RISK STRATIFICATION IN MULTIPLE MYELOMA IN INDIAN SETTINGS

Introduction

Multiple myeloma (MM) is characterized by malignant proliferation of plasma cells producing monoclonal protein. The outcome has drastically improved in the patients of MM in last decade due to a better understanding of the disease biology and evolution of newer therapies[1,2]. Prognosis can vary from patient to patient even with similar staging and host factors, which can be explained based on underlying disease biology[1]. Many prognostic factors which define the innate aggressiveness of the disease have been described and attempts have been made to risk stratify the patients using these prognostic factors. This is important for predicting the clinical aggressiveness of disease as well as the known fact that some of these factors can be overcome by the use of newer treatment modalities[2,3]. Thus with the changing paradigm of the treatment options, a uniform risk stratification system is the need of the time, to allow better comparisons of the patient's groups, given the heterogeneity in their outcomes.

Each nation or regional group has its own data system which is utilized for research purposes, inter-regional comparisons, clinical trials and therapeutic interventions. Though there is immense western data on genetic abnormalities in patients of MM, there is a paucity of Indian data despite the substantial burden of the disease, thus hampering the inter-study comparisons. Though large scale studies from India have considered prognostic impact of initial staging [4,] only small retrospective studies have studied cytogenetics as a prognostic tool [5]. Identification of genetic abnormalities has major prognostic and likely predictive impact; hence it becomes important to construct a set of Indian data. Need for standard guidelines required for minimal risk stratification in the Indian setting, becomes inevitable. Risk stratification is also very important for patient counselling, as the first few questions of most Indian patients after they are told about the diagnosis of MM is ‘How much time do I have?’, ‘How advanced is the cancer?’ and ‘What is the stage of this cancer?’. These questions can be addressed only after appropriate risk stratification.

In this paper, we will review the current knowledge about these factors and their utility for risk stratification as well as bare minimal risk stratification required to be done in resource constraint settings. We will also address the shortcomings of various methods of cytogenetics in terms of their sensitivity and technical aspects like sampling, processing and plasma cell enrichment for enhancing the ability of techniques like FISH to detect abnormalities. Last but not the least is to consider the extra financial burden and cost benefit analysis in carrying out complete recommended risk stratification in Indian setting.

Prognostic markers

Prognostic factors define the innate aggressiveness of the disease, irrespective of the treatment received. These include patient factors, tumor factors, disease biology and availability and response to therapy. Prognostic studies are essential for risk stratification of patients of MM. This is important to understand the aggressiveness of the disease, chances of survival, rational selection/sequencing of the therapy and to explore other research questions. Though cytogenetics explains a large fraction of disease heterogeneity, host factors form an important component in determining patient outcome[1,6]. Host factors such as age, performance status, and co-morbidities have a predominant role in determining prognosis at the onset of the disease. Trials have shown that young and fit patients do well despite high risk genetic profile presumptively because of better tolerability of the therapy[7]. Moreover, different prognostic factors may change with disease relapse/progression i.e. the factor that can predict the outcome at the onset of disease may have a reduced effect on relapse. Risk stratified therapy is considered as the backbone in hematological malignancies and is widely used in leukemias and lymphomas. Risk stratification in MM, based on available prognostic factors helps in minimizing the treatment related toxicity and maximizing the outcome in low risk patients[8]. It is of utmost importance that future trials should address the different risk groups for therapeutic consideration.

Prognostic classification

It incorporates variables affecting the outcome of newly diagnosed patients of MM treated with multiple treatment modalities[7,8,9]. Apart from patient factors, it includes determinants of disease burden (β2 microglobulin, lactate

dehydrogenase, high proliferation rates, extramedullary disease & hypercalcemia), and cytogenetics. Cytogenetics as an example, the t(4;14)(p16;q32) has been associated with more aggressive disease at baseline, and worse outcome as well as shortened survival even in the group of patients treated with conventional or high-dose chemotherapy[9,10]. Also, recent studies have shown that the worse prognostic significance of patients harboring t(4;14)(p16;q32) may be ameliorated by using bortezomib based therapy[11,12].

Staging

One of the important prognostic factors is tumor burden, which is evaluated by staging. Two main staging system exists: the International staging system (ISS)[13] and the Durie-Salmon staging (DSS) system[14]. Though studies have not proven any superiority of ISS over DSS in determining predictive ability, the former is being preferred in view of its simplicity and exclusion of subjectivity. These staging systems are predominantly used for risk stratification of patients enrolled in clinical trials for allowing its better interpretation and comparison of such trials.

