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Prognostic Biomarkers in the Progression from MGUS to Multiple Myeloma: a Systematic Review

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABSTRACT

Multiple myeloma (MM), characterized by malignant plasma cells in the bone marrow (BM), is consistently preceded by asymptomatic pre-malignant stage monoclonal gammopathy of undetermined significance (MGUS). These MGUS patients have an annual risk of 1% to progress to MM. Clinical, imaging and genomic (genetic and epigenetic) factors were identified, whose presence increased the risk of progression from MGUS to MM. This systematic review summarizes the currently identified clinical, imaging and genomic biomarkers, suggested to increase the progression risk or demonstrated to be differential expressed/presence between both cohorts of patients. Despite the wide range of proposed markers, there are still no reliable biomarkers to individually predict which MGUS patient will progress to MM and which will not. Research studying biomarkers in the progression from MGUS to MM will give more insight in the unknown pathogenesis of this hematological malignancy. This would improve both research by elucidating new pathways and potential therapeutic targets as well as clinical management by closer follow-up and earlier treatment of high-risk MGUS patients.

KEYWORDS: Hematological malignancy, Biological marker, Risk factors, Premalignant stage, Disease progression

INTRODUCTION

Multiple myeloma (MM), also known as Kahler's disease or plasma cell (PC) myeloma, is a clonal PC malignancy originating in the bone marrow (BM). The clonal expansion of PCs leads to a homogeneous monoclonal overproduction of a specific type of antibody, called monoclonal (M)–protein. (1, 2) In 2012, more than 114,000 patients were newly diagnosed worldwide with MM, making it one of the most common hematological malignancies. (3, 4)

This disease is associated with some typical clinical symptoms, summarized as the CRAB criteria standing for hypercalcemia, renal insufficiency, anemia and bone lesions. In 2014, the International Myeloma Working Group (IMWG) revised the definition and diagnostic criteria of MM (**Table 1**) such that asymptomatic patients without the typical CRAB criteria but with one of the new criteria are also diagnosed as MM and started treatment. These new criteria are based on percentage clonal plasma cells on BM biopsy, serum free light chain (sFLC) ratio and amount of focal lesions on advanced imaging. (5) The genetic background and disease course are highly heterogeneous. Despite important progress in elucidating the molecular mechanism of MM, its pathogenesis is still unknown. Disease initiation and progression is due to multistep and –factorial processes. This happens in a branching, non-linear fashion with somatic mutations, epigenetic alterations, translocations and chromosomal copy-number changes. Another level of heterogeneity is added by the PCs' intimate relation with the BM and its microenvironment for growth, survival etc. (1, 6-8)

Currently, MM still remains a largely incurable malignancy with poor prognosis. Treatment, such as chemo-and radiotherapy, and (autologous) stem cell transplantation is mostly focused on slowing down the disease course, suppressing clinical symptoms and preventing complications. Median survival in MM patients only amounts to five to seven years, depending on host factors (e.g. tobacco use, stress, comorbid conditions like diabetes etc.), tumor stage, biology (e.g. cytogenetic abnormalities) and therapeutic responses. (10, 11)

All cases of MM are consistently preceded by the asymptomatic pre-malignant stages monoclonal gammopathy of undetermined significance (MGUS) and/or smoldering (asymptomatic) myeloma (SMM). (12) The pre-stage MGUS is characterized by slightly elevated clonal PCs on the BM biopsy, presence of a small M-protein and absent CRAB criteria (**Table 1**). It is present in approximately 3.5% of the general population over the age of 50. (2, 12) The pre-stage SMM is characterized by higher cut-off values for the M-protein and percentage clonal PCs on BM biopsy but still absence of the CRAB criteria. (12, 13) However, SMM is still a relatively uncommon clinical entity and there is no population-based registry of these patients for prevalence. (14) Because the asymptotic character of both premalignant stages, detection is rather accidental. (15) Both MGUS and SMM have risks to progress ultimately to MM. For MGUS, the risk of progression is about 1% per year. (2, 12) For SMM, the risk of progression is higher. About 10% of SMM patients per year will progress to MM in the first five years, 3% per year for the next five years, and 1% per year for the subsequent ten years. (13, 16)

Currently, reliable biomarkers predicting which MGUS/SMM patients will progress to MM and which will not, are lacking. Consequently, it is impossible to discriminate and identify individuals with a high versus low risk of progression. Whereas only a select percentage of all MGUS and SMM patients will develop MM, all patients need close follow-up monitoring during their life. Therefore, clinical management of MGUS and SMM is based on the "watch and wait" strategy, being clinical follow-up of all patients without treatment until MM progression and, accordingly the presence of end-organ damage (i.e. CRAB).

(13) This strategy not only cause distress to the patients and their environment, but also economy (i.e. health care) encounters difficulties as a consequence of this approach.
Given the identification of biomarker(s), high risk individuals could benefit from close observation and early therapeutic intervention, whereas low risk individuals can have a reduced frequency of monitoring and avoidance of unnecessary treatments. (13, 17) Also from a research standpoint, opportunities will arise by new insights and disease understanding, leading to improved treatments and a possible future cure for MM. (12, 17, 18)

Conclusively, biomarker(s) could have a lot of potential with significant improvements for society (i.e. life quality, personalized follow-up schemes), economy (i.e. decreasing health care expenses), research (i.e. more insights into pathological pathways), clinical management (i.e. risk stratification, directed search instead of "watch and wait") and personalized medicine (i.e. targeted therapy). Accordingly, this definitely underlines the need and importance of biomarkers predicting which MGUS or SMM patients will progress to MM and which will not. Because of this need, a literature search (PubMed) was performed to summarize the currently known biomarkers in disease progression to MM. The Medical Subject Headings (MeSH) terms 'Multiple Myeloma', 'Monoclonal Gammopathy of Undermined Significance', 'Disease Progression' and 'Biomarkers, Tumor' were used to obtain the available literature. This systematic review addresses the following research question: "Which biomarkers are already available to predict the progression from MGUS to MM?". Our focus was mainly emphasized on the progression from the pre-stage MGUS to ultimately MM, since Muchtar *et al*. (19) recently published a review focusing on the management of SMM. Comparative studies that reported on markers in MGUS and MM separately were also included. The relevant information is summarized and divided according the different types of biomarkers: clinical, imaging and molecular (genetic and epigenetic) markers.

MATERIALS AND METHODS

RESEARCH AIMS

This systematic review intends to investigate the availability of biomarkers predicting the progression from the asymptomatic disease MGUS to the bone marrow cancer MM.

SEARCH STRATEGY

A systematic search of English-language literature using PubMed (**Table 2**) was performed along with a manual search of the cited references of the selected articles. We will include all studies performed on patient material, until June 2017 describing biomarkers predicting the progression from MGUS to MM. We will exclude studies for any of the following reasons: no abstract available, use of animal models and/or cell cultures and focus on therapy or diagnosis. A PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart (**Figure 1**) was created to illustrate the number of articles used for this systematic review.

