International Journal of Oncology Research

ISSN Print: 2664-665X
ISSN Online: 2664-6668
IJOR 2024; 4(1): 24-31
www.oncologyjournal.in
Received: 05-01-2024
Accepted: 09-02-2024

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Fluorescence *in situ* hybridization in molecular classification of multiple myeloma: Prediction of response to triplet standard combination therapy

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DOI: https://doi.org/10.33545/2664665X.2024.v4.i1a.15

Abstract
Background: Multiple myeloma is a cancer originates from plasma cell usually from bone marrow, divided into two subtypes: active or symptomatic multiple myeloma and smoldering multiple myeloma. The aim of this work was to study the role of translocation t(4:14), translocation t(14:16) and deletions (17p) by fluorescence *in situ* hybridization technique for risk stratification of newly diagnosed multiple myeloma patients, their correlation with clinico-laboratory features, and to explore their predictive value of disease response to triplet standard combination therapy.
Methods: 50 adults with newly diagnosed multiple myeloma and a performance status more than 2 were involved in this prospective study. Stages I and II were considered normal risk, stage III was considered high risk with a single hit, and super high risk with two or three hits was considered super high risk in the revised international staging system (R-ISS) with molecular risk stratification. Randomization of patients to VRD or VCD procedures was based on their degree of renal impairment, and they all received triplet conventional combination medicine. Patients in stages 3 and 4 started VCD treatments. Afterwards, the response was assessed in accordance with the IMWG Standard Answer Criteria.
Results: 7 patients (4: 14) had positive translocation t, 2 had positive t (14: 16), and 3 had positive del 17p. Two patients had 4:14 hit t and del 17p, one had 14:16. Serum IgG and IgA levels were significantly greater in the very high-risk group compared to high and normal risk groups (p<0.001). With substantial differences (p= 0.0014), the very high-risk group got Bortezomib, cyclophosphamide, and dexamethasone (VCD), whereas the high-risk and standard risk groups received VCD and VRD, respectively Most ultra-high-risk patients had stable disease (SD), more high-risk patients had partial response (PD), and practically all standard risk patients achieved CR or very strong partial response.
Conclusion: Comparing the ISS and R-ISS systems may misallocate a patient group with inferior response at the lower ISS stage. Multiple myeloma prognoses have improved with protease inhibitors and immunomodulators. However, extremely high- and high-risk patients responded less to traditional triplet combination therapy than normal risk patients.

Keywords: Fluorescence *in situ*, hybridization, molecular, multiple myeloma, triplet, ultra high, double hit, predictive, response

Introduction
In terms of hematologic malignancies, multiple myeloma ranks second worldwide, just behind non-Hodgkin lymphoma. In 2020, 117,077 people lost their lives and 176,404 were newly diagnosed. Everyone, regardless of age or gender, is susceptible to this illness. Despite the fact that HIV is still incurable, a lot has changed in the last fifteen years [1, 2].
Multiple myeloma is a cancer that originates from plasma cell usually from bone marrow, divided into two subtypes: active or symptomatic multiple myeloma and smoldering multiple myeloma.
At time of diagnosis multiple myeloma patient's symptoms and signs are often non-specific; including general fatigue, bony pain, unintentional significant weight loss, anemia of unknown origin and accidentally discovered impaired renal functions [3].
Diagnosis requires a minimum of 60% clonal bone marrow plasma cells in conjunction with a single focal bony lesion on magnetic resonance imaging, or a minimum of 10% clonal bone

marrow plasma cells in addition to one or more multiple myeloma defining events, such as hypercalcemia, renal failure, anemia, or lytic bone lesions [4].

Multiple myeloma results from sequential clonal cytogenetic abnormalities classified into two phases: Premalignant phase (Primary cytogenetic abnormalities); from normal plasma cells to MGUS or smoldering multiple myeloma that evolves to active multiple myeloma at an incidence roughly 1% annually. Malignant phase (Secondary cytogenetic abnormalities); from MGUS or smoldering multiple myeloma to active multiple myeloma [5].

Nowadays, Patients are classified into high-risk and standard-risk diseases according to the Revised International Staging System. This system is based on cytogenetic abnormalities found through fluorescence *in situ* hybridization on bone marrow samples and thorough laboratory investigations that include beta 2 microglobulin, lactate dehydrogenase, and serum albumin levels [6].

