CD 27 and CD 81 in Multiple Myeloma: Correlation with Clinicopathological Features and Response to Therapy

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Abstract

Multiple Myeloma (MM) is a neoplastic plasma cell (PC) disorder, characterized by clonal proliferation of malignant PCs in the bone marrow (BM) and is accompanied by increased level of monoclonal protein in the blood and/or urine. Myeloma PCs demonstrate phenotypes which deviate from those typically seen in normal BM PCs. Multiparametric flow cytometry (MFC) is a method that enables the characterization of malignant PCs immunophenotype. CD27 and CD81 are PC surface markers implicated in MM biology. Their prognostic and predictive value remains unclear. This study aimed to assess correlation between the expression of CD27 and CD81 on BM samples of adult Egyptian MM cases by flow cytometry, and different clinico-pathological features, and its impact on response to treatment. This retrospective-descriptive analytical study included 90 MM patients diagnosed at the Oncology Center, Mansoura University, between May 2018 and October 2024. MFC was used to assess CD27 and CD81 expression. Associations with clinicopathological features, response to treatment, disease-free survival (DFS), and overall survival (OS) were analyzed. CD27 negative expression correlated with elevated LDH levels. Leucopenia showed numerical but no statistical significance. CD81 negative and weak positive expression correlated with elevated LDH levels. No statistical significant difference was detected in response to treatment, relapse incidence, DFS and OS in correlation with CD27 and CD81 expression. It can be concluded that CD27 and CD81 are valuable immunophenotypic markers in MM with well-characterized biological functions. In this study, CD27 negativity was associated with elevated LDH levels and inferior early treatment response but did not predict long-term survival outcomes. CD81 expression did not demonstrate significant prognostic or predictive associations.

Keywords: Multiple Myeloma, CD27, CD81, Immuno-phenotype, Prognostic markers, Clinicopathological features, Treatment response

1. Introduction

Multiple myeloma is described as a malignant disease characterized by the abnormal proliferation of PCs in the BM, causing the formation of monoclonal immunoglobulin (Ig) or its fragments (M protein). This induces injury to the affected organs or tissues, presenting as manifestations which include bone damage, impaired renal functions, increased calcium (Ca) values, and anemia [(1)].

Myeloma PCs demonstrate phenotypes which deviate from those classically detected in normal BM PCs. Several markers are accompanied by informative aberrant antigen (Ag) expression profiles for Minimal Residual Disease (MRD) monitoring in MM including CD27 [(2)] and CD81 [(3)]. Thus, MFC has become an essential tool in diagnosing of MM and monitoring of response to treatment owing to its high sensitivity (Sn) and specificity (Sp) [(4)].

A combined use of several markers raises the precession of abnormal PCs (APC) recognition. CD19, CD45, CD56, CD117, CD27, and CD81 increase the Sp for APC and normal PCs (NPC) identification and enumeration [(5)].

CD27 Ag is a member of the tumor necrosis factor (TNF) receptor family and interacts with its ligand CD70 to encourage cell apoptosis (programmed single cell death). Such process is essential for the normal differentiation of memory B cells and PCs. It is recorded that throughout disease advancement (from MGUS to MM or with MM disease progression), the CD27 Ag level diminished in a gradual manner till it was finally lost. Compared with normal BM PCs, MM PCs show down regulated CD27 levels [(6) (7)].

CD81, a nonglycosylated tetraspanin, is a transmembrane protein that has an essential role in synapse formation between B cells and T cells. It, in addition, regulates CD19 expression in B lymphocytes and has a role in cellular growth, motion, signal transduction, and the homing of BM cells. It also suppresses the migration and invasion of PCM (Plasma Cell Myeloma) cells, which suggests that it is an inhibitor of PCM metastasis and is related to the infiltration of different immune cells, particularly of monocytes and macrophages. So, CD81 now plays a significant role in MM pathogenesis and represent a new adverse prognostic marker in myeloma [(8) (9).]

Wang et al. [(10)] explored the expression of CD27 antigen in patients with MM, and its clinical diagnostic value, as well as the correlation of CD27 with clinical features and genetic abnormalities. CD27 showed a unique expression profile, and its negative or weak expression

was highly suggestive for MM. The CD27 positive rate of abnormal plasma cells in MM patients was 48% (59/123), MFI was 31.3±35.6; while the positive rate of normal or reactive plasma cells were 100% (51/51), the MFI was 93.7±6.3. The positive rate and MFI of CD27 in MM patients were significantly lower than that in normal or reactive plasma cells (P<0.01). Laboratory examination of 58 cases of CD27 negative and 49 cases of CD27 positive MM patients indicated that no significant differences were shown on disease progress parameter, such as hemoglobin, albumin, serum calcium, serum creatinine, and no notable differences were involved in the analysis of prognostic factors between the 2 groups, such as β2-MG microglobulin and LDH levels (*P<0.05*).

In addition, the study by Si et al. [11] evaluated the prognostic significance of CD27 and CD117 expression in 160 newly diagnosed multiple myeloma patients. Flow cytometry was used to assess pretreatment expression of these markers. CD27-negative patients showed more severe disease (higher β2-MG, more marrow plasma cells, lower hemoglobin). CD27-positive patients had significantly longer progression-free survival (PFS) (78 vs. 33 months; P = 0.0078).CD117 alone was not prognostic, but combined CD27(+)CD117(+) expression predicted the best outcomes i.e. longer PFS, earlier-stage disease, higher remission rates, and fewer high-risk cytogenetic abnormalities.