CLINICAL MARKERS

One type of markers which could be helpful for the identification of MGUS patients with higher progression risk to transform to MM are the clinical markers (**Table 3**). Most of the time, these markers are easily, simply, fastly and with minimal invasiveness detected, making their use more applicable on a larger scale. Several studies described a wide range of different clinical markers, with the potential to discriminate and/or predict the progression from the non-malignant pre-stage to the malignancy itself.
Up until now, patients are risk stratified based on two models: one model proposed by the Mayo Clinic and the other by the Spanish Study group. (12, 18) The Mayo Clinic model focuses on the quantification

of the M-spike and BM PCs. The typical features listed by them as adverse risk factors for MGUS patients are: non-IgG isotype, M-protein concentration >1,5 g/dL and abnormal serum FLC ratio. (12, 18, 20, 21) With this model, the presence of all three risk factors is defined as high-risk MGUS. At 20 years of follow-up, these high-risk MGUS patients had 58% risk of MM progression. When MGUS patients showed two (high-intermediate risk MGUS), one (low-intermediate risk MGUS) and none (low-risk MGUS) of these risk factors, the risk of progression at 20 year follow-up decreased; the corresponding risks amounted to 37%, 21% and 5%, respectively. (12, 21)

The other model, developed by the Spanish Study group, incorporates the degree of clonality assessed by immunophenotyping and uses multi-parametric flow cytometry to differentiate aberrant from normal PCs. Normal PCs express CD138 and intense CD38, while aberrant PCs have decreased CD38 expression, increased CD56 expression and absence of CD19 and/or CD45. The proportion of BM aberrant PCs within the BMPC compartment (aPC/BMPC) is used as one of the criteria for differential diagnosis between MGUS and MM. The risk of MM progression at 5 years for MGUS and SMM cases having 95% aPC/BMPC amounted to 25% and 64%, respectively. When MGUS and SMM patients have less than 95% aPC/BMPC, the risks decreased to 5% and 8%, respectively. In addition to 95% aPC/BMPC, the DNA index has a prognostic value for MGUS patients (based on multivariate analysis). Prognostic stratification is divided into three risk categories, based on the presence of two, one or none of the risk factors. For MGUS patients, the risk of MM progression at five years for the three subgroups amounts to 46%, 10% and 2%, respectively. (12) Although these models for risk stratification are helpful, they are still not accurate and reliable for implementation in the clinical practice. Due to the heterogeneity of the disease, the risk of progression to MM is very individual-dependent. (12, 18)

PARAMETERS ASSOCIATED WITH IMWG CRITERIA

As earlier described, IMWG redefined myeloma and the diagnostic criteria. A lot of research has focused on these criteria in the search for biomarkers in the progression from MGUS to MM. Dhodapkar *et al*. (22) described clinical predictors of myeloma progression from 331 AMG patients (152 MGUS and 179 SMM patients who met the IMWG criteria). Different clinical factors were associated with increased risk of progression to clinical MM requiring therapy. In the univariate analysis, the risk of disease progression in the MGUS subgroup at two years amounted to 1.6%. The clinical predictors associated with progression from AMG to clinical MM requiring therapy were age ≥65 years, hemoglobin <12 g/dL, serum albumin <4 g/dL, serum β2-microglobulin >3 mg/L, elevated serum M- (≥3 g/dL) and urine M-protein (>0 g/dL) and low levels of uninvolved immunoglobulins, level of involved sFLC (>25 mg/dL), elevated ratio of involved/uninvolved sFLC (>10) and increased BM PCs (≥20%). In the multivariate model, three independent predictors of the progression risk the clinical MM requiring therapy were found: serum M-protein ≥3g/dL, BMPCs ≥20% and age ≥65 years. These results are consistent with prior data and studies identifying M-protein levels and type, percentage of BM plasmacytosis and sFLC ratio as important markers in MGUS, helpful to classify patients who are at higher versus lower risk of MM progression. (23-25)

As shown by Papanikolaou *et al*. (26) one of the variables already known to be associated with the progression into MM is the extent of the abnormality of the involved-to-uninvolved FLC ratio, also forming the basis for the new IMWG criteria for MM.

The serum heavy/light chain isotype (Hevylite), a laboratory blood test approved by the US Food and Drug Administration (FDA), focusses on the typical features of MM by measuring the intact immunoglobulins. It determines the suppression of the uninvolved heavy/light chain (HLC) pair. (18, 27)

In a 999 MGUS patient cohort study, it was found that HLC-pair suppression could predict progression in MGUS. This suppression was associated with a two-fold excess risk of progression to MM after adjusting for other known risk factors such as FLC ratio and serum M-protein type and size. (18, 28) In the univariate analysis, the effect of different variables was tested for their significance as prognostic factor in disease progression. The variables effect of HLC-pair suppression, abnormal HLC-pair ratio, uninvolved immunoglobulin suppression, as well as the previously identified variables M-protein size, heavy chain isotype and FLC ratio were significant. In the multivariate analyses, HLC-pair suppression was identified as independent risk factor in combination with serum M-spike size, heavy chain isotype and free light chain ratio. These findings suggest that HCL-pair suppression is an independent, significant risk factor for progression in MGUS. (21, 28)

Cytoplasmic immunoglobulin index (CIg), quantitated as described by Papanikolaou *et al.* (29), is a measure of plasma cell immunoglobulin production detected by two-color flow cytometry of nuclear DNA and CIg (DNA/CIG method). CIg was defined as a prognostic factor for disease progression. In a study of 110 patients with a median follow-up time of 7.8 years, median CIg values declined in the progression from MGUS to SMM to MM (10.5; 5.6 and 3.3 respectively). In order to exclude the decreasing percentage of highly secreting normal PCs as reason for this progressive decline, measurements are repeated for strictly aneuploidy cases. The same progressive CIg decline was seen in the transition from MGUS to SMM to MM (16; 9.1 and 3.5, respectively). This study concluded that there is an actual progressive decline in immunoglobulin production capacity in the progression to MM. (26)

ANGIOGENESIS

Other potential clinical markers of disease progression are related to angiogenesis and its mechanisms. One identified marker are the serum levels of sCD105 by Tsirakis *et al*.. (30) CD105 is a cell membrane glycoprotein, predominantly expressed in angiogenic endothelial cells. This glycoprotein is a powerful marker to quantify tumor angiogenesis, which is the formation of new vessels from pre-existing vasculature. In this study, 50 newly diagnosed MM patients and 20 MGUS patients were enrolled. Serum levels of sCD105 were significantly higher in advanced disease stages. The sCD105 levels in MM patients were significantly higher compared to MGUS patients and control group, with mean ± standard deviation values of 11.0±5.3 ng/mL, 9.0±2.7 ng/mL and 8.0±2.1 ng/mL, respectively. In addition, serum Transforming Growth Factor beta 1 (sTGF-β1) levels were measured, which were significantly lower in MM patients compared to the MGUS patients and control group, while higher in MGUS patients compared to control subjects. Additionally, IL-6 serum levels were changing with increasing disease stage. In the group of MM patients, IL-6 levels were significantly higher compared to the levels in MGUS patients and control group. Predominantly, the significance of sCD105 in the progression from MGUS to MM was highlighted in this study. It suggests that sCD105 levels may represent a marker of disease progression from MGUS to MM, addressing the importance of angiogenesis. Another study investigated serum levels of soluble Angiopoietin-2 (sAng-2) in patients with hematological neoplasms: 24 MM and six MGUS patients. sAng-2 is a growth factor important in neoplastic angiogenesis. The measured sAng-2 levels were lower but not statistically significant in MGUS patients compared to control subjects. In adversity, sAng-2 levels were elevated in MM patients. This study gives a first indication that serum sAng-2 levels may play a role in the progression from MGUS to MM. (31) Several studies correlated angiogenesis with disease activity. Kuehl *et al*. (32) demonstrated low levels of angiogenesis in MGUS, higher levels in SMM and markedly higher levels in MM. Rajkumar *et al*. (33) also demonstrated a gradual increase in degree of BM angiogenesis, based on microvessel density (MVD) estimation, along

the disease progression from MGUS to MM. These studies investigated angiogenesis on protein levels. However, *VEGF* expression patterns were also examined. This will be further elucidated in the molecular part.

