Describing IgA Myeloma: An Immunophenotypic and Molecular Approach

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ABSTRACT

Plasma cell myeloma (PCM) is defined as a clonal disease of terminally differentiated plasma cells that secrete immunoglobulin. The biologic underpinnings of IgA-type multiple myeloma's (IgAMM) aggressive nature, including its increased morbidity and mortality, have not been elucidated. We describe the clinical, phenotypic, and cytogenetic characteristics of IgA-MM. Flow-cytometry analysis was performed to phenotype clonal plasma cell populations, and interface with fluorescent in situ hybridization (iFISH) to exploit cytogenetics to determine risk stratification; 68.1% of cases were of intermediate or high risk. On flow cytometry, samples from our IgA-PCM cohort revealed less frequent CD56 expression when compared to samples with other PCM subtypes. Our study demonstrated lower frequency of CD56 expression (52.8%). We hypothesize that loss of CD56 may play a significant role in the aggressive behavior of IgA-PCM due to the loss of cell-to-cell adhesion resulting in a higher propensity for extramedullary presentation.

KEYWORDS: Multiple Myeloma, IgA Paraprotein, Cytogenetic, Flow Cytometry, CD56, Lytic Bone Lesion

INTRODUCTION

Plasma cell myeloma (PCM) is defined as clonal proliferation of immunoglobulin producing plasma cells within the bone marrow, comprising overall greater than 10% of marrow cellularity. PCM is typically associated with a detectable serum or urine paraprotein with or without secondary organ involvement. The incidence in the United States is over 15,000 annually with a steady increase since the 1990s.[1,2] PCM accounts for approximately 20% of hematological cancer deaths and 2% of all cancer deaths.[3,4]

PCM has been described as an incurable disease, with an overall median survival under 5 years.[5] In the United States, PCM affects males more frequently than females (3:2 male to female ratio). Most affected patients are diagnosed with PCM between the ages of 40 and 84 years with a median age of 65. Although monoclonal gammopathy of undetermined significance (MGUS) can be precursor of PCM, there are no other causal associations.[6] Family history of PCM is an established risk factor, as is African American descent. Factors such as lower socioeconomic status, alcohol usage, smoking, poor diet, obesity, and pesticide exposure are weakly associated with PCM.[7,8]

The etiology of the disease remains unknown. Common clinical and laboratory findings include hypercalcemia, renal insufficiency, anemia, and lytic bone lesions.[9,10] These are colloquially described as CRAB symptoms. However, occasional patients may be asymptomatic. PCM presentation, response to treatment, and prognosis are related to chromosomal abnormalities but currently, molecular classifications are not applied as PCM is largely recognized as a single disease entity. In addition to variation in cytogenetic abnormalities, PCM can be categorized by protein production subtype. The most common M-protein in myeloma is IgG, accounting for approximately 55% of cases, followed by IgA which accounts for roughly 22%.[11,12] Although less common than IgG myeloma, IgA multiple myeloma (IgA-MM) has a more aggressive clinical course and overall poorer prognosis.[13,14]

Treatment remains an area of debate as attempts to attain complete remission have been largely unsuccessful, while other approaches focusing on control and containment of disease have shown some success.[15] Disease attributes such as cytogenetic alterations and heavy/light chain ratios, among other parameters, have not been fully investigated as predictive markers for IgA-MM.[14,16-18]. Presently no specific factor has been shown to account for the aggressive behavior of IgA-MM.[19] Herein, we aim to elucidate the aggressive nature of IgA-MM through analysis of the clinical, morphologic, and phenotypic features in our study cohort.

MATERIALS AND METHODS

We retrospectively searched our pathology and clinical database (2011–2021) for patients with IgA-PCM following IRB approval. All available hematoxylin and eosin-stained (H&E) slides, immunohistochemical stains, and in situ hybridization preparations were reviewed for all cases. The diagnostic criteria used for the study were according to the revised 2016 World Health Organization (WHO) classification of hematolymphoid neoplasms.[4,20,21]

Immunohistochemical and in situ hybridization slides were prepared at our own institution on Leica BOND III

automated instruments. Fluorescence in situ hybridization (FISH) studies were performed at our institution using encircled areas of foci of interest gathered from formalin fixed, paraffin-embedded tissue sections. FISH was performed using the protocols recommended by the manufacturers of the FISH probes used. A total of 200 interphase cells were analyzed for each sample with images captured and stored using Applied Imaging/Cytovision system. Final results were reported using the cut-offs established in the laboratory for each of the probes tested.

Flow cytometry studies were also performed at our institution. Bone marrow aspirates were collected in EDTA anticoagulant. Cells were incubated with specific monoclonal antibodies for 20 minutes at room temperature in the dark. Plasma cells were first identified by live gating using CD138, CD38, and CD45 and analyzed for additional antigen expression using CD138 in combination with the other antibodies, specifically CD56, CD117 and CD20. CD56 positivity was defined as expression on CD138+ plasma cells that was greater than or equal to 20% of the above the cutoff.

