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Minimal Residual Disease Assessment by Flow Cytometry in Multiple Myeloma After Receiving Triple Combination Therapy

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ABSTRACT

This study aimed to measure minimal residual disease for fifty newly diagnosed multiple myeloma patients after receiving triple combination therapy who achieved complete response by multi-parameter flow cytometry and correlate with clinical and laboratory data of the patients. Most of patients included at the study were males with mean age about 56 years old and most of them were score 2 performance score. Regard disease characteristic about (58%)29 patients were anemic. Most of patients were normal renal function (62%)31 patients. About (20%)10 patients were high calcium level. Serum protein electrophoresis and immunofixation done and about (64%)32 patients were IgG. About (66%) 33 patients were with multiple bone lesions. Patients characteristic either age, sex and performance statues have no effect on MRD result of patients with newly diagnosed MM. There was significant association between B2M level and MRD result and the P Value was (0.010), high level was associated with more MRD positivity. Also there was significant association between ISS and MRD result. As regard patient with low risk ISS (64.7%) 11 patients achieved negative MRD, patients with high risk ISS (17.6%) 3 patients only achieved negative MRD and the p value was significant about (0.020). But there was no significant association between LDH level and MRD results. There were more MRD negativity with patients who received VRD protocol but was not significant so either VCD or VRD protocol is accepted for induction therapy regarding renal function. There was discordant between complete response and MRD result after induction therapy. Although all patient included achieved complete remission after induction therapy but only about one third of patients were negative MRD. This study included fifty newly diagnosed multiple myeloma patients was carried out at Clinical Oncology and Hematology Departments at Tanta and Alexandria Universities at the period from august 2022 to June 2023.

Keywords: Residual Disease, Diagnosed Multiple Myeloma, Serum protein electrophoresis,

1. Introduction

Multiple myeloma (MM) is characterized by the accumulation of clonal, malignant plasma cells in the bone marrow (Marcon *et al.,* 2023).

The cause of myeloma is unknown. Radiation may be a factor in some cases (although there is no association with therapeutic radiation). Exposure to industrial/agricultural toxins or viruses have all been considered, but proof is lacking (Ludwig and Kumar, 2023).

Chromosomal abnormalities have been identified, most commonly involving the immunoglobulin heavy chain switch region (on the long arm of chromosome 14), although these do not appear to be enough on their own to give rise to MM (Mcavera *et al.,* 2023).

Multiple myeloma accounts for 1% of all cancers and is the 2nd most common hematologic malignancy after lymphoma (Huang *et al.,* 2022).

The median age of patients at diagnosis is approximately 66–70 years with 37% of patients being younger than 65 years old (Waszczuk-Gajda *et al.,* 2023).

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The symptoms reported by patients with multiple myeloma on presentation are often non-specific and may already have been present for an extended period. Anemia of unknown origin is found in 73% of patients, bone pain in 58%, and fatigue in 32%. Around 25% of them report unexplained weight loss, and renal function is often impaired (Mikhael *et al.,* 2023).

In addition to history taking and physical examination, the diagnostic work-up for multiple myeloma comprises clinical chemistry, cytogenetic analysis of bone marrow, and radiological investigation to detect bone changes (Zeng *et al.,* 2023).

Multiple myeloma (MM) is a heterogeneous disease, with survival duration ranging from a few months to more than 10 years (Kazandjian, 2016).

The International Staging System (ISS) is a simple risk stratification algorithm based on two parameters; high serum 2-microglobulin level reflects high tumor mass and reduced renal function, and low serum albumin in MM is mainly caused by inflammatory cytokines such as interleukin-6 secreted by the myeloma microenvironment (Solimando *et al.,* 2023).

The ISS score, defined in 2005, identifies three patient groups with different prognoses; the median overall survival (OS) was 62 months in the ISS stage I, 44 months in the ISS stage II, and 29 months in the ISS stage III groups (Chen *et al.,* 2023).

Overall survival in myeloma has improved significantly in the last decade with the emergence of thalidomide, bortezomib, and lenalidomide (Chacon *et al.,* 2023).

