

Total Soluble HLA Class I and Soluble HLA-G in Multiple Myeloma and Monoclonal Gammopathy of Undetermined Significance

Xavier Leleu,^{1,3,4} Gaëlle Le Fricq,^{5,6} Thierry Facon,¹ Laurence Amiot,⁵ Renée Fauchet,⁵ Bernadette Hennache,² Valérie Coiteux,¹ Ibrahim Yakoub-Agha,¹ Sylvain Dubucquoi,² Hervé Avet-Loiseau,⁹ Claire Mathiot,⁷ Régis Bataille,⁹ and Jean-Yves Mary⁸
on behalf of the Intergroupe Francophone du Myélome

Abstract Serum β_2 -microglobulin, the light chain of the HLA class I molecular complex, remains one of the best survival prognostic factors in multiple myeloma, but other HLA class I molecules might be of interest in monoclonal gammopathies. In this study, we evaluate total soluble HLA class I (HLA-I_s) and soluble HLA-G (HLA-G_s) in 103 patients with newly diagnosed multiple myeloma, 30 patients with monoclonal gammopathy of undetermined significance (MGUS), and 30 healthy subjects, studying their prognostic value in multiple myeloma. In multiple myeloma patients, HLA-I_s and HLA-G_s median values were 0.8 μ g/mL and 28 ng/mL, respectively. Median HLA-I_s concentration was higher in stage II and III multiple myeloma patients than in stage I multiple myeloma, MGUS, and control patients. Median HLA-G_s was significantly lower in healthy controls than in MGUS and multiple myeloma patients. A high level of HLA-I_s (≥ 2.1 μ g/mL) was predictive of short survival ($P = 0.017$). For each given level of β_2 -microglobulin, the relative risk of death was higher for patients with HLA-I_s ≥ 2.1 μ g/mL than in patients with a lower level ($P = 0.047$). HLA-G_s, a marker of monoclonal gammopathy, was of no prognostic value, but the addition of HLA-I_s to β_2 -microglobulin produced an efficient prognostic score ($P < 0.0001$). HLA-I_s is a new marker of multiple myeloma tumor load and provides additional survival prognostic information to β_2 -microglobulin.

In multiple myeloma, β_2 -microglobulin is widely recognized as one of the most useful survival prognostic factors (1). Among several staging systems using β_2 -microglobulin, the β_2 -microglobulin and albumin combination is of particular interest (2) and has been recently validated in the context of the International Staging System (3). Other staging systems using more sophisticated variables such as cytogenetics could also emerge in the future (4).

The serum β_2 -microglobulin is the light chain of the HLA class I molecular complex, expressed on the surface of the majority of nucleated cells (5), and corresponds to the

serologic soluble HLA heavy chain-free β_2 -microglobulin (6). Because serum β_2 -microglobulin is an important feature in multiple myeloma, other HLA class I molecules may be of interest in monoclonal gammopathies. Soluble HLA class I molecules (HLA-I_s) as well as the main soluble HLA-G isoform (HLA-G_s) are known to have immunosuppressive properties (7). This immunomodulating role is partially explained by their capacity to protect target cells from natural killer and T cytotoxicity and to trigger apoptosis in activated T CD8⁺ cells (8, 9). The cell origin and pathway of HLA-I_s secretion in multiple myeloma remain unclear. Several soluble β_2 -microglobulin-associated HLA class I heavy chains are generated by different mechanisms: the full-length lipid-associated protein (44000 Da) generated by exosomal shedding, the truncated protein (39000 Da) resulting from alternative splicing, and the proteolytic form (35000 Da) released by metalloproteinase-mediated cleavage (10). However, as several metalloproteinases are produced by human myeloma cells and seem to play a critical role in the pathogenesis of multiple myeloma (11, 12), this suggests a critical proteolytic pathway involvement.

A recent study suggested that the soluble β_2 -microglobulin-free HLA class I heavy chain expression could be a disease marker in multiple myeloma (13). In a tumoral process, HLA-G expression could constitute a mechanism of tumor progression, and HLA-Gs expression has been shown in various cancer types and in some lymphoproliferative disorders (14–17).