Recently, the revised International Staging System (R-ISS), based on 3060 patients enrolled in the 11 international trials, incorporated the factors included in the original ISS (serum B2M and serum albumin), and added prognostic information obtained from serum lactate dehydrogenase (LDH) and high-risk chromosomal abnormalities detected by interphase fluorescence in situ hybridization (FISH)[15]. The information provided by R-ISS is more comprehensive and robust as compared to ISS. Patients with ISS stage III (B2M ≥5.5 mg/L) plus LDH above normal limits and/or detection of one of the following by FISH: del(17p), t(4;14), or t(14;16) are considered to be high risk as per R-ISS. Advantages and shortcomings of various staging systems have been summarized in table-1.

Table 1. Salient differences between the available staging systems

Characteristics	DSS[14]	ISS[13]	R-ISS[15]
Year of acceptance	1975	2005	2015
Data based on number of patients	70	11000	3060
Factors Included	Clinical Biochemical Ig levels Imaging	Host factor(Albumin) Tumor burden(β2 microglobulin)	LDH and Cytogenetics in addition to factors of ISS
Molecular factors	Not included	Not included	del(17p), t(4;14), or t(14;16) Included
Advantages/ Shortcomings	Subjective Requires multiple investigations, imaging	Simple and lack of subjectivity	More comprehensive and robust

Cytogenetics

A simplified risk stratification based on combined ISS-genetic prognostic system has been proposed by International myeloma working group (IMWG), dividing patients into low risk, standard risk and high risk categories (table 2)[16.]

t(11;14)(q13;q32): Of all newly diagnosed MM patients, 15% harbor t(11;14)(q13;q32) with resultant upregulation of cyclin D1. It is associated with typical characteristic features such as lymphoplasmacytic morphology, CD20expression, hyposecretory and λ light chain disease[17,18]. Though not strong enough to be statistically significant, in most of the studies this aberration is associated with favorable outcome except in cases of plasma cell leukemia where this translocation is associated with the aggressive disease.

t(4;14)(p16;q32): The unique clinicopathological features associated with this particular translocation are IgA heavy chain disease, λ light chain disease, and

Table 2. Initial Risk Stratification and outcome in Multiple Myeloma[16]

	High Risk	Intermediate risk	Low risk
Parameters	ISS II/III and t(4;14)a or 17p13 del	Others	ISS I/II and absence of t(4;14), 17p13 del and +1q21 and age <55 years
Median OS	2 years	7 years	>10 years
% Patients	20%	60%	20%

high prevalence of coexistent chromosome 13 abnormalities[10,19]. The translocation results in increased expression of FGFR3 and MM SET domain (MMSET) and is often not detectable by conventional karyotyping, and thus requiring FISH or RT-PCR. Several groups have shown that patients harboring t(4;14)(p16;q32) are associated with shorter remission and overall despite high dose chemotherapy with stem cell support[20,21].

t(14;16)(q32;q23): The translocation t(14;16)(q32;q23), arising from IgH gene rearrangements involving a fragile site in chromosome 16, is found in 5-7% of patients. Other characteristic features include higher frequency of associated chromosome 13 deletion, IgA isotype and more aggressive clinical course of the disease[9].

Chromosome 13 abnormalities: Multiple trials have shown the critical role of chromosome 13 as a pre-requisite for clonal expansion of tumor. Among all cases with chromosome 13 abnormalities, 85% constitute monosomy, and the remaining 15% are interstitial deletions. Though chromosome 13 abnormalities are associated with poor prognosis and shorter survival, there is no significant prognostic difference between monosomy and deletions. More than 90% of cases with t(4;14)(p16;q32) are co-associated chromosome 13 deletion [22,23].

17p13 deletion: 17p13 deletion (the locus for the tumor suppressor gene, p53) is a more important prognostic factor in patients with MM[8,9]. In multiple series 17p13 has been proven as a negative prognostic factor associated with aggressive disease, higher prevalence of extramedullary disease (such as plasmacytomas), hypercalcemia. Despite aggressive therapy and high dose

therapy (HDT), these patients have an inferior outcome and shorter overall survival [8,9,24]. It has been hypothesized that the plasma cells are indeed capable of surviving at extramedullary locations, but they will usually undergo apoptosis in the presence of an intact p53 response, thus 17p53 del is associated with extramedullary disease and plasma cell leukemia[25].