Bortezomib is a proteasome inhibitor; the mechanism of action of thalidomide and lenalidomide is unclear, but they are considered immunomodulatory agents and may require cereblon (the putative primary teratogenic target for thalidomide) expression for their anti-myeloma activity. More recently carfilzomib (a new proteasome inhibitor) and pomalidomide have been approved for the treatment of multiple myeloma (Kaiser *et al.,* 2023).

The depth of response has prognostic value in MM. Patients who achieve a minimal residual disease (MRD)-negative state have superior progression-free and overall survival compared with those in whom MRD testing shows residual disease (Das and Gupta, 2023)

In multiple myeloma (MM) multi-parameter flow cytometry assessment of minimal residual disease (flow-MRD) in bone marrow (BM) which done by monitoring CD38 and CD138 for the recognition of BM plasma cells; in contrast, other markers are used to further differentiation between normal/reactive plasma cells and myeloma cells in the MRD methods. Among the later markers, CD19, CD45, CD27, and CD81, also with CD56, CD117, CD200, and CD307, have been be also informative (Panakkal *et al.,* 2023).

The aim of this work is assessment of flow cytometry method for detection of minimal residual disease in multiple myeloma cases achieved complete response after triple combination therapy and its correlation with clinico-laboratory features.

2. Patients and Methods

This prospective study was carried out on fifty newly diagnosed multiple myeloma patients at Clinical Oncology and Hematology Departments at Tanta and Alexandria Universities from August 2022 to June 2023.

Inclusion criteria

Multiple myeloma patients achieved complete remission using International Myeloma Working Group (IMWG) Response Criteria after receiving triple combination therapy (Cavo *et al.,* 2015).

Exclusion criteria

- Cases with smoldering myeloma, monoclonal gammopathy of unknown significance and solitary myeloma**.**

I. Triplet therapy

- Bortezomib, lenalidomide, and "low dose" dexamethasone (VRd).
- Cycle length: 28 days
- Number of cycles: 4 cycles

Table 1: VRD protocol.

Drug	Dose and route	Administration	Given on days
Bortezomib	1.3 mg/m^2 SC or IV	Given as a single SC injection or as a rapid IV bolus over three to five seconds.	Days 1, 8,15 and 22
Lenalidomide	25 mg$^{\Delta}$ by mouth	Administer with water. Swallow capsule whole; do not break, open, or chew.	Daily, on days 1 through 21
Dexamethasone	40 mg by mouth	Take with food (after meals or with food or milk) in the morning.	Days 1, 8, 15and 22

- Bortezomib plus cyclophosphamide and dexamethasone (VCD or CyBorD).
- Cycle length: 28 days.
- Number of cycles: 4 cycles.

Table 2: VCD protocol.

Drug	Dose and route	Administration	Given on days
Bortezomib	1.5 mg/m^2 SC or IV	Given subcutaneously or as a rapid IV bolus over three to five seconds.	Days 1, 8, 15, and 22
Cyclophosphamide	300 mg/m^2 by mouth, once weekly	Dose rounding to the nearest 50 mg. Do not cut or crush. Take during or after meal in the morning.	Days 1, 8, 15, and 22
Dexamethasone	40 mg by mouth, once weekly	Take with food (after meals or with food or milk) in the morning.	Days 1, 8, 15, and 22

II. Asses the response

Using International Myeloma Working Group (IMWG) Standard Response Criteria, all patients included at our study achieved complete response (Kumar *et al.,* 2016).

Patient evaluation

I. Careful history taking:

- Personal history (age, sex, occupation, residence, marital status, personal habits, exposure to radiation).
- Present history including patient complaint
- Past history and others co-morbidity (e.g. Diabetes mellitus, hypertension, Cardiac disease, etc.)
- History of renal impairment.
- Family history.

II. Performance status according to ECOG score (Voorhees *et al.,* 2023).

III. Clinical examination

- The general examination includes general appearance, vital signs, head & neck, chest, upper and lower limb examination.
- The local examination include bone examination.

IV. Investigations

A. Routine investigation

- Complete blood picture .Anemia defined as Hemoglobin <10 g/dL (<100 g/L) or >2 g/dL (>20 g/L) below normal (Meriche *et al.,* 2023).
- Kidney function tests.
- Check electrolytes calcium total and ionized.
- Serum albumin, serum beta-2 microglobulin (B2M) and lactate dehydrogenase (LDH).
- Serum protein electrophoresis (SPEP) with immunofixation (IFIX).

