

Risk of progression in smouldering myeloma and monoclonal gammopathies of unknown significance: comparative analysis of the evolution of monoclonal component and multiparameter flow cytometry of bone marrow plasma cells

Ernesto Pérez-Persona,¹ Gema Mateo,¹ Ramón García-Sanz,^{1,2} María-Victoria Mateos,¹ Natalia de las Heras,³ Alfonso García de Coca,⁴ José M. Hernández,⁵ Josefina Galende,⁶ Guillermo Martín-Núñez,⁷ Abelardo Báñez,⁸ José M. Alonso,⁹ Alejandro Martín,¹⁰ Consuelo López-Berges,¹ Alberto Orfao,^{2,11} Jesús F. San Miguel^{1,2} and María-Belén Vidriales,^{1,2}

¹Department of Haematology, University Hospital, ²Centro de Investigación del Cáncer, Universidad de Salamanca/ CSIC, Salamanca, ³Department of Haematology, Complejo Hospitalario, León, ⁴Department of Haematology, University Hospital, Valladolid, ⁵Department of Haematology, General Hospital, Segovia, ⁶Department of Haematology, Hospital Comarcal del Bierzo, Ponferrada, León, ⁷Department of Haematology, Hospital Nuestra Señora del Puerto de Plasencia, Cáceres, ⁸Department of Haematology, Hospital Nuestra Señora de Sonsoles, ⁹Department of Haematology, Hospital Río Carrión, Palencia; ¹⁰Department of Haematology, Hospital Virgen de la Concha, Zamora, and ¹¹Department of Cytometry, University of Salamanca, Salamanca, Spain

Summary

The present study explored the impact of two novel criteria; having >95% abnormal plasma cells by flow cytometry at diagnosis and the evolving subtype of the disease, as predictors of progression in 61 smouldering multiple myeloma (SMM) and 311 monoclonal gammopathy of unknown significance (MGUS) patients. Although both criteria were of prognostic value, the risk of progression was better identified by immunophenotyping [Hazard Ratio (HR) 6.2 and 17.2 for SMM and MGUS, respectively] than evolving subtype, which had independent prognostic value only in MGUS (HR 3.6). Immunophenotyping discriminated the different risk of progression within the evolving and non-evolving subgroups of SMM ($P = 0.01$) and MGUS ($P < 0.001$).

Keywords: smouldering myeloma, MGUS, evolving pattern, flow cytometry, risk of progression.

Received 24 May 2009; accepted for publication 4 August 2009

Correspondence: María-Belén Vidriales, Department of Haematology, Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, 37007 Salamanca. Spain.
E-mail: mbvidri@usal.es

Monoclonal gammopathy of uncertain significance (MGUS) and smouldering multiple myeloma (SMM) are two forms of asymptomatic plasma cell disorders that may eventually progress to multiple myeloma MM (International Myeloma

Working Group, 2003; Rajkumar, 2005). Several reports identified factors for progression in both entities (Weber *et al*, 1997; Cesana *et al*, 2002; Kyle *et al*, 2002; Rajkumar, 2005) and recently, Rosiñol *et al* (2003, 2005) described evolving and

non-evolving SMMs based on their patterns of evolution. The evolving type was defined as an increase in the level of serum M protein of at least 10% during the first 6 months of follow-up, or a progressive and constant increase of the M component until overt MM developed. These patients had a shorter time to progression (TTP) and exhibited cytogenetic characteristics frequently observed in symptomatic MM (Rosñol *et al*, 2005). Although the concept of evolving type has not yet been applied to MGUS, it could also be useful.

Multiparameter flow cytometry (MFC) has been increasingly used in the study of plasma cell dyscrasias (Harada *et al*, 1993; Rawstron *et al*, 2008). A high proportion of abnormal plasma cells (aPC) within the bone marrow plasma cell (BMPC) compartment (>95% aPC/BMPC) has been shown to predict the risk of progression in both SMM and MGUS (Ocqueteau *et al*, 1998; Pérez-Persona *et al*, 2007).

The present study explored the impact of these two criteria (having >95% aPC/BMPC by MFC at diagnosis and the evolving subtype of the disease) as predictors of progression in SMM and MGUS. Theoretically, earlier and more accurate definition of the risk of progression in both entities should help to establish a better follow-up strategy adapted to the patient's individual risk.