Percentage of newly diagnosed patients with high molecular risk classification is about 25% with median survival two to three years despite the triplet standard therapy, on the other side the median survival of standard molecular risk classification is eight to ten years with the same standard therapy [7, 8].

Ultra-high-risk myeloma, which refers to patients who experience early disease progression and death, lacks a universally accepted definition. However, certain characteristics such as extramedullary disease or plasma cell leukemia, as well as the presence of multiple del(17p), 1q21 gain, t(4;14), t(14;16) cytogenetic abnormalities, have been identified as additional factors that contribute to further differentiation beyond the R-ISS [8].

Nevertheless, the clinician's perspective on the conventional approach to all patients, irrespective of their Risk Stratifications potential, remains an unresolved matter [9].

The aim of this work was to study the role of translocation t(4:14), translocation t(14:16) and deletions (17p) by fluorescence *in situ* hybridization technique for risk stratification of newly diagnosed multiple myeloma patients, their correlation with clinico-laboratory features, and to explore their predictive value of disease response to triplet standard combination therapy.

Patients and Methods

This research was conducted on a group of 50 patients, all of whom were at least 18 years old, of both genders, and had just been diagnosed with multiple myeloma. The patients had a performance level of at least 2 according to the ECOG performance status [10]. The research was conducted between August 2022 and April 2023, after the authorization of the Ethical Committee of Tanta University Hospitals in Tanta, Egypt. The patients provided informed written consent.

Cases with smoldering myeloma, monoclonal gammopathy of uncertain significance, solitary plasmacytoma, and other hematological or solid cancers were excluded based on the established exclusion criteria.

A comprehensive evaluation of each patient included a clinical examination, a review of their medical history, laboratory investigations (including complete blood count (CBC), kidney and liver function tests, serum electrolytes, lactate dehydrogenase (LDH), serum protein electrophoresis (SPEP), immunofixation, and serum beta 2 microglobulin (B2M)), and a bone marrow examination (including bone marrow aspirate and biopsy if necessary during the diagnosis and post-induction treatment assessment]. Radiological investigations were conducted using X-ray technology.

According to ISS: The patients were categorized into three phases based on B2M and albumin levels [11]. Stage I: B2M levels below 3.5 mg/L, with albumin levels below 3.5 g/dL. Stage II: B2M levels below 3.5 mg/L, with serum albumin levels below 3.5 g/dL. Stage III: B2M levels between 3.5 and 5.5 mg/L, regardless of serum albumin levels.

ISS The process of categorizing risk into three stages: low risk (stage I), moderate risk (stage II), and high risk (stage III).

According to R-ISS: The patients were categorized into three phases based on their B2M tests, albumin levels, lactate dehydrogenase (LDH) readings, and the presence of any chromosomal abnormalities. Palumbo *et al*. [12] [Stage I: B2M with a concentration of 3.5 mg/L, albumin levels of 3.5 g/dL, normal LDH, absence of del(17p), t(4;14), or t(14;16). Stage II: neither stage I nor stage III. Stage III: B2M with a concentration of 5.5 mg/L, and/or LDH levels more than the upper limit of normal (ULN) and/or del(17p), t(4;14), or t(14;16) as determined by FISH.

Risk stratification using R-ISS: The risk levels for myeloma are as follows: standard risk for stage I and stage II, high risk for stage III with Single Hit (representing the presence of only one of the known high-risk genetic abnormalities in myeloma), and ultra-high risk for stage III with Double or triple Hit (representing the presence of 2 or more of the known high-risk genetic abnormalities in myeloma) [13].

All patients received induction Triplet combination therapy, according to kidney function tests, patients were selected to start VRD or VCD protocol. Patients who are taught to have an increased risk of complication from the use of lenalidomide; patients with impaired kidney functions (stage 3 or stage 4) started (VCD) protocol.

Triplet therapy protocols

Bortezomib, lenalidomide, and "low dose" dexamethasone (VRD) four cycles [14]. Cycle length: 21 days. Bortezomib plus cyclophosphamide and dexamethasone (VCD or CyBorD) four cycles [15]. Cycle length: 28 days.

Response assessment

Using International Myeloma Working Group (IMWG) Standard Response Criteria [16].