Despite promising results, no strong data is available on CD27 and CD81 as predictive and prognostic markers in MM patient. In this study we will assess correlation between the expression of CD27 and CD81 on BM samples of adult Egyptian MM cases by flow cytometry, and different clinico-pathological features, and its impact on response to treatment.

2. Patients and Methods

This retrospective descriptive analytical study included 90 MM cases treated at Oncology Center, Mansoura University (OCMU) from May, 2018 to October, 2024. Clinical and laboratory data were collected. Bone marrow aspirates were analyzed by MFC for CD27 and CD81 expression. Patients received four cycles of VCD (bortezomib, cyclophosphamide, dexamethasone). Responses were assessed per IMWG criteria. Survival analysis was performed using Kaplan–Meier and Cox regression.

Inclusion Criteria

MM patients from both genders, above the age of 18 years who received their treatment at OCMU

Exclusion Criteria

MM patients who were previously treated or those with advanced medical comorbidities excluding renal failure

Pretreatment assessment

Thorough history taking and clinical assessment; sex, age, cervical, axillary, inguinal lymphadenopathy, hepatosplenomegaly, and toxic manifestations; weight loss, fever, and drenching night sweats, bone aches, features of anemia, bleeding or recurrent infections, abnormal body swellings, Routine laboratory investigations; CBC, blood film, liver function tests, and renal function tests, ESR, hepatitis B and C, and HIV testing. Specific laboratory investigation; BM aspiration (BMA) and biopsy (BMB), serum protein electrophoresis (SPEP), serum immunfixation, immunoglobulin assay, B2 microglobulin, cytogenetic testing, and serum free light chain (FLC).Immunophenotyping (IPT); routine IPT for MM including CD27 and CD 81.Pathological evaluation; BMB (e.g. immunohistochemistry (IHC); CD38, 56, 138) and biopsy from abnormal body swelling (when indicated).Radiological studies; whole body skeleton CT, and MRI used when indicated (e.g. spine lesions), Treatment; Documentation of different treatment protocols and different toxicities

CD27 and CD81 expression by multi-parameter flow cytometry

The fundamental principle of flow cytometry is the passage of cells in single file in front of the laser so they could be identified, counted and sorted. Cell components are fluorescently labeled and then excited by the laser to emit light at varying wave length. The fluorescence could then be measured to detect the amount and type of cells present in the sample. Up to thousands of particles per second could be analysed as they pass across the liquid stream. A beam of laser light is directed at a hydro dynamically-focused stream of fluid that carries the cells. Multiple detectors are cautiously positioned around the stream, at the point where the fluid passes through the light ray. One of these detectors is in line with the light beam and is used to measure forward scatter (FSC). Another detector is located perpendicular to the

stream and is utilized to measure side scatter (SCC). The suspended particles or cells pass across the beam of light and scatter the light beams. The fluorescently labeled cell component is excited by the laser and emits light at a longer wave length than the light source. This is then detected by the detectors. The detectors as a result pick up a combination of scattered and fluorescent light. This data is then analyzed by a computer that is linked to the flow cytometer using special software. The brightness of each detector (one for each fluorescent emission peak) is adjusted for this detection. Using the light measurements, different data could be gathered about the physical and chemical structure of the cells. In general, FSC could determine the cell volume while side scatter detect the inner complexity of the particle which include its cytoplasmic granule content or nuclear structure.

Statistical Analysis

A personal computer running SPSS© for windows (Statistical Package for Social Scientists) Release 15 was utilized to examine the data. A statistically significant result is defined as a two-tailed p value of less than or equal to 0.05. The frequency distribution approach was utilized to measure the number of cases and percentages for descriptive statistics of qualitative variables. In quantitative variable descriptive statistics, central tendency and dispersion will be described by the mean, standard deviation, median and range, if appropriate. The Chi Square Test was utilized to examine any associations between category variables. If the chi square assumptions were broken, Fisher's exact test would be applied. We will use the Kaplan-Meier Product-Limit Estimator to calculate survival and progression free survival analyses. Comparison of the survival will be performed by the Log-Rank Test. Exploring variables for their independent prognostic effect on survival will be carried out using the multivariate stepwise Cox's proportional regression hazard model.

Ethical Consideration

This study was ethically approved by Mansoura University's Research Ethics Committee (MS- IRB #23.09.2558). Written informed consent was obtained from all participants. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human subjects.

3. Results

Among the studied patients, CD27 expression was studied in 87 patients, results were 48 (55.2%), 13 (14.9%), 26 (29.9%), positive, weak positive and negative, respectively. CD81 expression was studied in 54 patients, results were 18 (33.3%), 13 (24.1%), 23 (42.6%), positive, weak positive and negative, respectively. Combined CD 27 and CD 81 expression was studied in 51 patients, 23 (45.1%) patients were CD27 positive and CD 81 positive, 5 (9.8%) patients were CD 27 negative and CD 81 negative ,and 23 (45.1%) others as shown in Table (1)

As shown in Table (2), regarding CD27 expression (negative and weak positive vs. positive), elevated LDH level showed statistical significance (*P= 0.019*) and thrombocytosis, too, showed statistical significance (*P= 0.0*). Leucopenia showed numerical but not statistical significance (*P= 0.06*). However, there was no statistical significant difference with other different clinico-pathological features such as; anemia (*P= 0.1*), hypercalcemia (*P =0.45*), hypoalbuminemia *(P= 0.76*), and also neutrophil lymphocyte ratio (*P= 0.8*).