BONE METABOLISM

Bone metabolism and their associated markers are one of the possible leads to search for progressive biomarkers. Politou *et al*. (34) evaluated the role of bone (remodeling) markers measured in serum samples of MGUS, MM patients and controls. By comparing MGUS and MM patients, soluble receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) ratio, N-telopeptide of collagen type-I (NTX), tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) and macrophage inflammatory protein-1alpha (MIP-1α) were significantly decreased, while osteoprotegerin (OPG) and bone-alkaline phosphatase (bALP) were increased in MGUS. When comparing MGUS patients and control subjects, only sRANKL/OPG ratio and NTX levels were significantly increased in the MGUS patients. This implies that bone resorption is an early event, already present in the pre-stage. Although bone resorption is increased in MGUS patients, they do not have any (osteo)lyic lesions or diffuse osteoporosis. This can be explained by the normal osteoblast function with compensatory mechanisms to deal with the increased bone loss. This is in contrast to MM, where bone formation by osteoblasts does not compensate for the increased bone loss by osteoclasts, leading to (osteo)lytic lesions. Politou *et al*. (34) concluded that the above mentioned markers could be useful in the differential diagnosis of MGUS and MM. Another study proposed osteopontin (OPN) production as possible implying factor in disease progression. OPN is a secreted phosphoglycoprotein, involved in normal tissue remodelling processes. By immunocytochemistry, abundant OPN was detected as a brown stain in BM cells from the MM patients, while no OPN could be detected in the BM cells from patients with other haematological disease, including MGUS patients. To confirm, OPN concentrations were also measured in culture supernatans. More OPN was detected in freshly prepared BM cells from MM patients compared to those from SMM and MGUS patients, suggesting an active production of OPN by MM BM cells. Additionally, plasma OPN levels showed a similar pattern: significantly higher OPN plasma levels in MM patients compared to patients with MGUS and healthy controls. This demonstrated that OPN plasma levels correlate with disease progression and may be a useful biomarker for the differential diagnosis of MGUS and MM. (35)

IMMUNE SYSTEM

The role of the immune system and host response in controlling the malignant transformation was investigated. Although the same immune reactivity was detected in MGUS, non-progressive myeloma, progressive myeloma and healthy controls indicating general immune competence, a reversible defect in natural killer T cell function characterizing the progression from premalignant to malignant MM was shown. The clinical progression in MGUS patients was associated with loss of ligand-dependent IFN-γ production by invariant natural killer T cells. However, there was no detectable difference in the antitumor killer T cell function between progressive and non-progressive myeloma patients. They hypothesized that natural killer T effector function plays a role in the control of malignant growth of transformed PCs in gammopathies. (36)

STROMAL CELL CYTO- AND CHEMOKINES

Stromal cell cytokine and chemokine profiles were measured within the context of disease progression. Kline *et al.* (37) aimed to investigate the factors secreted by stromal cells, independent of stimulation by cell-to-cell contact with BM cells, and the soluble BM proteins that trigger stromal cell protein

production. The supernatans isolated after culturing the BM samples from MM patients had increased levels of the proteins IL-6, interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein 1beta (MIP-1β) compared to cultured MGUS patient BM supernatans, in which those protein levels were low to undetectable. As stromal cell cultures alone produced nearly saturating IL-6 levels, comparison of IL-6 between different patient groups using antibody arrays was not informative without sample dilution, since saturation of the membranes of the antibody arrays complicates the evaluation of increased cytokine expression. Next, the co-cultures of patient BM cultured supernatans with stromal cells showed increased IL-8 and MCP-1 levels in MM, compared to MGUS samples. Further research by ELISA showed a correlation of IL-6 and IL-8 with clinical disease (from MGUS to MM). There was a significant increase in stromal cell IL-6 and IL-8 production stimulated by BM cells from MM patients compared to those stimulated by BM of MGUS patients. When studied in SMM population, the levels represented an intermediate group with features of both. While the contribution of IL-6 is still uncertain and further research with reliable analyses and techniques (e.g. ELISA) is required, IL-8 is a protein to keep in mind for further studies elaborating their role and importance in the progression from MGUS to MM.

IMAGING MARKERS

As previously mentioned, bone lesions are a common feature of MM patients, while they are absent in the pre-stages MGUS or SMM. This fundamental difference resulted in the idea of searching for markers in the field of imaging (**Table 4**). An earlier detection of these lesions would identify patients with a higher risk to progress from the non-malignant pre-stage to the hematological cancer.

One of the variables linked to disease progression are the magnetic resonance imaging (MRI)-defined focal lesion number and size. (18, 26) Hillengass *et al.* (38) studied 137 patients with MGUS using whole-body MRI. This imaging technique non-invasively detects and displays the clonal PC accumulation and infiltration in the BM compartment, even before bone destruction has occurred. Of the 137 studied MGUS patients, 32 (23%) patients had focal lesions (with 1-46 focal lesions per patient) and 52 (8%) patients showed images with a diffuse infiltration pattern. In the median follow-up of 4.8 years, four of the 32 (13%) MGUS patients with focal lesions, seven of the 105 (7%) MGUS patients without lesions, six of the 52 (12%) MGUS patients with diffuse infiltration pattern and five of the 85 (6%) MGUS patients without diffuse infiltration progressed. The univariate analysis revealed both the presence and number (with optimal cut point of 0-1 versus >1) of focal lesions as prognostic factors for MM progression. When linked to other factors, patients with a diffuse infiltration in MRI were significantly younger than those with a normal appearing signal and patients with elevated M-protein levels had significantly more focal lesions. Whole-body MRI can be of prognostic significance of MGUS patients and predicting their MM progression risk.

Also MRI of the spine appeared to be useful in the evaluation of the presence of BM infiltration, plasmacytoma and focal lesions. (22, 39) Dhodapkar *et al.* (22) examined 64 MGUS patients with available MRI data. Focal lesions >1 were detected by MRI imaging of the spine in three of the 64 (5%) MGUS patients. Univariate analysis revealed a significant association of the presence of focal lesions (>1) on MRI of the spine with an increased progression risk to clinical MM. The multivariate analysis revealed that the presence of >1 MRI-detected focal lesions was not significantly associated, which was probably due to the small study population. However, this study identified the presence of multiple (>1) MRI-detected focal lesions as an independent risk factor.

In an MV analysis restricted to patients with available MRI data, the presence of >1 MRI-detected focal lesions did not emerge as an independent variable (data not shown). The presence of multiple (>1) focal lesions was detected by MRI in only 9 (6%) patients. However, the presence of multiple (>1) MRI-detected focal lesions was an independent predictor of increased risk of disease progression

Beside bone and osteolytic lesions, angiogenesis is a possible factor playing a role in the progression from MGUS to MM. Increased angiogenesis leads to changes of BM microcirculation. Based on this, the use of dynamic contrast-enhanced MRI (DCE-MRI), an imaging technique able to visualize the changes in microcirculation, in predicting the disease progression was investigated. This study included 60 MGUS patients, 75 newly diagnosed symptomatic MM patients and 22 healthy controls with DCE-MRI of the lumbar spine. Beside the characterization of microcirculation patterns, also semi-quantitative microcirculation parameters were measured. The median of amplitude A and exchange rate constant *kep* was significantly higher in MGUS and MM patients compared to controls and was significantly different between MGUS and MM. A continuously increase in the median of both microcirculation parameters was observed from controls to symptomatic MM. (40) Hillengass *et al.* (40) hypothesized that in the process of microcirculation augmentation from MGUS to symptomatic MM, the impaired structure of the walls of neo-angiogenic blood vessels can be detected before an actual increase in vascular volume appears. So, these findings propose that angiogenesis in the BM increases with progression from premalignant to symptomatic disease. DCE-MRI might be clinical relevant for identifying MGUS patients with higher risk to progress to MM. (12, 40)

Although different imaging techniques (such as MRI) are used in the search for predictive imaging biomarkers, the IMWG defined criteria for bone lesions are still based on the amount of osteolytic lesions determined on conventional skeletal radiography, CT, or PET-CT. (2, 5, 38)