In this study, we evaluate the levels of soluble associated β_2 -microglobulin/HLA-G_s (HLA-G_s) and total HLA-I_s in patients with multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), and in age-matched

Authors' Affiliations: ¹Service d'Hématologie, ²Laboratoire de Biochimie, Lille, France; ³Department of Medical Oncology, Harvard Medical School; ⁴Bing Program for Waldenström's Macroglobulinemia and Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Boston, Massachusetts; ⁵Laboratoire d'Hématologie et de Biologie des Cellules Sanguines, Rennes, France; ⁶Washington University School of Medicine, St. Louis, Missouri; ⁷Laboratoire d'Hématologie, Institut Curie; ⁸Institut National de la Santé et de la Recherche Médicale U717, Hôpital St. Louis, Université Paris VII, Paris, France; and ⁹Laboratoire d'Hématologie, Nantes, France

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Note: X. Leleu and G. Le Fricq contributed equally to this work.

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Requests for reprints: Thierry Facon, Service des Maladies du Sang, Hôpital Huriez, Centre Hospitalier Régional Universitaire de Lille, rue Michel Polonovski, 59037 Lille, France. Phone: 33-3-20-44-57-13; Fax: 33-3-20-44-47-08; E-mail: t-facon@chru-lille.fr.

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healthy subjects. We also studied their potential prognostic value on survival in multiple myeloma, especially in relation to β_2 -microglobulin.

Patients and Methods

Patient selection. One hundred and three patients with multiple myeloma diagnosed between 1989 and 2000 in the center of Lille according the criteria of the International Myeloma Working Group (18) and staged according to the Durie/Salmon staging system (19) were included in the study. The patient characteristics are given in Table 1. The patients were always treated after serum collection according to the Intergroupe Francais du Myelome protocols, receiving either conventional chemotherapy ($n = 53$) or high-dose therapy with autologous peripheral blood stem cell support ($n = 38$). Twelve patients remained untreated at the date of point. For treated patients, the median time between serum collection and treatment was 0.3 month (interquartile range, 0.0-2.9).

Thirty patients with MGUS diagnosed according to the International Myeloma Working Group (ref. 18; 17 females and 13 males) and 30 age-matched healthy subjects were also studied. MGUS patients had a median age of 62 years (interquartile range, 56-68) and their median bone marrow plasmacytosis was 3% (interquartile range, 2-5). At the date of point, no patient had progressed to multiple myeloma.

Laboratory variables. The biological evaluations were done in Lille for usual variables and Rennes for soluble HLA class molecules. Chromosome 13 deletion ($\Delta 13$) was determined using fluorescence *in situ* hybridization analysis (20). HLA-Gs and HLA-Is concentrations were measured using a specific sandwich ELISA. Microtiter plates (Corning Costar, Brumath, France) were coated in PBS at pH 7.4 with MEM-G/9 (10 $\mu\text{g/mL}$), a monoclonal antibody detecting specifically HLA-Gs molecules associated with β_2 -microglobulin (Exbio, Prague, Czech Republic) or with W6/32 (5 $\mu\text{g/mL}$), monoclonal antibody recognizing a monomorphic determinant of β_2 -microglobulin associated to any of the HLA class I heavy chains (Harlan Sera-Lab Ltd., Leicestershire, England). After three washes in PBS containing 0.05% Tween 20, plates were saturated with 250 μL of PBS containing 2% bovine serum albumin for 30 minutes at room temperature. One hundred microliters of pure sera for HLA-Gs or diluted at 1/50 in PBS for HLA-Is were added to each well and tested in triplicate. After incubation for 1 hour at room temperature, plates were washed thrice in PBS with 0.05% Tween 20. Anti- β_2 -microglobulin/horseradish peroxidase (DAKO, Trappes, France; 100 μL) was added to each well and plates were incubated for 1 hour at room temperature. Plates were washed thrice and then incubated with the substrate (orthophenylenediamine dihydrochloride, DAKO) for 30 minutes in dark. After addition of H_2SO_4 (1 N), absorbencies were measured at 490 nm. Standard curves were done using serial dilutions of either calibrated supernatant of B-lymphoblastoid cell line LCL-721.221 (American Type Culture Collection, Rockville, MD) transfected with HLA-Gs cDNA (LCL721.221-G5), kindly provided by V. Rebmann (Essen, Germany). Soluble HLA-B7s molecules (Sangstat, Fremont, CA) were used as a standard to calculate the total amount of sHLA-I antigens. Thus, the concentrations of HLA-Gs and HLA-Is were determined from the value of absorbance according to the standard curves.

