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RISK FACTORS OF SHORT-TERM PROGRESSION IN PATIENTS WITH MONOCLONAL GAMMOPATHIES

Coordinatore:
Chiar.mo Prof. Carlo Ferrari

Tutore:
Chiar.mo Prof. Nicola Giuliani

Dottorando: Anna Benedetta Dalla Palma

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SUMMARY

ABSTRACT

Asymptomatic Monoclonal Gammopathies (AMG), including Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Multiple Myeloma (SMM), are pre-malignant conditions, characterized by a heterogeneous risk of progression to Multiple Myeloma (MM). The identification of risk factors for progression is crucial in the clinical management of patients with AMG, in order to properly stratify patients, plan an adequate follow-up and identify patients with short-term risk of progression, who could potentially benefit from an early treatment.

In this study, we retrospectively evaluated possible risk factors of short-term progression to active MM in a cohort of MGUS and SMM patients admitted to our haematological centre between 2010 and 2018. We analysed a total cohort of 220 patients diagnosed with AMG (73 MGUS and 147 SMM) according to the International Myeloma Working Group (IMWG) recently updated diagnostic criteria. All patients analysed underwent to Bone Marrow (BM) examination to confirm diagnosis and quantify the percentage of Bone Marrow Plasma Cells (BMPCs).

Median age of the total cohort was 68 years (range 35-93 years), and 58% were male. Light chain was kappa in 70% of patients (77% among the MGUS subgroup and 67% among the SMM subgroup, respectively). Median percentage of BM plasma cells (BMPCs) was 12% (range 2-55%) in the entire population, 7% (range 2-9) in MGUS and 15% (range 8-55) in SMM patients. Median serum M-protein was 1.7 g/dL (range: 0.17-4.5), 1.6 g/dL (range 0.5-3.3) in MGUS and 1.8 g/dL (range 0.17-4.5) in SMM patients. An abnormal serum free light chain (FLC) ratio was found in 68% of AMG patients, among the ones that performed the analysis (134/220 patients). The presence of immunoparesis in one or two of the uninvolved immunoglobulins occurred in 59% of the entire population, in 44% of MGUS and in 63% of SMM patients.

Median follow-up time was 34 months (range 3 – 126) for the whole population. Overall 52 patients of the entire cohort progressed to MM (47 SMM and 5 MGUS), with a median TTP of 16 months (range 4-75).

By univariate analysis we found that percentage of BMPCs, entity of M-protein and presence of immunoparesis were significantly correlated with progression to active MM (p<0.001 for each

variable). On the other hand, presence of an abnormal FLC ratio did not reach a statistical significance between progressed and non-progressed patients, as well as value of the involved serum FLC (p=0.078). Nevertheless, the presence of a FLC ratio < 0.125 or > 8 (as used in Mayo scoring system for SMM) showed a relationship at the limit of statistical significance in this subgroup of patients (p=0.055). Multivariate analysis confirmed percentage of BMPCs and entity of serum M-protein as independent risk factors of progression.

We therefore calculated risk of progression on the whole AMG population, showing that the two independent variables, when combined, could stratify patients in different risk groups based on the tumor burden of the gammopathy. Given that evidence, we create a "tumor-burden" score combining the two variables, and stratifying patients in 3 groups. Patients defined as low risk showed a probability of progression of 7% as compared to high risk patients, in which this probability was 70%.

Afterwards, we applied Kaplan Meier method on risk factors resulted significant in univariate analysis demonstrating that they also significantly influenced the Time To Progression (TTP) to MM. TTP was significantly different even when applied to the "tumor-burden" score (median TTP in high risk patients: 18 months, not reached in intermediate- and low-risk patients, p<0.001).

In conclusion, our work highlights the importance of entity of BMPCs and of serum M-protein, directly related to tumor burden, as risk factor in short-term progression of AMG. The development of a simple, reproducible score based on BMPCs and M-protein could allow to overcome the traditional distinction between MGUS and SMM in the evaluation of the progression of AMG patients to active MM.

1. INTRODUCTION

1.1 Asymptomatic monoclonal gammopathies.

Asymptomatic monoclonal gammopathies (AMG) are pre-malignant conditions characterized by the proliferation of a plasma cell clone that usually produces a monoclonal protein (M-protein), that can be highlighted on serum or urine.

The M-protein is an Immunoglobulin (Ig), and is made by a heavy chain, either IgG, IgA, IgM or less frequently, IgD or IgE, and a light chain, κ or λ. Sometimes the M-protein may consist of only the light chain κ or λ, which show up at urinary level as Bence Jones (BJ) proteinuria.

The definition of AMG includes two separate entities, divided substantially on the basis of the tumor burden of the gammopathy itself: Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Multiple Myeloma (SMM). AMG are moreover distinguished from symptomatic forms of monoclonal gammopathies by the absence of end-organ damage or Myeloma Defining Events (MDE).

Diagnostic criteria and definition of MGUS and SMM have varied upon years; MGUS has been first described as "essential hyperglobulinemia" in the early sixties(1). The term MGUS was coined by Robert A. Kyle in 1978(2), while the term SMM was first used in 1980(3). In 2003, the International Myeloma Working Group (IMWG) developed the first consensus definitions of monoclonal gammopathies(4): MGUS was defined by the presence of serum M-protein <3 g/dL, Bone Marrow Plasma Cells (BMPCs) <10%, no evidence of other B-cell lymphoproliferative disorder and absence of end-organ damage defined by CRAB criteria: hypercalcemia (serum calcium level >1 mg/dL above the upper limit of normal or >11 mg/dL), renal failure (creatinine level >1.95 mg/dL with no other cause of renal failure identified), anemia (hemoglobin level <10g/dL or of at least 2 g/dL below the lower limit of normal), or skeletal lesions (lytic lesions detected by skeletal survey, osteoporosis with pathological fractures or cord compression). Criteria of end-organ damage included also presence of symptomatic hyperviscosity, amyloidosis or recurrent bacterial infections. SMM was defined by the

presence of either serum M-protein ≥3 g/dL and/or monoclonal BMPCs ≥10%, and absence of end organ damage defined by CRAB criteria. Since then, diagnostic criteria of monoclonal gammopathies have been updated by IMWG in 2010 and in 2014(5); in those years, definition of AMG has changed not as regards cut-off levels of serum M-protein or percentage of BMPCs, but mostly concerning criteria for defining end-organ damage; this is in part due to the fact that clinical criteria for end-organ damage strictly depends on the sensitivity and specificity of the methods used to detect the damage, which is improved over years. Moreover, in the past few years several studies identified a series of biomarkers that were associated with a rapid and near inevitable development of CRAB criteria in SMM patients, who were then classified as ultra-high risk: these biomarkers have now been included in criteria for defining active MM, based on the observation that in patients who presented with one or more of those biomarkers, treatment delay could be detrimental.

1.1.1 Monoclonal Gammopathy of Undetermined Significance.

In the 2014 IMWG updated criteria for the diagnosis of Multiple Myeloma (MM) and related disorders(5), MGUS is defined by: (*i*) presence of serum M-component <3 g/dL, (*ii*) presence of BMPCs <10% and (*iii*) absence of end-organ damage that can be attributed to the underlying Plasma Cells (PCs) or lymphoproliferative disorders.

As mentioned above, 2014 IMWG criteria were updated mostly regarding criteria for defining and organ damage. CRAB criteria are now defined as: hypercalcemia (serum calcium level >1 mg/dL above the upper limit of normal or >11 mg/dL), renal failure (creatinine level >2 mg/dL or reatinine clearance <40 mL/min), anemia (hemoglobin level <10g/dL or of at least 2 g/dL below the lower limit of normal), or skeletal lesions (lytic lesions detected by skeletal survey, Computed Tomography -CT or Positron Emission Tomography-PET/CT). To note, the presence of osteoporosis is no more considered as bone disease, and the others signs/symptoms previously considered as related to end-organ damage (presence of symptomatic hyperviscosity or recurrent bacterial infections) are no mentioned anymore. Moreover, three highly specific biomarkers of malignancy (also known as slim-CRAB criteria) have been added to classical CRAB criteria to define the presence of active MM: (*i*) BMPCs ≥ 60, (*ii*) serum Free Light chain (FLC) ratio ≥ 100, and (*iii*) presence of > 1 focal lesion on Magnetic Resonance Imaging (MRI) studies.

MGUS can be classified according to the type of monoclonal component in: IgM-MGUS, non-IgM MGUS or light-chain MGUS (Table A).

Table A. MGUS classification.

	non-IgM MGUS	Light-chain MGUS	IgM MGUS
Serum M-protein	Serum non- IgM M-protein <3 g/dL	• Absence of heavy chain on immunofixation • Unbalanced FLC ratio & ↑ involved FLC •Urine M.protein <0.5 g/24 hours	Serum non- IgM M-protein <3 g/dL
Bone marrow infiltrate	Clonal BMPCs <10%		Clonal BM lymphoplasmacyte <10%
Absence of end-organ damage	E.g. anemia, ↑ serum calcium, ↓ renal function, presence of lytic lesions or MDE		E.g. anemia, constitutional symptoms, organomeagaly, lymphoadenomegaly
Progression rate	**1%/yr**	**0.3%/yr**	**1.5%/yr**

Abbreviations: FLC, free light chain; BMPCs, bone marrow plasmacells; MDE, myeloma defining events

Incidence of MGUS arises with age, from approximately 0.3% in patients <50 years(6), to 3.2%, 5.3% and 7.5% in patients ≥50, ≥75 and ≥85 years, respectively(7); moreover, incidence is higher in male than in female patients(8). A population-based study on 12.482 persons estimated a prevalence of 2.4%, and an adjusted prevalence higher in blacks compared with whites or Hispanic(9).

Presence of MGUS is associated with an increasing risk to develop MM or, less frequently, other lymphoproliferative disorders; however, MGUS is also associated with the presence of other conditions(10). In the past few years, the term Monoclonal Gammopathy of Clinical Significance (MGCS) have been introduced, to define a group of monoclonal gammopathies in which the organ damage is directly related to the presence of an M-protein through various mechanism: directly deposition and accumulation of the M-protein (e.g. in AL Amyloidosis, Type I Crioglobulinemia, Light Chain Deposition Disease), autoimmune activity of the M-protein against several antigens (e.g. in Mixed Crioglobulinemia, Cold Agglutinine Disease and in the MGUS-related polyneuropathies)

or complement activation or secretion of pro-inflammatory cytokines, like what happens in POEMS syndrome(11).

1.1.2 Smoldering Multiple Myeloma.

Smoldering Multiple Myeloma (SMM) is the other premalignant plasma cell disorder, characterized by a higher tumor burden and a higher risk of progression to symptomatic MM as compared to MGUS. Similar to MGUS, it is also characterized and distinguished from symptomatic forms of monoclonal gammopathies by the absence of end-organ damage(12). According to the 2014 International Myeloma Working Group (IMWG) updated criteria(5), definition of SMM requires the following parameters (both criteria must be met):

- Serum monoclonal protein (IgG or IgA) ≥3 g/dL or urinary monoclonal protein ≥500 mg per 24 h and/or clonal bone marrow plasma cells 10–60%;
- Absence of MDE or amyloidosis. MDE refer to the presence of the "classical" CRAB symptoms or to the presence of one or more of the biomarker of malignancy, previously enlisted.

SMM is usually IgG or IgA, others Igs are less frequently involved; could also be light-chain only(13). Incidence of SMM differs from one series to another; in a paper published in 2016 by Ravindran et al.(14) the incidence of MM in the United States was approximately 0.9 cases per 100.000 persons; among the cohort examined, SMM patients represented 13.4% of the total myeloma population. In a previous Swedish based-population study incidence was reported as 0.44 cases per 100.000 persons, and 14.4% of total myeloma patients were smoldering at diagnosis.

1.2 Pathogenesis and natural history of monoclonal gammopathies.

AMG consistently precedes MM: almost all cases of MM derives from the asymptomatic stage of SMM, either detected or not; SMM, in turn, derives from MGUS, which is characterized by a lower tumor burden(15,16). A recent review(17) proposed 3 different models to better understand progression from MGUS to SMM to MM. The first is the "clinical" model, that consider tumor burden as the principal factor that determines progression of monoclonal gammopathies. Several studies have

shown that increasing tumor burden is associated with risk of progression from MGUS and SMM to MM(18,19). However, this model has some limitations, since the fact that there are patients with low tumor burden that progresses among few years, and patients classified as SMM due to their higher tumor burden, that have a rate of progression similar to those with MGUS(20), suggesting the importance of detecting the underlying molecular alterations that could explain those differences.