MOLECULAR MARKERS

Several studies suggested that secondary mutational changes in oncogenic pathways are the driving force behind MM progression (**Table 5**). One such pathway is the deregulation of *MYC*. The shift from MGUS to MM might be driven by MYC, as MYC is activated during this transformation. (41) This finding is supported by a recent study, which has found that the MYC signaling was activated in 85% of MM patients, while being undetectable in MGUS patients. Using serial BM samples, Xiao *et al*. (42) demonstrated that 62.5% of MGUS patients who progressed to MM showed induced expression of *MYC*. These findings raised the idea that *MYC* expression is involved in the progression from MGUS to MM. Another study by Chng *et al*. (43) showed that *MYC* activation is likely to be an early event in myeloma pathogenesis. They also suggested an association between RAS mutations and *MYC* activation, whereby the MYC protein is stabilized by RAS mutants, prolonging the oncogenic activity of MYC. (43, 44) A different study revealed the presence of *RAS* mutations in about 30% of MM patients, while it was rarely seen in MGUS. This data suggests that activating *RAS* mutations play a role in disease progression. (32, 43)

Nagoshi *et al*. (45) investigated the role of the Deleted in Colorectal Carcinoma (*DCC*) gene in the pathophysiology of MM and identified four types of *DCC* transcripts (wild type (*wt).DCC* , splice variant (*sv).DCC*, and two types of mutant (*mt).DCCs*) in patient-derived PCs from MGUS and MM. The *mt.DCCs* transcripts were found in 25% and 57% of PCs from MGUS (n=8) and MM (n=30), respectively, indicating an increased presence along with disease progression. In one patient who progressed from MGUS to MM, a change of *DCC* expression pattern was demonstrated. In the MGUS phase, only *sv.DCC* was

expressed, whereas both *mt.DCC* and *sv.DCC* were expressed after transformation to MM. This study suggests that the detection of *mt.DCCs* could be one of the biomarkers indicative of disease progression.

Nestin (NES), a class VI intermediate filament protein, plays a role in several cell processes, such as proliferation, migration, and cell survival. Since NES levels correlate with tumor grade and represent a marker of cancer stem cells, it is presumed that NES also plays a role in carcinogenesis. Svachova *et al*. (44) analyzed the occurrence of NES throughout the pathogenesis of MM, including patients with no hematological malignancy and MGUS. They showed that *NES* is differentially expressed in both patients with MM and MGUS. A significant difference was found between MGUS and MM patients according to the %NES^+PCs and the intensity of NES fluorescence in $CD138^+38^+$ PCs of MM and $CD138^+38^+56^+19^-$ PCs of MGUS.

The Notch family of transmembrane proteins acts as receptors and transcription factors simultaneously. They provide a balance between self-renewal and differentiation both in normal and cancerous cells. Tumor cells do express the Notch ligands *Jagged1* and *Jagged2*. Interestingly, Colombo *et al*. showed that *Jagged2* is deregulated in the MGUS phase, while *Jagged1* deregulation is only found during progression to MM. They concluded that cancer cells express *Jagged1* and *Jagged2* through the activation of two different mechanisms in different phases of MM progression. (46, 47)

Human leukocyte antigen (HLA) class I and II molecules play a key role in the development of T-cell responses against myeloma, stopping the disease progression in early stages. Spanoudakis *et al*. (48) investigated the effect of CD1d – a HLA class I-like molecule – ligation on the survival of myeloma cells and expression of CD1d during disease progression. They demonstrated that *CD1d* is highly expressed in MGUS and even in early myeloma, while its expression was reduced and eventually lost in advanced stages. These findings suggest that CD1d expression is a negative predictor for MM cell survival. Another study of Robillard *et al*. (49) also focused on the role of T lymphocytes. They investigated the effect of *CD28* expression on PCs of MGUS and MM patients. CD28 is an antigen expressed on T cells and plays a role in T-cell activation and T-cell-B-cell interactions. $CD28^+$ PCs were detected in 19% of 31 MGUS samples and in 41% of 116 MM samples (i.e. 27% of 79 MM samples at diagnosis, 59% of 22 medullary relapse MM samples and 93% of 15 extramedullary relapse MM samples), which suggests that its expression correlates with disease progression.

As mentioned in the clinical part, angiogenesis may play a role in MM progression. Kumar *et al*. (50) examined the expression of *VEGF*, *basic fibroblast growth factor* (*bFGF*) and their receptors in PCs in patients with MGUS and MM. At mRNA level, they demonstrated no significant difference between MGUS and MM. However, they suggested that the gradual increase in angiogenesis, assessed by MVD, with disease progression may be related to the cumulative angiogenic effect of increasing numbers of PCs rather than increased *VEGF*/*bFGF* expression by individual PCs. Nevertheless, they maintained their hypothesis that increased angiogenesis is causally involved in the progression from MGUS to MM and addressed the need of serial BM samples from individual patients at different stages of the disease to shed more light on the biology behind this progression.

An upcoming topic in the search for molecular changes in MM is targeted gene expression profiling (GEP). The University of Arkansas for Medical Sciences (UAMS) group was the first to define a 70-gene classifier (GEP70) with genes linked to short survival, including seven gene expression clusters, identifying patients with a high risk for short progression-free survival (PFS) and overall survival (OS). (18, 51) A GEP70 risk score of >-0,26 seemed to correlate with an increased risk of disease progression and

emerged as a significant prognostic variable. The GEP70 associated risk was more evident in the SMM than in the MGUS cohort. These findings showed that an increased GEP70 score was an independent predictor of the risk of transformation from precursors to MM. (18, 22)

Zhan *et al*. (52) identified with whole genome microarrays 52 genes involved in cancer pathways, with diverse expression patterns in normal, MGUS and MM PCs. Unsupervised hierarchical cluster analysis of these genes identified MGUS with features of MM and MM with features of MGUS. Of these genes, 41 presented a progressive increased expression pattern along the transition from normal PCs, MGUS and MM, while four showed a reduced pattern. Six genes showed higher and one a lower expression level in MGUS compared with normal PCs or MM. They classified patients into four groups: MM-like MGUS, non-MM-like MGUS, MGUS-like MM, and non-MGUS-like MM. Based on their findings, they proposed that MM-like MGUS patients have an increased risk of progression and that these patients may benefit from closer follow-up and earlier treatments. However, this hypothesis needs to be tested on larger cohorts of patients. Other studies did find significant gene expression differences between MGUS and normal PCs, but not between MGUS and MM. (18, 22, 52) However, Ria *et al*. (53) detected 22 genes that were differentially expressed in the two latter groups. Of these, 14 genes were down-regulated and eight were up-regulated in MM when compared to MGUS. Although these data were interesting, GEP analysis of MGUS has the inherent problem that the percentage of PCs is low, by definition <10%, so there is significant contamination with other kinds of cells. Even monoclonal PCs are also likely to be contaminated with normal PCs, which can severely affect gene expression profiles. (12)

The group of Jungbluth *et al*. (54) detected cancer/Testis Antigens (CTAs) in tumor samples from patients with MM. More specifically, CTA members of the type-I melanoma antigen (*MAGE*) family appear to contribute to the malignant phenotype. (55) In a previous study from the same research group, three CTAs (*MAGE-C1/CT7, MAGE-A3/6, and LAGE-1*) were found to be often expressed in MM. The CTA *MAGE-C1/CT7* gene was most frequently expressed in MM and may have a prognostic impact in overall survival. (56) However, they only investigated the role of CTA *MAGE-C1/CT7* in three patients with MGUS. To fully understand the impact of *MAGE-C1/CT7* expression on disease progression, more MGUS patients must be investigated.

Using the Database for Annotation, Visualization and Integrated Discovery (DAVID) Functional Annotation analysis, we demonstrated gene clusters and common pathways between above mentioned genes. Gene Ontology analysis demonstrated two important biological processes affected by these genes. Seven genes (i.e. *CD1d*, *CD28*, *KRAS*, *JAG1*, *NES*, *TP53*, and *MYC*) were involved in the regulation of cell proliferation, while another cluster of seven genes (i.e. *CD28*, *DCC*, *KRAS*, *MAGEA3*, *NES*, *TP53*, and *MYC*) were involved in programmed cell death/apoptotic processes. Both processes are known to be dysregulated in several cancers.