Material and Methods

The study included 61 patients with SMM and 311 patients with MGUS. The International Myeloma Working Group (2003) criteria were used for diagnosis and evaluation of progression; all patients had more than 10% of PC BM infiltration and/or monoclonal component higher than 30 g/l at diagnosis. Smouldering multiple myeloma and MGUS patients had a minimum follow-up of 24 and 36 months, respectively, with measurement of monoclonal component (MC) at least four times a year for SMM and twice a year for MGUS. Evolving SMM was defined as having an increase of at least 10% of the MC in the first year, and evolving MGUS, which has an indolent course, was defined as an increase of MC of at least 10% by the third year, confirmed by two consecutive measurements separated by at least 1 month. Median follow-up for SMM was 42 months (range: 24–110 months) and 57 months (range: 36–168 months) for MGUS. Twenty-four MGUS (8%) and 17 SMM patients (28%) progressed. Median TTP for SMM patients was 28 months (range: 13–73 months), all evolving into symptomatic MM. In MGUS, median TTP was 49 months (range: 12–106 months), with 21 patients (87%) progressing to symptomatic MM, and three cases (13%) to non-Hodgkin lymphoma.

Immunophenotypic analyses were performed at diagnosis according to established methods (Mateo *et al*, 2005; Pérez-Persona *et al*, 2007; Paiva *et al*, 2008; Rawstron *et al*, 2008), using a direct immunofluorescence technique [CD38-fluorescein isothiocyanate (FITC)/ CD56-phycoerythrin (PE)/ CD19-peridinin chlorophyll-cyanin 5 (PerCP-Cy5)/

CD45-allphycocyanin (APC)]. Acquisition was performed in a FACSCalibur flow cytometer [BD Biosciences (BDB), San Jose, CA, USA] using the CELLQUEST software (BDB), providing information from $\geq 3 \times 10^5$ BM cells. Normal and aberrant phenotypes (polyclonal and clonal PC) were discriminated using a validated immunophenotypic approach (Mateo *et al*, 2005; Pérez-Persona *et al*, 2007; Paiva *et al*, 2008; Rawstron *et al*, 2008), i.e., absence of CD19 and/or CD45 expression, over expression of CD56, and weak expression of CD38, the most frequent aberrant phenotypes on myeloma-tous PC.

The total percentage of PCs from the whole BM cellularity and the percentage of abnormal plasma cells (aPC) within the BMPC compartment (referred to as aPC/BMPC) were recorded.

The Statistical Package for the Social Sciences (SPSS) software, version 12.5 (SPSS Inc., Chicago, IL) was used for statistical analyses, as elsewhere described (Mateo *et al*, 2005; Pérez-Persona *et al*, 2007; Paiva *et al*, 2008).

Results

The main clinical and biological characteristics of the patients at diagnosis are shown in Table I.

Within the SMM group, 31 out of 61 patients (51%) were identified as having an evolving SMM, while 30 patients (49%) had a stable MC and were considered non-evolving SMMs. Considering MGUS patients, 121 cases (39%) were classified as evolving, and 190 (61%) as non-evolving. The cumulative

Table I. Main clinical and biological characteristics of MGUS and SMM patients.

Characteristics	MGUS N: 311	SMM N: 61
Gender (male/female)	155/156	28/33
Age (years)*	71 (32–95)	69 (39–88)
Serum MC (g/l)*	16 (6–30)	21.3 (6.2–50.3)
Ig isotype		
IgG	76%	64%
IgA	24%	34%
Light chain isotype		
Kappa	55%	64%
Lambda	45%	36%
Cases with immunoparesis†	27 (9%)	14 (23%)
% BMPC by morphology (aspirate)*	4 (1.0–9.7)	12 (2.4–12.0)
% BMPC by FC*	0.98 (0.01–37)	2.86 (0.19–27)
% abnormal PC (among BMPC)*	73 (0–100)	95 (11–100)
% patients with >95% aPC	56 (18%)	32 (52%)
Aneuploidy (%)	72 (33)	24 (39)
% cases that progressed	24 (8%)	17 (28%)

MC, monoclonal compartment; Ig, immunoglobulin; BMPC, bone marrow plasma cells; aPC, atypical plasma cell.

*Mean (range).