Statistical analysis

The statistical study was conducted using SPSS v26, developed by IBM Inc. in Chicago, IL, USA. The quantitative variables were presented using the mean and standard deviation (SD), and thereafter compared across the three groups by an analysis of variance (ANOVA) with a post hoc test (Tukey). The Chi-square test was used to report and assess the frequency and percentage (%) of qualitative variables. A result was considered statistically significant if the two-tailed P value was less than 0.05.

Results

The mean of age 64.9±7.5 and with male predominance (58%). Most of patients had performance status 2(62%). Bony lesions were present in 43(86%) of patients and extra-medullary infiltration and plasma cell leukemia were present

in only 4(8%) and 2(4%) of patients respectively. Most of patients 38(76%) had impaired kidney function more than stage 1. One third of patients 16(32%) had hypercalcemia, half of patients 25(50%) had hypoalbuminemia and 19(38%) of patients had mild anemia while 23(46%) had moderate to severe anemia. Serum LDH was elevated in 12(24%) of patients. About half of patients had B2M below 3.5. Eighty percent of patients had M band IgG while 10(20%) had M band IgA and there was no patient with M band IgD. Twenty eight percent of patients were ISS stage I at time of diagnosis while 26(52%) and 10(20%) were ISS stage II and III respectively. Table 1

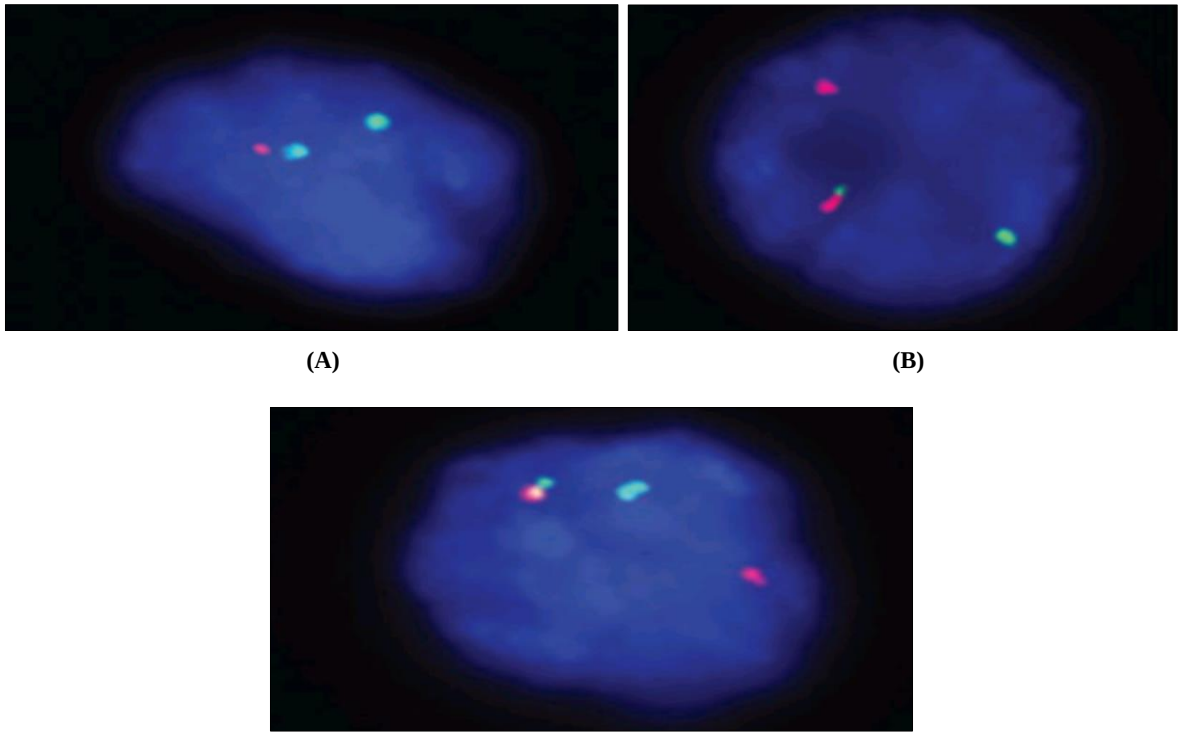
Table 1: Patients and disease characteristics of the included patients