Statistical analysis. The distributions of HLA-Is and HLA-Gs levels among the different groups (controls, MGUS, and multiple myeloma by stage) were characterized through median and interquartile range. Distribution comparison between these samples were done through Mann-Whitney or Kruskal-Wallis tests, according to the following strategy: normal versus MGUS, homogeneity among multiple myeloma patients according to stage, MGUS versus multiple myeloma in case of homogeneity among multiple myeloma patients across stage, otherwise MGUS versus stage I multiple myeloma, homogeneity between stage II and stage III multiple myeloma, and stage I versus stage II or III multiple myeloma if homogeneity among stage II and stage III multiple myeloma

Table 1. Patient characteristics at blood collection ($n = 103$, except if indicated)

	% Patients	Median (interquartile range)
Age (y)		64 (56-72)
>70	29	
Male sex	44	
Stage		
I	38	
II	19	
III	43	
Isotype		
IgG	64	
IgA	27	
Only light chain	6	
Other	3	
Kappa light chain ($n = 102$)	65	
HLA-Is ($\mu\text{g/mL}$)		0.8 (0.5-2.1)
≥ 2.1	25	
HLA-Gs (ng/mL)		28 (17-39)
Treatment		
No ($n = 12$)	12	
Yes ($n = 91$)		
Conventional	58	
Intensive	42	
Time to treatment (mo), $n = 91$		0.3 (0.0-2.9)
Bone marrow plasmacytosis (%)		23 (12-45)
<25	53	
25-50	27	
≥ 50	20	
Chromosome 13 deletion ($n = 70$)	31	
Hemoglobin (g/d)		10.8 (9.6-12.7)
≤ 12	71	
Creatinine (mg/L)		10 (8-12)
≥ 15	14	
Albumin (g/L)		39.5 (36.5-43.0)
≤ 35	17	
Calcium (mg/L)		94 (91-99)
β_2 -microglobulin (mg/L)		2.8 (2.0-3.9)
<2.5	42	
2.5-4.0	37	
≥ 4.0	21	
LDH (IU/L), $n = 102$		252 (207-304)
≥ 220	66	
CRP (mg/L)		3.0 (2.0-7.0)
≥ 6	29	

Abbreviations: LDH, low-density hydrocarbon; CRP, C-reactive protein.

patients, stage I versus stage II multiple myeloma otherwise. Correlations between HLA-Is and HLA-Gs levels with the level of other biological variables involved in multiple myeloma disease were tested through Spearman correlation coefficient, within the multiple myeloma sample, if homogeneous according to the methodology described above.

Date of point was May 1, 2004. Overall survival and progression-free survival were evaluated through Kaplan-Meier estimates as a function of

time from serum collection and after first conventional or intensive treatment, respectively (21). In addition to HLA-Is and HLA-Gs, all variables described in Table 1 were examined for their prognostic value on overall survival. In these prognostic analyses, continuous variables were categorized based on 25th, 50th, and 75th percentiles, as described previously (22). Usual limits (as 2.5, 4.0, and 6.0 mg/L for β_2 -microglobulin) were also tested. For univariate analyses, overall survival curves were compared through log-rank test (23). The estimate of the relative risk of death and its 95% confidence interval was estimated through proportional hazards model (24). Then, the ability of each variable, HLA-Is and HLA-Gs, to increase overall survival predictive ability of the β_2 -microglobulin was tested through backward stepwise proportional hazards model using likelihood ratio test (25). The subsequent prognostic models were simplified by using the same procedure, as the one described for univariate analysis of continuous prognostic variable, leading to final models with three or four categories only. No further improvement by adding another factor in the model was looked for according to the sample size and the number of deaths in our sample. The influence of patient treatment on these prognostic models was investigated by testing their prognostic ability through a proportional hazards model including conventional and intensive treatments as time-dependent covariates (26).