B. Bone marrow aspiration and or biopsy.
C. Radiological investigations including one of the following when needed:

- X-ray skeletal survey
- Whole body low dose CT or MRI.
- PET CT if needed.

D. Assessment of minimal residual disease on bone marrow sample with flow cytometry method with the following panel of monoclonal antibodies : CD19, CD45, CD56, CD38, CD138, CD81,CD27, κappa, and λ lambda (Bertamini *et al.,* 2021)

Methods of flow cytometry for detection of minimal residual disease at multiple myeloma

Immunophenotyping studies were carried out on the bone marrow aspirates using pretitrated volumes of the following monoclonal antibodies: CD19, CD45, CD56, CD38, CD138, CD8, CD27, κappa, and λ lambda.

Staining was done using standard whole blood lysis technique. For assessing surface antigens, an aliquot of cells containing 1 × 106 cells was labeled with pretitrated volumes of preconjugated monoclonal antibodies, and to study cytoplasmic κ and λ light chain expression, fixation and permeabilization before staining were carried out per the manufacturer's recommendation.

The cells were then washed with phosphatebuffered saline and suspended in 1% paraformaldehyde.

Acquisition was done on a flow cytometer (BD FACSCanto, BD Biosciences) equipped with facility for at least 10-color immunophenotyping, and at least 10^5 events were acquired in each tube.

Analyses were carried out using FacsDiva software. Negative limits were set using autofluorescence alone.

We compared various gating strategies using 2 antibodies (CD38 and CD138 for distinguishing PCs from other hematopoietic cells.

An aberrant immunophenotype was defined as expression of antigens not normally expressed or lack of expression of antigens normally expressed by the PCs.In a patient with MM, expression of an antigen was considered positive when at least 10% of the PCs expressed it at the time of diagnosis

Statistical Analysis

The collected data were organized and entered on Excel sheet and statistically analyzed using SPSS software statistical computer package for Windows, version 25 (IBM Corp., Armonk, N.Y., USA).

3. Results

This prospective study was carried out on fifty newly diagnosed multiple myeloma patients at Clinical Oncology and Hematology Departments at Tanta and Alexandria Universities, from August 2022 to June 2023.

A total of fifty patients with newly diagnosed multiple myeloma who achieved complete remission after triple combination therapy were included in the study.

Among fifty patients, 28 (56%) were males and 22 (44%) were females. The mean age at diagnosis was 56 years. Most of the patients (68%) 34 were performance status 2 (Table3).

Table 3: Patients' characteristics (n=50)

	No.	%
Sex		
Male	28	56.0
Female	22	44.0
Age		
Mean ± SD.	56.2 ± 9.35	
Min. – Max.	35.0 – 72.0	
PSS		
Mean ± SD.	1.7 ± 0.47	
Min. – Max.	1.0 – 2.0	
Median (IQR)	2.0 (1.0 – 2.0)	
Performance status 1	16	32.0
Performance status 2	34	68.0

IQR: Interquartile range

Serum albumin, B2M were measured to categorize patients for International scoring system, unfortunately cytogenetic analysis not done.

Among fifty newly diagnosed multiple myeloma patients, about (38%) 19 patients were low risk, (30%)15 patients were intermediate risk and (32%)16 patients were high risk.

About fifty newly diagnosed multiple myeloma patients, about Fifty-eight percent of patients suffering of different degree of anemia. Most of patients were normal renal function (62%)31 patients, most of patients were normal creatinine clearance, the mean creatinine clearance was 71.1. Approximately (20%)10 patients were high calcium level. Serum protein electrophoresis and immunofixation were done. the majority of our patients (64%)32 patients were IgG myeloma subtypes. Thirtythree patients (66%) were with multiple bone lesions. The mean plasma cells at BMA/B were 35.9 as listed at (Table 4).

Out of fifty patients no one had extra medullaryinfiltration nor plasma cell leukemia.

All patients received four cycles of triple combination therapy either VRD or VCD protocol. About (66%) 33 patients received VRD protocol and (34%) 17 patients received VCD protocol.