		N=50
Age (years)		64.9±7.5
Sex	Male	29 (58%)
	Female	21 (42%)
Performance status (%)	1	19 (38%)
	2	31 (62%)
Bony lesions		43 (86%)
Extra- medullary infiltration (%)		4 (8%)
Serum creatinine (mg/dL)		3.2±1.7
Creatinine clearance (ml/min/m^2)		64.7±24.3
Kidney function level	stage 1	12 (24%)
	stage2	17 (34%)
	stage3	12 (24%)
	Stage 4	9 (18%)
Serum total Calcium (mg/dL)		10.6±1.73
Hypocalcemia		6 (12%)
Normal		28 (56%)
Hypercalcemia		16 (32%)
Serum albumin (g/dL)		3.3±0.54
Normal		25(50%)
Hypoalbuminemia		25(50%)
Serum LDH (mg/dL)		245±90
Normal		38 (76%)
High		12(24%)
Hb(g/dL)		9.5±2.1
Level of anemia (%)	Normal	8(16%)
	Mild	19(38%)
	Moderate	12(24%)
	Severe	11 (22%)
Beta- 2 macroglobulin (mg/L)		3.8±1.3
<3.5		26 (52%)
3.5- 5.5		14(28%)
<5.5		10(20%)
Plasma cell leukemia (%)		2(4%)
Plasma cell count (%)		43.3±14.7
Involved serum IgG		3034±975
Involved serum IgA		5293±1994
M band subtype	IgG MM	40(80%)
	IgA MM	10(20%)
	IgD MM	0(0.0%)
ISS	Stage I	14(28%)
	Stage II	26(52%)
	Stage III	10(20%)

Out of fifty patients, 15(30%) patients had positive one or more Hit, 7 patients had positive translocation t (4: 14) while 2 patients were positive for t (14: 16) mutation and 3 patients were positive for del 17p mutation. Two patients had dual hit t (4:14) plus del 17p and one patient had dual hit t (14:16) plus del 17p. Table 2, Figure 1.

Table 2: Incidence of cytogenetic abnormalities of the included patients

	Have cytogenetic abnormalities					**No cytogenetic abnormalities**
	Single Hit			**Double Hit**		
	t (4:14)	t(14:16)	Del 17p	Del 17p + t (4:14)	Del 17p + t (14:16)	
No. of patients	7(14%)	2(4%)	3(6%)	2(4%)	1(2%)	35(70%)



(A) **(B)**

(C)

Fig 1: (A) Interphase FISH depicting 17P deletion (one red signal), (B) Interphase FISH with IGH/FGFR3 dual color dual fusion signals positive for t(4;14) and (C) Interphase FISH with IGH/MAF dual color dual fusion signals positive for t(14;16)

Patients were stratified according to R- ISS staging system. Regarding ISS, only 10 patients were stage 3 and stratified as high risk according to ISS risk stratification, while 3 patients low risk stage 1 plus 5 patients intermediate risk stage 2 according to ISS risk stratification were upgraded to stage 3 according to R-ISS with total 18 patients, three of 18 patients with R-ISS stage 3 were stratified as ultra-high risk and other 15 stage 3 patients were stratified as high risk according to R-ISS risk stratification, twelve of 15 high risk patients in RISS risk stratification had high risk cytogenetic abnormalities. Table 3.

Table 3: Staging according to R- ISS, R-ISS Risk stratification and distribution of patients between R-ISS and ISS stages

		N=50	
R- ISS staging	Stage I	11(22%)	
	Stage II	21(42%)	
	Stage III	18 (36%)	
R-ISS Risk stratification	Standard Risk	32(64%)	
	High Risk	15(30%)	
	Ultra-High Risk	3(6%)	
Distribution	ISS I	ISS II	ISS III
R-ISS I	11(22%)	0(0.0%)	0(0.0%)
R-ISS II	0(0.0%)	21(42%)	0(0.0%)
R-ISS III	3(6%)	5(10%)	10(20%)