The second model of progression is the "molecular model", that highlighted the fact that tumoral progression is determined by the progressive acquisition of genetic alterations. In the classical view of transformation from MGUS/SMM to MM, a initiating hit is required to immortalize a myeloma-propagating cell, which is destined to acquire additional genetic hits, through loss of heterozigosity, gene amplification, mutation or epigenetic changes: this additional hits deregulate the myeloma-propagating cell, leading to clinically recognized features of MM; however, emerging insights based on the currently best available technologies suggest that there is substantial complexity in the genetic basis of multiple myeloma and its precursor states(16,21). Several studies revealed that cytogenetics and genetic alterations detected in MM cells can also be found in precursor cells(22–24). Using single nucleotide polymorphism-based mapping arrays, a progressive increase in the incidence of copy number abnormalities from MGUS to SMM and to MM, as well as gains on chromosome 1q, and 1p, 16q and 22q deletions have been observed to be less frequent in MGUS than in MM. Although MM has more copy number abnormalities than its precursors, MGUS is as genetically aberrant as MM, and the progression from MGUS to MM, through the intermediate stage of SMM, seems to be related to an expansion of altered clones already present in MGUS(25). Recently, a study by our group(26) compared the transcriptome of plasma cells (PCs) from paired samples of SMM patients progressed to active MM with a short Time to Progression (TTP), without finding significant difference in the profile of SMM and their paired MM samples. The transition from MGUS to MM involves moreover changes due to the complex interaction between the malignant plasma cells and the BM microenvironment, which plays a key role in MGUS/SMM initiation and progression. Although the BM microenvironment is commonly considered as a "nontumor" entity, it is a complex network including a wide range of cells and factors; a definite pathogenic role for the BM niche in the progression of MGUS and SMM to active MM remains under investigation(27), but increasing evidences suggest that malignant transformation may depend in part by interactions of tumor cells

with the BM microenvironment(28). The third model of progression from AMG to MM is the "clonal" model. Clonal evolution has been first described by Morgan et al.(16). After the initial hit that originates the first clone of the disease, progression from MGUS to SMM to MM can follow 4 different patterns of clonal evolution: branching, differential, stable and linear(29)(figure A); recent data suggest that progression may also be mediated via competition between subclones, and outgrowth of the fittest clone(23).

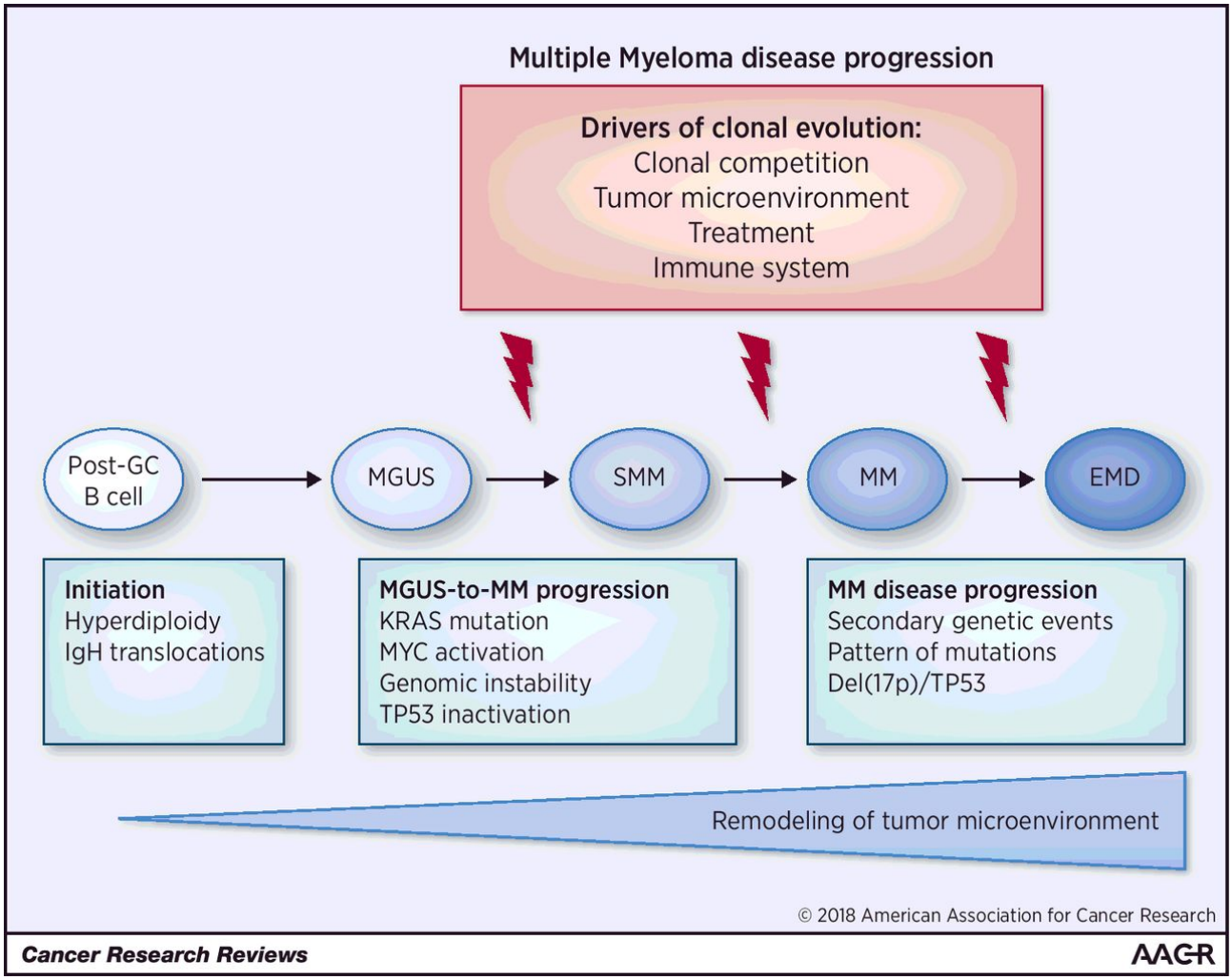


Figure A. Clonal evolution of monoclonal gammopathies. Reproduced from *Niels van Nieuwenhuijzen et al. Cancer Res, 2018.*

1.3 Stratifying monoclonal gammopathies: a neverending story.

Given the fact that all MM are consistently preceded by an asymptomatic phase of MGUS and SMM, risk-stratification of AMG, when detected and diagnosed, are crucial in the management of those

conditions, in order to properly plan an adequate follow-up and identify those patients who could eventually benefit from an earlier treatment strategy.

1.3.1 Risk stratification in MGUS.

MGUS progresses to MM with an average of about 1% per year(7,30). In the past years, three score systems have been validated in MGUS:

- The Mayo score(31), that was developed from a study on 1148 patients with a median follow-up of 15 years, in which three factors were related to a shorter time to progression: (*i*) type of MGUS (non-IgG MGUS), (*ii*) entity of serum M-protein (> 1.5 g/dL), and (*iii*) an unbalanced serum FLC ratio. Patients were stratified in 4 groups, according to the presence of none to all the three factors: risk of progression arises from 5% at 20 years with no risk factors, to 58% at 20 years with 3 risk factors. Entity of serum M-protein and the presence of an unbalanced serum FLC ratio have been confirmed as risk factor for progression in a recent publication, that analyzed data among 1384 patients with MGUS, with a median follow-up of 35 years(30). The paper, moreover, highlighted that IgM MGUS were characterized by a higher risk of progression than non-IgM MGUS: according to the presence of none, one or two factors, risk of progression at 20 years was respectively 7%, 20% and 30% for non-IgM MGUS, and 19%, 41% and 55% for IgM MGUS.
- The PETHEMA score(32), that has been developed from a study on 407 patients, with a median follow-up of 56 months. The study identified 2 factors significantly related to progression: (*i*) presence of BMPCs with aberrant phenotype (defined as absence of CD19 and/or CD45, reduced expression of CD38, positivity of CD56) >95%, and (*ii*) presence of DNA aneuploidy. Risk of progression at 5 years, according to the presence of none, one or two risk factors was 4%, 46% and 72%, respectively.
- The "Danish" score, published by Turesson et al.(33), that combines the three factors considered by the Mayo score with the presence of immunoparesis. The study was conducted on 728 MGUS patients, with a median follow-up of 10 years: the cumulative probability of progression at 10 years ranged from 4% with no risk factors, to 40% with all 4 risk factors.

There are many other factors that have been related to progression in MGUS, both clinical and molecular. Cesana C. et al(34) evaluated a cohort of 1104 MGUS patients, with a median follow-up of 65 months, and showed that clonal BMPCs >5%, immunoparesis, elevated erytocyte sedimentation rate (ESR) and presence of BJ proteinuria were independent factors for progression. The Spanish Pethema group(35) highlighted the role of the pattern of evolution of M-protein in MGUS progression; more recently, in a prospective study on AMG, the presence of a 70 gene-expression profiling signature has been related with an increased risk of progression to symptomatic disease, and when combined with elevated serum FLC and entity of serum M-protein identified a subset of patients with high risk of progression (67% at 2 years) (36).

The presence of bone disease is one of the hallmark of symptomatic MM(37); few studies have evaluated the possible role of markers of bone remodeling, and osteoclast activation/function in progression from MGUS to MM. Politou et al.(38) found higher serum levels of N-telopeptide of Collagen-1 (NTX), a marker of bone resorbtion, soluble RANKL (sRANKL) and sRANKL/osteoprotegerin (OPG) ratio (both markers of osteoclast activation) in MGUS patients as compared to controls. Ng et al.(39) found high serum levels of the Wnt pathway inhibitor Dickkopf-related protein 1 (DKK1) and of the osteoclast-activating factor MIP-1α in MGUS patients. More recently, a study from our group found that BM levels of several cytokines involved in the altered bone remodeling of MM were significantly different in SMM, MGUS and MM patients(40).

Unfortunately, there are no study comparing head-to-head the different risk-stratification scores; however, in routine clinical practice the most used score is probably the Mayo score, given its easy reproducibility.

1.3.2 Risk stratification in SMM.

SMM patients are characterized by ah higher risk of progression as compared to MGUS, not stable among years: is approximately 10% per year for the first 5 years, then 3% for the next 5 years, and 1% for the subsequent 10 years and is influenced by the proportion of BMPCs and the serum M-protein level at diagnosis(41). However, SMM are very heterogeneous, including patients with very different risk of progression to MM: patients similar to those with MGUS, with a very low progression rate, as well as patients with a more rapid pattern of progression to MM.

A better stratification of patient could help to differentiate low-risk patients, that could be approached like MGUS patients, from high-risk patients, that could be followed up more frequently and possibly considered from early treatment strategies.

Factors related to a ≥50% risk of progression at 2 years can be grouped as follows(42):

1) Features related to tumor burden; ≥10% clonal plasma cell bone marrow infiltration plus ≥3 g/dL of serum M-protein and:
    a. Serum free light-chain ratio of 0.125 or less or 8 or more
    b. Bence-Jones (BJ) proteinuria positive from 24-h urine sample
    c. Peripheral blood circulating plasma cells (CPCs) >$5x10^6$/L
2) Immunophenotyping Characterization and Immunoparesis: ≥95% of aberrant plasma cells by flow cytometry within the plasma cell bone marrow compartment plus immunoparesis (>25% decrease in one or both uninvolved immunoglobulins relative to the lowest normal value)
3) Cytogenetic and Molecular Abnormalities:
    a. Presence of t(4;14)
    b. Presence of del(17p)
    c. Gain of 1q24
    d. Hyperdiploidy
    e. Gene Expression Profiling risk score >-0.26
4) Pattern of serum M-Component Evolution:
    a. Evolving type: if M-protein ≥3 g/dL, increase of at least 10% within the first 6 months. If M-protein ≥3 g/dL, annual increase of M- protein for 3 years
    b. Increase in the M-protein to 3 g/dL over the three months since the previous determination
5) Imaging Assessments:
    a. MRI: Radiologic progressive disease (MRI-PD) defined as newly detected focal lesions (FLs) or increase in diameter of existing FL and a novel or progressive diffuse infiltration.
    b. Positive PET/CT with no underlying osteolytic lesion.