As shown in Table (3), CD81 expression (negative and weak positive vs. positive), elevated sFLC showed statistical significance (*P= 0.043*). However, there was no statistical significant difference with other different clinico-pathological features; anemia (*P= 0.42*), hypercalcemia (*P= 0.39*), hypoalbuminemia (P= 0.52), and also neutrophil lymphocyte ratio (*P= 0.56*)

As regard response assessment among whole 48 patients evaluated after 4 cycles VCD, 26 (54.2%) achieved CR, 3 (6.2%) achieved VGPR, 11 (22.9%) achieved PR, 8 (16.7%) achieved SD or PD. Among whole 48 patients who received 4 cycles VCD, 14 (29.2%) patients relapsed, as shown in Table (4).

As show in Table (5), as regard CD81 expression (negative vs. positive vs. weak positive), there was no statistical significant difference with relapse (*P=0.76*). As regard CD81 expression (positive vs. negative and weak positive), there was no statistical significant difference with relapse (*P =0.46*). As regard CD27 expression (negative vs. positive vs. weak positive), there was no statistical significant difference with relapse (*P =0.42*). As regard CD27 expression (positive vs. negative and weak positive), there was no statistical significant difference with relapse (*P =0.64*)

According to response evaluation as regard CD27 and CD 81 expression among 47 patients for CD27 and 31 patients for CD81 after 4 cycles VCD (CR and VGPR vs. PR, SD and PD) as shown in Table (6). As regard CD27 expression (negative vs. positive and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P= 0.77*). As regard CD27 expression (positive vs. negative and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =0.59*). As regard CD81 expression (negative vs. positive and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =0.67*). As regard CD81 expression (positive vs. negative and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =0.62*).

Response evaluation as regard CD27 and CD 81 expression among 47patients for CD27 and 31 patients for CD81 after 4 cycles VCD (CR, VGPR, and PR vs. SD, and PD) as shown in Table (7). As regard CD27 expression (negative vs. positive and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =0.06*). As regard CD27 expression (positive vs. negative and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =0.123*). As regard CD81 expression (negative vs. positive and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =1.0*). As regard CD81 expression (positive vs. negative and weak positive), there was no statistical significant difference with response after 4 VCD cycles (*P =1.0*)

Regarding survival functions, There was no statistical significant difference in DFS as regard CD 27 expression (Negative vs. Positive vs. Weak Positive) (*P= 0.78*) as shown in Figure (1). There was no statistical significant difference in DFS as regard CD81 expression (Negative vs. Positive vs. Weak Positive). (*P= 0.63*) as shown in Figure (2). There was no statistical significant difference in OS as regard CD 27 expression (Negative vs. Positive vs. Weak Positive) (*P= 0.68*) as shown in Figure (3). There was no statistical significant difference in OS as regard CD81 expression (Negative vs. Positive vs. Weak Positive) (*P= 0.68*) as shown in Figure (4).

Table (1): CD27 and CD 81 expression among studied multiple myeloma patients

Marker	Expression	Number of patients (percentage)
CD 27 (done in 87 patients)	Positive	48 (55.2%)
	Weak positive	13 (14.9%)
	Negative	26 (29.9%)
CD 81 (done in 54 patients)	Positive	18 (33.3%)
	Weak positive	13 (24.1%)
	Negative	23 (42.6%)
Combined CD 27 and CD 81 (done in 51 patients)	Both positive	23 (45.1%)
	Both negative	5 (9.8%)
	Others	23 (45.1%)

Table (2): CD 27 expression in correlation with different clinico-pathological features

Features	Value	CD27 Negative and weak positive	CD27 positive	P
Bone lytic lesions	**Absent**	9 (23.1%)	10 (20.8%)	**0.8**
	Present	30 (76.9%)	38 (79.2%)	
Pathological fracture	**Absent**	22 (56.4%)	29 (60.4%)	**0.7**
	Present	17 (43.6%)	19 (39.6%)	
Plasmacytoma	**Absent**	18 (46.2%)	31 (64.6%)	**0.08**
	Present	21 (53.8%)	17 (35.4%)	
Anemia	**Absent**	17 (43.6%)	13 (27.1%)	**0.1**
	Present	22 (56.4%)	35 (72.9%)	
High Creatinine	**Absent**	13 (33.3%)	24 (50%)	**0.12**
	Present	26 (66.7%)	24(50%)	
Hypercalcemia	**Absent**	23 (69.7%)	34 (77.3%)	**0.45**
	Present	10 (30.3%)	10 (22.7%)	
Positive BJP	**Absent**	6 (28.6%)	12 (38.7%)	**0.45**