Mean age was significantly higher among standard risk group than high and ultra-high-risk group and in high-risk group than Ultra- high-risk group (p<0.001). Sex, performance status, bony lesions, extra- medullary infiltration and plasma cell leukemia were insignificantly different between the three groups. Mean s. creatinine was significantly higher and creatinine clearance was lower among high risk and ultra- high-risk groups than standard risk groups (p<0.001). Most of patients in standard risk had impaired kidney function stage 1 or 2 while most of high risk and ultra- high- risk patients had impaired kidney function stage 3 or 4 with statistically significant differences (p= 0.001). Mean serum total calcium levels were significantly higher among ultra high-risk group than high risk and standard risk group (p= 0.001). All patients in Ultra high-risk group were hypercalcemic. Also, higher percent of patients in high-risk group were hypercalcemic than standard risk group (p<0.001). Mean Hb levels were significantly lower among ultra-high-risk group than standard risk and high-risk groups. Also, all patients in ultra high-risk group had severe anemia, most of patients in high-risk group had moderate to severe anemia and most of patients in standard risk group had mild anemia (p<0.001). Mean plasma cell counts significantly different between three groups (p= 0.004). Involved serum IgG and IgA levels were significantly higher among ultra high-risk group than high risk and standard risk groups (p<0.001). Most of patient in ultra-high-risk group had M band of IgA while most of standard risk and high-risk groups had M band of IgG with significantly differences (p<0.001). All patients in ultra high-risk group received VCD and seventy three percent of patients in high-risk group received VCD while most of patients in standard risk group received VRD with significantly differences (p= 0.0014). Table 4

Table 4: Patients and disease characteristics according to R-ISS Risk stratification

		Standard Risk (n=32)	High Risk (n=15)	Ultra- High Risk (n=3)	P
Age (years)		69.2±2.8	59±5.2a	46.6±5.5 a, b	**<0.001***
Sex	Male	18(65.2%)	11 (73%)	0(0%)	0.19
	Female	14 (43.8%)	4 (27%)	3 (100%)	
Performance status (%)	1	12 (37.5%)	7(47%)	0 (0%)	0.69
	2	20 (62.5%)	8 (53%)	3 (100%)	
Bony lesions		26 (81.3%)	14 (93.4%)	3 (100%)	0.49
Extra- medullary infiltration (%)	STMs from skeletal lytic lesions	0 (0%)	3 (20%)	0 (0%)	0.3
	Pleural effusions	0 (0%)	0 (0%)	1 (33.3%)	
Serum creatinine (mg/dL)		2.6±1.7	4.43±0.95a	4.6±0.7a	<0.001*
Creatinine clearance (ml/min/m^2)		77±17.3	43.9±18.7a	34±11.3a	<0.001*
Kidney function level	Stage 1	12 (37.5%)	0 (0%)	0 (0%)	0.001*
	Stage 2	13(40.6%)	1 (6.6%)	0 (0%)	
	Stage 3	7 (21.8%)	7 (46.6%)	1 (3.33%)	
	Stage 4	0 (0%)	7 (46.6%)	2 (66.6%)	
Serum total Calcium (mg/dL)		9.9±1.7	11.12±1.3 a	13.4±0.6 a	0.001*
Hypocalcemia		5 (15.6%)	1 (6.6%)	0 (0%)	<0.001*
Normal		21 (65.6%)	7(46.6%)	0 (0%)	
Hypercalcemia		6 (18.8%)	7 (46.6%)	3 (100%)	
Hb(g/dL)		10.3±2	8.3±1.1 a	6.6±0.77 a, b	<0.001*
Level of anemia (%)	Normal	8 (25%)	0 (0%)	0 (0%)	<0.001*
	Mild	18 (56.2%)	1 (6.6%)	0 (0%)	
	Moderate	5 (15.6%)	7 (46.6%)	0 (0%)	
	Severe	1 (3.2%)	7 (46.6%)	3(100%)	
Plasma cell leukemia (%)		0 (0%)	0 (0%)	2 (4%)	0.1
Plasma cell count (%)		37.5±13.2	51.6±11 a	43.3±14.7	0.004*
Involved serum IgG		2351±917	3448±968	6342	<0.001*
Involved serum IgA		507±512	5737±1439 a	6946±814 a	<0.001*
M band subtype	IgG MM	30 (93.7%)	9 (60%)	1 (33.3%)	<0.001*
	IgA MM	2 (6.3%)	6 (40%)	2 (66.6%)	
	IgD MM	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Standard triplet combination therapy	VRD	25 (78.2%)	4 (26.6%)	0 (0.0%)	0.0014*
	VCD	7 (21.8%)	11 (73.3%)	3 (100%)	

There were statistically significant differences between ultra-high risk, high risk and standard risk groups as regard response to treatment as most of patients in ultra-high-risk group had stable disease, higher percent of patients in high-risk group had partial response while most of patients in standard risk group had either complete response or very good partial response (p= 0.0005). Table 5.