Over the past years, several models of progression have been developed, based on those different features. Until a few years ago, the two most used models were the Mayo score and the Pethema score. The Mayo score(19) considers factors related to tumor burden, and is based upon a retrospective evaluation of 273 patients, with a median follow-up of 12.4 years; risk of progression to symptomatic MM was stratified by the presence of one or more of 3 characteristic: serum M-protein ≥3 g/dL, serum FLC ratio ≤0.125 or ≥8, and ≥10% of BMPCs. At 5 years of follow-up, SMM patients with all three risk factors had a cumulative risk of progression of 76% (with a median TTP of 1.9 years); for patients with two or one risk factors, the progression risk was 51% (median TTP 5.1 years) and 25% (median TTP 10 years), respectively. The Pethema score(32), conducted on 93 patients with SMM, identified two factors that were associated with progression: immunoparesis (defined as a reduction in the levels of 1 or 2 Ig, with respect to the values of the corresponding uninvolved Ig) and the presence of ≥95% aberrant plasma cells by multiparametric flow cytometry on BM aspirates (defined by the absence of CD19 and/or CD45, the decreased expression of CD38, and overexpression of CD56 on plasma cells). In SMM patients, Progression Free Survival (PFS) at 5 years was 4%, 46% and 72% for patients with respectively no, one or two risk factors. To note, the two models showed significant discordance in a prospective study on risk stratification in SMM(43).

Recently, a novel prognostic score has been proposed(44), incorporating the 2014 updated IMWG classifications of monoclonal gammopathies and based upon the presence or absence of the following criteria: (*i*) serum M-protein ≥2 g/dL, (*ii*) BMPCs ≥20%, and (*iii*) serum FLC ratio ≥20. The initial study included 421 patients; median TTP was 110, 68 and 29 months, respectively, for patients with none, one or ≥2 risk factors. The same parameters have been adopted by a larger study, conducted on 1996 patients(45): 2-years progression rate in the 3 risk groups were 6%, 18% and 44% respectively. Main SMM prognostic scores are summarized in Table B.

Table B. Scoring systems in SMM patients.

	Risk factors	Probability of progression (yrs)
Mayo Score	- serum M-protein ≥3 g/dL - serum FLC ratio ≤0.125 or ≥8 - BMPCs ≥10%	- 1 risk factor: 25% (5 yrs) - 2 risk factors: 51% (5 yrs) - 3 risk factors: 76% (5 yrs)
Pethema score	- Presence of immunoparesis - presence of ≥95% aberrant BM plasma cells	- 0 risk factor: 4% (5 yrs) - 1 risk factors: 46% (5 yrs) - 2 risk factors: 72% (5 yrs)
20-2-20 score	- serum M-protein ≥2 g/dL - BMPCs ≥20% - serum FLC ratio ≥20	- 0 risk factor: 6% (2 yrs) - 1 risk factors: 18% (2 yrs) - 2+ risk factors: 44% (2 yrs)

Abbreviations: yrs, years; FLC, free light chain; BMPCs, Bone Marrow Plasmacells.

Regarding the other aforementioned factors, several studies have validated their prognostic role in SMM patients. The role of BJ proteinuria has been outlined in a Spanish retrospective trial on 152 SMM patients, that showed a 51% risk of progression at 2 years in patients presenting with BJ proteinuria and a shorter TTP on the basis of the amount of the BJ proteinuria(46). The role of peripheric circulating plasma cells (CPCs) has been highlighted by two different studies: the first observed a higher of 2-year progression rate (71% *versus* 24%, $p<0.001$) in patients with CPCs $>5x10^6$/L, detected by an immunofluorescence assay(47); a more recent paper evaluated the presence of CPCs by flow cytometry, and correlates their levels with TTP to symptomatic MM(48).

There are few studies evaluating the impact of cytogenetic abnormalities, detected by Fluorescent in Situ Hybridization (FISH) on SMM progression. Rajkumar et al.(49) showed that patients carrying t(4;14) had a significantly shorter TTP than patients with t(11;14). Neben et al.(50) confirmed the prognostic role of t(4;14) and found that also del(17p), gain 1q21 and hyperdiploidia were associated with shorter TTP to active myeloma. More recently, FISH alterations have been associated with clinical factors (BMPCs≥20% and serum FLC ratio ≥20)(44) showing that patients carrying high-risk FISH alterations (defined as presence of t(4;14), del(17p) or hyperdiploidia) in combinations with the other 2 factors (BMPCs ≥20%and FLC ratio ≥20) had a significantly shorter TTP to symptomatic myeloma.

Two studies explored Gene Expression Profile (GEP) in SMM; one is the previously reported study on AMG, in which the presence of a 70 gene-expression profiling signature has been related with an

increased risk of progression to symptomatic disease, and when combined with elevated serum FLC and entity of serum M-protein identified a subset of patients with high risk of progression (67% at 2 years)(36). GEP of SMM patients has also been evaluated by Khan R et al.(51): in this work, a risk stratification model based on expression levels of four genes, serum M-protein and albumin levels was generated: high-risk, intermediate-risk, and low-risk groups had a 2-year progression probability of 85.7%, 44.8% and 5.0%, and respectively.

As far as in MGUS, role of evolving change in monoclonal protein level (eMP) and in hemoglobin in progression of SMM have emerged in a recent paper(52): the 2-year progression risk was 81.5% in individuals who demonstrated both eMP and eHb.

Imaging techniques, especially MRI and Positron Emission Tomography/Computed Tomography (PET/CT) emerged as risk factors for progression in several studies; a retrospective evaluation conducted at Mayo Clinic on 122 patients with suspected SMM found that the probability of progression to MM within 2 years was 75% in patients with a positive PET/CT compared with 30% in patients with a negative PET/CT; median time to progression was 21 months versus 60 months, respectively (p=0.0008); probability of progression, moreover, was higher in the positive group, in the presence of underlying osteolysis(53). In an Italian series of 120 SMM patients evaluated with PET/CT, 16% presented with focal lesion, and exhibited a probability of progression of 58% within 2 years, as compare to 33% probability of progression in PET/CT negative patients(54). The prognostic role of spinal or whole-body MRI in progression of smoldering myeloma has emerged in several studies(36,55,56). Interestingly, a recent publication outlined that different MRI protocols (spinal *versus* spinal + pelvic *versus* whole-body) can leads to different staging decisions, according to slim-CRAB criteria; MRI protocols limited to the spine or spine + pelvis, indeed, lead to underdiagnoses of patients who actually have >1 focal lesion; however, in the study a probability of progression of 80% at 2 years (who is actually the goal of the slim-CRAB criteria) was observed in patients with >3 focal lesions in spine + pelvis or >4 focal lesions on whole body MRI, suggesting a revision of the cutoff for the number of focal lesions to be considered as slimCRAB criteria(57). Finally, regarding the role of cytokines involved in bone disease in MM, our group has recently showed the role of BM levels of DKK1 as independent risk factors for progression in SMM patients(58).

1.4 Follow-up of monoclonal gammopathies.

No prospective data supporting utility of routine follow-up of MGUS are available; however, follow-up of MGUS is still recommended given the seriousness of potential complications at progression(59). MGUS patients should be followed-up on the basis of their risk stratification. In 2014, the European Myeloma Network (EMN) suggested a first follow-up at 6 months for all patients; thereafter, patients should be followed every 1-2 years if low risk, or every year if intermediate or high risk (based on Mayo score)(10). The previously reported paper(59) suggests that, after a first reassessment at 6 months, confirmed low-risk patients shouldn't need subsequent controls in the absence of suspect of progression of the gammopathy, whereas intermediate or high-risk patients should be followed up every year. Since the low progression rate of MGUS, subsequent controls, moreover, may be avoided in patients with life expectancy < 5 years.

SMM follow-up must be more frequently, given its higher risk of progression to symptomatic MM, especially in the first 5 years from diagnosis. The Mayo Clinic group recently proposed the following approach for SMM follow-up(60): all patients, despite of their risk, should undergone a first follow-up at 3 months from diagnosis; then, in confirmed high-risk patients, they recommend to continue a 3 months follow-up, while in intermediate or low-risk patients subsequent controls could be deferred to twice a year. After 5 years, if patients remain stable, controls may be delayed to once a year.

1.5 Emerging treatment for SMM.

Actually, patients with SMM have no clear indication to start treatment, and current approach is observation until development of CRAB or slimCRAB symptoms. In the past few years, however, many trials investigated the possibility of an early treatment approach in those patients, given the fact that several new drugs have become available, and in order to avoid the potentially detrimental complication which could follow development of CRAB symptoms and, at the end, prolong survival in MM patients.

Treatment approaches in SMM can be substantially divided in two categories: an "immunomodulatory" strategy, aimed to restore the control of the immune system of the patient upon

the plasma cell clone, and a “curative” strategy, aimed to completely eradicate the plasma cell clone, in order to avoid risk of subsequent progression of the disease, and possibly “cure” the patient.

The immunomodulatory drug Lenalidomide has been tested in clinical trials alone or in combination with Dexamethasone. The recently published updated results of the QuiReDex trial(61) showed that treatment with Lenalidomide and Dexamethasone significantly prolonged TTP to active MM as compared to observation only (median TTP at 6 years of follow-up: not reached versus 23 months); similarly, treatment with Lenalidomide alone significantly delayed progression to symptomatic myeloma in another randomized trial(62) (1-, 2-, and 3-year Progression Free Survival: 98%, 93%, and 91% for the lenalidomide arm versus 89%, 76%, and 66% for the observation arm, respectively).

On the other side, a more intensive approach with 2 or 3-drugs combinations followed by Autologous Stem Cell Transplantation (ASCT) has been tested in few trials; the preliminary results of the GEM-CESAR trial showed a 30-months PFS of 93% after treatment with Carfilzomib, Lenalidomide ad Dexamethasone (KRd) as induction followed by ASCT and KRd consolidation; moreover, each phase of therapy was associated with increasing rates of MRD negativity (31% after induction, 56% after ASCT, 63% after consolidation)(63).

2. AIM OF THE STUDY.

AMG are composed by a heterogeneous group of patients, with different risk of progression. A huge effort has been made in the past few years, in order to identify factors that could precisely stratify patients, and then help hematologists or other specialists to plan an adequate follow-up, or address patient to starting treatment. However, there are still medical needs: some scores utilize parameters and technologies that aren't available in all hematology centers, and therefore couldn't be applied widely. Among the clinical risk factors identified, moreover, serum FLC and FLC ratio can be measured with different laboratory kits, and the use of these different methodologies could potentially affect the results, and so their importance as risk factors.

Given those assumptions, aims of this observational, retrospective, single-centre study were:

- validate in a real-life perspective the main known risk factors for AMG, in order to identify those factors that mostly impact on short-term progression to symptomatic MM;
- propose a simply and reproducible score that could help clinicians to overcome conventional distinction between MGUS and SMM, properly stratify patients only on the basis of their actual risk of progression to MM, and subsequently plan an adequate follow-up for those patients
- identify patients at higher risk of progression that, given the recent evidences regarding early treatment of high-risk SMM patients, could potentially benefit from starting therapy earlier, avoiding risk of potentially severe complications.

3. SUBJECTS AND METHODS.

3.1 Study cohort.

This is a single-center, retrospective observational study in which we included patients who underwent bone marrow aspirate and/or biopsy at our Institution between January 2010 and November 2018 by routine clinical practice. All patients had an AMG detected by hematochemical exams; after bone marrow biopsy, were classified as MGUS or SMM according to the 2014 revised IMWG criteria for the diagnosis of monoclonal gammopathies. Study protocol was approved by Ethic Committee of the "Area Vasta Emilia Nord" (Modena, Italy).

Baseline laboratoristic parameters evaluated included hemogram with hemoglobin value (reference ranges – r.r. 13.5-17.5 g/dL), renal function with serum creatinine levels (r.r. 0.5-1.4 mg/dL), serum calcium (r.r. 8.3-10.5 mg/dL), β_2-microglobulin (r.r. 1.2-2.5 mg/L), LDH (r.r. 250-500 U/L), serum protein electrophoresis (SPEP), serum or urine M-protein levels, involved and uninvolved immunoglobulins levels (r.r. IgG 700-1600 mg/dL, IgA 70-400 mg/dL, IgM 40-230 mg/dl), serum and urine immunofixation, with detection of heavy and light chain involved. Serum FLC assay was performed mostly at the central laboratory of our Institution, starting from 2011, with the N Latex FLC reagents (Siemens Healthineers, Eschborn, Germany, r.r.: k = 6,7-22,4 mg/L; λ = 8.3-27.0 mg/L, ratio 0.31-1.56).

