those with febrile episodes was 7 per 100 person year (95% confidence interval: 5.0-11.1). Positive cultures were rare and the most frequently isolated bacteria were *E. coli* and pneumococci. Herpes viruses were frequent, but rarely caused severe infections and never death. We found a few severe opportunistic infections (five episodes), usually in patients with multiple risk factors, such as treatments including fludarabine, hypogammaglobulinemia or advanced stage of disease. Associations between risk factors recorded at the time of infection aand the severity of the infection were searched for by univariate analysis (Table 2). In our experience, fludarabine treatment (previous or current) and prophylaxis with acyclovir and cotrimoxazole, even if involved in the pathogenesis of opportunistic infections, were not associated *per se* with development of severe infections.

Similarly, neutropenia was not found to be a significant risk factor, although in a prospective surveillance study more than 50% of the cases of nosocomial bacteremia in CLL patients occurred with neutrophils $<0.1\times10^9$/L.[10] Only patients submitted to more than one regimen of chemotherapy developed a significantly higher proportion of severe infections. This result was also confirmed by multivariate analysis (odds ratio of the same magnitude as that in univariate analysis: OR= 3.25 95% CI: 1.61-6.55, p=0.001). Our result is similar to that recently reported by Hensel *et al.*[9] who described a series of 187 patients, in whom only disease aggressiveness and previous lines of therapy proved to be major risk factors for infections at multivariate analysis.

Until now it has been debatable whether and when antibacterial prophylaxis could be useful in CLL patients, particularly during the neutropenic period. It is a current strategy to administer antibacterial prophylaxis to CLL patients with previous severe and/or relapsing bacterial infections. Even with all the limits of a retrospective study, our data suggest that patients submitted to more than one line of chemotherapy may run the risk of severe infections, indicating the possible utility of antibacterial prophylaxis also in this subset of patients, and not only in severely neutropenic patients or in those previously affected by severe bacterial infections. Prospective studies are warranted to confirm this point.

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Multiple Myeloma

Bone marrow plasma cell microaggregates detected by immunohistology predict earlier relapse in patients with minimal disease after high-dose therapy for myeloma

Plasma cell microaggregates detected by CD138 immunohistology were demonstrated in 22% of patients achieving morphologic remission 3 months after high-dose therapy for myeloma. Microaggregates were predictive of earlier disease progression, indicating that immunohistology may represent a useful tool in the assessment of minimal disease in patients after high-dose therapy for myeloma.

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The treatment of chemoresponsive plasma cell myeloma with high-dose chemotherapy and autologous stem cell replacement achieves complete response (CR) in one-third of patients.[1] CR requires the sustained absence of serum and urine paraprotein for at least 6 weeks, together with less than 5% plasma cells in the bone marrow as assessed by morphologic analysis.[2] Estimation of plasma cell infiltrates in marrow aspirates and hematoxylin and eosin-stained trephine sections can be subjective and dependent on observer expertise.[3] Immunohistological staining of plasma cells using highly specific antibodies (e.g anti-CD138/syndecan-1) enhances the detection of small plasma cell clusters, or *microaggregates* in the bone marrow (Figure 1A). Microaggregates have been defined as focal and contiguous interstitial collections of at least 10 plasma cells in a non-perivascular location.[4]

In a preliminary analysis, interstitial plasma cell microaggregates were detected in 100% of patients with asymptomatic myeloma (with 10-15% plasma cells in the bone marrow; n=21), in 25% with monoclonal gammopathy of undetermined significance (n=32) and in none of the patients with non-malignant plasmacytosis (n=11). As interstitial plasma cell microaggregates were always present in patients with myeloma, we hypothesized that their presence might be a useful indicator of disease after treatment, particularly in patients fulfilling the criteria for complete morphologic remission (<5%

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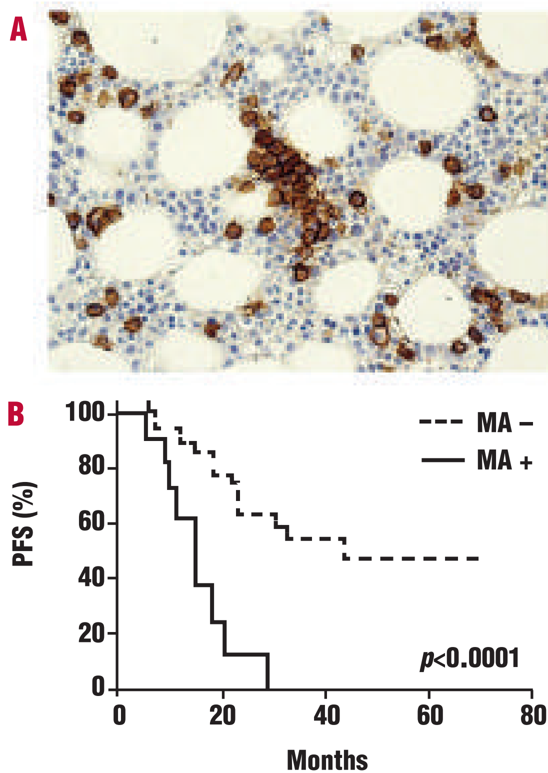