†Immunoparesis defined as reduction under the lower normal limit of the uninvolved immunoglobulins.

probability of progression (CPP) at 3 years was 27% and 11% for evolving and non-evolving SMM cases, respectively ($P = 0.003$) (Fig 1A), and for MGUS patients the CPP at 7 years was 19% for evolving and 7% for non-evolving cases, respectively ($P = 0.05$) (Fig 1D).

MFC showed that 52% of SMM patients had $\geq 95\%$ of aPC/BMPC, while only 18% of cases reached this threshold in the MGUS group. This cut-off level distinguished the different risks groups in the two entities (Fig 1B and E).

Interestingly, considering evolving and non-evolving subgroups within the separate groups of SMM and MGUS patients, the evaluation of the BMPC compartment by MFC discriminated the different risks of progression. Thus, in the evolving SMM group, the risk of progression at 3 years for patients with $\geq 95\%$ aPC/BMPC and $<95\%$ aPC/BMPC was 46% and 8% ($P = 0.01$), respectively. This was also observed in the non-evolving SMM cases, in which the risk of progression at 3 years was 15% for cases with $\geq 95\%$ aPC/BMPC, whereas no progressions were observed in the $<95\%$ aPC/BMPC group ($P = 0.01$). Considering evolving MGUS patients, the risk of

progression for cases with $\geq 95\%$ aPC/BMPC and $<95\%$ aPC/BMPC at 7 years was 72% and 12% ($P < 0.0001$), respectively. Similarly, among the non-evolving MGUS patients, the risk of progression at 7 years was 29% and 2% for cases with $\geq 95\%$ and $<95\%$ aPC/BMPC, respectively ($P < 0.0001$).

Based on these two variables, we designed a prognostic index that clearly identified three risk groups. Among SMM patients, the CPP at 3 years was 0% for cases with no risk factors ($<95\%$ aPC/BM and non-evolving), 12% for cases with one risk factor ($\geq 95\%$ aPC/BM or evolving subtype) and 46% for cases with both factors ($\geq 95\%$ aPC/BM and evolving subtype) ($P < 0.001$). The same approach was used for MGUS patients, with a CPP at 7 years of 2%, 16% and 72% for patients with none, one and two risk factors, respectively ($P < 0.001$) (Fig 1E and F).

Finally, we performed a multivariate analysis for PFS including, in addition to the already mentioned variables, those with significant impact in the univariate analysis (percentage of BMPC by morphology and by flow cytometry, and presence of immunoparesis; for MGUS we also included Bence-Jones proteinuria >0.2 g/24 h, DNA aneuploidy and