The respective qualities of our final models and of some well-known previously published models (2, 3, 22, 27, 28) applied to our sample were described through Kaplan-Meier estimates of survival curves and quantified through the value in $-2\log$ (likelihood), the lower value corresponding to the best fit of data for a fixed number of prognostic categories and the same sample size. All analyses were done with the SPSS software (29).

Results

HLA-Is and HLA-Gs serum levels and correlations to other variables. As described in Table 2A, HLA-Is concentration varied across stages in multiple myeloma patients ($P = 0.02$) but not between stages II and III ($P = 0.88$). A statistically significant difference was observed between stage I and stage II

to III multiple myeloma ($P = 0.005$), with higher values for the latter group. In contrast, no significant differences were observed between controls and MGUS and between MGUS and stage I multiple myeloma. Regarding HLA-Gs (Table 2B), there was no variation across stages in multiple myeloma patients and between MGUS and multiple myeloma patients. The only difference was evidenced between controls and MGUS ($P = 0.03$) or multiple myeloma ($P < 0.001$). No variation of HLA-Is and HLA-Gs with age could be evidenced among multiple myeloma patients. No correlation was observed between HLA-Is and HLA-Gs. HLA-Is showed a minor correlation with β_2 -microglobulin in stage I ($r = 0.44$, $P = 0.005$), stage II to III multiple myeloma ($r = 0.27$, $P = 0.03$), and in the entire multiple myeloma population ($r = 0.29$, $P = 0.003$). In contrast, HLA-Gs did not correlate with β_2 -microglobulin in multiple myeloma regardless of disease stage. Minor correlations of HLA-Is with hemoglobin ($r = -0.23$, $P = 0.02$) or bone marrow plasmacytosis ($r = 0.23$, $P = 0.02$) were observed but only globally in multiple myeloma patients, as a consequence of their variations across stages. Both HLA markers did not correlate to creatinine and to chromosome 13 deletion.

Survival analyses. In multiple myeloma, the median follow-up time was 73.2 ± 5.4 months and the median survival time was 51.4 ± 5.8 months (65 deaths). Among the 91 treated multiple myeloma patients, 64 progressions were observed and the median progression-free survival time was 18.8 ± 1.7 months. Variables significantly affecting overall survival in univariate analysis are presented in Table 3. A high level of HLA-Is (≥ 2.1 $\mu\text{g/mL}$) was predictive of a short overall survival, as shown on Fig. 1. In contrast, HLA-Gs level had no effect on overall survival. There was a trend in patients with increasing β_2 -microglobulin level but not in those with high HLA-Is level to shorter progression-free survival ($P = 0.08$ and $P = 0.55$, respectively).

Table 2. Values of total HLA-Is and HLA-Gs among the different groups

	Controls	MGUS	Stage I MM	Stage II MM	Stage III MM
A. HLA-Is ($\mu\text{g/mL}$)					
Mean \pm SD	1.04 \pm 0.92	0.66 \pm 0.32	0.93 \pm 0.83	1.87 \pm 1.70	1.79 \pm 1.70
Median	0.70	0.52	0.73	1.34	1.03
Interquartile range	0.42-1.15	0.39-0.89	0.39-1.03	0.48-2.68	0.58-2.79
No. patients ($n = 64$)	30	30	39	20	44
B. HLA-Gs (ng/mL)					
Mean \pm SD	18.6 \pm 9.3	28.7 \pm 17.6	32.2 \pm 23.4	28.2 \pm 13.3	32.0 \pm 19.6
Median	19.5	28.7	26.7	30.9	26.9
Interquartile range	9.8-25.2	11.6-41.6	16.0-39.5	14.8-38.4	18.1-43.6
No. patients ($n = 103$)	30	30	39	20	44
				103	

NOTE: Stages I, II, and III are according to the Durie and Salmon classification.
Abbreviation: MM, multiple myeloma.