	Present	15 (71.4%)	19 (61.3%)	
M-band	**Absent**	5 (13.9%)	5 (10.4%)	**0.63**
	Present	31 (86.1%)	43 (89.6%)	
Elevated sFLC	**Absent**	6 (16.7%)	6 (12.5%)	**0.59**
	Present	30 (83.3%)	42 (87.5%)	
Hypoalbuminemia	**Absent**	11 (37.9%)	17 (41.5%)	**0.76**
	Present	18 (62.1%)	24 (58.5%)	
Elevated LDH	**Absent**	6 (24%)	20 (54.1%)	**0.019**
	Present	19 (76%)	17 (45.9%)	
Leucopenia	**Absent**	39 (100%)	43 (89.6%)	**0.06***
	Present	0	5 (10.4%)	
Leucocytosis	**Absent**	32 (82.1%)	40 (83.3%)	**0.87**
	Present	7 (17.9%)	8 (16.7%)	
Thrombocytopenia	**Absent**	37 (94.9%)	48 (100%)	**0.2***
	Present	2 (5.1%)	0	
Thrombocytosis	**Absent**	35 (89.7%)	26 (54.2%)	**0.0**
	Present	4 (10.3%)	22 (45.8%)	
Neutrophil Number	**Low**	3 (7.7%)	6 (12.5%)	**0.76**
	Normal	35 (89.7%)	41 (85.4%)	
	High	1 (2.6%)	1 (2.1%)	
Lymphocyte Number	**Low**	3 (7.7%)	8 (16.7%)	**0.45**
	Normal	30 (76.9%)	33 (68.8%)	
	High	6 (15.4%)	7 (14.6%)	
NLR	**Normal**	27 (73%)	31 (70.5%)	**0.8**
	High (>3)	10 (27%)	13 (29.5%)	
BMA PC	**(≥ 34.5)**	24 (61.5%)	25 (52.1%)	**0.37**
	(< 34.5)	15 (38.5%)	23(47.9%)	
MCV	**Below the mean**	19 (48.7%)	19 (39.6%)	**0.39**
	Above the	20 (51.3%)	29 (60.4%)	

	mean			
MCH	**Below the mean** **Above the mean**	20 (51.3%) 19 (48.7 %)	17 (35.4 %) 31 (64.6%)	**0.14**
RDW	**Below the mean** **Above the mean**	12 (54.5%) 10 (45.5%)	12 (50%) 12(50%)	**0.76**

***fisher exact test**

Table (3): CD 81 expression in correlation with different clinico-pathological features

Features	**Value**	**CD81 Negative and weak Positive**	**CD81 Positive**	**P**
Bone lytic lesions	**Absent** **Present**	8 (22.2%) 28 (77.8%)	6 (33.3%) 12 (66.7%)	**0.38**
Pathological fracture	**Absent** **Present**	20 (55.6%) 16 (44.4%)	11 (61.1%) 7 (38.9%)	**0.69**
Plasmacytoma	**Absent** **Present**	22 (61.1%) 14 (38.9%)	10 (55.6%) 8 (44.4%)	**0.69**
Anemia	**Absent** **Present**	12 (33.3%) 24 (66.7%)	8 (44.4%) 10 (55.6%)	**0.42**
High Creatinine	**Absent** **Present**	16 (44.4%) 20 (55.6%)	7 (38.9%) 11 (61.1%)	**0.69**
Hypercalcemia	**Absent** **Present**	24 (80%) 6 (20%)	11 (68.8%) 5 (31.2%)	**0.39**
Positive BJP	**Absent** **Present**	5 (22.7%) 17 (77.3%)	2 (20%) 8 (80%)	**1.0***

M-band	**Absent**	2 (5.7%)	2 (11.8%)	**0.59***
	Present	33 (94.3%)	15 (88.2%)	
Elevated sFLC	**Absent**	1 (2.9%)	4 (22.2%)	**0.043**
	Present	33 (97.1%)	14 (77.8%)	
Hypoalbuminemia	**Absent**	10 (35.7%)	6 (46.2%)	**0.52**
	Present	18 (64.3%)	7 (53.8%)	
Elevated LDH	**Absent**	12 (46.2%)	6 (50%)	**0.82**
	Present	14 (53.8%)	6 (50%)	
Leucopenia	**Absent**	35 (97.2%)	17 (94.4%)	**1.0***
	Present	1 (2.8%)	1 (5.6%)	
Leucocytosis	**Absent**	32 (88.9%)	13 (72.2%)	**0.12**
	Present	4 (11.1%)	5 (27.8%)	
Thrombocytopenia	**Absent**	34 (94.4%)	18 (100%)	**0.55**
	Present	2 (5.6%)	0	
Thrombocytosis	**Absent**	25 (69.4%)	11 (61.1%)	**0.54**
	Present	11 (30.6%)	7 (38.9%)	
Neutrophil Number	**Low**	4 (11.1%)	2 (11.1%)	**0.73**
	Normal	27 (75%)	12 (66.7%)	
	High	5 (13.9%)	4 (22.2%)	
Lymphocyte Number	**Low**	3 (8.3%)	0	**0.54**
	Normal	33 (91.7%)	18 (100%)	
NLR	**Normal**	26 (72.2%)	12 (80%)	**0.56**
	High (>3)	10 (27.8%)	3 (20%)	
BMA PC	**(≥ 34.5)**	18 (50%)	11 (61.1%)	**0.44**
	(< 34.5)	18 (50%)	7 (38.9%)	
MCV	**Below the mean**	14 (38.9%)	8 (44.4%)	**0.69**
	Above the mean	22 (61.1%)	10 (55.6%)	
MCH	**Below the mean**	11 (30.6%)	9 (50%)	**0.16**
	Above the mean	25 (69.4%)	9 (50%)	

RDW	**Below the mean**	6 (35.3%)	1 (25%)	**1.0***
	Above the mean	11 (64.7%)	3 (75%)	

***fisher exact test**

Table (4): Response assessment among whole 48 patients evaluated after induction with 4 cycles VCD and percent of relapse

Parameter	Response	Number of patients (percentage)
Response	CR	26 (54.2%)
	VGPR	3 (6.2%)
	PR	11 (22.9%)
	SD and PD	8 (16.7%)
	Total	**48 (100%)**
Relapse		**14 (29.2%)**