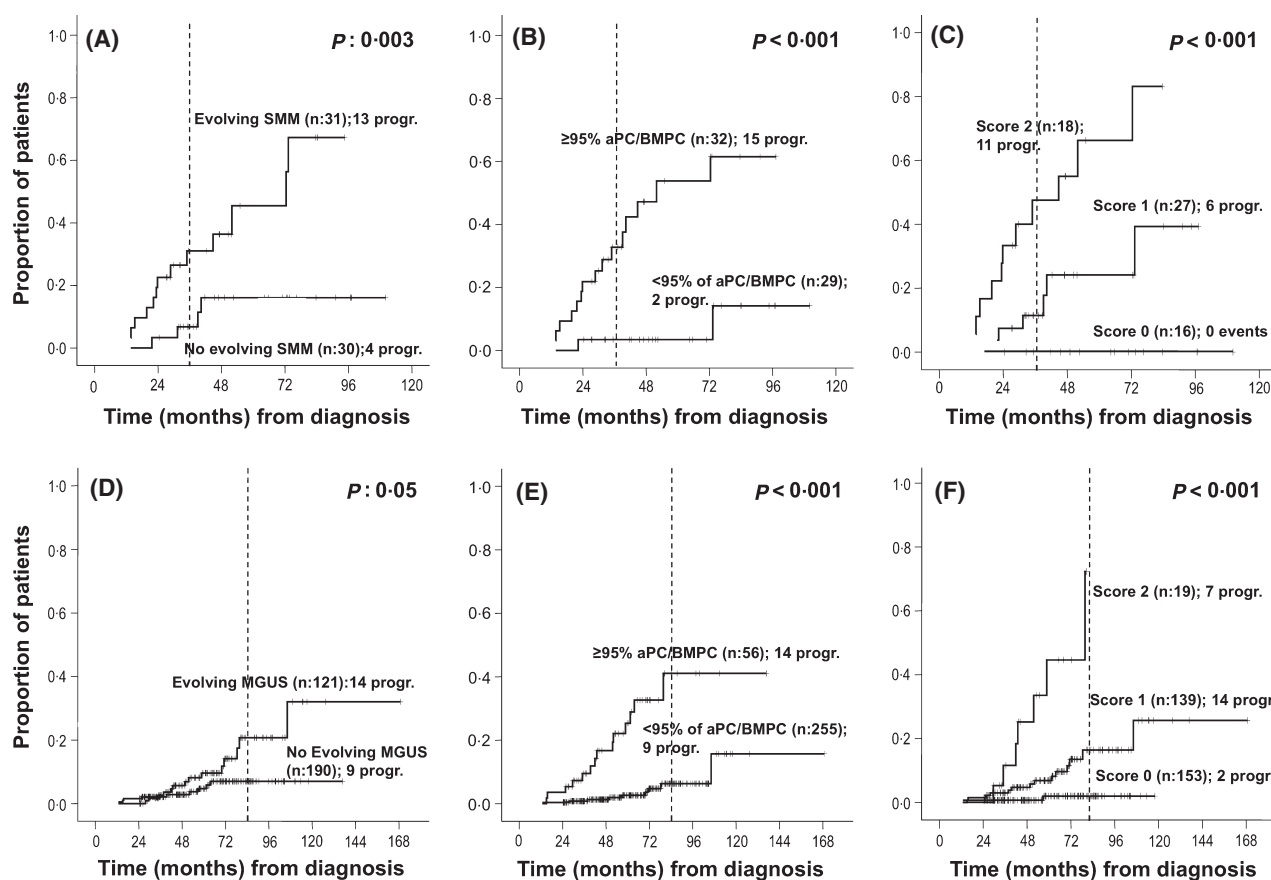


Fig 1. Time to progression (TTP) in SMM and MGUS with respect to percentage of aPC/BMPC, evolving concept and a new score. Upper panels: Risk of progression for SMM. Panel A: 27% vs. 11% for evolving SMM vs. non-evolving SMM ($P = 0.003$). Panel B: 23% vs. 3% for patients with $\geq 95\%$ vs. $<95\%$ aPC/BMPC ($P < 0.001$). Panel C: Based on the score system (see text) of 0%, 12% and 46% for patients with 0, 1 or 2 points ($P < 0.001$). Lower panels: Risk of progression for MGUS. Panel D: 19% vs. 7% for evolving MGUS vs. non-evolving MGUS ($P = 0.05$). Panel E: 41% vs. 6% for patients with $\geq 95\%$ vs. $<95\%$ aPC/BMPC ($P < 0.001$). Panel F: Based on the score system (see text) of 2%, 16% and 72% for patients with 0, 1 or 2 points ($P < 0.001$).

MC levels ≥ 20 g/l). In MGUS patients, having $\geq 95\%$ aPC/BMPC (HR: 17.2; $P < 0.001$) and the evolving concept (HR: 3.6; $P = 0.008$) together with aneuploidy (HR: 5.07; $P = 0.001$) were of independent prognostic value. However, for SMM patients only, having $\geq 95\%$ aPC/BMPC (HR: 6.2; $P = 0.02$) and immunoparesis (HR: 4.0; $P = 0.02$) were selected in the model. Interestingly, the greater effect on risk of progression was shown by $\geq 95\%$ aPC/BMPC in both entities.

Discussion

Asymptomatic gammopathies are characterised by the presence of a MC without evidence of organ damage (International Myeloma Working Group, 2003), MGUS being the paradigm of such entities. Several efforts have been made to identify variables that predict progression (Kyle *et al*, 2002), because the risk of transformation (Cesana *et al*, 2002) is life-long and patients should be monitored throughout their lives. Therefore, a better knowledge of risk factors associated with progression would help to individualise follow-up strategies. Accordingly, Rosiñol *et al* (2003, 2005) described two types of SMM on the basis of the MC dynamics observed in early phases of evolution. However, no other group has confirmed the existence of these two types of SMM. Our results support the different prognosis of these two categories of SMM, and we also showed that the same concept could be applied to patients diagnosed with MGUS.