Percentage of clonal plasma cells in the bone marrow (BMPCs) was quantified either by aspirate and/or biopsy and the higher value was reported; interphase Fluorescence in Situ Hybridization (FISH) analysis has been performed in those patients in which levels of plasma cells were enough to allow the examination.

The following factors were collected given their known role as risk factors of progression in AMG.

- Percentage of BMPCs, considered either as a continuous variable or a categorical variable, therefore stratifying patients into 2 classes (patients with BMPCs < 20% and patients with BMPCs ≥ 20%).

- Entity of serum-M protein, also considered either as continuous or categorical variable. Patients were stratified according to serum M-protein levels in 3 different groups: *i*) serum M-protein < 1.5 g/dL (or < 200 mg/24 hours urine output), *ii*) serum M-protein ≥ 1.5 and < 3 g/dL (or between 200 and 500 mg/24 hours urine output), and *iii*) serum M-protein ≥ 3 g/dL (or < 500 mg/24 hours urine output). The 3 g/dl cutoff was used also to stratify patients into 2 classes, to obtain another categorical variable.
- Presence or absence of immunoparesis (defined as reduction of levels of at least one uninvolved immunoglobulin).
- Involved serum FLC levels, and the ratio between involved and uninvolved serum FLC. Serum FLC ratio has been codified as categorical variable too, on the basis of the presence/absence of an unbalanced FLC ratio. In SMM subpopulation, FLC ratio has been stratify using the Mayo score cutoff: *i*) FLC ratio between 0.125 and 8; *ii*) FLC ratio < 0.125 or > 8, and the "20-2-20" score cutoff (serum FLC ratio < 20 or ≥20).
- For SMM subpopulation, we calculated, when applicable, Mayo score(19) and "20-2-20" score(44).

Imaging was performed in order to determine the presence of bone involvement. Standard exams to detect bone lytic lesion was whole-body skeletal x-ray; given its known low specificity, it has been replaced at our institution, since 2015, by whole-body low-dose CT (WBLDCT). Spinal Magnetic Resonance Imaging (MRI) was used to demonstrate the presence focal lesions, but also to describe other patterns of involvement of bone marrow, such as diffuse pattern, mixed pattern or "salt and pepper" pattern. ^{18}-Fluorodeoxyglucose (FDG) Positron Emission Tomography (PET)/Computed Tomography (CT) was used to identify the presence of hypercaptation at bone level, corresponding to an area with an higher cellular metabolism, with or without lysis.

Instrumental examinations were considered only if performed within three months from BM evaluation. Presence of focal lesion at MRI studies were considered as SMM-associated risk factors. All features were obtained analyzing patients' medical records, and data considered if performed within one month from execution of the bone marrow biopsy/aspirate.

All patients were subsequently followed at 3, 6 or 12 months, on the basis on their risk of progression;

to detect signs of progression, by routine clinical practice we usually execute hemogram, serum creatinine and calcium, SPEP, serum and urine M-protein levels, serum FLC and FLC ratio (data not collected).

Time to progression (TTP) was defined as duration between confirmed diagnosis of SMM and progression to symptomatic MM, determined by onset of CRAB criteria (Hypercalcemia: serum calcium >1 mg/dL higher than the upper limit of normal or >11 mg/dL; Renal insufficiency: creatinine clearance <40 mL/min or serum creatinine >2 mg/dL; Anemia: hemoglobin value of >2 g/dL below the lower limit of normal, or a hemoglobin value <10 g/dL; Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET/CT) or slim-CRAB criteria (clonal BMPCs percentage >60%, involved/uninvolved serum free light chain ratio ≥100, >1 focal lesions on MRI studies), or censored at last follow-up. For patients progressed, data regarding clinical features at progression (presence or absence of CRAB or slimCRAB symptoms, and more specifically presence and number of bone lytic lesions) were also collected.

3.2 Sample collection and purification of CD138+ plasmacells.

BM aspirates and biopsies were obtained from the iliac crest of all patients, in order to quantify the percentage of clonal BMPCs after informed consent according to the Declaration of Helsinki.

BM mononuclear cells were obtained from BM samples of patients by Ficoll-Hypaque (Bichrome AG, Berlin, Germany) density sedimentation. $CD138^+$ plasma cells were purified from mononuclear cells through an immunomagnetic method, using anti-CD138 monoclonal antibody-coated microbreads (MACS, Miltenyi Biotec, Bergisch-Gladbach, Germany).

3.3 BMPCs phenotype analysis.

Multiparameter Flow Citometry (MFC) immunophenotyping has been performed on EDTA-anticoagulant bone marrow aspirate sample. The samples have been processed with the general staining procedure and lyse&wash protocol. Staining panels of the following monoclonal antibodies conjugated with chromophores have been employed: CD45, CD19, CD56, CD38, CD138.

Approximately 1 million cells have been acquired for each sample. Acquisition and analysis of samples has been performed on a two-laser FACSCanto II or on a FACSCelesta instrument (both BD Biosciences, Franklin Lakes, NJ, USA), using FACSDiva software. The analysis focused on the identification and enumeration of the normal and aberrant PCs, and the panel of monoclonal antibodies expressed. Moreover, PETHEMA score for SMM has been calculated(32).

3.4 Cytogenetic and FISH analysis.

Cytogenetic and FISH analysis has been performed on fresh CD138+ plasma cells purified by immunomagnetic method (Miletnyi), testing the presence of: del(13q) (D13 S319SO/CEP 12SG, Metasystems, Altlussheim, Germany); del(17p) (LSI ATM SG/p53SO, Metasystems, Altlussheim, Germany); chromosome 14 translocation (14 BREAK-APART, Metasystems, Altlussheim, Germany), if positive, t(4;14) (FGFR3SO/IGHSG, Abbott Laboratories, Abbott Park, IL), t(11;14) (LSI IGH/CCND1XT, Laboratories, Abbott Park, IL) and t(14;16) (IGH/MAF, Abbott Laboratories, Abbott Park, IL); hyperdiploidy (ON9RED/15GREEN, Kreatech, Diagnostics, Durham, NC); amp(1q21) and del(1p32) (XL1p32SG/1q21SO, Metasystems, Altlussheim, Germany).

Patients were divided into two prognostic groups with either del(17p), t(4;14) or t(14;16) considered to be high-risk and others considered to be standard risk.

3.5 Statistical analysis

Quantitative variables were compared by non – parametric Mann-Whitney test. Categorical variables were analyzed by contingency tests as appropriate: Fisher's test is used to examine the significance of the association between only two kinds of classification; Chi-Square is used to determine whether there is a significant difference between the expected and the observed frequencies in one or more categories if alternatives were more than 2.

Firstly, we did a general descriptive analysis to obtain averages of quantitative variables and distribution for categorical variables for total population and for each subpopulation (MGUS and SMM).

Afterwards we correlated main clinical features and risk factors to progression to active MM. Variables achieving a significant level on univariate analysis were subsequently included in a multivariate Cox regression analysis to identify significant independent predictors of progression to active MM.

The influence of single variables on TTP to symptomatic MM was studied with Kaplan-Meier method, and the log-rank test was used to make comparisons between groups.

Moreover, for variables resulted significant on univariate analysis we calculated the probability of progression with binomial logistic regression.

Results had been considered significant at $p \leq 0.05$. The tests were performed using SPSS software.

4. RESULTS

4.1 Main charachteristics of the cohort of patients: descriptive analysis.

We initially selected a total number of 235 patients (154 SMM and 81 MGUS), who underwent a bone marrow biopsy at our centre between January 2010 and November 2018. 15 of them had a follow-up of less than 3 months, and were subsequently excluded from the study. So, the final analysis was conducted on 220 patients (147 SMM and 73 MGUS).

Median age at histologic diagnosis of monoclonal gammopathy was 68 years (range 35-93); male represented the majority (58%) of patients, in line with literature data. Light chain was kappa in 70% of patients and lambda in the remaining 30%; heavy chain was IgG in 77% of patients, IgA in 19%, IgM in 2% and biclonal (IgG/IgA) and IgD in 1% of patients; 3 patients had a light-chain only monoclonal gammopathy. Median BMPCs was 12% (range 2-55%), and median serum M-protein was 1.7 g/dL (range 0.17-4.5 g/dL). Immunoparesis was present in 59% of patients. Regarding serum FLC, the data was available for 134 patients: median involved FLC value was 54.1 mg/L, and 68% of patients had an unbalanced FLC ratio.

We also collected data regarding Hb value (*n*=209, median value 13.3 g/dL, range 7.4-17.8 g/dL), serum creatinine (*n*=198, median value 0.9 g/dL, range 0.5-11.5 mg/dL) and serum calcium (*n*=143, median value 9.2 mg/dL, range 7.3, 10.8 mg/dL). Beta2-microglobuline was available in 98 out of the 220 patients, with a median value of 2.6 mg/L (range 1.1-11.2 mg/L).

Regarding the SMM proportion of the cohort, median age at diagnosis was 68 years (range 36-93); 60% of patients were male. Light chain was kappa in 67% of patients, and lambda in 33%; heavy chain was IgG in 74% of cases, IgA in 25%, IgM, IgD or bi-clonal in 1%; 2 patients had light-chain SMM. Median BMPCs was 15% (range 8-55), and median level of M-protein was 1.8 g/dL (range 0.17 – 4.5 g/dL). 63% of patients had immunoparesis. FLC was determinated in 88 patients (FLC ratio was available, therefore, in 95 patients), with a median involved FLC of 56.8 mg/L (range 7.06-2360 mg/L), and FLC was unbalanced in 74% of patients. Regarding SMM risk stratification scores, Mayo score was calculated in 89 patients: 50 had 1 risk factor, 36 had 2 risk factors and only 3 had 3

risk factors. 20-2-20 score was available in 86 patients: 43% were classified as low-risk, 31% as intermediate-risk and 26% as high risk.

Median follow-up for the entire cohort was 34 months. 52 patients (5 MGUS and 47 SMM) progressed to symptomatic myeloma, with a median Time To Progression (TTP) of 16 months. 67% of patients progressed with anemia, 54% with bone disease, 10% with renal insufficiency and 8% with hypercalcemia.

We also collected data on imaging; given the fact that patients were diagnosed with asymptomatic monoclonal gammopathy, only a small proportion of them underwent bone imaging. 62 patients (15 MGUS and 47 SMM) underwent a skeletal x-ray survey. The exam was negative in all patients with the exception of 2, both with skull small lytic lesions. Whole-body low-dose TC was performed in 19 patients (17 SMM and 2 MGUS) and all resulted negative for lytic lesions attributable to the monoclonal gammopathy, whereas 97 patients (91 SMM and 6 MGUS) underwent spinal MRI. Regarding MRI, 8 patients with SMM had a positive MRI; 5 of them subsequently progressed to symptomatic MM. Of the progressed patients, 3 had a single focal lesion on MRI pattern and 2 had a positive RMN, without a clear description of the MRI pattern. 22 patients (18 SMM and 4 MGUS) performed a PET/TC scan: 3 of them had a positive exam, all without lytic lesion and with a SUV < 3; 2 of them subsequently progressed to symptomatic MM. However, given the small number of patients with a skeletal imaging, and the very low incidence of positive exams, no further analysis was conducted on bone imaging features. Table 1 summarizes the main characteristic of the entire cohort, and of the SMM proportion of the population.

4.2 Univariate analysis.

4.2.1 Total population.

Firstly, we performed univariate analysis on general characteristic of the cohort of patients, to compare progressed and non-progressed patients. No statistically significant differences were seen among the two cohorts regarding median age at diagnosis, sex, light and heavy chain distribution. We also collected data on median serum Hb, creatinine, calcium, LDH and beta-2-microglobuline level,

without finding any significant difference in progressed *versus* non-progressed patients (data not shown).

We then analyze known prognostic factors in monoclonal gammopathies. BMPCs resulted significantly higher in progressed patients (median percentage of BMPCs: 23% vs 11%, $p<0.001$). Serum M-protein, as previously described, was considered in 3 different ways, always resulting significantly different in progressed *versus* non progressed patients.: as a continuous variable (median serum M-protein levels: 2.3 versus 1.6 g/dL, $p<0.001$), stratifying population in two cohorts with the cut-off of 3 g/dL (or 500 mg/24 hours for light-chain only gammopathy, $p=0.007$), and in three cohorts as previously described ($p<0.001$). Immunoparesis was confirmed as a statistically significant parameter too ($p<0.001$).