Table (5): CD 27 and CD 81 expression in correlation relapse

CD 81	No relapse	Relapse	P
Negative	13 (40.6%)	3 (33.3%)	0.76
Positive	10 (31.2%)	4 (44.5%)	
Weak positive	9 (28.2%)	2 (22.2%)	
Positive	10 (31.2%)	4 (44.4%)	0.46
Negative and weak positive	22 (68.8%)	5 (55.6%)	
CD 27	No relapse	Relapse	P
Negative	14 (31.8%)	4 (25%)	0.42
Positive	25 (56.8%)	8 (50%)	
Weak positive	5 (11.4%)	4 (25%)	
Positive	25 (56.8%)	8 (50%)	0.64

Negative and weak positive	19 (43.2%)	8 (50%)	

Table (6): CD 27 and CD81 expression in correlation with the response (evaluation after 4 VCD cycles) – CR and VGPR vs. PR, SD, and PD

CD 27	**CR and VGPR**	**PR, SD, and PD**	**P**
Negative	10 (35.7%)	6 (31.6%)	**0.77**
Positive and Weak positive	18 (64.3%)	13 (68.4%)	
Negative and weak positive	14 (50%)	8 (42.1%)	**0.59**
Positive	14 (50%)	11 (57.9%)	
CD 81	**CR and VGPR**	**PR, SD, and PD**	**P**
Negative	4 (21.1%)	4 (33.3%)	**0.67***
Positive and Weak positive	15 (78.9%)	8 (66.7%)	
Negative and weak positive	11 (57.9%)	8 (66.7%)	**0.62**
Positive	8 (42.1%)	4 (33.3%)	

*fisher exact test

Table (7): CD 27 and CD 81 expression in correlation with the response (evaluation after 4 VCD cycles) – CR, VGPR, and PR vs. SD and PD

CD 27	CR, VGPR, and PR	SD and PD	P
Negative	11 (28.2%)	5 (62.5%)	**0.06**
Positive and Weak positive	28 (71.8%)	3 (37.5%)	
Negative and weak positive	16 (41%)	6 (75%)	**0.123***
Positive	23 (59%)	2 (25%)	
CD 81	**CR, VGPR, and PR**	**SD and PD**	**P**
Negative	8 (27.6%)	0	**1.0***
Positive Weak positive	21 (72.4%)	2 (100%)	
Negative and weak positive	18 (62.1%)	1 (50%)	**1.0***
Positive	11 (37.9%)	1 (50%)	

*fisher exact test

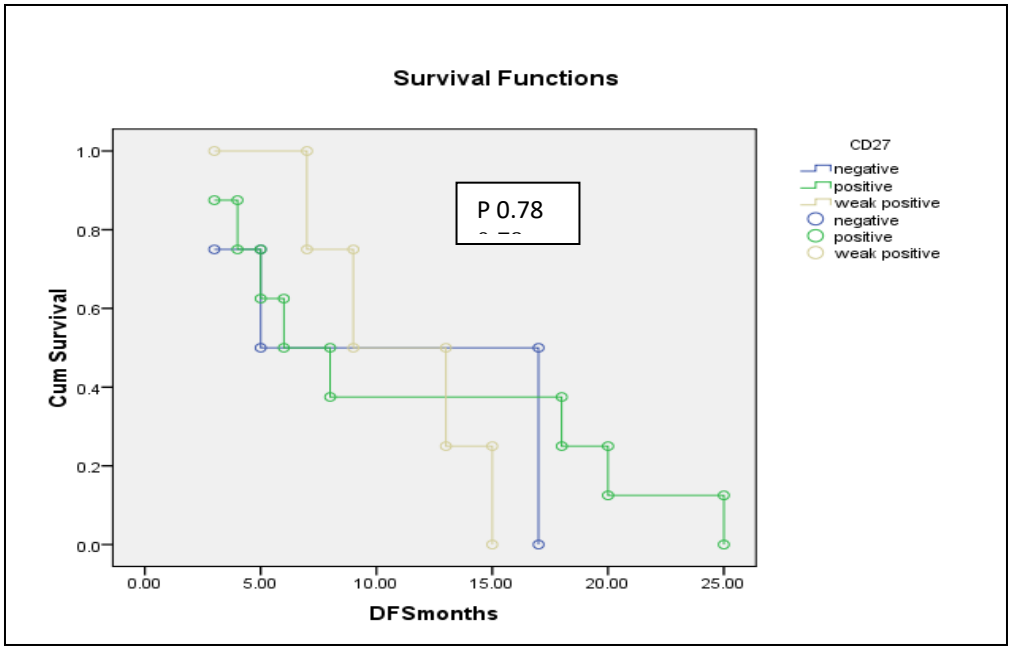

Figure (1): DFS in correlation with CD27 expression (negative vs. positive vs. weak positive)