EPIGENETICS

There is a considerable amount of evidence supporting the idea that epigenetic changes, including DNA methylation, histone acetylation, and microRNAs (miRNAs) are important for MM development and progression (**Table 6**). (18) During the progression from MGUS to MM, global DNA methylation and gene-specific DNA hypermethylation are the most important epigenetic changes identified so far. Normal B cells, PCs, and MGUS cells have a methylation pattern that is different from that of malignant cells in patients with newly diagnosed MM. (1, 18)

Several studies report on the role of promoter methylation in patients with MGUS and MM. (12, 57-59) Based on small numbers, the promoters of *p16*, E-cadherin (*ECAD*), death-associated protein kinase (*DAPK*), human mutL homolog 1 *(hMLH-1*) and suppressor of cytokine signaling 1 (*SOCS-1*) have been shown to be increasingly methylated in MM compared with MGUS. (12) One study showed that the prevalence of p16 methylation was increased as the disease progressed; 24% in MGUS and 34% in MM. However, p16 methylation may not contribute in the transformation process from MGUS to MM as 24% of MGUS patients already harbors p16 methylation. (57)

Seidl *et al*. (58) investigated the percentage of methylation of the genes *p16*, tissue inhibitor of metalloproteinase 3 (*TIMP3*), *p15, ECAD, DAPK, p73*, RAS-association domain family 1A (*RASSF1A*), p14, O6-methylguanine DNA methyltransferase (*MGMT*), and retinoid acid receptor β2 (*RARβ*) in large numbers of patients with MGUS and MM. Except for *p15* and *ECAD*, most methylation patterns were present in both MGUS and MM. For *p15*, methylation was detected in a greater proportion in MM samples. In contrast, they found that *ECAD* was unmethylated in all MGUS samples, while a significant percentage of ECAD methylation was observed in SMM (20%) and MM (27%). This suggests that *ECAD* methylation is a potential biomarker for disease progression. They also demonstrated in two patients with follow-up samples that *ECAD* was unmethylated at the time of diagnosis but methylated at the time of disease progression.

Walker *et al*. (59) analyzed the methylation status of more than 27,000 CpG sites in normal PCs, MGUS, and myeloma samples. They revealed that at the transformation from MGUS to MM, 1428 genes were hypomethylated. In contrast, they identified gene-specific hypermethylation involving 77 genes only, suggesting the importance of both hypo- and hypermethylation.

A common DNA methylation change is seen in patients with the t(4;14) translocation, whose myeloma samples have increased gene-specific DNA hypermethylation compared with samples of other cytogenetic subgroups. This t(4;14) subgroup overexpresses *MMSET*, which encodes a histone methyltransferase transcriptional repressor. MMSET mediates histone H3 lysine 36 (H3K36) methylation and its deregulation leads to global changes in histone modifications that promote cell survival, cell cycle progression and DNA repair. (1) They did not investigate these methylation levels in MGUS patients with t(4;14).

miRNAs are an abundant class of regulatory noncoding single-stranded RNA molecules involved in the regulation of gene expression. They play crucial roles in biological processes, including cell motility, differentiation, proliferation and apoptosis; they also contribute to tumor formation and progression. (60, 61) Divers studies, using BM PCs, found several deregulated miRNAs in MM and MGUS. However, since taking a BM sample is an invasive procedure, Kubiczkova *et al*. (61) identified a profile of five miRNAs, which are deregulated in MM and MGUS sera. Levels of miR-744, miR-130a, let-7d and let-7e were significantly decreased whereas miR-34a was increased in MM and MGUS. However, they did not find miRNAs predicting the progression from MGUS to MM. (61) Jones *et al*. (62) performed miRNA expression profiling of serum samples as well. They showed that the combination of miR-1246 and miR-1308 could distinguish myeloma from MGUS patients, with a sensitivity of 79.2% and specificity of 66.7%. To improve the sensitivity and specificity, analyses of more miRNAs are required. (60, 62)

Another study from Pichiorri *et al*. (63) found 41 miRNAs up-regulated and seven down-regulated in MGUS compared with $CD138^{+}$ healthy controls. Also, analysis of PCs in patients with MM revealed up-regulation of 60 and down-regulation of 36 miRNAs when compared to CD138+ healthy controls.

However, unlike MGUS, the miR-17-92 cluster (in particular miR-19a and b) was significantly up-regulated only in MM samples. (63) Two other studies detected miR-32 and the cluster miR-17-92 to be up-regulated in myeloma, but not in MGUS, suggesting a possible role in the progression of MGUS to MM. (1, 12, 63)

CRITICAL NOTE AND DISCUSSION

Despite the targeted Pubmed search for tumor biomarkers in disease progression from MGUS to MM, still a lot of the selected references needed to be excluded because the focus was elsewhere, i.e. therapy, without MGUS and/or MM etc. Although the use of 'biomarkers' as search term, descriptive studies, purely defining differences between the groups without mentioning their role as potential biomarkers, were recorded too. In fact, the identified biomarkers could be distinguished into two different types: factors associated with higher likelihood of progression (predictors) versus markers with only a differential expression or presence when compared between the two disease entities. Therefore, an extra column was added to the tables to clearly delineate the identified findings. As both the pre-stages as MM self are still very complex and heterogeneous diseases, it is important to clearly delineate and describe the different study groups in papers. However, this was not always the case. In order to draw unambiguous conclusions, clearly defined study populations are needed. Furthermore, the use of the same units (i.e. g/L, g/dL) and terms would be very valuable. However, the reasons for these difficulties are due to the constant revising of clinical and laboratory parameters.

For the part of clinical markers, a wide range of different potential markers were listed. There was little overlap between the studies but also barely no contradictions or inconsistencies were identified. So, most studies described very diverse parameters, also accentuating/emphasising the complexness and heterogeneity of the disease. Where the clinical markers were very broad and diverse, the part of imaging markers was more limited and bordered. For the molecular part, several genes (e.g. *DCC*) were only mentioned once in the literature search for this systematic review. Although these studies showed promising results, more research is necessary to fully unravel the role of those specific genes in the pathogenesis of MM. Some of the included studies did not mention the exact number of patients/samples for each clinical stage, making it difficult to draw clear conclusions. Remarkable, whereas a broad range of potential biomarkers (i.e. mutations, epigenetics, serum levels) were identified, no biomarkers directly/closely related to the BM microenvironment were found in this literature search, even though this microenvironment is known to play an important role in PC growth and survival. (8)

Currently, lifelong annual (routine) follow-up is recommended for patients with the asymptomatic, non-malignant pre-stages. However, retrospectively, it was already determined that only a minority of the MM diagnoses is attributable to serial follow-up (laboratory testing) of MGUS patients. The majority of MM is still diagnosed by the clinical presence of serious MM-related complications and other symptoms. (64, 65) These findings imply that the current watch-and-wait strategy is too standardized and does not work efficiently. On the other hand, it has been shown that MM patients with prior knowledge of MGUS have significantly better overall survival than MM patients without this awareness. Even though the watch and wait strategy does not work optimally, these findings stress the importance of clinical follow-up in MGUS patients. (66) Therefore, an adapted approach of personalized follow-up is highly required. The identification of biomarkers with a predictive value and associated with a higher likelihood of progression to MM would be of importance in the prediction of which individual MGUS patients will progress to MM and which will not. Further strategies regarding their follow-up and treatment regimens

can be guided, based on the early biomarker data. The MGUS patients with identified high-risk markers could have more regular and close follow-up. Additionally, the potential for earlier therapeutic intervention and its usefulness within MGUS may be explored. As this malignancy is still incurable, an early selection of individuals having the highest chance to progress would/may be of critical importance to be able to treat those patients as early as possible. This approach already showed significant benefits on time to progression and overall survival in SMM patients. However, larger patient cohorts need to be studied in order to investigate the effect of early treatment on long-term toxicities and outgrowth of more resistant clones. (67, 68)