Chromosome 1 abnormalities: Chromosome 1 abnormalities, most of which involve rearrangements involving the pericentromeric region, are highly prevalent in patients of MM. Though two series have failed to confirm the overriding negative prognostic association with chromosome 1 amplification detected by FISH, recent data confirms the worse prognosis in patients of MM, with gain of chromosome 1q despite novel agents based therapies[26,27]

Hyperdiploid and non hyperdiploid-MM: MM can be broadly divided into two major groups, hyperdiploid MM (h-MM) (harboring numerous chromosomal trisomies and a low prevalence of IgH translocations) and non-hyperdiploid MM (nh-MM) (encompassing hypodiploid, pseudodiploid and near tetraploid MM, and highly enriched for IgH translocations). Though this classification has no major clinical implication, h-MM is associated with more advanced age at diagnosis, bone predominant disease, and favorable outcome[28,29]. In one series h-MM, patients with chromosome 13 deletion were associated with shorter survival whereas in a larger series this prognostication was not apparent. Further h-MM has been divided based on gene expression profiling into four different groups, to discriminate the patients with poor prognosis and shorter survival.

Circulating tumor cells: Circulating plasma cells (PC) detected by either immunofluorescence assay or via flow cytometry by gating on CD38+/CD45- cells, have an adverse prognostic implication and act as a surrogate of high risk disease[30,31,32]. It can be detected in all patients with plasma cell leukemia, in 80% of patients with newly diagnosed MM and 90% of those with relapsed disease[30]. Studies have confirmed shorter median survival for patients with more than 10 circulating PC versus those with less than 10 circulating PC per 50000 mononuclear cells[31,32].

A significant correlation has been found between number of circulating PC with levels of serum β2 microglobin. Prognostic value of ISS incorporated with circulating PC (β2 microglobin, Albumin, circulating PC) has been proven to be superior to ISS alone. Patients with no (low risk), one (low-intermediate risk), two (high-intermediate risk), or three (high risk) of these adverse factors had median survivals of >79, 48, 32, and 13 months, respectively. This is useful in predicting poor outcome in a subset of patients prior to chemotherapy and stem cell transplant[33,34].

Technical aspects

Clonal proliferation in MM is unique and differs from other hematologic malignancies in possessing high fraction of low proliferating malignant PC, posing a great challenge for standardization of cytogenetic analysis. Due to the same reason, conventional karyotyping is able to detect genetic abnormalities in only up to 30% of patients whereas, comparative genomic hybridization has confirmed that almost all myelomas have karyotypic changes[35,36,37]. Beyond doubt, Interphase FISH has emerged as a highly sensitive technique for cytogenetic assessment, appropriate sampling, timely transportation, processing selection of the malignant cells by morphology, and immunophenotyping. However, sorting of PC is required before FISH probes can give reliable results[38,39].

Cytogenetics is hampered not only by low proliferation activity of plasma cell in BM aspirates but also owing to the low median proportion of malignant plasma cells in the samples. FISH technique could not be directly performed as in other hematological malignancies. The PC need to be selected, either by flow cytometry or CD138 immunomagnetic-bead based PC sorting or by the concomitant labeling of the cytoplasmic immunoglobulin light chain, so as to improve the sensitivity of FISH and produce reliable results[40,41].Studies have shown that in the enriched bone marrow aspirate samples, the plasma cell percentage ranged from 28 to 96% (median, 72.5%), as compared to 1 to 28% (median, 8%) in non-enriched samples, resulting in detection of genetic abnormalities by FISH is three times more in PC enriched samples[41]. Availability of high resolution array comparative genomic hybridization (aCGH) has lead to identification of new prognostic markers like 12p deletion and 5q

amplification. Advantage of aCGH is that these new markers can be easily converted to other diagnostic tools like FISH [42].

Drawbacks of plasma cell enrichment techniques are that, they are expensive and labor intensive. The RQ-PCR based detection of overexpressed partner genes involved in IGH translocation is an alternative in such cases. Recently multiplex ligation-dependent probe amplification (MLPA) was developed as a fast and robust alternative method to analyze copy number changes in a wide set of loci. Few studies have shown that it can be applied in routine laboratories [43].

Role of imaging studies: Though few small single institution studies have indicate role of imaging studies such as MRI and FDG-PET CT in risk stratification, further independent confirmation is required before they are widely applied [44].