Table 5: Response after end of induction with Triplet Standard Combination Therapy according to R-ISS Risk stratification

	Standard Risk (n=32)	High Risk (n=15)	Ultra-High Risk (n=3)	P
Complete response	13(40.6%)	1(6.6%)	0(0.0%)	0.0005*
Very good partial response	17 (53.1%)	4(26.6%)	0(0.0%)	
Partial response	2(6.3%)	8 (53.3%)	1 (33.3%)	
Stable disease	0(0.0%)	2 (13.3%)	2 (66.6%)	
Progressive disease	0(0.0%)	0(0.0%)	0(0.0%)	

Discussion

Multiple myeloma is the most aggressive plasma dyscrasias and still considered incurable disease, accounts for 20% of hematological malignancy deaths and 2% of all cancer deaths [9].

Serum LDH was above the normal limit in 24% of our patients with a mean 245 mg/dL and (SD±90), which was close to Lopes *et al.* [17] with 20% of Brazilian newly diagnosed multiple myeloma patients had high LDH level.

In this study, almost half of patients 46% presented with moderate to severe anemia and the mean Hb level was 9.5 g/dL with (SD±2.1), which was similar to Hussain *et al.* [18] found that 50% of patients were found to have moderate to severe anemia.

Regarding Beta 2 macroglobulin in our study, almost 48% of patients have a high B2M above 3.5 mg/L with a mean 3.8 mg/L and (SD±1.3). In comparison with Hussain *et al.* [18] the B2M mean was 2.7 mg/L with (SD±0.95), reflecting more disease burden in our patients.

The incidence of hypoalbuminemia was 50% in our patients with a mean 3.3 g/dL similar to (Shaikh *et al*., 2019) that mean value of albumin level was 3.36 g/dL, while in comparison with (Hussainet *et al*., 2019) there were only 30% of their patients presented by hypoalbuminemia in Lybia.

By bone marrow examination, the mean value of plasma cells was 43.3% with (SD±14.7), which was lower than the reported by Hussain *et al.* [18] the mean plasma cells value was 66%.

In this study, the most common type of M protein was IgG in 80% of patients, followed by IgA in 20%. In comparison with Sidana *et al.* [19] IgG accounted for 52% of cases while

IgA, light chain and non-secretory myeloma accounted for 24%, 23% and 1% respectively.

International staging system, in present study, the distribution was 28%, 52%, and 20% for ISS stages I, II, and III, respectively. In Shaikh *et al*. [20] the distribution was 33.7%, 28.7%, and 37.7% for ISS stages I, II, and III respectively in Pakistani newly diagnosed multiple myeloma patients.

In this study, FISH analysis detected genetic abnormalities in 30% of total patients distributed as 24% had single hit and only 6% had double hit. In comparison with Panopoulou *et al*. [21] 40% of total 139 newly diagnosed multiple myeloma patients had single hit and only 9% had double hit, this difference is related to their expanded panel of cytogenetic abnormalities and larger sample size.

The translocation t (4:14) was the most frequent type of anomaly with 18%, followed by Del 17p with 12%, then t (14:16) with 6%. That was similar to the cytogenetic abnormalities of Hamdaoui *et al*. [22] in 99 newly diagnosed multiple myeloma Moroccan patients, as 14% and 12% of patients had t (4:14) and Del 17p, respectively.

Revised-International staging system, in the present study, 22% had R-ISS I, 42% had R-ISS II, and 36% had R-ISS III. In comparison with R-ISS of Kastritis *et al*. [23] the distribution by group, 18% had R-ISS I, 64% had R-ISS II, and 18% had R-ISS III, as there study involved a larger sample size (n = 475), plus the majority of patients that included in our study were presented with moderate to severe organ damage, which doubles the percentage of stage III patients in our study according to R-ISS.

According to R-ISS risk stratification in our study, the distribution by group was as follows: 64% were standard risk, 30% were high risk, and only 6% were ultra-high risk. The 15 high-risk patients according to R-ISS were 20% ISS I, 33.3% ISS II, and 46.7% ISS III at the time of diagnosis. That was close to the study of Baysal *et al*. [24] the distribution group according to R-ISS risk stratification was 71% standard risk, 23% high risk, and only 5.6% ultra-high risk were 5% ISS I, 35% ISS II, and 60% ISS III at the time of diagnosis, and the difference may be related to the smaller sample size in our study and the different population.