Additionally, given the heterogeneity and broad range of the different identified markers (clinical, molecular), this will also determine future treatment strategies. Similar to the identified markers, the clinical onset of MM is highly heterogeneous. Disease initiation and progression is due to multistep and –factorial transformational processes. In MM, cytogenetics and fluorescence in situ hybridization (FISH) results have prognostic significance. High-risk abnormalities such as hyperdiploidy, deletions (i.e. del(13), del(17p)) and translocations (i.e. t(4;14), t(14;16)) are mostly associated with adverse outcome. In contrast, hypodiploidy and translocation t(11;14) are indicated as rather standard risk features. (9)

Although this literature search defined a lot of potential markers, it is currently not possible to classify or define individual patients unequivocally in high- or low-risk groups and thus to predict which MGUS/SMM patients will progress to MM. When validated in a larger cohort of patients, the classification of Zhan et al. (52) may be used to evaluate MGUS and MM patients in order to go towards personalized therapy. Treatment may be postponed in patients with MGUS-like MM, whereas subjects with MM-like MGUS may benefit from early therapeutic intervention. This categorization could be useful for SMM as well, since it may distinct high-risk SMM patients from SMM patients with MGUS-like signatures. A subset of MGUS-like MM was identified and validated with favorable clinical features and longer survival. These results are supported by the observation of MGUS-like signatures in most patients surviving more than 10 years after initiation of total therapy 1. (69)

CONCLUSION

The clonal PC malignancy MM is a very complex disease. Despite many studies, the knowledge about disease initiation and progression is still limited and incomplete. It is known that MM is preceded in practically all cases by an asymptomatic, non-malignant pre-stage, called MGUS. The progression risk from MGUS to MM is about 1% per year. Reliable biomarkers to discriminate an individual MGUS patient with high risk to progress to MM, are not available. This would improve clinical management of MGUS patients dramatically. Whereas high-risk patients would benefit from frequent monitoring with close follow-up and earlier treatment, low-risk patients should not undergo unnecessary BM and other examinations. More understanding of the pathogenesis underlying both the premalignant as malignant disease will give insights in new molecular pathways. Additionally, new therapeutic targets can arise from these findings. Until now, no biomarkers predicting the progression from MGUS to MM are identified.

Conclusively, in this systematic review, we discussed the current clinical, imaging and molecular markers. A whole range of different possible implying factors were identified, increasing the risk of progression from MGUS to MM. However, no reliable markers are identified to use in clinical practice. Confirmation of findings in independent cohort studies are necessary and future research should focus on larger cohorts and study populations to account for the inter- and intra-clonal character of this highly

heterogeneous disease. Additionally, serial samples, which are follow-up samples from the same patient at different time-points in the disease progression from MGUS to MM, would be a very valuable source of information, both improving molecular understanding as finding predictive biomarkers.

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TABLES

Table 1: Criteria defining MGUS, SMM, and MM. (2, 5) The asymptomatic, non-malignant pre-stages MGUS and SMM are collectively referred to as AMG. Each subgroup has typical cut-off values and diagnostic criteria. AMG, asymptomatic monoclonal gammopathy; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma; MM, multiple myeloma.

Disorder			Definition	Reference
AMG	MGUS		<3 g/dL M-protein	(70)
		AND	<10% clonal plasma cells on BM biopsy	
		AND	Absence of CRAB-criteria*	
	SMM		≥ 3 g/dL M-protein	(5)
		OR	≥ 500 mg/24 hours urinary M-protein	
		AND/OR	10-60% clonal plasma cells on BM biopsy	
		AND	Absence of CRAB-criteria	
MM			≥10% clonal plasma cells on BM biopsy	(5)
		AND	Myeloma defining events: evidence of end organ damage attributed to underlying plasma cell proliferative disorder = presence of CRAB-criteria*	
		OR	Any one or more of the following biomarkers of malignancy:	
			≥60% clonal plasma cells on BM biopsy	
			AND/OR Involved:uninvolved sFLC ratio ≥ 100	
			AND/OR >1 focal lesion on MRI studies (≥5 mm in size)	

**The CRAB-criteria are clearly defined events of end organ damage (5):*

- Hypercalcemia: serum calcium >0, 25 mmol/l (>1 mg/dl) higher than the upper limit of normal or >2, 75 mmol/l (>11 mg/dl)

- Renal insufficiency: creatinine clearance <40 ml per min or serum creatinine >177 µmol/l (>2 mg/dl)

- Anemia: hemoglobin value of >20 g/l below the lower limit of normal or a hemoglobin value <100 g/l

- Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT

Table 2: PubMed search strategy, performed on June 27th, 2017. The combination (#5) of following MeSH terms was used: 'Multiple Myeloma' (#1) OR 'Monoclonal Gammopathy of Undermined Significance' (#2) AND 'Disease Progression' (#3) AND 'Biomarkers, Tumor' (#4). A Title/Abstract [tiab] search (search terms have to be in the title and/or abstract) was performed in order to avoid the detection of abstracts that do not contain the terms you were searching for.

Search	Query
#5	Search (((((“Multiple Myeloma”[MeSH] OR Multiple Myelomas[tiab] OR Myelomas, Multiple[tiab] OR Myeloma, Multiple[tiab] OR Myeloma, Plasma-Cell[tiab] OR Myeloma, Plasma Cell[tiab] OR Myelomas, Plasma-Cell[tiab] OR Plasma-Cell Myeloma[tiab] OR Plasma-Cell Myelomas[tiab] OR Myelomatosis[tiab] OR Myelomatoses[tiab] OR Plasma Cell Myeloma[tiab] OR Cell Myeloma, Plasma[tiab] OR Cell Myelomas, Plasma[tiab] OR Myelomas, Plasma Cell[tiab] OR Plasma Cell Myelomas[tiab] OR Kahler Disease[tiab] OR Disease, Kahler[tiab] OR Myeloma-Multiple[tiab] OR Myeloma Multiple[tiab] OR Myeloma-Multiples[tiab])))) OR ((“Monoclonal Gammopathy of Undetermined Significance”[MeSH] OR Monoclonal Gammapathies, Benign[tiab] OR Benign Monoclonal Gammapathies[tiab] OR Benign Monoclonal Gammapathy[tiab] OR Gammapathies, Benign Monoclonal[tiab] OR Gammapathy, Benign Monoclonal[tiab] OR Monoclonal Gammapathy, Benign[tiab] OR Monoclonal Gammopathies, Benign[tiab] OR Benign Monoclonal Gammopathies[tiab] OR Benign Monoclonal Gammopathy[tiab] OR