The choice of treatment protocol was according to creatinine clearance of the patients, patients with low creatinine levels received VCD protocol, while the remaining patients received VRD protocol.

The Correlation between MRD results with patients' characteristics was listed at (Table 5), on fifty newly diagnosed multiple myeloma patients there was no significant association between MRD results with sex, age and performance status.

There was significant association between B2M level and MRD results with P Value was (0.010), high level B2M was associated with more MRD positivity as listed at (Table 6).

Also there was significant association between ISS and MRD results. regarding patients with low risk ISS (64.7%) eleven patients achieved negative MRD, patients with high risk ISS (17.6%) 3 patients only attend MRD negativity and the p value was significant about (0.020) as listed at (Table 6).

However, there was no significant association between serum LDH level and MRD results among fifty newly diagnosed patients

Table 4: Tumor characteristics (n=50)

	No.	%
Serum albumin		
Normal	40	80.0
Low	10	20.0
B2m		
Normal	19	38.0
Intermediate	13	26.0
High	18	36.0
ISS		
Stage I	19	38.0
Stage II	15	30.0
Stage III	16	32.0
LDH		
Normal	34	68.0
High	16	32.0
Anemia		
Normal	21	42.0
Anemic	29	58.0
Renal functions		
Normal	31	62.0
High	19	38.0
Calcium level		
Normal	40	80.0
High	10	20.0
Myeloma subtypes		
IgA	10	20.0
IgD	5	10.0
IgG	32	64.0
IgM	3	6.0
Bone lesions		
Normal	17	34.0
Multiple bone lesions	33	66.0
BMA/B plasma cell		
Mean ± SD.	35.9 ± 19.48	
Min. – Max.	14.0 – 100.0	
Median (IQR)	30.0 (20.0 – 50.5)	
Creatinine clearance		
Mean ± SD.	71.1 ± 23.19	
Min. – Max.	32.0 – 120.0	

IQR: Interquartile range

Table 5: Correlation between negative and positive MRD regarding patients' characteristics

	MRD		Test of sig.	P
	Negative (n=17)	**Positive (n=33)**		
Sex				
Male	11 64.7%	17 51.5%	χ^2 **0.792**	**0.373**
Female	6 35.3%	16 48.5%		
Age				
Mean ± SD.	52.9 ± 8.95	57.8 ± 9.22	**t** **1.833**	**0.073**
Min. – Max.	38.0 – 68.0	35.0 – 72.0		
PSS				
Mean ± SD.	1.6 ± 0.51	1.7 ± 0.45	**U** **241.5**	**0.323**
Min. – Max.	1.0 – 2.0	1.0 – 2.0		
Median (IQR)	2.0 (1.0 – 2.0)	2.0 (1.0 – 2.0)		
Score 1	7 41.2%	9 27.3%	χ^2 **0.997**	**0.318**
Score 2	10 58.8%	24 72.7%		

χ^2: Chi square test t: Independent t test
U: Mann Whitney U test IQR: Interquartile range

Table 6: Correlation between negative and positive MRD regarding International Scoring System

	MRD		χ^2	P
	Negative (n=17)	**Positive (n=33)**		
Albumin				
Normal	15 88.2%	25 75.8%	**1.092**	**0.296**
Low	2 11.8%	8 24.2%		
B2m				
Normal	11 64.7%	8 24.2%		
Intermediate	1 5.9%	12 36.4%	**9.154**	**0.010***
High	5 29.4%	13 39.4%		
LDH				
Normal	14 82.4%	20 60.6%	**2.439**	**0.118**
High	3 17.6%	13 39.4%		
ISS				
Stage 1	11 64.7%	8 24.2%		
Stage 2	3 17.6%	12 36.4%	**7.803**	**0.020***
Stage 3	3 17.6%	13 39.4%		

χ^2: Chi square test *p ≤ 0.05 (Statistically significant)

Also there was no significant association between the different degrees of anemia, renal function, and calcium level with MRD results of our fifty newly diagnosed patients as listed at (Table 7).