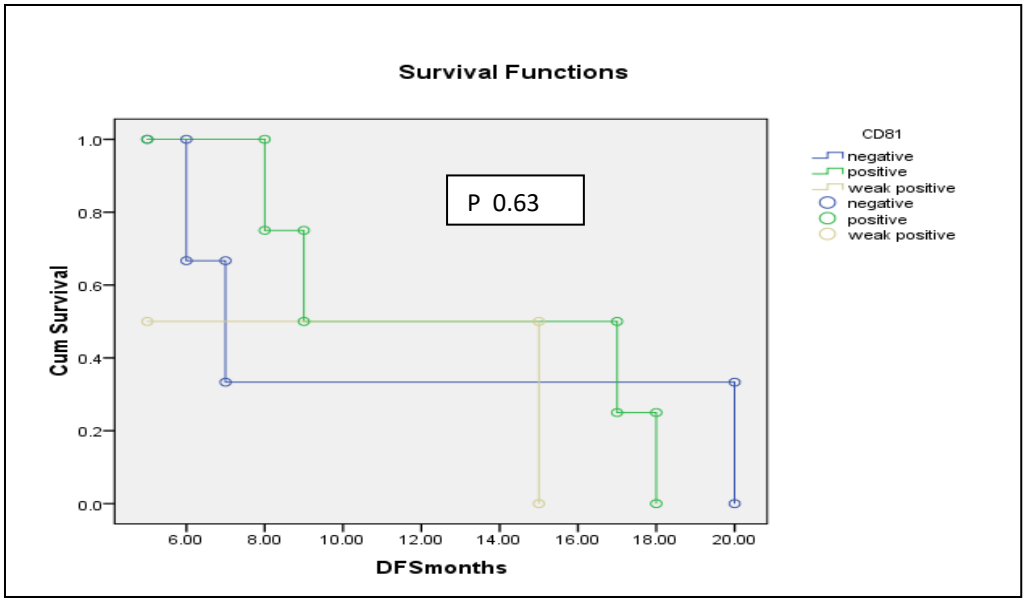

Figure (2): DFS in correlation with CD81 expression (negative vs. positive vs. weak positive)

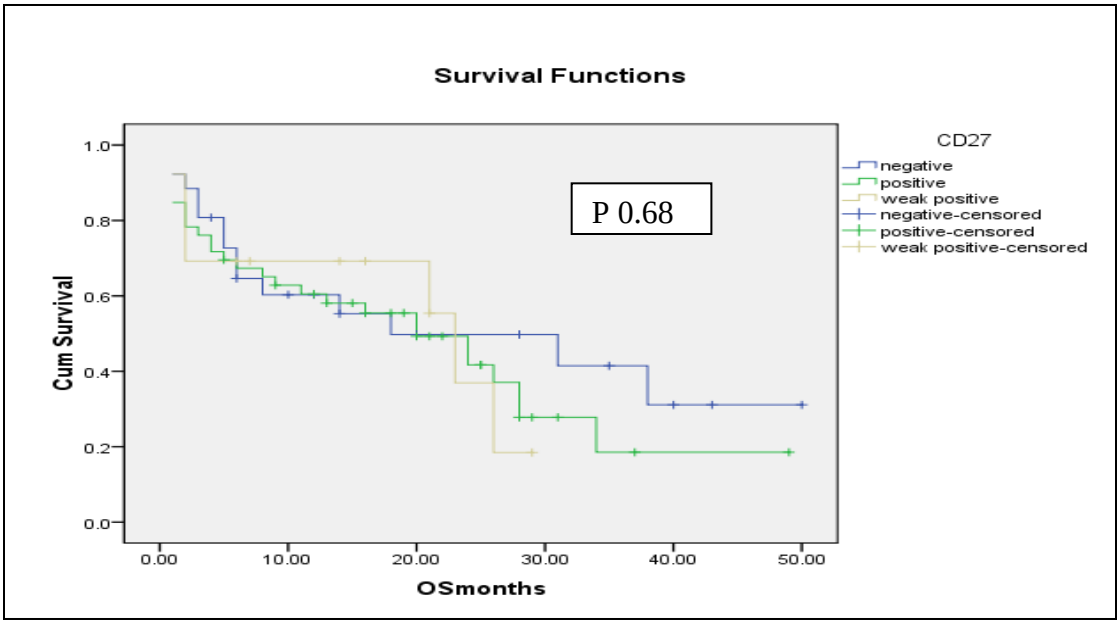

Figure (3): OS in correlation with CD27 expression (negative vs. positive vs. weak positive)