	Gammopathies, Benign Monoclonal[tiab] OR Gammopathy, Benign Monoclonal[tiab] OR Monoclonal Gammopathy, Benign[tiab] OR Monoclonal Gammapathy of Undetermined Significance[tiab]))) AND (("Disease Progression"[MeSH] OR Disease Progressions[tiab] OR Progression, Disease[tiab] OR Progressions, Disease[tiab] OR Disease Exacerbation[tiab]))) AND (("Biomarkers, Tumor"[MeSH] OR Tumor Biomarkers[tiab] OR Markers, Biological Tumor[tiab] OR Tumor Markers, Biological[tiab] OR Markers, Tumor Metabolite[tiab] OR Tumor Metabolite Markers[tiab] OR Metabolite Markers, Tumor[tiab] OR Marker, Tumor Metabolite[tiab] OR Metabolite Marker, Tumor[tiab] OR Tumor Metabolite Marker[tiab] OR Tumor Markers, Biologic[tiab] OR Biologic Tumor Markers[tiab] OR Markers, Biologic Tumor[tiab] OR Marker, Biologic Tumor[tiab] OR Biologic Tumor Marker[tiab] OR Tumor Marker, Biologic[tiab] OR Biochemical Tumor Markers[tiab] OR Markers, Biochemical Tumor[tiab] OR Marker, Biochemical Tumor[tiab] OR Biochemical Tumor Marker[tiab] OR Tumor Marker, Biochemical[tiab] OR Tumor Markers, Biochemical[tiab] OR Carcinogen Markers[tiab] OR Markers, Carcinogen[tiab] OR Markers, Neoplasm Metabolite[tiab] OR Neoplasm Metabolite Markers[tiab] OR Marker, Neoplasm Metabolite[tiab] OR Metabolite Marker, Neoplasm[tiab] OR Neoplasm Metabolite Marker[tiab] OR Metabolite Markers, Neoplasm[tiab] OR Biological Tumor Markers[tiab] OR Biological Tumor Marker[tiab] OR Tumor Marker, Biological[tiab] OR Marker, Biological Tumor[tiab] OR Markers, Tumor[tiab] OR Tumor Markers[tiab] OR Biomarkers, Cancer[tiab] OR Cancer Biomarkers[tiab]))
#4	Search ("Biomarkers, Tumor"[MeSH] OR Tumor Biomarkers[tiab] OR Markers, Biological Tumor[tiab] OR Tumor Markers, Biological[tiab] OR Markers, Tumor Metabolite[tiab] OR Tumor Metabolite Markers[tiab] OR Metabolite Markers, Tumor[tiab] OR Marker, Tumor Metabolite[tiab] OR Metabolite Marker, Tumor[tiab] OR Tumor Metabolite Marker[tiab] OR Tumor Markers, Biologic[tiab] OR Biologic Tumor Markers[tiab] OR Markers, Biologic Tumor[tiab] OR Marker, Biologic Tumor[tiab] OR Biologic Tumor Marker[tiab] OR Tumor Marker, Biologic[tiab] OR Biochemical Tumor Markers[tiab] OR Markers, Biochemical Tumor[tiab] OR Marker, Biochemical Tumor[tiab] OR Biochemical Tumor Marker[tiab] OR Tumor Marker, Biochemical[tiab] OR Tumor Markers, Biochemical[tiab] OR Carcinogen Markers[tiab] OR Markers, Carcinogen[tiab] OR Markers, Neoplasm Metabolite[tiab] OR Neoplasm Metabolite Markers[tiab] OR Marker, Neoplasm Metabolite[tiab] OR Metabolite Marker, Neoplasm[tiab] OR Neoplasm Metabolite Marker[tiab] OR Metabolite Markers, Neoplasm[tiab] OR Biological Tumor Markers[tiab] OR Biological Tumor Marker[tiab] OR Tumor Marker, Biological[tiab] OR Marker, Biological Tumor[tiab] OR Markers, Tumor[tiab] OR Tumor Markers[tiab] OR Biomarkers, Cancer[tiab] OR Cancer Biomarkers[tiab])
#3	Search ("Disease Progression"[MeSH] OR Disease Progressions[tiab] OR Progression, Disease[tiab] OR Progressions, Disease[tiab] OR Disease Exacerbation[tiab])
#2	Search ("Monoclonal Gammopathy of Undetermined Significance"[MeSH] OR Monoclonal Gammapathies, Benign[tiab] OR Benign Monoclonal Gammapathies[tiab] OR Benign Monoclonal Gammapathy[tiab] OR Gammapathies, Benign Monoclonal[tiab] OR Gammapathy, Benign Monoclonal[tiab] OR Monoclonal Gammapathy, Benign[tiab] OR Monoclonal Gammopathies, Benign[tiab] OR Benign Monoclonal Gammopathies[tiab] OR Benign Monoclonal Gammopathy[tiab] OR Gammopathies, Benign Monoclonal[tiab] OR Gammopathy, Benign Monoclonal[tiab] OR Monoclonal Gammopathy, Benign[tiab] OR Monoclonal Gammapathy of Undetermined Significance[tiab])
#1	Search ("Multiple Myeloma"[MeSH] OR Multiple Myelomas[tiab] OR Myelomas, Multiple[tiab] OR Myeloma, Multiple[tiab] OR Myeloma, Plasma-Cell[tiab] OR Myeloma, Plasma Cell[tiab] OR Myelomas, Plasma-Cell[tiab] OR Plasma-Cell Myeloma[tiab] OR Plasma-Cell Myelomas[tiab] OR Myelomatosis[tiab] OR Myelomatoses[tiab] OR Plasma Cell Myeloma[tiab] OR Cell Myeloma, Plasma[tiab] OR Cell Myelomas, Plasma[tiab] OR Myelomas, Plasma Cell[tiab] OR Plasma Cell Myelomas[tiab] OR Kahler Disease[tiab] OR Disease, Kahler[tiab] OR Myeloma-Multiple[tiab] OR Myeloma Multiple[tiab] OR Myeloma-Multiples[tiab])

Table 3: Summary clinical markers. HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma; MM, multiple myeloma; BL, blood; UR, urine; BM, bone marrow; PCs, plasma cells; D, differential presence/expression; L, likelihood/predictor

Marker		Patients	Sample	D or L	Ref
M protein	> 1.5 g/dL	MGUS (n=1384)	BL, UR and BM	L	(20)
		MGUS (n=1148)	Cryo-preserved BL, UR and BM	L	(21)
	≥ 3 g/dL	MGUS (n=152)	BL, UR and BM	L	(22)
	Concentration	MGUS (n=407)	BL, UR and BM	L	(24)
	Size	MGUS (n=999)	Cryo-preserved BL	L	(28)
	IgM	MGUS (n=213)	BL and BM	L	(23)
	Non-IgG	MGUS (n=1384)	BL, UR and BM	L	(20)
	Non-IgG	MGUS (n=1148)	Cryo-preserved BL, UR and BM	L	(21)
	IgA	MGUS (n=407)	BL, UR and BM	L	(24)

	Heavy chain isotype	MGUS (n=999)	Cryo-preserved BL	L	(28)
		MGUS (n=1148)	Cryo-preserved BL, UR and BM	L	(21)
Bone marrow plasma cells	Not mentioned	MGUS (n=1384)	BL, UR and BM	L	(20)
		MGUS (n=1148)	Cryo-preserved BL, UR and BM	L	(21)
	≥ 20%	MGUS (n=152)	BL, UR and BM	L	(22)
		MGUS (n=407)	BL, UR and BM	L	(24)
FLC ratio	Abnormal	MGUS (n=1384)	BL, UR and BM	L	(20)
	Abnormal	MGUS (n=1148)	Cryo-preserved BL, UR and BM	L	(21)
	Abnormal	MGUS (n=999)	Cryo-preserved BL	L	(28)
	Abnormal	MGUS (n=407)	BL, UR and BM	L	(24)
	> 25 mg/dL	MGUS (n=152)	BL, UR and BM	L	(22)
Involved/uninvolved sFLC	> 10	MGUS (n=152)	BL, UR and BM	L	(22)
aPC/BMPC	95%	MGUS (n=407)	BL, UR and BM $CD138^+$ PCs	L	(24)
DNA index		MGUS (n=407)	BL, UR and BM	L	(24)
HLC-pair suppression		MGUS (n=999)	Cryo-preserved BL	L	(28)
Uninvolved Ig suppression		MGUS (n=999)	Cryo-preserved BL	L	(28)
		MGUS (n=152)	BL, UR and BM	L	(22)
Age	≥ 65 years	MGUS (n=152)	BL, UR and BM	L	(22)
Hemoglobin	< 12 g/dL	MGUS (n=152)	BL, UR and BM	L	(22)
Serum albumin	< 4 g/dL	MGUS (n=152)	BL, UR and BM	L	(22)
Serum β2-microglobulin	> 3 mg/L	MGUS (n=152)	BL, UR and BM	L	(22)
sCD105		HC (n=28), MGUS (n=20), MM (n=50)	BL and BM	D	(30)
sTGF-β1		HC (n=28), MGUS (n=20), MM (n=50)	BL and BM	D	(30)
sAng		HC (n=15), MGUS (n=6), MM (n=24)	BL	D	(31)
Microvessel density		HC (n=42), MGUS (n=76), MM (n=99)	BM	D	(33)
IL-6 serum levels		HC (n=28), MGUS (n=20), MM (n=50)	BL and BM	D	(30)
		Not mentioned	BM	D	(37)
IL-8		Not mentioned	BM	D	(37)
IFN-γ	Loss of production	MGUS/MM (n=23)	BL and BM	D	(36)
MIP-1α		HC (n=45), MGUS (n=40), MM (n=42)	BL	D	(34)
MIP-1β		Not mentioned	BM	D	(37)
NTX		HC (n=45), MGUS (n=40), MM (n=42)	BL	D	(34)
TRACP-5b		HC (n=45), MGUS (n=40),	BL	D	(34)