Subsequently, we analyzed data on serum FLC and FLC ratio. Both median serum FLC and FLC ratio was higher in progressed patients, however without reaching a statistical significance (serum FLC median levels: 104 *versus* 49.1 mg/L, $p=0.078$; FLC ratio median levels: 6.6 *versus* 3.12, $p=0.076$). FLC ratio were considered also as a categorical variable, and patients stratified according to the presence or absence of an unbalanced serum FLC ratio; however, this parameter was not associated with a shorter TTP to symptomatic myeloma ($p=0.313$).

Given the fact that few patients progressed from MGUS, we conducted separate analysis only for SMM cohort.

4.2.2 SMM cohort.

The same univariate analysis were therefore conducted on SMM patients. We confirmed that age, sex, heavy or light chain were not statistically different in progressed *versus* non-progressed SMM, as far as median serum Hb, creatinine, calcium, LDH and beta2-microglobuline levels (data not shown).

BMPCs was higher in progressed patients (median BMPCs 25% *versus* 12%, $p<0.001$), as far as serum M-protein (median levels 2.35 *versus* 1.6 g/dL, $p<0.001$). The two factors remained significant even if considered as categorical variable, as previously described.

Immunoparesis was more represented in progressed patients (p<0.001); regarding serum FLC and FLC ratio, levels were higher in progressed patients, however without reacing a statistically significant difference (mediam serum FLC levels: 109 *versus* 49.6 mg/L, p=0.143; median FLC value 8.72 *versus* 4, p=0.337). Moreover, for SMM patients we stratified population according to Mayo(19) and "20-2-20"(44) FLC ratio cutoff: an FLC ratio ≤0.125 or ≥8 was more frequent in progressed patients (p=0.055); an FLC ratio ≥20 was present in a very small number of SMM, and did not resulted statistically significant in progressed *versus* non progressed patients (p=0.395).

Thereafter, we applied the Mayo Score and the "20-2-20" score, confirming their value on stratifying patients with SMM (p=0.006 and p<0.001, respectively). Table 2 summarizes results of the univariate analysis, for the entire cohort and for SMM patients.

4.2.3 BM phenotype and FISH analysis.

As previously specified, data on BMPC phenotype and FISH were collected if available. FISH data were collected in 40 patients (39 SMM). No statistically significant difference were seen regarding neither the presence or absence of del(13q), del(17p), hyperdiploidy, t(4;14) or t(14;16), nor the presence or absence of one or more high-risk FISH alterations, between progressed and non progressed patient, both in total population and in SMM patients. To note, the presence of t(11;14) was higher in non-progressed patients (p=0.042 in total population and p=0.047 in SMM).

Regarding BMPCs phenotype, data were collected in 99/147 SMM patients. The presence of an abnormal PC phenotype >95% was more frequent in progressed patients, even if it didn't reach a statistic significance (p=0.067). No statistically significant difference was found regarding the presence or absence of CD138, CD38, CD45, CD19 and CD56 in progressed or non-progressed patients. Pethema(32) score was applied and confirmed his value in stratifying patients (p=0.008).

4.3 Multivariate analysis

Multivariate Cox regression analysis combining parameters that resulted significant in univariate analysis (percentage of BMPCs, serum M-protein, immunoparesis) showed that percentage of

BMPCs and serum M-protein remained independent risk factors of progression (p<0.001 for both parameters respectively, data not shown).

4.4 Risk of progression.

We evaluated the risk of progression in the whole AMG population through binomial logistic regression.

By combining the two indipendent variables, we obtained an increasing risk of progression on the basis of percentage of BMPCs and serum M-protein, substantially based on the tumor burden of the gammopathy (data not shown).

Given the results of multivariate analysis and the increasing risk of progression highlighted by binomial logistic regression, we created a "tumor-burden" score, based on entity of serum M-protein (< 2 g/dL, 0 points, and ≥2 g/dL, 1 point) and percentage of BMPCs (<10%, 0 points, 10-20%, 1 point, ≥20%, 2 points), and therefore stratified patients into 3 classes: low-risk (score: 0-1), intermediate risk (score:2) and high-risk (score: 3).

With the use of the score combining percentage of BMPCs and serum M-protein, risk of progression resulted 7.9% for low-risk patients, 39% for intermediate-risk patients and 70% for high-risk patients (Table 3, Figure 1).

4.5 Survival analysis

We finally performed survival analysis on both total population and SMM patients, on factors that resulted statistically significant in univariate analysis.

- Percentage of BMPCs: patients with BMPCs ≥20% had a shorter TTP to MM (median TTP: 23 months *versus* not reached, p<0.001 in total population, and 23 months *versus* not reached, p<0.001 in SMM) (Figure 2A and 3A).
- Entity of serum M-protein: when divided into 2 classis on the basis of M-protein levels, patients with higher levels showed a shorter TTP to symptomatic myeloma (median TTP: 50

months in patients with M-protein ≥3 g/dL *versus* not reached in patients with M-protein <3 g/dL, *p*=0.002 in total population; median TTP: 34 months in patients with M-protein ≥3 g/dL *versus* not reached in patients with M-protein <3 g/dL, *p*=0.002 in SMM patients) (Figure 2B and 3B).

- Immunoparesis: the presence of immunoparesis was associated with a significantly shorter TTP in the whole population (median TTP 56 months *versus* not reached, *p*<0.001) and also in SMM patients (median TTP 53 months *versus* not reached, *p*<0.001) (Figure 2C and 3C).

Given the results of multivariate analysis we therefore created a "tumor-burden" score, based on entity of serum M-protein (< 2 g/dL, 0 points, and ≥2 g/dL, 1 point) and percentage of BMPCs (<10%, 0 points, 10-20%, 1 point, ≥20%, 2 points), and we therefore stratified patients into 3 classes: low-risk (score: 0-1), intermediate risk (score:2) and high-risk (score=3). High risk patients had a significantly shorter time to progression to MM (median TTP 18 months *versus* not reached in the other two classes, *p*<0.001 in both total population and SMM patients) (Figure 2D and 3D).

5. DISCUSSION.

In the last years, several studies have evaluated different scoring system that could potentially improve stratification in MGUS and SMM patients and eventually lead to anticipate treatment in very high-risk patients, thus avoiding risk of potentially severe complications. Since attention is mainly focused on patients at higher risk of progression, with the shorter TTP, in this study, we tried to identify in a real-life perspective those factors that significantly impact on short-term progression in AMG.

This is a single-centre, retrospective study, and these probably are the principal limitations that could affect our results. However, the number of patients enrolled is quite high, and could be representative of a larger proportion of patients: indeed, median age at diagnosis of monoclonal gammopathy, sex and general characteristic of the cohort (e.g. distribution of light and heavy chain) are similar to those of larger studies, either considering MGUS or SMM patients(19,30,44).

Median FU for the entire cohort was 34 months, and is similar for MGUS (32 months) and SMM patients (36 months). Median TTP in progressed patients was 16 months for patients with SMM; 32% of SMM patients globally progressed within the follow-up, and this is in line with literature data, that reported risk of progression for SMM of 10% for the first 5 years(41). 5 patients with MGUS (3.6% of the cohort) globally evolve to active MM, and this is also consistent with previously reported data(30).

Our data globally confirm previously reported data on risk factors of progression for AMG: entity of BMPCs infiltrate, amount serum M-protein and presence of immunoparesis are consistently related with a shorter TTP to symptomatic MM, both in the entire cohort and in SMM subpopulation. All these 3 factors are related to tumoral mass in monoclonal gammopathies. Moreover, we applied Mayo score, 20-2-20 score and Pethema score in SMM patients, that resulted significantly related with a shorter TTP to MM.

In our cohort, involved serum FLC and FLC ratio did not results significantly related to progression; this could be attributable to at least 2 different motivations: first of all, the data was available only in a proportion of patients (61% of the total cohort, n= 134/220; 65% of SMM patients, n=95/147), and

the small number of patients could negatively impact on the results. This hypothesis is supported by the fact that involved serum FLC and serum FLC ratio are considerably higher in patients progressed *versus* non-progressed (p=0.078 for involved FLC and p=0.079 for FLC ratio in total population). On the other hand, in our study FLC were measured by N-latex, wheter the large majority of studies regarding FLC role in stratifying MGUS and SMM were conducted using serum FLC assay by Freelite. The two methods have been compared by few studies, showing a good correlation between the two assays(64,65). A recent paper(66) confirmed, as previously reported, that the two assays are comparable when values are in normal ranges, but diverge for high values; the paper noticed also that, regarding the role of serum FLC in predicting risk of progression of SMM to MM, an FLC ratio measured by N-latex ≥70 appears to provide similar performances to a Freelite sFLC ratio ≥100, with a better positive predictive value. Finally, the role of serum FLC as predictors of progression in AMG is still under debate; for example, a paper by Sorrig et al(67) did not confirm the predictive value of FLC ratio>100, included in the slimCRAB criteira.

There are few studies evaluating the impact of cytogenetic and FISH analysis on progression. Rajkumar et al.(49) showed that patients carrying t(4;14) had a significantly shorter TTP than patients with t(11;14). Neben et al.(50) confirmed the prognostic role of t(4;14) and found that also del(17p), gain 1q21 and hyperdiploidia were associated qith a shorter TTP to active myeloma. More recently, FISH alterations have been associated with clinical factors (BMPCs≥20% and serum FLC ratio ≥20)(44) showed that patients carrying high-risk FISH alterations (defined as presence of t(4;14), del(17p) or hyperdiploidia) in combinations with the other 2 factors had a significantly shorter TTP to symptomatic myeloma. In our analysis we failed to found a correlation statistically significant between FISH alterations (considered separately) and progression to myeloma; we then divided patients into high-risk (as defined by the aforementioned paper) and low-risk, but didn't find a correlation between presence of such alterations and progression to MM (p=0.196); however, despite the very low number of patients that underwent the examination, we found that incidence of patients with t(11;14) was higher among non-progressed patients, in line with studies that found that t(11;14) is associated with a longer TTP to symptomatic myeloma.

Finally, given the fact that serum M-protein and percentage of BMPCs remained independent factors for progression even in multivariate analysis, we create a "tumor-burden" score that could possibly predict the risk of progression of AMG, based on three different ranges of percentage of BMPCs (2 division points at values of 10% and 20%) combined with 2 different range of M-protein (1 division point at the value of 2 g/dL), and divided patients into 3 categories. Low-risk patients had a 7.9% risk of progression, intermediate-risk a 39% risk of progression, whereas high-risk patients had a 70% risk of progression. Log-rank test confirmed that high-risk patients either in the whole population and in SMM had a significantly shorter time to progression to symptomatic MM as compared to intermediate and low-risk patients (p<0.001 for both curves). To note, this is the first time that a score used to calculate risk of progression in AMG is applicate either to MGUS and SMM, defined by updated IMWG criteria.

In conclusion, our results show that in patients with AMG clinical factors which mostly impact on the short-term risk of progression to active MM are the entity of the PCs infiltrate and the monoclonal component, directly related to the tumoral mass. The development of a clinical score based on BMPCs and M-protein will permit to overcome the traditional distinction between MGUS and SMM in the evaluation of the progression of AMG patients to active MM.

6. TABLES.

Table 1. Main characteristic of the cohort of patients.

		Total Population	SMM
N° of patients		220	147
Median age (range), years		68 (35-93)	68 (36-93)
Sex	M	128 (58%)	88 (60%)
	F	92 (42%)	59 (40%)
Median Hb (range), g/dL		13.1 (7.4-17.8) [*n*=209]	13.2 (7.4-17.8) [*n*=143]
Light chain	κ	154 (70%)	98 (67%)
	λ	66 (30%)	49 (43%)
Heavy chain	IgG	168 (77%)	107 (74%)
	IgA	42 (19%)	36 (25%)
	Others (IgM, IgD, biclonal)	7 (4%)	2 (1%)
	Light-chain	*n*=3	*n*=2
Median BMPCs (range)		12% (2-55)	15% (8-55)
Median serum M-protein (range), g/dL		1.7 (0.17-4.5) [*n*=173]	1.8 (0.17-4.5) [*n*=133]
Immunoparesis		105 (59%) [*n*=178]	88 (63%) [*n*=139]
Median involved FLC value (range), mg/L		54.1 (7.06-2360) [*n*=118]	56.8 (7.06-2360) [*n*=88]
FLC ratio	unbalanced	91 (68%) [*n*=134]	70 (74%) [*n*=95]
	≤ 0.125 or ≥ 8	/	37 (39%)
	> 20	/	14 (15%)
Median follow-up (range), months		34 (3-126)	36 (4-126)
Progressed		52 (23.6%)	47 (32%)
Median TTP (range), months		16 (4-75)	16 (4-75)

Abbreviations: SMM, Smoldering Multiple Myeloma; Hb, Hemoglobin; BMPCs, Bone Marrow Plasmacells; FLC, Free Light Chain; TTP, Time To Progression.