In the multivariate analysis, β_2 -microglobulin ($P < 0.001$) and HLA-Is ($P = 0.047$) were independent prognostic factors affecting overall survival. Relative risk of death (95% confidence interval) was 2.1 (1.2-3.7) for patients with β_2 -microglobulin between 2.5 and 4.0 mg/L and 3.6 (1.9-6.9) for patients with β_2 -microglobulin ≥ 4.0 mg/L compared with patients with β_2 -microglobulin < 2.5 mg/L and 1.8 (1.0-3.2) for patients with HLA-Is ≥ 2.1 $\mu\text{g/mL}$ compared with patients with HLA-Is < 2.1 $\mu\text{g/mL}$. Based on these results, a prognostic model with four categories was derived (Table 4; Fig. 2A): 1 point was given for a β_2 -microglobulin level between 2.5 and 4.0 mg/L; 2 points if β_2 -microglobulin level was ≥ 4.0 mg/L; and 1 point if HLA-Is level was ≥ 2.1 $\mu\text{g/mL}$, the risk of death increasing with the score or total of points. This classification seemed clearly different from the one obtained with β_2 -microglobulin alone (Table 3; Fig. 2B).

The β_2 -microglobulin/HLA-Is model still strongly affected overall survival when taking into account conventional and intensive treatments as time-dependent covariates ($P = 0.009$). This prognostic classification, as well as the one based on β_2 -microglobulin alone, had only a borderline significance on progression-free survival ($P = 0.15$ and 0.08 , respectively).

Comparison between prognostic models. We did the comparison of the β_2 -microglobulin/HLA-Is model with some previously published models. Our previous model using β_2 -microglobulin, fluorescence *in situ* hybridization $\Delta 13$, and IgA isotype (22) was superior to the β_2 -microglobulin/HLA-Is model, the latter was superior to the Southwest Oncology Group β_2 -microglobulin/albumin model (28), $-2\log$ (likelihood) being estimated to be 279.700, 286.813, and 292.362, respectively. To compare our model with previous models

Table 3. Significant prognostic factors for survival (univariate Cox analysis)

	O/N*	Relative risk of death estimate (95% confidence interval)	Survival time (mo, median \pm SE)	P [†]
β_2 -microglobulin (mg/L)				0.0001
<2.5	20/43	1 [‡]	78.6 \pm 7.1	
2.5-4.0	27/38	2.1 (1.2-3.7)	47.3 \pm 5.3	
≥ 4.0	18/22	3.8 (2.0-7.3)	18.2 \pm 7.2	
Stage				0.001
I	21/39	1 [‡]	70.9 \pm 7.0	
II/III	44/64	2.4 (1.4-4.2)	32.0 \pm 5.4	
Hemoglobin (g/d)				0.002
>12	14/30	1 [‡]	78.6 \pm 7.0	
≤ 12	51/73	2.5 (1.4-4.6)	36.6 \pm 6.4	
Bone marrow plasmacytosis (%)				0.002
<25	30/55	1 [‡]	69.6 \pm 10.1	
≥ 25	35/48	2.2 (1.3-3.5)	31.9 \pm 4.8	
Albumin (g/L)				0.004
>35	51/86	1 [‡]	57.8 \pm 8.1	
≤ 35	14/17	2.3 (1.3-4.2)	18.2 \pm 8.5	
LDH (IU/L)				0.011
<220	17/35	1 [‡]	95.2 \pm 29.9	
≥ 220	47/67	2.1 (1.2-3.7)	45.9 \pm 8.4	
Age (y)				0.013
≤ 70	41/73	1 [‡]	68.4 \pm 7.3	
>70	24/30	1.9 (1.1-3.1)	32.0 \pm 8.0	
HLA-Is ($\mu\text{g/mL}$)				0.017
<2.1	47/77	1 [‡]	63.6 \pm 8.9	
≥ 2.1	18/26	2.0 (1.1-3.5)	31.3 \pm 6.4	
Chromosome 13 deletion				0.029
No	26/48	1 [‡]	63.6 \pm 8.1	
Yes	16/22	2.0 (1.1-3.7)	25.6 \pm 4.0	
Treatment [§]				
No	5/12	1 [‡]	79.7 \pm 1.3	
Intensive	18/38	4.3 (1.5-11.7)	68.4 \pm 8.7	0.002
Conventional	42/53	9.1 (3.6-23.2)	32.0 \pm 5.9	<0.0001