Clonal plasma cells were stratified by fluorescent in situ hybridization. The high-risk group was classified as patients with samples harboring t(4;14)(p16;q32)FGFR3-IGH, t(14;16;)(q32;q23)IGH-MAF,17p13 (TP53) deletion, or 1q21 gain. The intermediate-risk group was classified as patients whose samples showed 13q deletion, and the low-risk group was classified as patients whose samples showed t(11;14)(q13;q32)CCND1-IGH. All others were classified as normal.[22]

The clinical and standard laboratory variables collected for our study included age at diagnosis, gender, race, levels of hemoglobin (in g/dL), serum creatinine, and serum lactate dehydrogenase. Beta-2 microglobulin, M-spike extracted from SPEP, clonality from immunofixation, and immunoglobulin quantification by nephelometry, and free light chain assay were also evaluated. We further reviewed and collected any other data pertaining to cytogenetic and flow cytometry analysis when available. These findings were compared to those of non-IgA PCM, namely IgG-PCM. SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Fisher's exact test was used in the flow cytometry statistical comparison between our data and the literature data.

RESULTS

Demographics of Patient Population

We identified 61 patients with IgA-MM treated at our institution. These included 34 men (56%) and 27 women (44%) (M:F ratio 1.26:1.00) with mean age of 64 years (range: 40–90 years). Self-reported ethnicities included Hispanic patients (n=29, 48%), followed by White (n=22, 36%) and Black (n=10, 16%). Demographics for all patients appear in **Table 1**.

Table 1. Demographics and Laboratory Findings

Demographics	Frequency
Mean Age at Diagnosis	63.7 +/- 10.2 (Range: 40.0–90.0)
Gender	
Male	n=34 (56%)
Female	n=27 (44%)
Ethnicity (self-identified)	
Hispanic	n=29 (48%)
White	n=22 (36%)
Black	n=10 (16%)
Laboratory Findings	Frequency
Lytic Bone Lesions	n=41 (67.2%)
Anemia (Hb < 10 g/dl)	n=20 (32.8%)
Renal Involvement	n=18 (29.5%)
Serum ß globulin < 3.5 g/dl	n=14 (23.0%)
Solitary (extramedullary) Plasmacytoma	n=12 (19.8%)
Amyloid Deposits	n=5 (8.2%)

Hb = hemoglobin

Clinical and Laboratory Findings

Lytic bone lesions, indicative of osteolytic damage due to plasma cell burden in bone marrow, were the most characteristic finding, occurring in 67.2% of patients (n=41). Anemia, defined as a hemoglobin under 10.0 g/dl in both males and females, was found in 33% of patients (n=20). Renal involvement was found in 29% of patients (n=18). Serum ß globulin, a marker of inflammatory diseases, infection, or immune disorders, was low, defined as below 3.5 g/dl, in 23% of patients. Less common findings included solitary, or extramedullary plasmacytoma present in 20% of patients (n=12) and amyloid deposits, found in 8% of patients (n=5). Laboratory and clinical findings are detailed in **Table 1**.

Serum Protein Electrophoresis (SPEP)

SPEP revealed hypogammaglobinemia as a common abnormality (92%). Although universal hypogammaglobinemia was present, the predominant location of the spike occurred in the β-1/β-2 regions (80%) and most of the patients (56%) had an abnormal free light chain ratio. SPEP results are summarized in **Table 2**.

Table 2. Serum Protein Electrophoresis (SPEP) Results

Parameter	Frequency
Hypogammaglobulinemia	56 (92.0%)
Abnormal free light chain ratio	34 (55.7%)
M-spike on ß1, ß2 region or bridge between ß and δ confirmed by IFE	49 (80.3%)

IFE = Immunofixation Blood Test

Flow Cytometry

Flow-cytometry data were available for 53 (n=53, 87%) patients in our cohort; CD56 was expressed in the neoplasms from 28 (53%). This is in contrast to similar studies, where flow-cytometry analysis detected higher percentage of CD56 expression (60–75%).[23] CD20 was co-expressed in samples from only 13.2% of the patients. Expression of CD117 was observed in samples from 24.5% (n=13). Co-expression of CD56 and CD117 was identified in samples from 9 (17.0%) patients. Only one lesion showed co-expression of CD56, CD117 and CD20. Samples from 18 patients (34.0%) showed no aberrant expression of CD20, CD56 or CD117. CD20 expression without CD56 and CD117 was not observed. Flow-cytometry results are highlighted in **Table 3.**

Table 3. Summary of Flow Cytometry Results of IgA Multiple Myeloma

High frequency protein expression identified with flow cytometry	IgA MM cases	MM cases	P-Value overall*
CD56	28 (52.8%)	70–80%	0.0099
CD117	13 (24.5%)	30–32%	0.1959
CD56+CD117	9 (17.0%)	28%	0.0268
CD56/CD117 +CD20	7 (13.2%)	17–30%	0.0324
CD56+CD117+CD20	1 (1.9%)	—	
No aberrant expression of CD56, CD117, and CD20	18 (34.0%)	—	