Table 2. Univariate analysis on known risk factors for progression.

	P vs NP – total population	P vs NP - SMM
BMPCs	***p*<0.001**	***p*<0.001**
Monoclonal Component	***p*<0.001**	***p*<0.001**
Immunoparesis	***p*<0.001**	***p*<0.001**
Involved FLC	*p*=0.078	*p=0.*143
Unbalanced FLC ratio	*p* =0.079	*p*=0.337
MAYO	/	***p*=0.015**
2-20-20	/	***p*<0.001**
PETHEMA	/	***p*=0.022**

Abbreviations: P, progressed; NP, non-progressed; SMM, Smoldering Multiple Myeloma; BMPCs, Bone Marrow Plasmacells; FLC, Free Light Chain.

Table 3. Relative risk of progression according to "tumor-burden" score.

			95% Confidence Interval	
"Tumor-burden" score	**Probability**	**SE**	**Lower**	**Upper**
Low-risk	**7,9%**	0,0269	4,0%	15,0%
Intermediate-risk	**39,0%**	0,0762	25,5%	54,5%
High-risk	**70,0%**	0,0837	51,7%	83,6%

7. LEGEND OF FIGURES.

Figure 1. Probability of progression according to percentage of BMPCs and entity of serum M-protein, combined in the "tumor-burden" score (p<0.001).

Figure 2. Log-rank tests for the whole population on: (**a**) Percentage of BMPCs (p<0.001); (**b**) Entity of serum M-protein (p=0.002); (**c**) immunoparesis (p<0.001); (**d**) "tumor-burden" score.

Figure 3. Log-rank tests for SMM patients on: (**a**) Percentage of BMPCs (p<0.001); (**b**) Entity of serum M-protein (p=0.002); (**c**) immunoparesis (p<0.001); (**d**) "tumor-burden" score (p<0.001).

8. FIGURES.

Figure 1

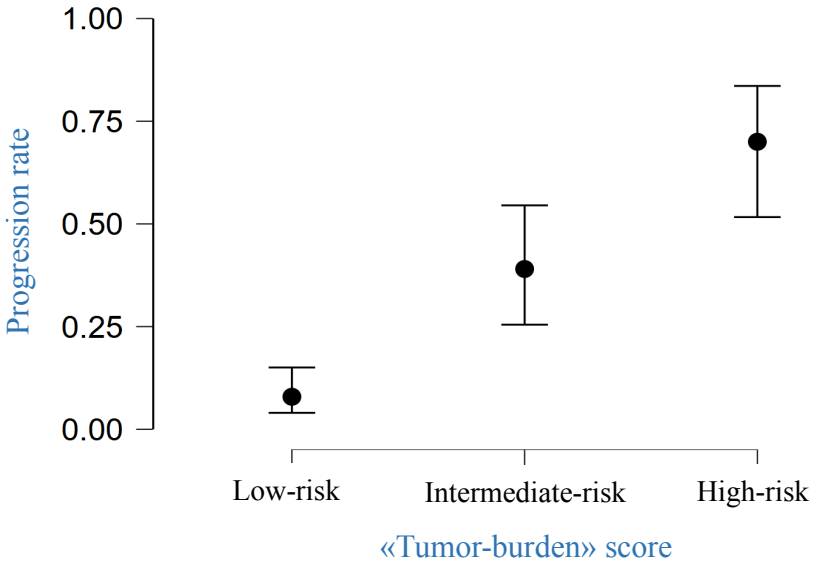


Figure 2.

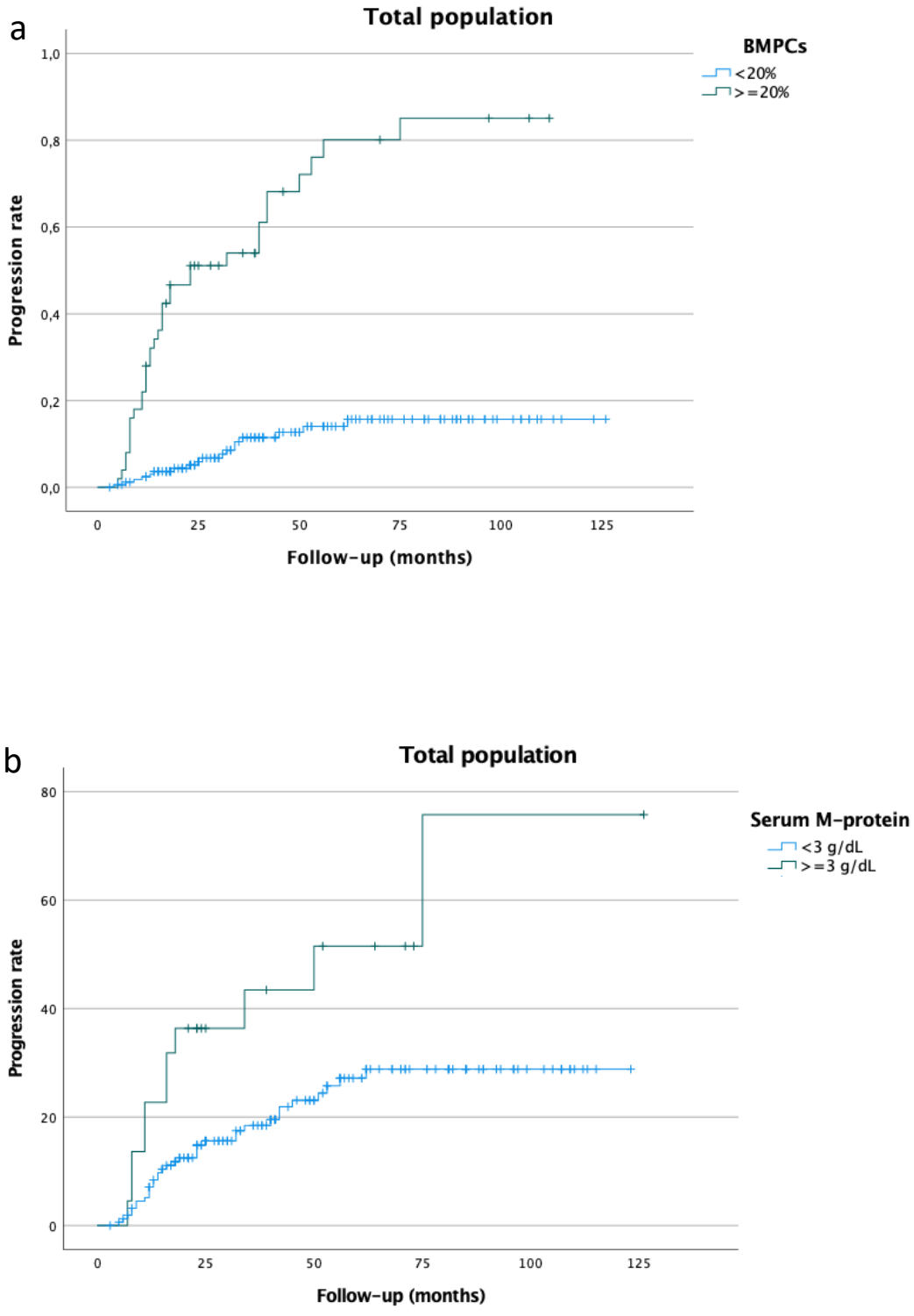
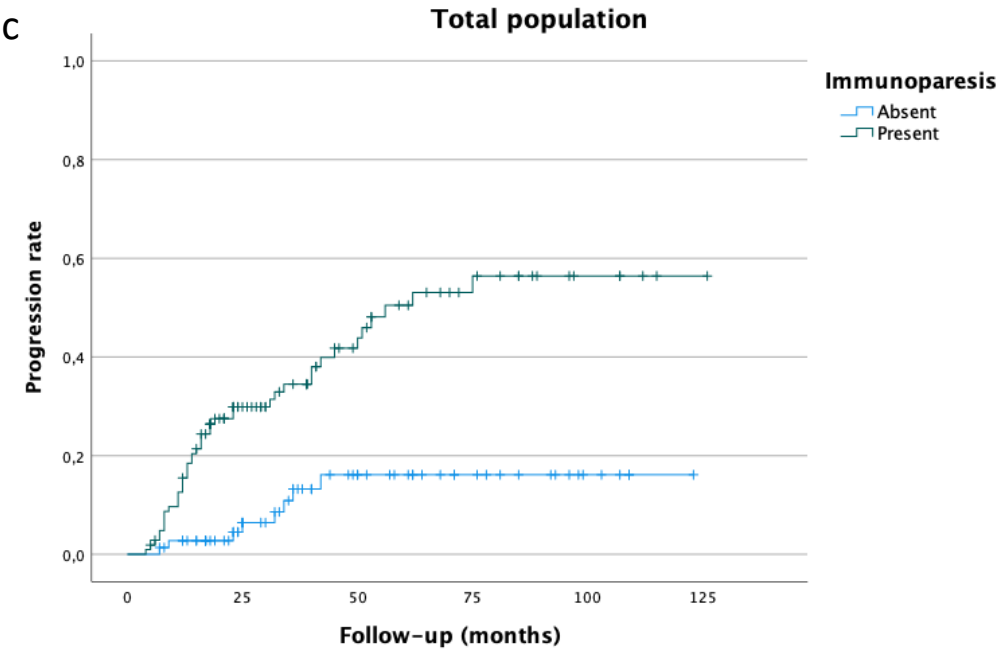

a
Total population
BMPCs
<20%
>=20%
Progression rate
1,0
0,8
0,6
0,4
0,2
0,0
0
25
50
75
100
125
Follow-up (months)
b
Total population
Serum M-protein
<3 g/dL
>=3 g/dL
Progression rate
80
60
40
20
0
0
25
50
75
100
125
Follow-up (months)

Figure 2 (cont.).

C



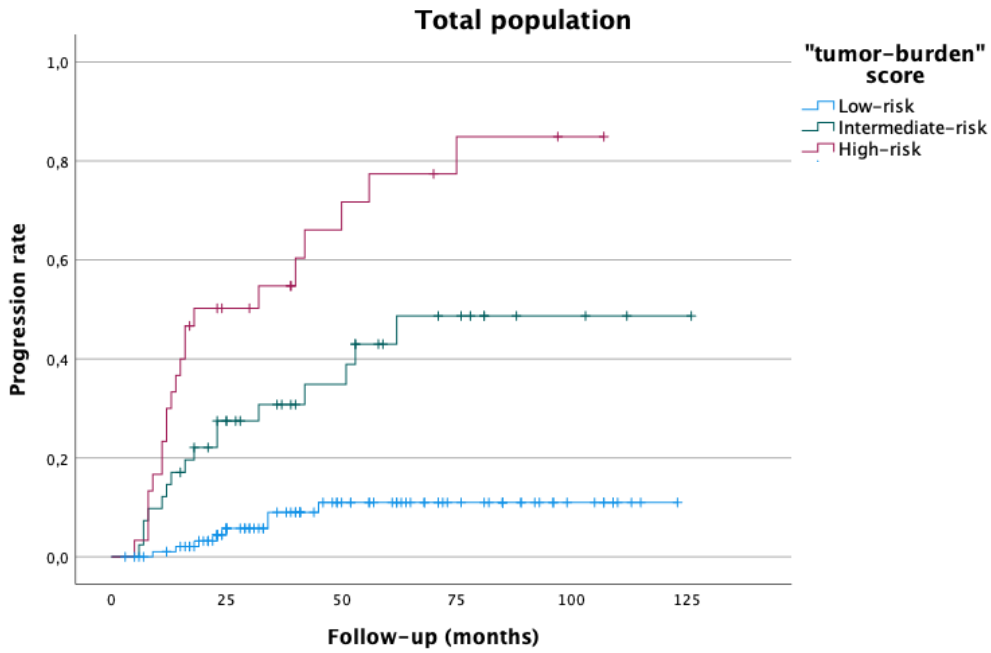


Figure 3.