Free light chain (FLC) assay: This has well established utility in diagnosis and treatment response assessment in MM. Altered ratio predicts progression in smoldering MM as well as monoclonal gammopathy of undetermined significance (MGUS) [45,46]. Though elevated FLC levels and ratios at diagnosis have been associated with poor progression free survival in MM patients, whether they have prognostic impact independent of cytogenetic risk factors has to be established [47,48,49].

Recommendations

We concur with the recent literature, that all newly diagnosed patients should undergo risk stratification based on available good and robust prognostic markers. These include serum albumin and beta-2 microglobulin for ISS staging, LDH, and cytogenetics for Revised-ISS staging.

Staging: Traditionally the Durie and Salmon system (DSS) and the International Staging system (ISS) have been used to assess the tumor burden in patients with newly diagnosed MM. ISS based on the serum beta-2 microglobulin (β2M) and albumin due to simplicity of use was the first to gain wide acceptance and superseded the DSS. However, in patients who are

aggressively treated using upfront ASCT, the ISS does not improve the prediction of post-transplant outcomes compared to the DSS. DSS is a good tool to indicate tumor burden , hence its utility is more for supplementing diagnostic criteria rather than being used for risk stratification. The R-ISS provides prognostic information that is more robust than that from the original ISS. Hence we recommend that R-ISS to be used for initial staging in all newly diagnosed MM patients. However, in the absence of cytogenetic evaluation, ISS should be used for staging purpose.

We recommend baseline cytogenetic evaluation in all newly diagnosed patients with MM, for risk stratification. Wherever, feasible, cytogenetic evaluation should include both conventional karyotyping and iFISH.

Conventional karyotype can detect only 20-30 % of the abnormalities due to a low number of metaphases in collected specimens. Considering this drawback, interphase FISH should be performed in all cases at initial diagnosis. FISH in unsorted samples results in low sensitivity and thus a lower yield. So, we strongly recommend FISH to be done in purified plasma cells or in combination with immunofluorescent detection of light chain-restricted plasma cells cytoplasmic immunoglobulin enhanced FISH (cIg-FISH to improve the rate of abnormality detection.

Minimal FISH panel for MM should include testing for t(4;14)(p16;q32), t(14;16)(q32;q23) and –17p13. These three probes have been proposed to be most useful in the stratification of cases into high- and standard-risk disease.

An optional panel may include testing for t(11;14)(q13;q32), chromosome 13 deletion, ploidy changes and chromosome 1 abnormalities.

There is paucity of data regarding prognostic implication of the risk stratification in Indian patients. It is imperative to design multi centeric collaborative projects where risk stratification is done for all patients as per the guidelines and long term follow up data is analyzed to see whether these prognostic markers are useful in Indian patients. With the availability of newer drugs in MM, it becomes

important that risk stratification is incorporated in decision making tools for deciding on best treatment for a given patient. A simplified algorithm (Fig-1) and costing considerations (Table-3) for deciding on risk stratification on initial diagnosis of MM would be useful for the clinicians.

Summary of recommendations

1. We strongly recommend that Revised International Staging system to be used to risk stratify all newly diagnosed patients of MM. ISS should be used in case cytogenetic evaluation is not feasible,.
2. Both conventional karyotyping and FISH should be done at baseline.
3. A minimum FISH panel should include testing for t(4;14)(p16;q32), t(14;16)(q32;q23) and 17p13. An optional comprehensive panel may include testing for t(11;14)(q13;q32), chromosome 13 deletion, ploidy changes and chromosome 1 abnormalities.

Recommendations (Technical aspects)

1. We strongly recommend that the sample for conventional karyotype and FISH studies should be part of the second draw of aspirate or first draw of the repositioned BM aspiration needle to improve the yield.
2. Minimum 02 ml of bone marrow aspirate sample should be collected in lithium heparinized vacutainers for conventional karyotyping and FISH evaluation.
3. Samples should not be frozen. A cool pack may be used to ensure that samples are not exposed to temperatures more than 30^0 C. For cytogenetic evaluation samples should be processed within 24 hours of collection. In case of long distance transportation, appropriate transport media should be used. The pre-processing holdup time of the same should not be more than 07 days.
4. Plasma cell enrichment should be done in the bone marrow sample to improve the yield before FISH testing.
5. Regular accreditation of the laboratories should be done to allow uniform interpretation of results.