Abbreviation: LDH, low-density hydrocarbon.

*O/N = number of dead patients/total number of patients.

[†]Significance level.

[‡]Baseline group.

[§]Analysis of treatment effect was done through time-dependent covariates (see text) and compared with the no-treatment group.

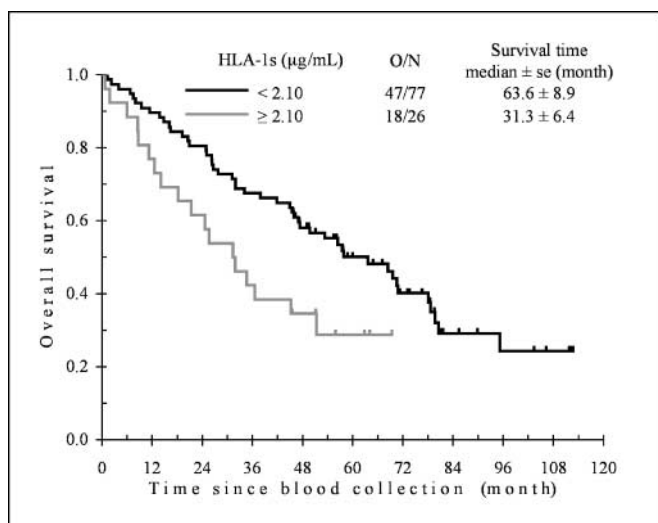


Fig. 1. Overall survival in 103 multiple myeloma patients according to total HLA-Is serum level. O/N = number of deaths/total number of patients.

with only three categories, the two intermediate categories of the β_2 -microglobulin/HLA-Is model were mixed (Table 4). In these conditions, the combination of β_2 -microglobulin and $\Delta 13$ was the most effective model followed by the combination of β_2 -microglobulin and HLA-Is with $-2\log$ (likelihood) estimated on 70 patients to be 294.023 and 296.721, respectively. The combination of β_2 -microglobulin and C-reactive protein (27) provided inferior models (data not

shown). The β_2 -microglobulin and albumin model which constitutes the basis of the simplified International Staging System (3) was also inferior in this series of patients, $-2\log$ (likelihood) being estimated on 103 patients to be 516.638 compared with 507.451 for the β_2 -microglobulin/HLA-Is model.

Discussion

This study suggests that HLA-Is is a new marker of multiple myeloma tumor load, with higher levels found in Durie Salmon stage II/III multiple myeloma compared with stage I multiple myeloma, MGUS, and healthy controls. HLA-Gs was higher in all kinds of monoclonal gammopathies compared with controls. Correlations between these markers and usual multiple myeloma variables were poor, except between HLA-Is and β_2 -microglobulin. In multiple myeloma, a high level of HLA-Is but not of HLA-Gs was associated with a poor survival. The combination of β_2 -microglobulin and HLA-Is provided a powerful prognostic model, a high level of HLA-Is identifying patients with a more severe prognosis, compared with a low level, for any level of serum β_2 -microglobulin. This prognostic model seemed more efficient than other previously published models, except the combination of β_2 -microglobulin and chromosome 13 deletion (22).