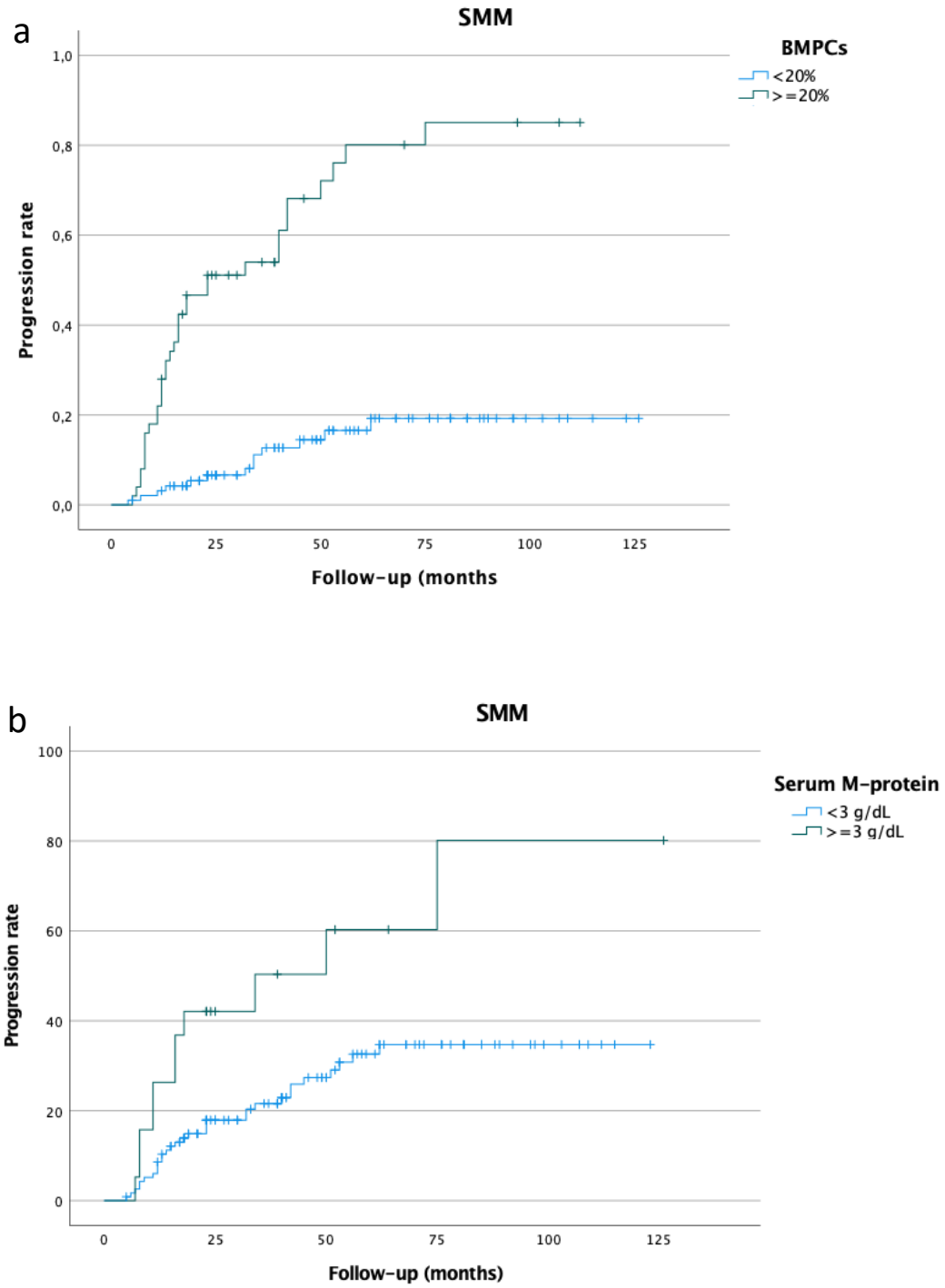
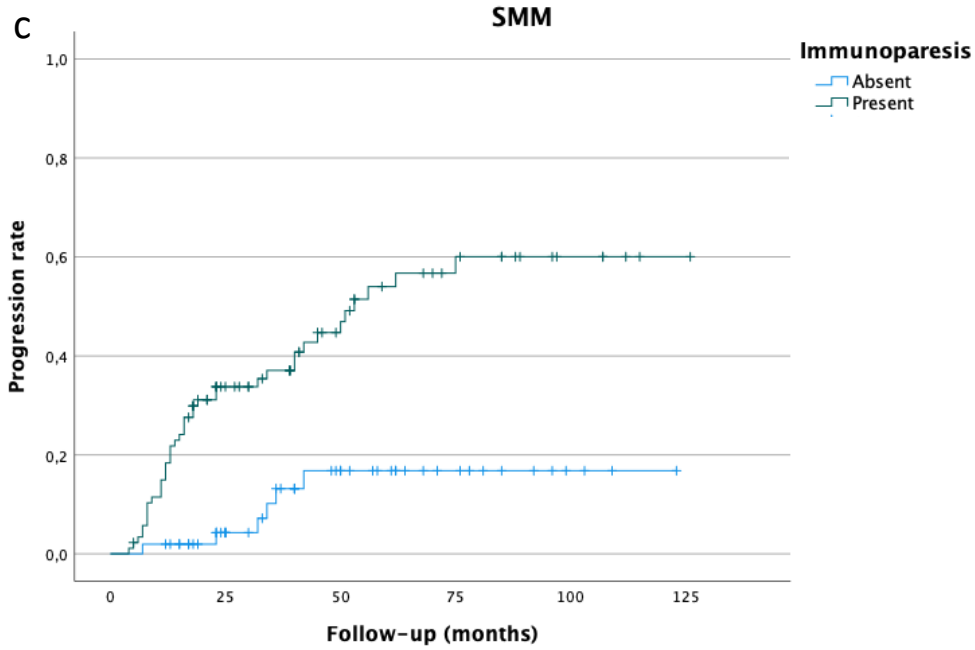
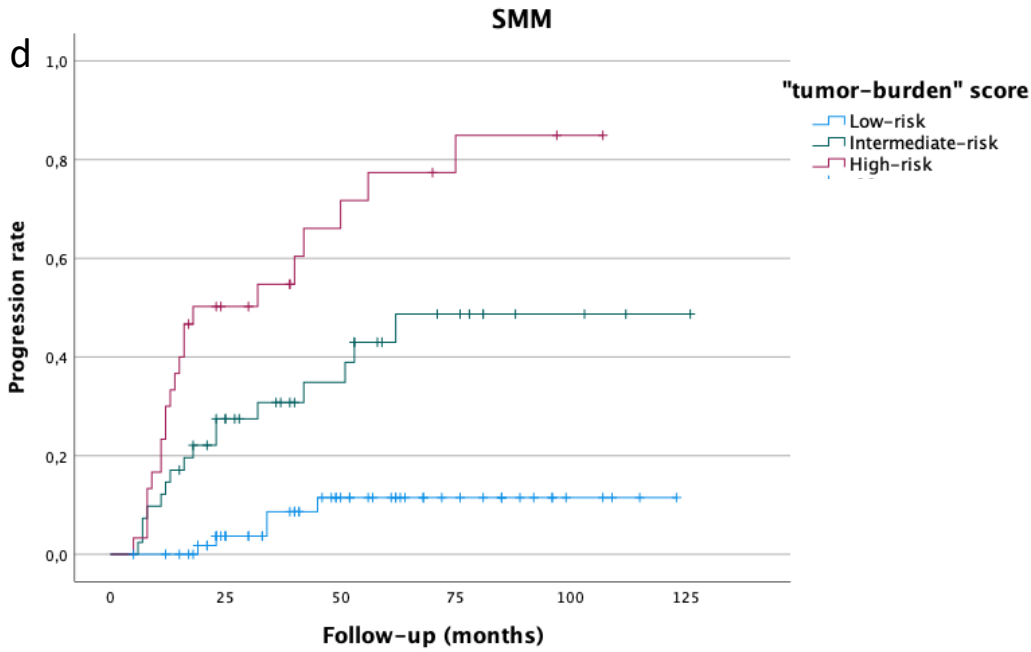

a
SMM
BMPCs
<20%
>=20%
Progression rate
1,0
0,8
0,6
0,4
0,2
0,0
0
25
50
75
100
125
Follow-up (months
b
SMM
Serum M-protein
<3 g/dL
>=3 g/dL
Progression rate
100
80
60
40
20
0
0
25
50
75
100
125
Follow-up (months)

Figure 3 (cont.)





9. BIBLIOGRAPHY.

1. Waldenström J. Disturbances of Gamma Globulin Synthesis. Ann Intern Med. 1962 Apr 1;56(4):695.
2. Kyle RA. Monoclonal gammopathy of undetermined significance. Natural history in 241 cases. Vol. 64, The American Journal of Medicine. Elsevier; 1978. p. 814–26.
3. Kyle RA, Greipp PR. Smoldering Multiple Myeloma. N Engl J Med. 1980 Jun 12;302(24):1347–9.
4. Kyle RA, Group IMW. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders. Br J Haematol. 2003;121:749–57.
5. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos M-V, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014 Nov;15(12):e538–48.
6. Landgren O, Graubard BI, Kumar S, Kyle RA, Katzmann JA, Murata K, et al. Prevalence of myeloma precursor state monoclonal gammopathy of undetermined significance in 12372 individuals 10-49 years old: a population-based study from the National Health and Nutrition Examination Survey. Blood Cancer J. 2017 Oct 20;7(10):e618.
7. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of Monoclonal Gammopathy of Undetermined Significance. N Engl J Med. 2006 Mar 30;354(13):1362–9.
8. Therneau TM, Kyle RA, Melton LJ, Larson DR, Benson JT, Colby CL, et al. Incidence of monoclonal gammopathy of undetermined significance and estimation of duration before first clinical recognition. Mayo Clin Proc. 2012;87(11):1071–9.
9. Landgren O, Graubard BI, Katzmann JA, Kyle RA, Ahmadizadeh I, Clark R, et al. Racial disparities in the prevalence of monoclonal gammopathies: A population-based study of 12 482 persons from the national health and nutritional examination survey. Vol. 28, Leukemia. Nature Publishing Group; 2014. p. 1537–42.
10. van de Donk NWCJ, Palumbo A, Johnsen HE, Engelhardt M, Gay F, Gregersen H, et al. The

clinical relevance and management of monoclonal gammopathy of undetermined significance and related disorders: Recommendations from the European Myeloma Network. Haematologica. 2014 Jun 1;99(6):984–96.

11. Fermand JP, Bridoux F, Dispenzieri A, Jaccard A, Kyle RA, Leung N, et al. Monoclonal gammopathy of clinical significance: A novel concept with therapeutic implications. Blood. 2018 Oct 4;132(14):1478–85.

12. Rajkumar SV, Landgren O, Mateos MV. Smoldering multiple myeloma. Vol. 125, Blood. American Society of Hematology; 2015. p. 3069–75.

13. Kyle RA, Larson DR, Therneau TM, Dispenzieri A, Melton LJ, Benson JT, et al. Clinical course of light-chain smouldering multiple myeloma (idiopathic Bence Jones proteinuria): A retrospective cohort study. Lancet Haematol. 2014;1(1):e28–36.

14. Ravindran A, Bartley AC, Holton SJ, Gonsalves WI, Kapoor P, Siddiqui MA, et al. Prevalence, incidence and survival of smoldering multiple myeloma in the United States. Blood Cancer J. 2016;6(10):e486.

15. Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: A prospective study. Blood. 2009;113(22):5412–7.

16. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Vol. 12, Nature Reviews Cancer. Nat Rev Cancer; 2012. p. 335–48.

17. Ho M, Patel A, Goh CY, Moscvin M, Zhang L, Bianchi G. Changing paradigms in diagnosis and treatment of monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM). Leukemia. Springer Nature; 2020.

18. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, et al. Clinical Course and Prognosis of Smoldering (Asymptomatic) Multiple Myeloma. N Engl J Med. 2007 Jun 21;356(25):2582–90.

19. Dispenzieri A, Kyle RA, Katzmann JA, Therneau TM, Larson D, Benson J, et al. Immunoglobulin free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. Blood. 2008 Jan 15;111(2):785–9.