Table 7: Correlation between negative and positive MRD regarding Anemia, Renal functions and calcium

	MRD		χ^2	P
	Negative (n=17)	**Positive (n=33)**		
Anemia				
Normal	7 41.2%	14 42.4%	**0.007**	**0.933**
Anemic	10 58.8%	19 57.6%		
Renal functions				
Normal	12 70.6%	19 57.6%	**0.806**	**0.369**
High	5 29.4%	14 42.4%		
Calcium level				
Normal	16 94.1%	24 72.7%	**3.209**	**0.073**
High	1 5.9%	9 27.3%		

χ^2: Chi square test

Regarding serum protein electrophoresis/immunofixation, there was no significant association between myeloma subtypes and MRD results (Table 8). However there was significant association between MRD status and bone lesions, the majority of patients were with multiple bone lesions about (78.8%) 26 patient were MRD positive with P value (0.008), while patients with no lytic lesion about (58.8%) 10 patients were negative MRD (Table 8).

Table 6: Correlation between negative and positive MRD regarding SPEP/immunofixation and Bone lesions**.**

	MRD		Test of sig.	P
	Negative (n=17)	**Positive (n=33)**		
Myeloma subtypes				
IgA	3 17.6%	7 21.2%		
IgD	1 5.9%	4 12.1%	**MC**	**0.482**
IgG	13 76.5%	19 57.6%		
IgM	0 0.0%	3 9.1%		
Bone lesions				
No lytic lesion	10 58.8%	7 21.2%	χ^2 **7.073**	**0.008***
Multiple bone lesions	7 41.2%	26 78.8%		

MC: Monte Carlo Exact test χ^2: Chi square test *p ≤ 0.05 (Statistically significant)

For fifty newly diagnosed multiple myeloma patients, there was no significant association between MRD results and creatinine clearance as listed at (Table 9).

There was significant association between plasma cell percent detected at bone marrow at diagnosis and MRD results, the median plasma cells was 25 for MRD negative and 36 for MRD positive patients with the P value was (0.018) as listed at (Table 9).

Table 9: Correlation between negative and positive MRD regarding BMB/BMA plasma cell and creatinine clearance

	MRD		Test of sig.	P
	Negative (n=17)	Positive (n=33)		
BMA/B plasma cell				
Mean ± SD.	27.2 ± 15.56	40.4 ± 19.96	**U**	**0.018***
Min. – Max.	15.0 – 78.0	14.0 – 100.0	**165.5**	
Median (IQR)	25.0 (15.5 – 32.0)	36.0 (25.0 – 55.0)		
Creatinine clearance				
Mean ± SD.	72.1 ± 19.56	70.5 ± 25.13	**T**	**0.820**
Min. – Max.	38.0 – 115.0	32.0 – 120.0	**0.229**	

U: Mann Whitney U test IQR: Interquartile range t: Independent t test
*p ≤ 0.05 (Statistically significant)

All the included patients had achieved complete remission after triple combination therapy before assessment of MRD.

Patients received VRD protocol were 33 patients, (39.4%) 13 patients were MRD negative and (60.6%) 20 patients were MRD positive.

Patients received VCD protocol were 17 patients, (23.5%) only three patient were negative MRD and (76.5)13 patients were positive MRD, there was no significant association between negative and positive MRD results and treatment protocol.

There was discordant between MRD results and complete remission, although all patients achieved complete remission after triple combination therapy only about one third (34%) of patients were negative MRD, and about two third (66%) of patients were positive MRD (Table 10).

Table 10: Discordant between complete remission and minimal residual disease (MRD) after receiving triple combination therapy.

	MRD		χ^2	p
	Negative (n=17)	Positive (n=33)		
Protocol				
VCD	4	13	**1.258**	**0.262**
	23.5%	76.5%		
VRD	13	20		
	39.4%	60.6%		

χ^2: Chi square test

4. Discussion

Multiple myeloma is a malignancy of plasma cells. Plasma cells are mature antibody-producing B cells which reside in the bone marrow and are essential for maintaining humoral immunity. Multiple myeloma is characterized by a monoclonal proliferation of plasma cells resulting in the production of monoclonal antibody and end-organ damage (Jurczyszyn and Suska, 2019).

This can damage bone marrow, resulting in cytopenia and frail, brittle bones, or renal failure (Colmone *et al.,* 2008).