MM = multiple myeloma; *Obtained from (22, 27, 31, 33)

Interphase Fluorescence In Situ Hybridization (iFISH) Analysis

Interphase fluorescence in situ hybridization (FISH) was performed on specimens from 47 patients and identified: n=5 (10.6%) chromosome 1q21 gain, n=4 (8.5%) 17p deletion, n=5 (10.5%) (4;14)(p16;q32) translocation of FGFR3 and IGH, n=18 (38.3%) 13q deletion, and n= 5 (10.6%) t(11;14)(q13;q32) translocation of CCND1 and IGH. Material from six patients (12.8%) had normal cytogenetics. Specimens from the remaining patients showed other abnormalities classified as low risk by the cytogenetic risk stratification schemes. According to the cytogenetic and molecular risk stratification schemes provided by International Myeloma Working Group IMWG, 22 (46.8%) patients were either classified as intermediate or high risk. A summary of interphase fluorescence in situ hybridization (iFISH) analysis is provided in **Table 4**.

Table 4. Summary of Interphase Fluorescence In Situ Hybridization (iFISH) Analysis

Abnormalities identified with interphase fluorescence *in situ* hybridization analysis	IgA MM	MM overall*
1q21 gain	5 (10.6%)	35–40%
(17p) deletion	4 (8.5%)	10%
t(4;14) translocation	5 (10.5%)	15%
del 13	18 (38.3%)	45–50%
t(11;14) translocation	5 (10.6%)	15%
Intermediate or high risk	22 (46.8%)	50–60%
Normal cytogenetics	10 (21.3%)	—

MM = multiple myeloma; IgA = Immunoglobulin A; *Obtained from (2, 3).

DISCUSSION

Plasma cell myeloma subtypes are identified using serum protein electrophoresis.[24] However, the identification of the IgA subtype is usually complex. While over 90% of the IgG and IgM paraproteins migrate in the γ globulin region of the SPEP gel, allowing easy identification and quantification, IgA paraprotein frequently migrates in the β region, where other β region proteins such as transferrin, β-lipoprotein, and complement components can conceal them.[25] As such, identification and quantification of IgA paraproteins has intrinsic challenges that further complicate monitoring treatment of patients with IgA-MM. For these individuals, the recommended approach by the IMWG is the use of nephelometry for IgA quantification. However, if the IgA concentration by nephelometry is within the reference range, the presence of an IgA paraprotein cannot be assessed and patients cannot be monitored.[21,26]

In this retrospective study of 61 patients in a routine clinical setting, we assessed patients with hypogammaglobulinemia with additional studies that included immunosubtraction and/or immunofixation. Using available cytogenetic risk stratification schemes,[22,27-29] 68.1% of our studied patients were classified as either intermediate or high risk. The aggressive clinical behavior described in the literature[14] for IgA-MM may be related to the high frequency of intermediate/high-risk genetic lesions such as 1q21 gain, del 17, and del13. However, our results were not significantly different from those found for other subtypes of myelomas. Consequently, cytogenetic aberrations alone may not explain the aggressive clinical course of IgA myeloma. On the other hand, our IgA-MM cases demonstrated less frequent CD56 expression when compared to that reported for the other subtypes of plasma cell myeloma (52.8% vs 70–80%).[23,30-32] CD56 is a homophilic binding glycoprotein that plays a role in cell-to-cell adhesion.[31] It is not expressed in non-neoplastic plasma cells; however, aberrant CD56 expression is a relatively common finding in neoplastic plasma cells, reported in up to 80% of cases.[17] There is growing evidence supporting an association between absent CD56 expression and poor prognosis in multiple myeloma patients with an increase in extramedullary disease.[33] Lack of CD56 expression in myeloma decreases the average patient lifespan by over six months.

As a typical median survival for patients with MM is under two years, this can be an extremely important prognostic indicator.[34] Our study demonstrated a lower frequency of

CD56 expression (52.8%) in IgA-MM, which, we hypothesize, may play a significant role in the aggressive clinical behavior due to the loss of cell-to-cell adhesion. No statistically significant ($p<0.05$) link was present between the CD56 expression or lack of expression and specific cytogenetics risk groups. Expression of CD117 and/or CD20 with or without CD56 expression did not significantly correlate with any other data in our limited cohort; however, it should be further investigated in a larger cohort study.

We also analyzed FISH results in samples with no aberrant expression of CD56, CD117, and CD20. Of these, FISH was not performed on samples from three patients. Twelve patients' specimens demonstrated low-risk FISH abnormalities. Myeloma cells from three patients harbored high-risk cytogenetic abnormalities. Thus, our study underscores the utility of multiparameter flow cytometry analysis as it appears to predict aggressive behavior of IgA plasma cell myeloma.

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