Multiparametric flow cytometry has proved to be a useful technique in the study of monoclonal gammopathies (Ocqueteau *et al*, 1998; Davies *et al*, 2002; Mateo *et al*, 2005), particularly because it allows myelomatous plasma cells to be discriminated from their normal counterparts (Harada *et al*, 1993; Ocqueteau *et al*, 1998), which may be of use in differentiating the diagnoses of MGUS and MM patients (Ocqueteau *et al*, 1998), predicting the risk of progression in SMM and MGUS (Pérez-Persona *et al*, 2007), and in studying minimal residual disease in MM (Davies *et al*, 2002; San Miguel *et al*, 2002; Mateo *et al*, 2005; Paiva *et al*, 2008).

The present study showed that both factors ($\geq 95\%$ aPC/BMPC and the evolving type) had prognostic value in predicting the risk of progression in MGUS and SMM patients. The risk was clearly and more accurately identified by the immunophenotypic analyses compared with the evolving concept. Moreover, MFC allows the discrimination of two groups with different risks of progression, based on the percentage aPC/BMPC in evolving and non-evolving gammopathies. Given that both variables were independent, we decided to test the value of a scoring system based on the presence or absence of these two characteristics that discriminate different risks of progression in SMM and MGUS. It should be noted that these are complementary, since the MFC analysis provides the information upfront while the evolving concept yields information about the dynamic evolution of the MC.

In summary, we confirmed not only the existence of two prognostic groups of SMM in accordance with the evolution of MC during the early phases of follow-up, but also that the same concept can be applied to MGUS. Nevertheless, MFC analysis of the BMPC compartment is a more powerful prognostic variable for predicting progression in both SMM and MGUS. It is also important to consider that the MFC information can be obtained at the time of diagnosis, which would help to establish an individually adapted follow-up strategy from the early stages, with a lower emotional cost to patients.

Acknowledgements

This work was supported in part by a national Grant from the Spanish 'Instituto de Salud Carlos III' (Ref. PI50575) and 'Ministerio de Educación y Ciencia' (Ref. SAF: 2004-06587). We would also like to thank M. Anderson and M.J. Rodrigo for their help with the English language. This work was supported by the Cooperative Research Thematic Network (RTICs; RD06/0020/0006), MM Jevitt, SL firm, and Instituto de Salud Carlos III/ Subdirección General de Investigación Sanitaria (FIS: PI060339; 02/0905; 01/0089/01-02; and PI060339).

Author contributions

MB Vidriales, JF San Miguel and A Orfao conceived the idea and designed the study protocol. E Pérez Persona, MB Vidriales, G Mateo and C López-Berges analysed the flow cytometry data. E Pérez Persona, MB Vidriales were involved in the data analysis and co-wrote the paper, together with JF San Miguel. The paper was reviewed and corrected by JF San Miguel and A Orfao. R García-Sanz and MV Mateos were responsible for the databases of clinical cases and follow-up of patients. A. García de Coca A, J Galende, G Martín-Núñez, JM Alonso, N. de las Heras, JM Hernández JM, A Barez and A Martín were responsible for clinical cases and follow-up of patients, and contributed to the clinical data entry.

Conflict of interest

We declare that we have no conflict of interest.

References

- Cesana, C., Klersy, C., Barbarano, L., Nosari, A.M., Crugnola, M., Pungolino, E., Gargantini, L., Granata, S., Valentini, M. & Morra, E. (2002) Prognostic factors for malignant transformation in monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. *Journal of Clinical Oncology*, **20**, 1625–1634.
- Davies, F.E., Rawstron, A.C., Owen, R.G. & Morgan, G.J. (2002) Minimal residual disease monitoring in multiple myeloma. *Best Practice & Research. Clinical Haematology*, **15**, 197–222.
- Harada, H., Kawano, M.M., Huang, N., Harada, Y., Iwato, K., Tanabe, O., Tanaka, H., Sakai, A., Asaoku, H. & Kuramoto, A. (1993) Phenotypic difference of normal plasma cells from mature myeloma cells. *Blood*, **81**, 2658–2663.

