A monoclonal gammopathy precedes multiple myeloma in most patients

Short title: MGUS precedes myeloma

Brendan M. Weiss M.D.¹, Jude Abadie Ph.D.², Pramvir Verma M.D.³, Robin S. Howard M.A.⁴, W. Michael Kuehl M.D.⁵

¹Hematology-Oncology Service, Department of Medicine, Walter Reed Army Medical Center, Washington, DC
²Department of Pathology and Area Laboratory Services, Walter Reed Army Medical Center, Washington, DC
³Hematology-Oncology Service, Department of Medicine, Womack Army Medical Center, Fort Bragg, NC
⁴Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC
⁵Genetics Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD

Corresponding author: Brendan M. Weiss, MD, Hematology-Oncology Service, Walter Reed Army Medical Center, 6900 Georgia Ave, N.W., Washington, DC 20307, tel. 202-782-5773, fax 202-782-8196, email: brendan.weiss@us.army.mil

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Abstract

Pre-existing plasma cell disorders, monoclonal gammopathy of undetermined significance or smoldering myeloma, are present in at least one-third of multiple myeloma patients. However, the proportion of patients with a pre-existing plasma cell disorder has never been determined by laboratory testing on pre-diagnostic sera. We cross-referenced our autologous stem cell transplant database with the Department of Defense Serum Repository. Serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE) and serum free light chain analysis (sFLC) were performed on all sera collected two or more years prior to diagnosis to detect a monoclonal gammopathy (M-Ig). In 30 of 90 patients, 110 pre-diagnostic samples were available from 2.2-15.3 years prior to diagnosis. A M-Ig was detected in 27 of 30 patients (90%, 95% CI 74-97%); by SPEP and/or IFE in 21 patients (77.8%), and only by sFLC in six patients (22.2%). Four patients had only one positive sample within four years prior to diagnosis, with all preceding sera negative. All four patients with light-chain/non-secretory myeloma evolved from a light-chain M-Ig. A pre-existing M-Ig is present in most MM patients prior to diagnosis. Some patients progress rapidly through a pre-malignant phase. Light-chain detected M-Ig is a new entity that requires further study.
Introduction

Multiple myeloma (MM) is a mostly incurable malignant disorder of plasma cells diagnosed in approximately 20,000 patients in the United States annually.\textsuperscript{1} Multiple myeloma is known to evolve from pre-malignant plasma cell disorders, such as monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM), in at least one-third of patients.\textsuperscript{2} The progression to MM occurs at average rates of 1\% per year for MGUS,\textsuperscript{2} and 10\% per year for SMM.\textsuperscript{3} The risk of progression from these pre-malignant conditions to MM is affected by the level of monoclonal immunoglobulin, the presence of non-IgG gammopathy, an abnormal serum free light chain ratio, the fraction of bone marrow plasma cells bearing an aberrant phenotype, increased bone marrow plasma cells, decreased levels of polyclonal immunoglobulin, and aneuploidy.\textsuperscript{2-7} However, the proportion of MM that develops from a pre-existing MGUS or SMM is unknown and remains an important unresolved issue in the understanding of the pathogenesis of myeloma.\textsuperscript{8}

These premalignant plasma cell disorders are asymptomatic and usually discovered during investigation for unrelated symptoms or laboratory abnormalities.\textsuperscript{9} Therefore, it is likely that previous studies have substantially underestimated the true proportion of MM patients with a pre-existing plasma cell disorder. Epidemiologic studies support the notion that a pre-existing plasma cell disorder is nearly always present.\textsuperscript{10} Others have suggested that a proportion of MM arises \textit{de novo} without a premalignant plasma cell disorder.\textsuperscript{11} It has also been postulated that MM that arises from a pre-existing plasma cell disorder has distinct genomic features, a unique pattern of response to therapy and a more favorable outcome.\textsuperscript{12-15}

We sought to determine the proportion of patients with newly diagnosed myeloma who had a pre-existing plasma cell disorder (PPCD) – as manifested by a monoclonal gammopathy - by using serum collected prior to their diagnosis. We retrieved samples from the Department of
Defense Serum Repository, which contains the unused sera from the mandatory, periodic blood tests performed on active duty U.S. military servicemembers.