Plasma cell leukemia and extra medullary infiltration, there was no statistical difference due to the small sample size, although all of them were positive in either the high risk or ultra-high risk group, as extramedullary infiltration was in only 8% (4 patients) and all of them had Del 17p, while plasma cell leukemia was in only 4% (2 patients) and both of them were ultra-high risk with an incidence of 66.6% in this risk group. another result found by Singh *et al*. [25] as 40% of ultra-high-risk patients were presented with plasma cell leukemia.

Regarding renal impairment in this study, there was a significant difference between the R-ISS risk stratification groups as almost all high and ultra-high-risk patients 95% were presented with a stage 3 or 4 kidney function level, while only 20% of the standard risk had stage 3 and no one had a stage 4 kidney function level. In comparison with Zhuang *et al*. [26] only 54% of high and ultra-high risk patients were presented with a stage 3 or 4 kidney function level.

All ultra high-risk patients presented with hypercalcemia with a higher mean value than high risk patients; 13.4 mg/dL and 11.12 mg/dL respectively. Hypercalcemia was significantly more common in high-risk patients than in standard risk with 46.5% and 18.8% respectively. While in Zhuang *et al*. [26] only 11% of high-risk patients were present high-risk hypercalcemia.

In our study, the majority of patients 95% that presented with normal hemoglobin level or mild degree of anemia were stratified in the standard risk group, While the majority of ultra-high- and high-risk patients 94% presented with moderate to severe anemia, as the Hb was lower among ultra-high-risk patients than high risk patients, which was lower than the standard risk group. Compared with Singh *et al*. [25] 70% of ultra-high-risk patients were presented with moderate to severe anemia.

Plasma cell percentage was significantly higher in the high-risk group than the ultra-high and standard risk groups, which was not expected to be higher than the ultra-high-risk group, and it may be related to the small number of ultra-high-risk patients in our study (only 3 patients).

According to the M protein subtype, IgA was closely related to the ultra-high-risk group, as 2 of 3 patients were IgA with incidence 66.6%, while most high and standard risk patients 83% were IgG. In Geng *et al*. [27] with regard to the M protein subtype in the ultra-high-risk group, only 24% had IgA, while the majority 61% had IgG, 23% had a light chain, and 3% were non-secretory.

In our study, we noted that the serum level of involved IgG and IgA were significantly correlated to risk groups, as a higher risk group related to a higher serum level.

Regarding the response in our study, 28%, 42%, 22%, and 8% of the total included patients achieved complete response, very good partial response, partial response, and stable disease respectively to standard triplet combination therapy and no one showed progressive disease. In comparison with Sidana *et al*. [28] achieved a complete response, 45% achieved a very good partial response, and 38% achieved a partial response, and no patient had stable or progressive disease.

No one in the ultra high risk group achieved either a complete response or a very good partial response and most of them 66.6% showed stable disease. On the other hand, 93% of standard risk patients achieved either a complete response or a very good partial response and no one showed stable disease, while in the high-risk group, the majority 53% had a partial response and 27% had a very good partial response. In comparison with Geng *et al*. [27] as regard response of the ultra-high-risk group to triple standard therapy, there was a different distribution as 13% had CR, 12% had VGPR, 33% had PR, 21% had SD, and 19% had PD.

Limitations of this study include the sample size was relatively small. So, we recommended that add MRD status and free light chain assay to the panel of assessment (they were not available for all our patients) to detect the depth of response as not every patient with a complete response has a signet complete response which affects the PFS. Longer follow up is needed to assess the effect of initial therapy on PFS and OS. Expand the panel of cytogenetic analysis, as multiple myeloma is a genetically complex and heterogeneous neoplasm in which cytogenetic abnormalities are major factors in the prediction of disease.

Conclusion

Comparing the ISS and R-ISS systems may misallocate a patient group with inferior response at the lower ISS stage.

Multiple myeloma prognoses have improved with protease inhibitors and immunomodulators. However, extremely high- and high-risk patients responded less to traditional triplet combination therapy than normal risk patients.

Financial support and sponsorship: Nil

Conflict of Interest: Nil

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How to Cite This Article
Helmy AH, Elgandor AH, Alameldin MA, Tawfik HA. Fluorescence *in situ* hybridization in molecular classification of multiple myeloma: Prediction of response to triplet standard combination therapy. International Journal of Orthopaedics Sciences. 2024;4(1):24-31.