- International Myeloma Working Group (2003) Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *British Journal of Haematology*, **121**, 749–757.
- Kyle, R.A., Therneau, T.M., Rajkumar, S.V., Offord, J.R., Larson, D.R., Plevak, M.F. & Melton, L.J. III (2002) A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *New England Journal of Medicine*, **346**, 564–569.
- Mateo, M.G., Miguel Izquierdo, J.F. & Orfao, A. (2005) Immunophenotyping of plasma cells in multiple myeloma. *Methods in Molecular Medicine*, **113**, 5–24.
- Ocqueteau, M., Orfao, A., Almeida, J., Bladé, J., González, M., García-Sanz, R., López-Berges, C., Moro, M.J., Hernández, J., Escribano, L., Caballero, D., Rozman, M. & San Miguel, J.F. (1998) Immunophenotypic characterization of plasma cells from monoclonal gammopathy of undetermined significance patients. Implications for the differential diagnosis between MGUS and multiple myeloma. *American Journal of Pathology*, **152**, 1655–1665.
- Paiva, B., Vidriales, M.B., Cerveró, J., Mateo, G., Pérez, J.J., Montalbán, M.A., Sureda, A., Montejano, L., Gutiérrez, N., García de Coca, A., Heras, N., Mateos, M.V., López-Berges, C., García-Boyer, R., Galende, J., Hernández, J., Palomera, L., Carrera, D., Martínez, R., de la Rubia, J., Martín, A., Bladé, C.J., Orfao, A. & San Miguel, J.F. (2008) Multiparameter flow cytometry remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. *Blood*, **112**, 4017–4023.
- Pérez-Persona, E., Vidriales, M.B., Mateo, G., García-Sanz, R., Mateos, M.V., García de Coca, A., Galende, J., Martín-Núñez, G., Alonso, J.M., de las Heras, N., Hernández, J.M., Martín, A., López-Berges, C., Orfao, A. & San Miguel, J.F. (2007) New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. *Blood*, **110**, 2586–2592.
- Rajkumar, S.V. (2005) MGUS and smoldering multiple myeloma: update on pathogenesis, natural history, and management. *Hematology*, **2005**, 340–345.
- Rawstron, A.C., Orfao, A., Beksac, M., Bezdicikova, L., Brooimans, R.A., Bumbea, H., Dalva, K., Fuhler, G., Gratama, J., Hose, D., Kovarova, L., Lioznov, M., Mateo, G., Morilla, R., Mylin, A.K., Omede, P., Pellat-Deceunynck, C., Pérez, A.M., Petrucci, M., Ruggeri, M., Rymkiewicz, G., Schmitz, A., Schreder, M., Seynaeve, C., Spacek, M., de Tute, R.M., Van Valckenborgh, E., Weston-Bell, N., Owen, R.G., San Miguel, J.F., Sonneveld, P. & Johnsen, H.E. (2008) Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. *Haematologica*, **93**, 431–438.
- Rosiñol, L., Bladé, J., Esteve, J., Aymerich, M., Rozman, M., Montoto, S., Gine, E., Nadal, E., Filella, X., Queralt, R., Carrio, A. & Montserrat, E. (2003) Smoldering multiple myeloma: natural history and recognition of an evolving type. *British Journal of Haematology*, **123**, 631–636.
- Rosiñol, L., Carrio, A., Bladé, J., Queralt, R., Aymerich, M., Cibeira, M.T., Esteve, J., Rozman, M., Campo, E. & Montserrat, E. (2005) Comparative genomic hybridisation identifies two variants of smoldering multiple myeloma. *British Journal of Haematology*, **130**, 729–732.
- San Miguel, J.F., Almeida, J., Mateo, G., Bladé, J., López-Berges, C., Caballero, D., Hernández, J., Moro, M.J., Fernández-Calvo, J., Díaz-Mediavilla, J., Palomera, L. & Orfao, A. (2002) Immunophenotypic evaluation of the plasma cell compartment in multiple myeloma: a tool for comparing the efficacy of different treatment strategies and predicting outcome. *Blood*, **99**, 1853–1856.
- Weber, D.M., Dimopoulos, M.A., Moullopoulos, L.A., Delasalle, K.B., Smith, T. & Alexanian, R. (1997) Prognostic features of asymptomatic multiple myeloma. *British Journal of Haematology*, **97**, 810–814.