**Materials and Methods**

A database of patients who underwent high dose chemotherapy and autologous stem cell transplantation for MM at Walter Reed Army Medical Center was cross referenced with the Department of Defense Serum Repository (DoDSR) by the Armed Forces Health Surveillance Agency. The DoDSR prospectively collects the unused sera from periodic mandatory blood tests performed on active duty U.S. military servicemembers for medical surveillance purposes. The repository contains 27 million samples on over 7 million individuals who have served since 1990.\(^{16}\) We intentionally chose a transplant population as these younger than average myeloma patients would have been most likely to have been serving on active duty after 1990 when the repository began. All available sera collected two or more years prior to the diagnosis of MM were retrieved. Serum samples less than two years prior to diagnosis were not tested as these patients were likely to have undetected multiple myeloma. Serum protein electrophoresis (SPEP) was performed using agarose gels and inspected by a technician and one of the investigators (J.A.) (Helena Laboratories, Beaumont, TX). Immunofixation electrophoresis (IFE) with antisera to IgA, IgM, IgG, kappa, and lambda were performed (Helena Laboratories) on all cases, and IgD antisera for selected cases. Serum free light chain (sFLC) levels were determined by automated immunoturbidimetric assays for free kappa (normal range, 3.3-9.4 mg/L) and lambda (normal range, 5.7-26.3) on a Bayer Advia 1650 (Bayer Diagnostics, Tarrytown, NY) using commercial reagents (Freelite™, The Binding Site, Ltd., Birmingham, UK). The kappa/lambda ratio (normal 0.26-1.65) was calculated. Subjects with ratios below the normal range have clonal lambda disorders and subjects with ratios above the normal range have clonal kappa disorders. A pre-existing plasma cell disorder (PPCD) was defined as a monoclonal gammopathy on SPEP, a positive IFE for IgG, IgA, IgD, kappa and/or lambda, or an
abnormal sFLC ratio. At the time of initial detection of a PPCD and at the immediate pre-diagnostic sample, the risk of progression to MM was determined by the Mayo Clinic Risk Stratification Model which uses the following three factors to define four risk groups: monoclonal immunoglobulin level greater than 1.5 gm/dL, abnormal serum free light chain ratio and non-IgG gammopathy.

Using SPSS for Windows (version 14.0) for all data analyses, categorical variables were compared between groups using Fisher’s exact test (2-tailed) and continuous data were compared using the Wilcoxon rank sum test. Ninety-five percent confidence intervals (95% CI) for proportions were estimated using the modified Wald method. This retrospective study was approved by the Human Use Committee at Walter Reed Army Medical Center and informed consent was waived.

Results

Serum samples were available for 30 of 90 (33%) patients. The median number of samples per patient was 3.5 (range 1-14), ranging from 2.2-15.3 years prior to the diagnosis of MM. The characteristics of the patients are shown in table 1. Patients with available sera were younger at myeloma diagnosis (48.1 years vs. 58.6 years, p<0.001), and overwhelmingly male (96% vs. 53%, p<0.001). Caucasian and African-American patients comprised 53% and 47%, respectively, of the serum cohort.

A PPCD was detected in 27 of 30 patients (90%, 95% CI 74-97%); by SPEP and/or IFE in 21 patients (78%), and sFLC in 6 patients (22%). The PPCD was initially detected by sFLC alone in 6/27 (22.2%), IFE alone in 1/27 (7.4%), SPEP+IFE in 5/27 (18.5%), IFE+sFLC in 1/27 (3.7%), and by all three assays in 14/27 (51.2%). The Mayo Clinic Risk stratification for MGUS was determined for all evaluable patients at their initial (n=18) and immediate pre-diagnostic (n=20) samples. At initial detection of a PPCD, 7 (39%) were low risk, 9 (50%) were low-
intermediate, and 2 (11%) were high intermediate. Immediately prior to diagnosis, 4 (20%) were low risk, 10 were low-intermediate (50%), and 6 (30%) were high intermediate.

The pattern of serum results prior to the diagnosis of myeloma is shown in figure 1. For patients 1-23 (excepting one sample from patient 21), all sera, up to 15 years before diagnosis, were positive. There were four patients (20-23) with light chain or non-secretory myeloma. All of these patients had a PPCD initially detected by sFLC alone. Patients 24-27 had a PPCD detected initially within 2.6-3.7 years prior to the diagnosis, but with all preceding sera testing negative. Patients 24 and 25 had monoclonal IgG-immunoglobulin levels of 0.36 gm/dL and 0.40 gm/dL, but only patient 24 had an abnormal sFLC ratio (2.05). Patient 26 (IgG-myeloma) was detected by an abnormal serum free light chain ratio (2.71) alone and patient 27 (IgD-myeloma) was detected by serum free light chain assay and immunofixation. Three patients (28-30) had no evidence of a PPCD. One patient (29) with IgG-myeloma had only one pre-diagnostic sample available 9.5 years prior to diagnosis. The two remaining patients without a PPCD were IgD-myeloma patients, with their most recent pre-diagnostic samples at 5.2 and 3.3 years, respectively.