To our knowledge, the main soluble HLA-G isoform, a nonclassical MHC class I molecule, have not been studied in multiple myeloma, but it has been investigated in other lymphoproliferative disorders: chronic lymphocytic leukemia and B and T non-Hodgkin lymphoma (14–17). In all these

Table 4. Survival prognostic models in 103 multiple myeloma patients according to β_2 -microglobulin and total HLA-Is

Variables		O/N*	Relative risk of death		Survival time (mo, median ± SE)	P [†]
β_2 -microglobulin (mg/L)	HLA-Is (µg/mL)		Estimate	95% confidence interval		
Four-category model						
<2.5	<2.1	16/35	1 [‡]		80.5 ± 9.4	<0.0001
<2.5	≥2.1	25/37	2.0	1.1-3.8	53.3 ± 5.8	
2.5-4.0	<2.1					
2.5-4.0	≥2.1	16/22	3.2	1.5-6.4	27.8 ± 3.7	
≥4.0	<2.1					
≥4.0	≥2.1	8/9	7.7	3.2-18.8	14.1 ± 4.3	
Three-category model						
<2.5	<2.1	16/35	1 [‡]		80.5 ± 9.4	<0.0001
<2.5	≥2.1	41/59	2.3	1.3-4.2	47.0 ± 8.5	
2.5-4.0	—					
≥4.0	<2.1	8/9	7.7	3.2-18.8	14.1 ± 4.3	
≥4.0	≥2.1					

*O/N: number of dead patients/total number of patients.

[†]Significance level.

[‡]Baseline group.

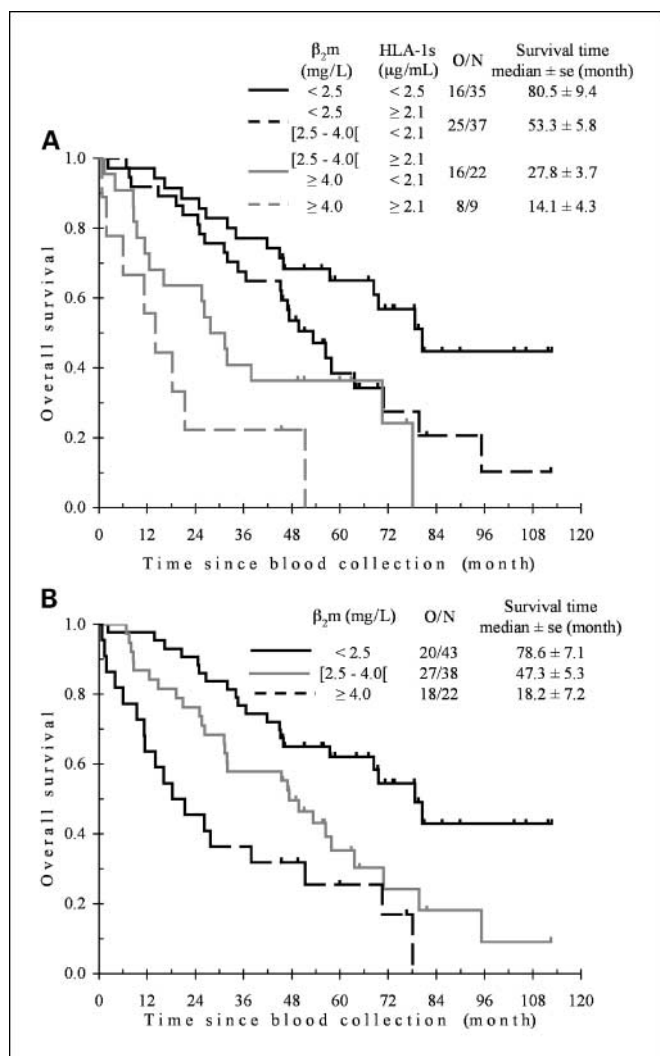


Fig. 2. Overall survival according to serum levels of (A) β_2 -microglobulin (β_2m) and total HLA-Is and (B) β_2 -microglobulin alone. O/N = number of deaths/total number of patients.

cases, the HLA-G molecules were at low density on the tumoral cell surface contrasting with the frequent detection of the soluble isoform. Approximately 60% of B- and T-lymphoid malignancies have significantly increased HLA-Gs levels compared with healthy subjects with mean levels equal to 49 and 47 ng/mL in chronic lymphocytic leukemia and B non-Hodgkin lymphoma, respectively (17). A study reported an increased level of HLA-G expression at the cell surface of T- and B-primary cutaneous lymphoma, but they did not consider the soluble isoform (30). These differences between cutaneous lymphoma and their nodal or leukemic counterparts could be partially explained by differences regarding the microenvironment and especially the cytokines involved (17).