Figure 1. Plasma cell microaggregates are typical in plasma cell dyscrasia. A. Immunohistology with antibody to CD138 showing a plasma cell microaggregate as defined by an interstitial collection of at least 10 contiguous plasma cells. The CD138 antibody stains the surface membrane of plasma cells. Other isolated interstitial plasma cells are also seen (x40 magnification). B. The progression-free survival (PFS) using the method of Kaplan and Meier of patients according to the presence (MA$^+$) or absence (MA-) of plasma cell microaggregates in the bone marrow 3 months after high-dose chemotherapy. All patients analyzed had less than 5% plasma cells by morphology. The curves were statistically compared using the log-rank test.

plasma cells in the bone marrow). The prognostic value of interstitial plasma cell microaggregates in patients with <5% plasma cells in the bone marrow after high-dose therapy (HDT) has not been described.

To determine the clinical significance of microaggregates after high-dose melphalan (140 or 200 mg/m^2) and autologous stem cell re-infusion (at least 2.5×10^6 CD34$^+$ cells/kg) for symptomatic myeloma, 49 patients were identified who had less than 5% plasma cells in the bone marrow 3 months after high-dose chemotherapy; 47% of these patients were also in CR with no detectable paraprotein in the serum or urine. The median age of the patients studied was 57 years (range 33-70), the male:female ratio 3.1:1, M-protein type was IgG (51%), IgA (19%), or light chain (30%; κ:λ 2:1), median lactate dehydrogenase 348 IU/L (range 202-893), median β2-microglobulin at diagnosis 2.5 mg/L (range 1.5-11), median albumin level at diagnosis 36 g/L (range 13-49), median bone marrow plasma cells pre-transplant 3% (range 0-41) and median paraprotein level pre-transplant 7 g/L (range 0-60). Immunohistology was performed on B5-fixed, de-paraffinized bone marrow trephine sections using antibody to CD138 (Clone MI15; DAKO). Interstitial plasma cell microaggregates were present in

Table 1. Univariate analysis of factors affecting progression-free survival.

Variable	Reference	Coefficient	p value
Microaggregates present	Absent	-1.3052	0.0024
Non-complete response	Complete response	-1.0729	0.0328
Age > 60 years	≤ 60	-0.1213	0.7807
Male	Female	-0.4723	0.2651
IgA disease	Non-IgA	0.5247	0.2678
Pre-transplant > 10 g/L paraprotein	≤ 10 g/L	0.2230	0.6164
Pre-transplant plasma cells >5%	≤5%	0.3924	0.3618
Lactate dehydrogenase elevated	Normal	0.3169	0.4821
Albumin <35 g/L	≥35 g/L	0.2277	0.5902
β$_2$ microglobulin ≥5.5 mg/L	< 5.5 mg/L	0.1282	0.7863

22% of patients 3 months after HDT. Remarkably, patients with at least one microaggregate 3 months after HDT had a significantly shorter median progression-free survival (15 months) compared to those without plasma cell microaggregates (44 months; relative hazard ratio: 0.21, 95% confidence interval: 0.01-0.21, *p* value <0.0001) after a mean follow-up time of 35 months (range 9 to 72 months; Figure 1B). Disease progression was defined using standard criteria.[2] Follow-up was inadequate to demonstrate a survival difference in patients with or without microaggregates after HDT. A univariate analysis of factors influencing progression-free survival showed that only interstitial plasma cell microaggregates and persistent paraprotein (non-CR) were both associated with earlier disease progression in patients without morphologic evidence of disease 3 months after HDT (Table 1). The study was inadequately powered to demonstrate an independent prognostic significance of microaggregates by multivariate analysis. Compared to other techniques for detecting minimal disease, such as fluorescent *in situ* hybridization for gene rearrangements, flow cytometry for aberrant plasma cell populations, free light-chain analysis and polymerase chain reaction detection of clonal IgH transcripts, immunohistology can be applied to all patients, does not require high-quality fresh aspirate specimens and can be readily performed on archival tissue. The lack of prognostic significance of many of the factors listed in Table 1 suggests that these parameters are less useful in patients with minimal levels of disease after HDT. Immunohistology may therefore be most useful in detecting plasma cell neoplasia when the disease burden is at the limit of routine morphologic sensitivity and suggests that immunohistological assessment of the trephine biopsy should be performed in addition to the marrow aspirate for response evaluation. In conclusion, immunohistological detection of pathological interstitial plasma cell microaggregates is a low-cost, widely available technology that may complement standard methods for defining complete response after high-dose treatment. Although the microaggregates appear to predict for earlier relapse in patients with minimal disease after high dose therapy for myeloma, studies including a larger number of uniformly treated patients are required for confirmation.