The changes in monoclonal immunoglobulin (M-Ig) levels during progression to MM in patients with available immunoglobulin levels at the time of diagnosis of MM are shown in figure 2. Two patterns were evident. In panel A, generally stable, low (<1.5 gm/dL) monoclonal immunoglobulin (M-Ig) levels are present for years prior to diagnosis, with a gradual increase resulting in a subsequent diagnostic M-Ig level of greater than 3 gm/dL. In panel B, generally stable, low (<1.5 gm/dL) M-Ig levels again are present for years prior to diagnosis followed by a gentle rise towards a diagnostic M-Ig level of less than 3 gm/dL.

The temporal changes in M-Ig and/or sFLC ratios were fully evaluable in ten individual patients. Seven examples are illustrated in figure 3. For one patient (panel A), there was a
gradual increase in M-Ig and the sFLC ratio, but a sharp increase only in the sFLC ratio 3.5 years prior to the diagnosis of MM. For a second patient (panel B), there was no M-Ig detectable until the time of diagnosis, but the sFLC ratio was abnormal up to eight years before the diagnosis of MM, and showed a dramatic increase four years before the diagnosis of MM. For a third patient (panel C), there was a gradual increase in both M-Ig and the sFLC ratio for several years and then a more substantial increase, particularly for the sFLC ratio, that occurred 2.5 years before the diagnosis of MM. Finally, panel G illustrates a patient with light-chain MM, in whom the sFLC ratio and free kappa light chain increased substantially three to four years prior to the diagnosis of MM. Altogether, there appeared to be a substantial increase in the sFLC ratio two or more years prior to the diagnosis of MM in seven of 10 evaluable patients (Fig. 3).

Discussion

In this study using sera available prior to the diagnosis of multiple myeloma, we demonstrated that at least 27 of 30 (90%) of patients with MM evolve from a PPCD. We suspect that the proportion of patients with a PPCD may have been higher than 90%, as the characteristics of the negative cases may have reduced our ability to detect a PPCD. One case of IgG-myeloma had only one pre-diagnostic serum sample available at 9.5 years prior to diagnosis. The remaining two negative cases were IgD-myeloma with sera available only three and five years prior to the diagnosis. This rare isotype is characterized by the secretion of very low levels of monoclonal immunoglobulins. An additional case of IgD-myeloma in our study was positive by serum free light chain assay and immunofixation without a quantifiable monoclonal immunoglobulin. Although three cases of IgD MGUS have been reported, the laboratory and clinical features, including measurement of serum free light chains, are not well known. Therefore, with the availability of samples closer to the diagnosis and perhaps more
sensitive detection methods for patients with IgD-myeloma, we suspect these patients might have had a PPCD.

It is clear from this study that a fraction of patients progress rapidly through a pre-malignant phase to MM. This is consistent with recent studies stratifying MGUS patients into groups with differing rates of annual progression, from 0.25% to 11.2%, based on several factors: an abnormal serum free light chain assay, non-IgG gammopathy, the level of bone marrow plasmacytosis, and the immunophenotypic features of bone marrow plasma cells.\textsuperscript{5,7} However, it is notable that in our study there were no patients who satisfied the criteria for high-risk MGUS by the Mayo Clinic model. In fact, two of the four rapidly progressing patients were low or low-intermediate risk by the Mayo Clinic risk stratification model. Unfortunately, there are no published data on progression rates for light chain MGUS.

A new entity called light-chain MGUS, defined as an abnormal serum free light chain ratio without detectable immunoglobulin heavy chain, has been described by the Mayo Clinic in a population-based prevalence study performed in Olmsted County, Minnesota.\textsuperscript{20} The investigators reported a prevalence of about 2% in those over the age of 50. This is close to the prevalence of intact immunoglobulin MGUS, and thus more information on the natural history of this condition is needed. Our study is the first detailed report of the natural history of this entity. Of six patients, four patients progressed to light chain/non-secretory myeloma, and the remaining to intact immunoglobulin myeloma. For light chain only or non-secretory myeloma, it has been unclear to what extent this occurs \textit{de novo}, is preceded by MGUS expressing an intact immunoglobulin, or is preceded by MGUS that expresses only a light chain. Therefore, it is significant that all four cases of light-chain/non-secretory myeloma in our study were preceded by light chain MGUS.
This is the first study documenting the serial changes in both the level of monoclonal immunoglobulin and the serum free light chain assay prior to the diagnosis of multiple myeloma. In seven of ten evaluable patients, there appeared to be a several fold increase in the involved free light chain ratio, either with or without a corresponding change in the intact immunoglobulin level, a few years prior to the onset of multiple myeloma (Fig. 3). This discordance of light-chain to heavy-chain production may be a harbinger of myeloma in MGUS or SMM patients and would be consistent with prior studies showing that an increasingly abnormal serum free light ratio increases the risk of progression. This finding needs confirmation in larger studies that include MGUS patients who do not progress to MM.