The accumulation of the monoclonal antibody, Bence–Jones proteins, can precipitate in the urine resulting in kidney damage and renal failure. Multiple myeloma also activates osteoclasts in the bones resulting in the destruction of bone via lytic lesions that predispose to pain, fractures (Michels and Petersen, 2017).

Patients often present with CRAB symptoms. When MM is suspected, blood and urine electrophoresis should be performed to look for the monoclonal light-chain secreted by the neoplasm (Padala *et al.,* 2021).

One of the most commonly used front-line triple therapies is bortezomib (a proteasome inhibitor), lenalidomide (an immunomodulator that downregulates inflammatory and proliferative

cytokines), and dexamethasone (a long-acting steroid), (called VRd after the tradenames Velcade, Revlimid, and Dexamethasone, respectively) (Moreau *et al.,* 2015).

Minimal residual disease (MRD) assessment in light of the effectiveness of new multiple myeloma (MM) treatment modalities and related to it increasing ratios of achieved complete remissions (CR), becomes an important tool in recognition of the depth of the response.

Multiparametric flow cytometry (MFC) is currently the most popular method for monitoring of MRD presence in bone marrow of MM patients (Rihova and Hajekm 2017).

MRD is characterized by presence of limited number of malignant cells that remain during or after therapy and are not detectable by serological or cytological approaches (Wijnands *et al.,* 2023).

The need for ultra-sensitive ways to detect minute remaining disease (MRD) is important in multiple myeloma, where the disease will ultimately recur in spite of reaching complete remission, which is usually common due to evident advance of treatment, its therapeutic significance is to analyze the efficacy-depth of a chosen management and hence predict an upcoming recurrence (Coffey *et al.,* 2023).

Flow Cytometry is the process whereby such measurements are made upon cells/particles as they pass through a measuring apparatus (in single file) suspended in a fluid stream. The basic building blocks of a Flow cytometer are Fluidics, Optics, and Electronics (Adan *et al.,* 2017).

Normal plasma cell exhibit variable expression of CD19 and CD45 and are generally negative for CD20 and CD117 also always have uniformly brilliant expression of CD81. In addition, CD28 and CD56 expression is found in a modest percentage (5–20%) of normal plasma cell (Bisharat, 2015).

As a result of normal plasma cells which frequently exhibit diverse or multimodal expression for several frequently utilized markers (for example, CD19, CD56, and CD45), whereas clonal plasma cells from patients of multiple myeloma typically exhibit more uniform antigen expression (Soh, 2020).

This prospective study was conducted at Clinical Oncology and Hematology Departments at Tanta and Alexandria Universities.

Through the period from August 2022 to June 2023. This study was conducted on fifty newly diagnosed multiple myeloma patients who achieved complete remission after triple combination therapy aiming to measure minimal residual disease and correlate to clinico-laboratory of the patients.

The males were (56%) and females were (44%) in agreement with western study Young *et al.* (2016) found that the males were (58%) and females were (44%).

In our study the mean age at diagnosis was 56 year but in another study San-Miguel *et al.* (2011) who found that median age was 64 years (range 32–79 years), and 45% of patients were ≥ 65 years of age, that difference may be due to small numbers included at the current study or may be awareness and early detection of disease.

At current study, after measuring serum albumin and B2m to categorize patients for ISS, about (38%) patients were low risk, (30%) patient were intermediate risk and (32%) patients were high risk unlike other study. Young *et al.* (2016) found that low risk were 16%, intermediate risk were 35% and high risk 49% ISS score at diagnosis (among those with known status, n = 815), small sample numbers with different population and disease characteristics may decrease percent of high risk ISS at our study.

About (66%) of our patients were with multiple bone lesions at the other study, (Devaraj and Al-Sader, 2023) noted that about (90%) of patients were with lytic bone lesions .

About (58%) of patients were anaemic unlike Michels and Petersen, (2017) reported that anaemic patients were about (36%).

At this study serum protein electrophoresis and immunofixation showed that most of patients about (64%) patients were IgG and this agreement with Kraj *et al.* (2015) who found IgG 66%.

About 34% of patients have low creatinine clearance less than 60 which was lower than that reported by Szabo *et al.* (2021). where low creatinine clearance were about (48%) of patients .