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Disorders of Hemostasis

Factor XI deficiency: identification of six novel missense mutations (P23L, P69T, C92G, E243D, W497C and E547K)

Factor XI (FXI) deficiency is a rare coagulation disorder associated with bleeding of variable severity but without a clear relationship between bleeding and FXI levels. This study reports the molecular genetic analysis of FXI deficiencies in thirteen patients. Six novel missense mutations were identified: P23L, P69T, C92G, E243D, W497C and E547K.

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Factor XI (FXI) is the zymogen of a serine protease that participates in the early phase of blood coagulation.[1] Congenital FXI deficiency is a rare autosomal disorder found predominantly but not exclusively in the Ashkenazi Jewish population and occurs rarely in other populations.[2] So far, around 80 mutations causing FXI deficiency have been reported (available at URL: *http://archive.uwcm.ac.uk/uwcm/mg/search/119891.html*).[3]

Thirteen French patients originating from nine unrelated families and various ethnic groups were studied (Table 1). All 15 exons and exon-intron boundaries of the FXI gene were sequenced. Six novel missense mutations have been identified: P23L, P69T, C92G, E243D, W497C and E547K.

Patient #1 is heterozygous for a novel c8220t substitution in exon 3, leading to the P23L mutation, consistent with his partial FXI deficiency.

In family II (patients #2 and #3), a novel t10663g substitution in exon 5, which predicts a C92G mutation, has been characterized. The C92G mutation involves the disappearance of the C92-C175 disulfide bond and probably impairs the structure of the second apple domain. Therefore, this mutation could alter the assembly or the secretion of the protein. The two affected members presented a different bleeding tendency. This discrepancy could be explained because patient #3 has others hemostatic abnormalities, including an alteration of von Willebrand factor antigen (40%) and of factor VIII (50%). This combined deficiency increases the bleeding tendency.[4] Two novel mutations have been characterized in the catalytic domain in two unrelated patients (patients #4 and #5) who have a partial FXI deficiency. Patient #4 is heterozygous for the W497C mutation which involves the appearance of a new cysteine residue close to a disulfide bond (C496-C563). Patient 5 #bears a heterozygous E547K mutation, affecting the charge of the molecule. These mutations are associated with a reduction of both FXI antigen and activity below the expected values for heterozygous subjects (Table 1). It is possible that these two mutations affect wild-type FXI secretion consistent with a dominant negative effect, as recently demonstrated for two others mutations in the catalytic domain.[5]

In the two patients of family V, a novel g16584c substitution leading to an E243D mutation has been identified. Moreover, the father bears a second mutation, P520L, which has been previously described as a mutation with a modest catalytic defect in functional assays.[6] The contribution of each of these mutations to the deficiency is unclear.

In family VI, the mother was homozygous (patient #8) and her two sons were heterozygous (patients #9 and #10) for the G460R mutation which has been recently described by Mitchell *et al*.[7] This mutation induces a change in the charge and occurs in the vicinity of one of the residues forming the catalytic triad of FXIa (D462), so the activity of the protein could be altered. Moreover, patient 10 had a more severe deficiency than his brother (patient 9). This discrepancy could be explained by the compound heterozygosity of patient #10 who carries, in addition to the G460R mutation, another novel mutation in exon 4, P69T.

Patient #11 is a compound heterozygote for two previously described mutations: the C38R[8] and the P382L[9] mutations. The C38R mutation contributes to FXI deficiency, as this change induces the absence of the disulfide bond between cysteines 32 and 38 in the apple 1 domain.[8] As previously demonstrated,[8,9] these two mutations resulted in the absence of secretion (FXI:C< 1U/dL; FXI:Ag < 1U/dL).

For patient 12 an E297K mutation was predicted from exon 9 sequencing. The patient was heterozygous for this substitution, consistent with his partial FXI deficiency. Two other unrelated cases of E297K mutation have also been found (*Quélin et al., unpublished data*). Patient #13 was found to be homozygous for the T304I mutation previously described by Pugh.[10] This patient has low levels of both FXI:Ag (26 U/dL) and FXI activity (11 U/dL). This is consistent with the findings of Pugh[10] who demonstrated that the T304I substitution causes a reduction of factor XI secretion *in vitro*.[10] Moreover, this patient suffered important bleeding manifestations but other hemostatic abnormalities, such as von Willebrand's disease or platelet dysfunctions, have not been searched for.

Altogether, six novel mutations and five previously described mutations were identified in patients living in the suburbs of Paris. None of these mutations was found in a total of 67 normal chromosomes screened, indicating that they are not common polymorphisms. No recurrent mutation was found, perhaps because there is more intense population mixing in Paris than in other areas of France.[8] Unfortunately, additional family studies are not

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