20. Kumar S, Rajkumar S V. Will the real myeloma please stand up? Vol. 27, Leukemia. Nature Publishing Group; 2013. p. 760–1.

21. Landgren O. Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma: biological insights and early treatment strategies. Vol. 2013, Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program. Hematology Am Soc Hematol Educ Program; 2013. p. 478–87.

22. Kaufmann H, Ackermann J, Baldia C, Nösslinger T, Wieser R, Seidl S, et al. Both IGH translocations and chromosome 13q deletions are early events in monoclonal gammopathy of undetermined significance and do not evolve during transition to multiple myeloma. Leukemia. 2004;18(11):1879–82.

23. Walker BA, Wardell CP, Melchor L, Brioli A, Johnson DC, Kaiser MF, et al. Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. Leukemia. 2014 Feb;28(2):384–90.

24. Pawlyn C, Morgan GJ. Evolutionary biology of high-risk multiple myeloma. Vol. 17, Nature Reviews Cancer. Nature Publishing Group; 2017. p. 543–56.

25. López-Corral L, Sarasquete ME, Beà S, García-Sanz R, Mateos M V., Corchete LA, et al. SNP-based mapping arrays reveal high genomic complexity in monoclonal gammopathies, from MGUS to myeloma status. Leukemia. 2012;26(12):2521–9.

26. Storti P, Agnelli L, Palma BD, Todoerti K, Marchica V, Accardi F, et al. The transcriptomic profile of CD138+ cells from patients with early progression from smoldering to active multiple myeloma remains substantially unchanged. Vol. 104, Haematologica. Ferrata Storti Foundation; 2019. p. E465–9.

27. Bianchi G, Munshi NC. Pathogenesis beyond the cancer clone(s) in multiple myeloma. Blood. 2015 May 14;125(20):3049–58.

28. Dhodapkar M V. MGUS to myeloma: A mysterious gammopathy of underexplored significance. Blood. 2016;128(23):2599–606.

29. Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I, et al.

Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. Nat Commun. 2014 Jan 16;5(1):1–13.

30. Kyle RA, Larson DR, Therneau TM, Dispenzieri A, Kumar S, Cerhan JR, et al. Long-Term Follow-up of Monoclonal Gammopathy of Undetermined Significance. N Engl J Med. 2018 Jan 18;378(3):241–9.

31. Rajkumar SV, Kyle RA, Therneau TM, Melton LJ, Bradwell AR, Clark RJ, et al. Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. 2005;

32. Perez-Persona E, Vidriales M-B, Mateo G, Garcia-Sanz R, Mateos M-V, de Coca AG, et al. 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. 2007 Oct 1;110(7):2586–92.

33. Turesson I, Kovalchik SA, Pfeiffer RM, Kristinsson SY, Goldin LR, Drayson MT, et al. Monoclonal gammopathy of undetermined significance and risk of lymphoid and myeloid malignancies: 728 cases followed up to 30 years in Sweden. Blood. 2014 Jan 16;123(3):338–45.

34. Cesana C, Klersy C, Barbarano L, Nosari AM, Crugnola M, Pungolino E, et al. Prognostic Factors for Malignant Transformation in Monoclonal Gammopathy of Undetermined Significance and Smoldering Multiple Myeloma. J Clin Oncol. 2002 Mar 15;20(6):1625–34.

35. Pérez-Persona E, Mateo G, García-Sanz R, Mateos MV, De Las Heras N, De Coca AG, et al. 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. Br J Haematol. 2010 Jan;148(1):110–4.

36. M.V. D, R. S, S. W, S. U, X. P, B. N, et al. Clinical, genomic, and imaging predictors of myeloma progression from asymptomatic monoclonal gammopathies (swog s0120). Blood. 2014;123(1):78–85.

37. Giuliani N, Rizzoli V. Myeloma cells and bone marrow osteoblast interactions: Role in the

development of osteolytic lesions in multiple myeloma. Vol. 48, Leukemia and Lymphoma. Leuk Lymphoma; 2007. p. 2323–9.

38. Politou M, Terpos E, Anagnostopoulos A, Szydlo R, Laffan M, Layton M, et al. Role of receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin and macrophage protein 1-alpha (MIP-1a) in monoclonal gammopathy of undetermined significance (MGUS). Br J Haematol. 2004 Sep 1;126(5):686–9.

39. Ng AC, Khosla S, Charatcharoenwitthaya N, Kumar SK, Achenbach SJ, Holets MF, et al. Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1α levels in patients with MGUS. Blood. 2011 Dec 15;118(25):6529–34.

40. Dalla Palma B, Guasco D, Pedrazzoni M, Bolzoni M, Accardi F, Costa F, et al. Osteolytic lesions, cytogenetic features and bone marrow levels of cytokines and chemokines in multiple myeloma patients: Role of chemokine (C-C motif) ligand 20. Leukemia. 2016;30(2):409–16.

41. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, et al. Clinical Course and Prognosis of Smoldering (Asymptomatic) Multiple Myeloma. N Engl J Med. 2007;356(25):2582–90.

42. Mateos M-V, San Miguel J-F. Smoldering Multiple Myeloma: When to Observe and When to Treat? Am Soc Clin Oncol Educ B. 2015;35:e484–92.

43. Cherry BM, Korde N, Kwok M, Manasanch EE, Bhutani M, Mulquin M, et al. Modeling progression risk for smoldering multiple myeloma: results from a prospective clinical study. Leuk Lymphoma. 2013 Oct 29;54(10):2215–8.

44. Lakshman A, Vincent Rajkumar S, Buadi FK, Binder M, Gertz MA, Lacy MQ, et al. Risk stratification of smoldering multiple myeloma incorporating revised IMWG diagnostic criteria. Blood Cancer J. 2018 Jun 1;8(6):59.

45. Mateos MV, Kumar S, Dimopoulos MA, González-Calle V, Kastritis E, Hajek R, et al. International Myeloma Working Group risk stratification model for smoldering multiple myeloma (SMM). Blood Cancer J. 2020 Oct 1;10(10):102.

46. González-Calle V, Dávila J, Escalante F, De Coca AG, Aguilera C, López R, et al. Bence Jones

proteinuria in smoldering multiple myeloma as a predictor marker of progression to symptomatic multiple myeloma. Leukemia. 2016;30(10):2026–31.

47. Bianchi G, Kyle RA, Larson DR, Witzig TE, Kumar S, Dispenzieri A, et al. High levels of peripheral blood circulating plasma cells as a specific risk factor for progression of smoldering multiple myeloma. Leukemia. 2013 Mar;27(3):680–5.

48. Gonsalves WI, Rajkumar S V., Dispenzieri A, Dingli D, Timm MM, Morice WG, et al. Quantification of circulating clonal plasma cells via multiparametric flow cytometry identifies patients with smoldering multiple myeloma at high risk of progression. Leukemia. 2017 Jan 1;31(1):130–5.

49. Rajkumar S V., Gupta V, Fonseca R, Dispenzieri A, Gonsalves WI, Larson D, et al. Impact of primary molecular cytogenetic abnormalities and risk of progression in smoldering multiple myeloma. Leukemia. 2013 Aug 21;27(8):1738–44.

50. Neben K, Jauch A, Hielscher T, Hillengass J, Lehners N, Seckinger A, et al. Progression in smoldering myeloma is independently determined by the chromosomal abnormalities del(17p), t(4;14), gain 1q, hyperdiploidy, and tumor load. J Clin Oncol. 2013;31(34):4325–32.

51. Khan R, Dhodapkar M, Rosentha A, Heuck C, Papanikolaou X, Qu P, et al. Four genes predict high risk of progression from smoldering to symptomatic multiple myeloma (SWOG s0120). Haematologica. 2015;100(9):1214–21.

52. Ravi P, Kumar S, Larsen JT, Gonsalves W, Buadi F, Lacy MQ, et al. Evolving changes in disease biomarkers and risk of early progression in smoldering multiple myeloma. Blood Cancer J. 2016;6(7):e454.

53. Siontis B, Kumar S, Dispenzieri A, Drake MT, Lacy MQ, Buadi F, et al. Positron emission tomography-computed tomography in the diagnostic evaluation of smoldering multiple myeloma: Identification of patients needing therapy. Blood Cancer J. 2015;5(10):e364-4.

54. Zamagni E, Nanni C, Gay F, Pezzi A, Patriarca F, Bellò M, et al. 18F-FDG PET/CT focal, but not osteolytic, lesions predict the progression of smoldering myeloma to active disease. Leukemia. 2016;30(2):417–22.

55. Kastritis E, Moulopoulos LA, Terpos E, Koutoulidis V, Dimopoulos MA. The prognostic

importance of the presence of more than one focal lesion in spine MRI of patients with asymptomatic (smoldering) multiple myeloma. Vol. 28, Leukemia. Nature Publishing Group; 2014. p. 2402–3.

56. Bäuerle T, Hielscher T, Hillengass J, Fechtner K, Weber M-A, Ayyaz S, et al. Prognostic Significance of Focal Lesions in Whole-Body Magnetic Resonance Imaging in Patients With Asymptomatic Multiple Myeloma. Artic J Clin Oncol. 2010;28.

57. Wennmann M, Hielscher T, Kintzelé L, Menze BH, Langs G, Merz M, et al. Spatial Distribution of Focal Lesions in Whole-Body MRI and Influence of MRI Protocol on Staging in Patients with Smoldering Multiple Myeloma According to the New SLiM-CRAB-Criteria. Cancers (Basel). 2020 Sep 7;12(9):2537.

58. Dalla Palma B, Marchica V, Pedrazzoni M, Accardi F, Notarfranchi L, Goldoni M, et al. Bone marrow Dikkopf-1 levels are a new independent risk factor for progression in patients with smouldering myeloma. Br J Haematol. 2017;

59. Go RS, Vincent Rajkumar S. How i manage monoclonal gammopathy of undetermined significance. Blood. 2018 Jan 11;131(2):163–73.

60. Muchtar E, Kumar SK, Magen H, Gertz MA. Diagnosis and management of smoldering multiple myeloma: the razor's edge between clonality and cancer. Leuk Lymphoma. 2018 Feb 1;59(2):288–99.

61. Mateos MV, Hernández MT, Giraldo P, de la Rubia J, de Arriba F, Corral LL, et al. Lenalidomide plus dexamethasone versus observation in patients with high-risk smouldering multiple myeloma (QuiRedex): long-term follow-up of a randomised, controlled, phase 3 trial. Lancet Oncol. 2016 Aug 1;17(8):1127–36.

62. Lonial S, Jacobus S, Fonseca R, Weiss M, Kumar S, Orlowski RZ, et al. Randomized Trial of Lenalidomide Versus Observation in Smoldering Multiple Myeloma. J Clin Oncol. 2019 Oct 25;JCO1901740.

63. Mateos M-V, Martínez-López J, Rodríguez-Otero P, de la Calle G V., González M-S, Oriol A, et al. CURATIVE STRATEGY (GEM-CESAR) FOR HIGH-RISK SMOLDERING MYELOMA: CARFILZOMIB, LENALIDOMIDE AND DEXAMETHASONE (KRD) AS

INDUCTION FOLLOWED BY HDT-ASCT, CONSOLIDATION WITH KRD AND MAINTENANCE WITH RD. HemaSphere. 2019 Jun;3:390.

64. White-Al Habeeb NMA, Earle T, Spencer M, Blasutig IM. Evaluation of the N-latex serum free light chain assay on the Siemens BNII analyzer and agreement with The Binding Site FreeLite assay on the SPAPlus. Clin Biochem. 2018 Jan 1;51:90–6.

65. Te Velthuis H, Knop I, Stam P, Van Den Broek M, Bos HK, Hol S, et al. N Latex FLC - New monoclonal high-performance assays for the determination of free light chain kappa and lambda. Clin Chem Lab Med. 2011 Aug 1;49(8):1323–32.

66. Henriot B, Rouger E, Rousseau C, Escoffre M, Sebillot M, Bendavid C, et al. Prognostic value of involved/uninvolved free light chain ratio determined by Freelite and N Latex FLC assays for identification of high-risk smoldering myeloma patients. Clin Chem Lab Med. 2019 Sep 1;57(9):1397–405.

67. Sørrig R, Klausen TW, Salomo M, Vangsted AJ, Østergaard B, Gregersen H, et al. Smoldering multiple myeloma risk factors for progression: a Danish population-based cohort study. Eur J Haematol. 2016;97(3):303–9.