		MM (n=42)			
bALP		HC (n=45), MGUS (n=40), MM (n=42)	BL	D	(34)
OPN		HC (n=30), MGUS (n=21), MM (n=30)	BL and BM	D	(35)
MCP-1		Not mentioned	BM	D	(37)
OPG		HC (n=45), MGUS (n=40), MM (n=42)	BL	D	(34)
RANKL/OPG ratio		HC (n=45), MGUS (n=40), MM (n=42)	BL	D	(34)
Urine M protein	> 0 g/dL	MGUS (n=152)	BL, UR and BM	L	(22)

Table 4: Summary imaging markers. HC, healthy control; AMG, asymptomatic monoclonal gammopathy; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; D, differential presence/expression; L, likelihood/predictor

Marker		Patients	Sample	D or L	Ref
Focal lesions	Not mentioned	MGUS (n=137)	Whole body MRI	L	(38)
	> 1 or ≥ 1	MGUS (n=64)	MRI of the spine	L	(22)
Microcirculation (angiogenesis)	Amplitude A	HC (n=22), MGUS (n=60), MM (n=75)	DCE-MRI of the lumbar spine	D	(40)
	Exchange constant *kep*			D	

Table 5: Summary genetic and transcriptomic markers. HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma; MM, multiple myeloma; BM, bone marrow; PCs, plasma cells; BL, blood; D, differential presence/expression; L, likelihood/predictor

Marker		Patients	Sample	D or L	Ref
MYC	Expression	MGUS (n=29), MM (n=26)	BM	L	(42)
	Activation	HC (n=15), MGUS (n=22), MM (n=101)	$CD138^+$ PCs	D	(43)
RAS	Mutation	HC (n=15), MGUS (n=22), MM (n=101)	$CD138^+$ PCs	D	(43)
DCC (wt.DCC, sv.DCC, and mt.DCC)	Mutation	MGUS (n=8), MM (n=30)	BM	D	(45)
NES	Expression	MGUS (n=9), MM (n=163)	BM	D	(44)
Jagged 1 (JAG1)	Expression	HC (n=4), MGUS/SMM/MM (n=14)	BM	L	(47)
JAG2	Expression	HC (n=4), MGUS/SMM/MM (n=14)	BM	D	(47)
CD1d	Expression	MGUS (n=8), MM (n=35)	BM	D	(48)
CD28	Expression	MGUS (n=31), MM (n=116)	PCs	D	(49)
VEGF	Expression	MGUS (n=10), MM (n=7)	PCs	D	(50)
bFGF	Expression	MGUS (n=10), MM (n=20)	PCs	D	(50)
GEP70	Expression	HC/MGUS/MM (n=532)	PCs	L	(51)
	Expression	MGUS (n=152)	$CD138^+$ PCs	L	(22)
52 gene cluster	Expression	HC (n=22), MGUS (n=44), MM (n=367)	Not mentioned	D	(52)
22 gene cluster	Expression	MGUS (n=5), MM (n=5)	BM	D	(53)

MAGE-C1 and MAGE-A3/6	Expression	MGUS (n=14), MM (n=44)	BM	D	(54)
	Expression	HC (n=10), MGUS (n=3), MM (n=39)	BM	D	(56)

Table 6: Summary epigenetic markers. HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma; MM, multiple myeloma; BM, bone marrow; PCs, plasma cells; BL, blood; D, differential presence/expression; L, likelihood/predictor

Marker		Patients	Sample	D or L	Ref
Deletion	**p16 (*CDKN2A*)**	MGUS (n=20), MM (n=37)	Cytospin slides	D	(57)
Methylation	**p16 (*CDKN2A*)**	MGUS (n=5), MM (n=37)	BM	D	(57)
		MGUS (n=12), MM (n=46)	$CD138^+$ PCs	D	
	ECAD (*CDH1*)	HC (n=7), MGUS (n=29), MM (n=113)	BM	D	(58)
	p15 (*CDKN2B*)	HC (n=3), MGUS (n=4), MM (n=161)	$CD138^+$ PCs	D	(59)
		HC (n=7), MGUS (n=29), MM (n=113)	BM	D	(58)
	MMSET	Not mentioned	Not mentioned	D	(1)
miRNAs	**miR-744**	HC (n=30), MGUS (n=57), MM (n=121)	BL	D	(61)
	miR-130a	HC (n=30), MGUS (n=57), MM (n=121)	BL	D	
	miR-34a	HC (n=30), MGUS (n=57), MM (n=121)	BL	D	
	let-7d	HC (n=30), MGUS (n=57), MM (n=121)	BL	D	
	let-7e	HC (n=30), MGUS (n=57), MM (n=121)	BL	D	
	miR-1246	HC (n=33), MGUS (n=15), MM (n=24)	BL	D	(62)
	miR-1308	HC (n=33), MGUS (n=15), MM (n=24)	BL	D	
	miR-17-92	HC (n=6), MGUS (n=6), MM (n=16)	$CD138^+$ PCs	D	(63)

FIGURES

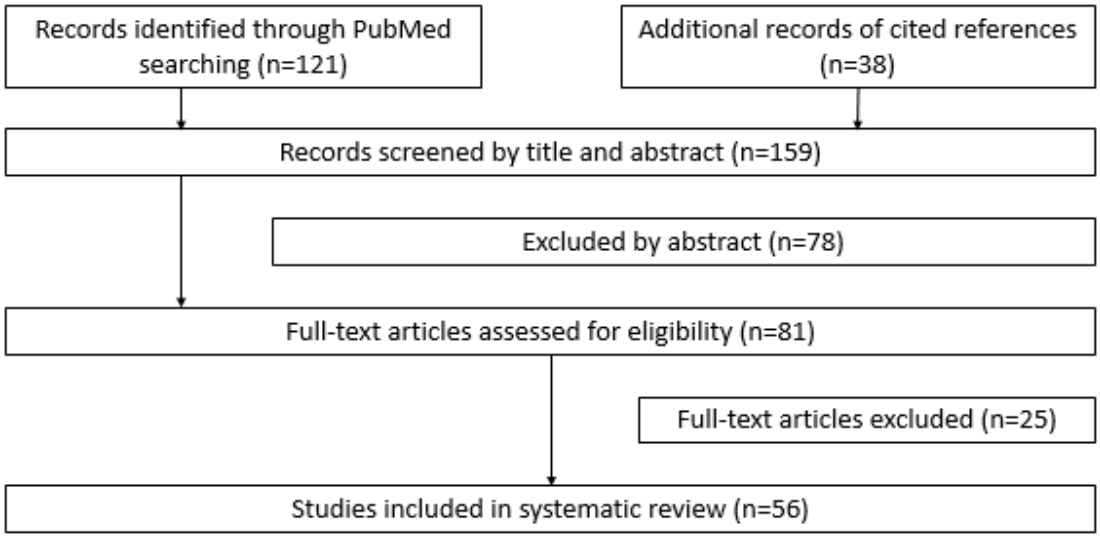

Figure 1: PRISMA flowchart. Articles were excluded due to (i) no abstract available, (ii) the use of animal models and/or cell cultures, (iii) the focus on therapy or diagnosis