Future prospects

We recommend that high through-put genomic tools such as gene expression profiling (GEP) and comparative genomic hybridization to be used whenever feasible, as they are highly capable of predicting high risk MM. It is conceivable that novel GEP derived signatures could be developed in the future and that will not only predict patient outcomes but will also be important and relevant in the journey of the evolution of newer anti-MM therapies.

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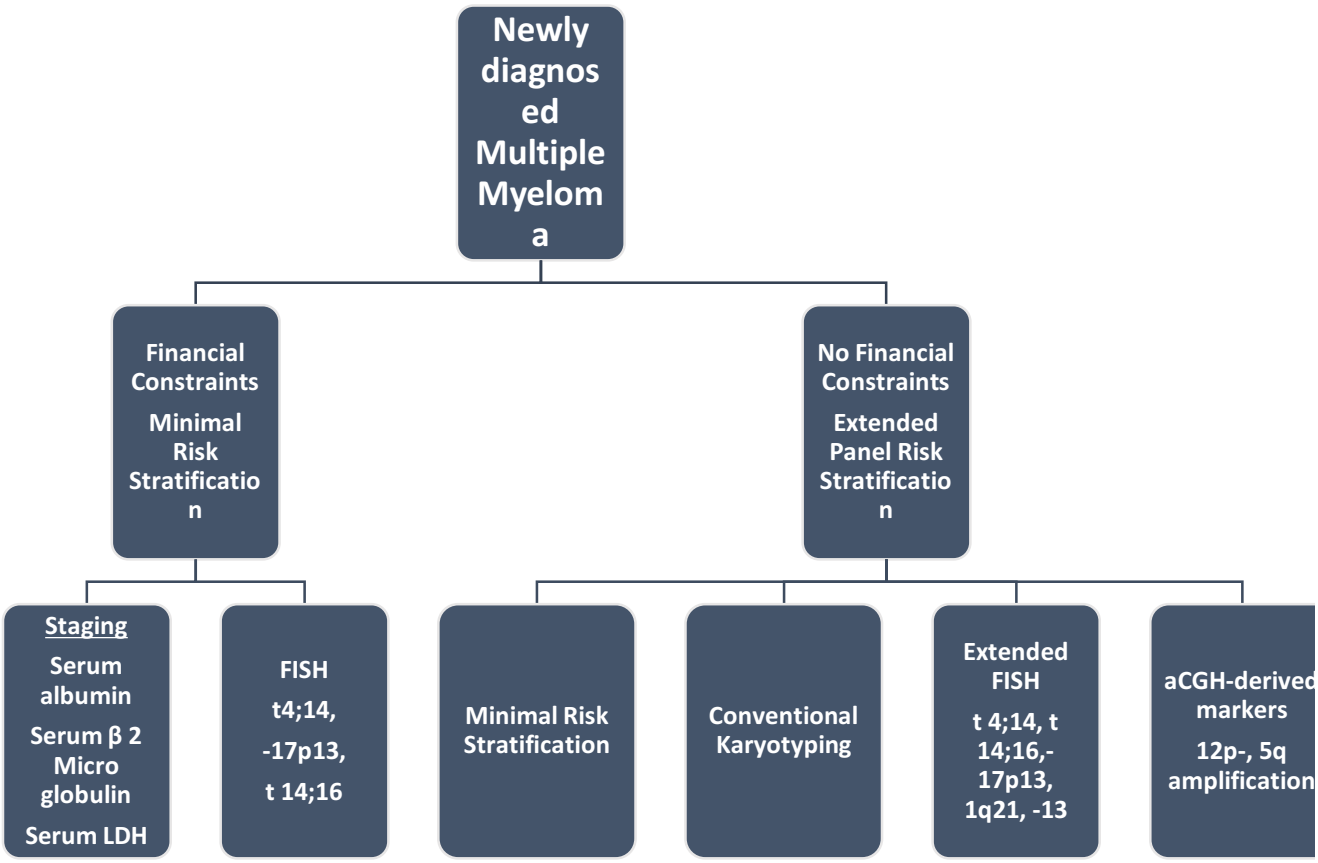

Fig-1 simplified algorithm towards initial risk stratification in patients of multiple myeloma

A CGH array comparative genomic hybridization

Table 1 Costing of Risk stratification in Indian setting

Risk stratification category*	Approximate cost in INR
Minimal	8420.00
Extended panel	22000.00

*See Fig-1