. Patients with acute CAD were admitted to the Cardiology Department of our hospital between 1998 and 2003 at their first episode of acute CAD. Acute CAD was confirmed on the basis of definitive ischemia or necrosis of the myocardium. Control subjects were friends and spouses of patients; they were included only if they had no personal history of thromboembolic disease, including venous and arterial thrombosis, cirrhosis, nephrotic syndrome or active cancer. Patients and controls gave informed consent to participation in the study. A limitation of our study is that it included the survivors of the acute event. Blood samples were obtained from the antecubital vein no earlier than 6 months after the acute episode. Fibrinogen was measured by the Clauss method as described elsewhere. Assays for factor VIII were performed on fresh plasma samples. The remaining plasma samples were stored at –80°C until used. Factor VIIIc and factor XIIc were assayed using deficient plasma from Diagnostica Stago (Asnières, France).[9,10] The 46 C→T variant was determined using the primers described previously , with minor modification in the reaction conditions.[10] For statistical analysis, using ROC curves, we considered factor VIII levels to be elevated if they were higher than 151% and fibrinogen levels elevated if they were higher than 3.5 g/L. For factor XIIc, we used levels lower than the 10th percentile (lower than 68%) as a cut-off. Odds ratios (OR) were calculated as risk for acute CAD adjusted for age and sex and other co-variables by logistic regression. We considered the genotypes C/C and C/T as the reference group. *p* values <0.05 were considered statistically significant.

The basic characteristics of the sample population are given in Table 1. All of the major cardiovascular conventional risk factors, such as hypercholesterolemia, hypertension, and diabetes mellitus, were associated with a signifi-

Table 1. Basic characteristics of patients and controls.

	ACAD (n=174) n (%)	*Controls (n=211) n (%)*	*Unadjusted OR (95% CI) and p values*
Sex (female/male)	54/120	91/120	NS
Age (years, mean, range)	57 (21-78)	57 (26-80)	NS
Smoking	96 (55)	78 (37)	2.1 (1.4-3.2)*
Hypercholesterolemia	92 (53)	44 (21)	4.3 (2.7-6.7)*
Family history of arterial thrombosis	84 (48)	38 (18)	4.3 (2.7-6.7)*
Hypertension	92 (53)	40 (19)	4.8 (3.0-7.5)*
Obesity	19 (11)	7 (3)	3.5 (1.5-8.7)*
Alcohol intake	11 (6)	11 (5)	1.7 (0.5-2.9)
Diabetes mellitus	34 (20)	11 (5)	4.4 (2,2-9.0)*
Factor VIIIc levels, % (mean, range)	186.9 (74-526)	159.4 (48-360)	*p*<0.0001
Fibrinogen, g/L, mean, range)	3.7 (1.9-6.9)	3.5 (2.1-7.8)	*P*=0.05

**Differences are statistically significant (p<0.05).*