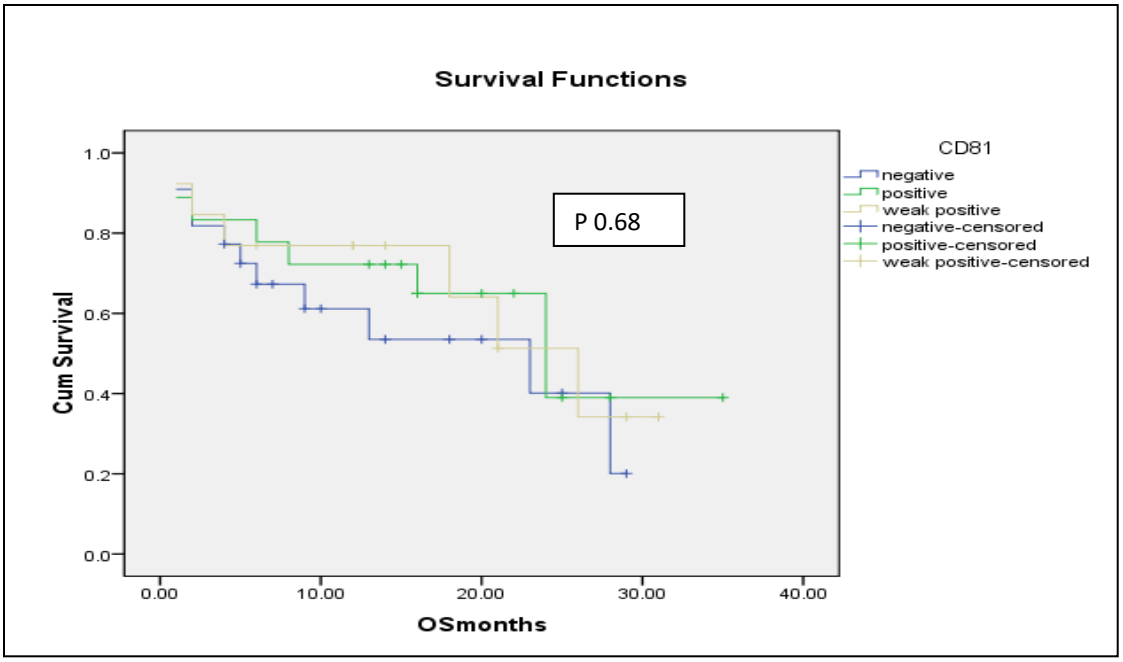

Figure (4): OS in correlation with CD81 expression (negative vs. positive vs. weak positive)

4. Discussion

Multiple Myeloma is a malignant PC neoplasm identified by clonal proliferation in the BM and increased level of monoclonal protein in the blood and/or urine, leading to end-organ damage. In assessing our patients regarding clinico-pathological features, CD27 expression was assessed in 87 cases, with 48 (55.2 %) being positive, 13 (14.9 %) weak positive, and 26 (29.9 %) negative. The negative and weak positive CD27 group displayed significantly higher LDH levels compared to CD27-positive cases (76% vs. 45.9 %, $P = 0.019$), a marker linked to higher tumor burden and more aggressive disease, indicating a possible link between CD27 negativity and aggressive disease biology. On the other hand, other clinico-pathological features such as anemia ($P = 0.1$), hypercalcemia ($P = 0.45$), hypoalbuminemia ($P = 0.76$), and high creatinine level ($P = 0.12$) did not show significant associations.

These results are partially concordant with Wang et al. (2017) [7] and Wang et al. (2024) [10] where factors such as gender, age, Hb level ($P= 0.145$), Ca level ($P= 0.787$), albumin ($P= 0.182$), creatinine ($P= 0.583$), and LDH ($P= 0.240$), too, didn't display significant changes between the two groups ($P > 0.05$).

A study conducted in 2025 is partially concordant with our study. In Si et al. [11] study, parameters such as hypercalcemia ($P= 0.735$), elevated LDH ($P= 0.826$), high creatinine level ($P= 0.290$) and hypoalbuminemia ($P= 0.884$) didn't differ significantly between CD27 positive and CD27 negative groups. The only significant value was lower levels of Hb ($P = 0.010$) in CD27 negative cases.

The PC proportion in our study was higher in CD27 negative and weak positive cases (61.5 %) compared to CD27 positive cases (52.1 %), although not statistically significant ($P = 0.37$). These findings resonate with Chu et al. [12], which found CD27 (-) MM cases to harbor more advanced disease stages and higher tumor burden (37 % vs. 22.5 %, $P <0.05$). These results are concordant with Si et al. [11] study, where CD27 negative patients were significantly associated with more severe BMPC infiltration ($P < 0.0001$). Both of these results are, on the other hand, dis-concordant with Wang et al. [7], where CD27 (+) group exhibited significantly elevated BMPC proportion (60.00 % vs. 27.27 %, $P = 0.009$) in comparison to CD 27(-) group.

CD81 was studied in 54 cases, with 18 (33.3 %) being positive, 13 (24.1 %) weak positive, and 23 (42.6 %) negative. The negative and weak positive CD81 group displayed elevated sFLC levels compared to CD81-positive cases (97.1 % vs. 77.8 %, *P=0.043*), which reflects disease activity. On the other hand, CD81 expression displayed insignificant correlation with most clinico-pathological variables including anemia (*P = 0.42*), hypercalcemia (*P = 0.39*), hypoalbuminemia (*P = 0.52*), or high creatinine level (*P = 0.69*).

These results are generally concordant with Zhao et al.[9] where no association was found between CD81 expression and sex (*P=0.4*), age (*P=0.530*), hemoglobin (*P=0.123*), subtype (*P=0.161*), stage (*P=0.191*), creatinine (*P=0.341*), Ca level (*P= 0.152*), LDH concentration (*P=0.584*), serum-FLC ratio (*P=0.149*), and extramedullary infiltration (*P=0.696*).

Among 47 cases evaluated after 4 VCD cycles, the ORR (ORR: CR, VGPR, PR) in CD27 (-) cases was 28.2 %, compared to 71.8 % in CD27 (+) and weak (+) cases (*P= 0.06*). Although numerically significant, these results displayed no statistical significance. These results are concordant with Chu et al. [12] where after 4 cycles of chemotherapy, the ORR in CD27 (−) group was lower than CD27 (+) group (56.67 % vs. 73.02 %, *P < 0.05*). On the other hand, results of another study [7] are dis-concordant were the ORR in CD27 (-) group was superior at 81.82 % compared to 55.00 % in the CD27 (+) group (*P= 0.026*).

In our study, the CD27 (-) and weak (+) group exhibited a higher rate of ineffective responses (SD/PD) at 75 %, compared to 25 % in CD27 (+) cases (*P= 0.123*). These results are disconcordant with Wang et al. [7] were the CD27 (+) group registered a greater ineffective rate (combining SD and PD) than the CD27 (-) group, with percentages standing at 45.00% and 18.18% respectively (*P= 0.026*).