Our study includes a unique group of patients. As we analyzed a military population from a serum repository that began in 1990, our study population is distinctly different from other studies of MGUS and MM. First, the median age of our patients was about 20 years younger than the median age of 70 years in general MM population, which allowed us to examine the relationship of MM and MGUS in younger patients. Second, nearly 50% of our patients were African-Americans, a population that is under-represented in most studies on MGUS and MM despite a more than twofold increase of both of these conditions in this group. Although it is clear that a larger population of patients needs to be analyzed, our present results show no obvious differences based on age or race for either the frequency of MM without a preceding monoclonal gammopathy or for rapid progression from MGUS to MM.

In conclusion, myeloma is nearly always preceded by a pre-malignant plasma cell disorder, most commonly MGUS. MGUS affects at least 3% of adults over the age of 50. The current approach to MGUS is for annual clinical follow-up with serum and urine protein electrophoresis, complete blood counts, serum creatinine and calcium concentrations. It has been suggested that patients at very low risk for progression based on monoclonal immunoglobulin levels below 1.5 gm/dL, with or without an abnormal serum free light chain
assay, may not warrant serial follow-up. Our findings suggest that current stratification models are insufficient to change the current practice of careful clinical follow-up. Improved biomarkers are needed to determine the significance of monoclonal gammopathies and future studies should explore the role of serum free light analysis in addition to proteomic and genomic approaches. Lastly, light-chain-detected MGUS is an important new pre-malignant entity for which further study is needed.

Acknowledgments

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Author Contributions

B.M.W., P.V. and W.M.K designed the study, J.A. assisted with study implementation and supervised laboratory procedures, P.V. developed the clinical database, R.S.H. performed statistical analysis, B.M.W., J.A., and W.M.K. analyzed the data and wrote the paper.

Conflicts of Interest

The Binding Site, Inc. provided reagents for the serum free light chain assays and is supporting other studies being performed by J.A. and B.M.W.
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undetermined significance (MGUS) and subsequent multiple myeloma among African American
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Figure Legends

Figure 1. Serum results prior to the diagnosis of myeloma. Positive results are shown by filled symbols (circle, square, triangle, x, +). Samples that were negative for all three tests, i.e. no monoclonal gammapathy present, are shown by open circles. Filled circle = positive serum protein electrophoresis, immunofixation electrophoresis and serum free light chain assay, filled square = positive serum protein electrophoresis and immunofixation, X = positive serum free light chain assay only, triangle = positive immunofixation electrophoresis only, + = serum free light assay and immunofixation. The color indicates the myeloma isotype: red = IgG, green = light chain/non-secretory, blue=IgD. Because the serum free light chain assay was not available at diagnosis, all patients are depicted with a filled circle at the time of diagnosis for clarity.

Figure 2. The change in monoclonal immunoglobulin level prior to the diagnosis of myeloma in patients with a diagnostic monoclonal immunoglobulin greater than 3 gm/dL (Panel A). The change in monoclonal immunoglobulin level prior to the diagnosis of myeloma in patients with a diagnostic monoclonal immunoglobulin less than 3 gm/dL (Panel B).

Figure 3. The temporal changes in monoclonal immunoglobulin level (M-Ig) and involved serum free light chain ratio (iFLC) are shown for six patients with intact immunoglobulin myeloma (Panels A-F) and one patient with light-chain myeloma (Panel G). The M-Ig is plotted on the outside axis and the iFLC ratio on the inside axis. The involved serum free light chain ratio (iFLC ratio) is expressed as lambda/kappa for patients with clonal lambda MM. For the one patient with light chain myeloma (Panel G) the involved serum free light chain is plotted on the outside axis and the involved free light chain ratio is plotted on the inside axis.
Figure 1
Figure 2, Panel A.
Figure 2, Panel B.
Figure 3, Panel A.
Figure 3, Panel B.
Figure 3, Panel C.
Figure 3, Panel D.
Figure 3, Panel E.
Figure 3, Panel F.
Figure 3, Panel G.
A monoclonal gammopathy precedes multiple myeloma in most patients

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