The serologic β_2 -microglobulin-free HLA class I heavy chain (FHC) has been recently studied (13). The total soluble HLA class I complex, a couple of β_2 -microglobulin and the HLA class I heavy chain, seems more stable than the β_2 -microglobulin-free HLA class I heavy chain (31). FHC levels were significantly higher in multiple myeloma compared with MGUS and controls but did not vary across multiple myeloma stages

(13). Similar minor correlations were observed between HLA-Is or FHC and β_2 -microglobulin ($r = 0.29$ or 0.31 , respectively) and bone marrow plasmacytosis ($r = 0.29$ and 0.36 , respectively). HLA molecules levels, total soluble or FHC, were not correlated to creatinine ($r = 0.09$ and 0.15 , respectively). This result was expected as the HLA-I heavy chain is heavier than the β_2 -microglobulin molecule and is more susceptible to catabolism rather than renal filtration. The importance of finding a new marker independent of creatinine in multiple myeloma has been stressed because β_2 -microglobulin is influenced by renal insufficiency and is normal in about 10% of patients (6, 32). No real prognostic factor analysis was done in the study reported by Perosa et al. (13). In our study, the 2.1 $\mu\text{g/L}$ threshold, the upper limit of the inter-quartile range, was derived from overall survival prognostic analysis. We did not do a systematic research of the most effective limit as provided by ROC curve analysis, because it is known to provide limit highly dependent on the studied sample. For every β_2 -microglobulin level, <2.5 mg/L, between 2.5 and 4 mg/L, and ≥ 4.0 mg/L, an HLA-Is level of ≥ 2.1 $\mu\text{g/L}$ reclassified patients in a worse prognostic group, concerning one third of patients within each level of β_2 -microglobulin.

The HLA-Is/ β_2 -microglobulin model was shown to be more effective than previously proposed models, except the $\Delta 13/\beta_2$ -microglobulin model (22). Of note, all the models including β_2 -microglobulin and albumin (2, 3, 28) seemed much less effective than the β_2 -microglobulin/HLA-Is model. This needs to be confirmed in other studies because the comparison between these models was done on a sample that was optimal for the HLA-Is/ β_2 -microglobulin model, because it was used to construct this model. Furthermore, it should be valuable to test on a sufficiently large sample the prognostic ability of the combination HLA-Is/ $\Delta 13/\beta_2$ -microglobulin. Anyway, the HLA-Is/ β_2 -microglobulin model seemed an effective alternative to the $\Delta 13/\beta_2$ -microglobulin model for investigators lacking cytogenetics.

HLA-Gs was of no survival prognostic value in multiple myeloma, contrarily to HLA-Is. The reason for the discrepancy observed by others that described a prognostic role of HLA-Gs in other B-lymphoid malignancies (14–17) underlined the particularity of the multiple myeloma pathogenesis. The reasons why HLA-Is seemed of prognostic value in multiple myeloma remain unclear. Its prognostic role could be related to its biological activities, especially its immunosuppressive properties. On the other hand, our HLA-Is ELISA evaluation, measuring a couple of β_2 -microglobulin and the HLA class I heavy chain, could be a way to measure the complementary part of β_2 -microglobulin not quantified by the dosage of β_2 -microglobulin alone (free β_2 -microglobulin).

In conclusion, in our study, HLA-Gs had no survival prognostic effect in multiple myeloma but was a marker of clonality. In contrast, HLA-Is gave strong additional survival prognostic information to β_2 -microglobulin alone, leading to a powerful prognostic score. This new prognostic classification needed to be confirmed in other patient cohorts.

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