In the present study all patients received triple combination therapy either VRD or VCD protocol. About (66%) patients received VRD protocol and (34%) patients received VCD protocol. The choice of the protocol was according to creatinine clearance and thromboembolic risk.

In agreement with Afrough *et al.* (2022) found that induction with VCD Versus VRD about 34.4% received VCD protocol and about 65.6% received VRD protocol, median age at auto-HCT was 61.9 years (range 33.9-79.6), with 35.4% (114/322) of the cohort being 65 years of age or older.

There was significant association between ISS and MRD results. As regard patient with low risk ISS (64.7%) patients achieved negative MRD. patients with high risk ISS (17.6%) patients only achieved negative MRD .

There was no significant association between anemia, renal function, creatinine clearance and calcium level of included patients, there was no significant association between negative and positive MRD results in agreement with (Caers *et al.,* 2018) which demonstrate that anemia may predict positive MRD after therapy not before induction.

There was significant association between negative and positive MRD result and bone lesions, patient with multiple bone lesions about 78.8%) 26 patients were MRD positive. Patients with no lytic lesion about (58.8%) 10 patients were negative MRD.

As regard triple combination therapy received. Patients received VRD about (39.4%) were MRD negative and (60.6%) patients were MRD positive.

At the present study patients who achieved MRD negativity were (34%). Patients received VCD protocol about (23.5%) achieved MRD negativity and (76.5%) patients were positive MRD ,there was no significant association between MRD result and treatment protocol although high percent of patient received VRD protocol were negative MRD (39%) .

Langerhorst *et al.* (2021) reported only 20% of patients achieved negative MRD after 8 cycles of VRD and 30% were MRD negative after ASCT.

At CASSIOPEIA trial about 37 % of patients achieved negative MRD after VTD+ ASCT+VTD but with transplant were higher MRD negativity about 57% of patient achieved negative MRD (Moreau *et al.,* 2019).

At the GRIFFIN trial MRD negativity was high at the group who received Daratumomab with triple combination therapy about 51% of patients achieved negative MRD, but the group who didn't receive Daratumomab only 20.4% of patients were negative MRD (Voorhees *et al.,* 2023).

At MYELOMA XI trial highlights that maintenance leinalidamide was associated with higher MRD negativity, 65.6% of patients were negative MRD in comparison to 34.4% of patients who not received maintenance therapy (De Tute *et al.,* 2022).

At the MAIA trial comparing Dara-Vd vs Vd, the group who received Dara-Vd about 24.2% of patients were negative MRD but the group who received Rd about 7.3% of patients were negative MRD (San-Miguel *et al.,* 2022).

Paiva *et al.* (2022) reported that discordant between complete remission and negative MRD, at GEM2000 study there were 25% of patient were MRD positive although they achieved complete response.

Paiva *et al.* (2022) reported that about 38.5 % of patients were MRD positive although all included patient at the study achieved complete response.

This result should be analysed carefully as the trials has different patient populations with different characteristics and number of cycles and different therapy at induction either triple or quadrate therapy in addition different methods of detection between different trials.

5. Conclusion and Recommendations

Fifty newly diagnosed multiple myeloma patient who achieved complete remission after four cycles of triple combination therapy we found discordant between complete response and MRD result. Although all patient included at the study achieved complete response after four cycles of triple combination therapy but about 66% of patients were positive MRD. Complete remission after induction therapy is not enough for assessment of response and we should go for MRD assay. All patients with newly diagnosed multiple myeloma must do expending cytogenetics analysis before starting treatment to get perfect risk stratification. More sample size is recommended to asses effect of induction triple combination therapy on MRD in multiple myeloma patients and may strengthen the results. Longer duration of follow up to detect effect of the interval therapy protocol on the PFS and OS. Although all patients included at our study achieved complete response after triple combination therapy but only 34% were MRD negative so more therapy is needed for patients to get MRD negativity either with adding monoclonal antibody therapy to the triple combination therapy or increase number of cycles. Assessment of minimal residual disease by another methods like PCR and NGS may be useful to get more perfect assay. Multiple myeloma is still an incurable disease so maintenance therapy is mandatory and maintenance therapy achieve more negative MRD.

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