Interestingly, in our study, when evaluating deep responses (CR/VGPR), it was observed in 14 cases for CD27 (-) and weak (+) group (n=22) and 14 cases for CD27 (+) group (n=25), but again this did not reach statistical significance (P = 0.59). On the other hand, in the study by Wang et al. [7], deep remission (encompassing sCR, CR, and VGPR) was noticed in twelve cases for the CD27 (-) group (n=22) and 14 for the CD27 (+) group (n= 60), marking a significant divergence between them (54.55 % vs. 23.33 %, *P = 0.007*). In Bao et al. [13], CR rate in the CD27 (-) group was lower than those in the CD27 (+) group (7.81% vs. 16.67%, *P <0.05*).

In short, CD27 (+) cases exhibited a notably higher ORR (71.8 %) following four cycles of VCD therapy compared to CD27 (-) patients (28.2%). However, this did not translate into statistically significant differences in deep remission (CR or VGPR) or ineffective responses (SD or PD), suggesting that CD27 status might be more reflective of treatment responsiveness rather than depth of response.

There was no statistically significant correlation between CD81 expression and treatment response after 4 VCD cycles. The CR/VGPR rates were 42.1 % in CD81 (+) vs. 57.9 %in CD81 (-) and weak (+) (*P = 0.62*), and broader response category (CR/VGPR/PR vs. SD/PD) comparisons even failed to yield significant differences (*P = 1.0*).

Our results are concordant with Paiva et al. [8] where the treatment arm didn't influence cases' outcome according to CD81 expression, where CR rates were 18 % in CD 81 (+) vs. 29 % in CD 81 (-) with (*P= 0.06*) showing no statistical significance. Although marginally associated with elevated sFLC levels (*P = 0.043*), CD81 failed to demonstrate prognostic value in multivariate clinical contexts.

In survival analysis, prior reports suggested a survival disadvantage in CD27 (-) phenotypes as in the study by Chu et al.[12] where in median FU of 18 mo., PFS was significantly shorter in CD27(−) group than in CD27 (+) group (22 vs. 40 mo., *p <0 .05*), so was OS (median OS not reached, *p < .05*), and also in the study by Bao et al. [13] with a median follow-up of 17 months, PFS and OS were both shorter in CD27 (-) group than in CD27(+) group (15 months vs. 27 months and 31 months versus not reached, respectively; *P < 0.05*). Our cohort, however, displayed no statistically significant difference in DFS (DFS, *P = 0.78*) or OS (OS, *P = 0.68*) based on CD27 status. A plausible explanation may lie in population heterogeneity or therapeutic variation. On the other hand, in the study by Wang et al. [7], the median PFS and OS of the CD27 (+) group were shorter than those of the CD27 (-) group (13 vs. 24 months, *P = 0.02*, and both OS not reached, *P = 0.002*)

Like CD27, CD81 expression did not influence survival metrics significantly. Among the 54 cases analyzed, neither DFS (*P = 0.63*) nor OS (*P = 0.68*) varied significantly by CD81 expression status. These results are dis-concordant with Paiva et al. [8] where CD81(+) expression displayed a significant shorter PFS compared to CD81(-) cases (3-year rates of 26 % vs. 52 %, *P=0.001*). OS between (+) vs. (-) CD81 cases was (3-year rates of 63 % vs. 78 %, *P= 0.007*).

In Zhao et al. study [9], the median FU period was 44.46 months. In cases with CD81(+), the median PFS was 34.48 mo. and the median OS was 68.06 months, compared to 43.71 months ($P = 0.200$) and 100.61 months ($P = 0.039$) for cases with CD81(-). Both of these outcomes are further concordant with Chen et al. [14] where CD81 (+) cases had shorter PFS ($P = 0.001$) and OS ($P = 0.002$).

These findings contribute to an emerging body of literature questioning the standalone prognostic value of immunophenotypic markers in MM. Our results encourage a multimodal approach integrating flow cytometry with cytogenetic, molecular, and minimal residual disease (MRD) profiling to enhance risk prediction and therapeutic personalization.

5. Conclusion

In this retrospective study of 90 cases with NDMM treated at the OCMU, we evaluated the expression patterns of CD27 and CD81 using MFC and correlated them with clinicopathological features, response to induction therapy, and survival outcomes.

CD27 was expressed in 55.2% of cases and CD81 in 33.3%. CD27 (-) cases demonstrated significantly higher LDH levels (P = 0.019) and exhibited less ORRs to VCD induction (28.2% vs. 71.8%). However, neither CD27 nor CD81 expression levels displayed significant associations with deep response, relapse incidence, or OS metrics.

Survival analysis displayed insignificant differences in DFS or OS based on expression of either marker. These results suggest that while CD27 and CD81 provide valuable immunophenotypic characterization, their role as independent prognostic tools remains limited.

Our findings highlight the complexity of MM biology and support the integration of surface marker profiling with cytogenetic and molecular risk stratification to guide treatment planning and prognostication and suggest that neither marker alone is sufficient for risk stratification or treatment guidance in NDMM. Nevertheless, the trends observed—particularly the link between CD27 loss and aggressive disease markers—merit further investigation. The relatively small sample size of our study is also considered as a limitation. And so, it is recommended that future multicenter prospective studies with larger cohorts, standardized treatment protocols, and integration of genetic and MRD analyses are necessary

to clarify the utility of CD27 and CD81 in clinical practice and potentially establish their value within composite prognostic models.

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