The Frequency and clinical Implications of Lymphocyte Subsets and Circulating Plasma Cells in Newly Diagnosed Multiple Myeloma Patients

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Multiple myeloma (MM) is characterized by clonal proliferation of plasma cells (PCs) in the bone marrow (BM) leading to end organ damage. Recent interests are increasingly focusing on the quantitative and functional profiles of T-cell subsets, natural killer cells (NK) and natural killer T-cells (NK T), due to their importance in the development of MM. Herein we tried to evaluate the frequency of different lymphocyte subsets and cPCs in newly diagnosed MM patients and their impact on survival. This prospective case–control study included 40 newly diagnosed MM patients presented to South Egypt Cancer Institute (SECI), Assiut University and 20 apparently healthy controls. Flow cytometry was used for evaluation of CD4+ T helper cells, CD8+ T cytotoxic cells, natural NK cells, NK T cells and cPCs. CD4+ T helper cells were significantly decreased in MM patients while, cytotoxic CD8+ T cells were significantly increased in comparison to the controls leading to a significant decrease in the CD4+/CD8+ ratio in MM patients. In addition, MM patients had deficiency in NK cells and NK-T cells. The median number of cPCs was 8 (range: 0 - 477) per 50,000 cells in the MM patients. The median OS for those with <8 cPCs was 22.5 months compared with 18 months for patients with ≥8 cPCs. In conclusion, alterations in the immune cells homeostasis in MM patients could play a role in the development of MM and may be associated with the release of plasma cells in the peripheral blood. Also, the quantitative estimation of cPCs in patients with newly diagnosed MM may be used as a predictor of survival.

Multiple myeloma (MM) is characterized by clonal proliferation of plasma cells (PCs) in the bone marrow (BM) leading to organ damage such as anemia, bone destruction, hypercalcemia or renal insufficiency [1]. Genetic and immune-related factors were considered to have roles in the pathogenesis of MM [2]. B-cell precursors and normal plasma cells are compromised, and immune paresis is a consistent finding in newly-diagnosed MM patients. Beyond the decrease in humoral immunity, there are also significant changes in other components of the immune system in MM [3]. Phenotypic alterations in T-cell subsets, manifested as an increased number of activated T cells, expressing the HLA-DR, have been also described. T cells seem unable to mediate normal cytotoxic activities. Cytotoxic $CD8^+$ T and natural killer (NK) cells may expand in both the bone marrow and peripheral blood, but they are unable to control disease progression, suggesting a marked immunosuppressive microenvironment [4-8].

Natural killer T (NKT) cells are capable of rapidly releasing cytokines that affect a wide range of innate and adaptive immune responses against cancer [9-11]. Defects in the NKT cell pool were associated with autoimmune diseases, [9, 12, 13], allergies and many forms of cancer [14-17]. There is an emerging focus on NKT cells in the context of MM, where immune dysfunction has been identified as a potentially important factor in disease predisposition and progression. A common finding for NK-cell cytotoxicity in humans is the lack of human leukocyte antigen (HLA) expression on the target cell [18]. Interestingly, in contrast to many tumor types, MM cells often do not show loss of HLA class I, suggesting a mechanism by which they may evade targeting by NK-cells [19]. NK-cells from MM patients are often dysfunctional compared with NK-cells from healthy individuals [20]. An initial look at studies of NK-cell counts in the peripheral blood of MM patients shows discordant findings [21-24].

Circulating plasma cells (cPCs) also can be detected in the peripheral blood of a significant proportion of patients with MM [25, 26]. Although the appearance of cPCs in the blood may be simply a reflection of a tumor mass, it could also represent differences in the disease biology [27]. The appearance of cPCs in the blood may signify more aggressive disease. The presence of circulating PCs in peripheral blood has been shown to be a biomarker to detect patients at high risk of progression to MM in patients with smoldering myeloma [26]. High number of cPCs predict early relapse of the MM after stem cell transplantation.[28] How the levels of lymphocyte subsets in the peripheral blood (PB) and circulating plasma cell levels may affect the outcome in newly diagnosed MM has not been clearly defined.

The aim of this study was to evaluate the frequency of cPCs, and different lymphocyte subsets in patients with newly diagnosed MM and evaluate their impact on overall survival of our patients.

Materials and Methods

This study was a prospective case–control study, included 40 newly diagnosed patients with multiple myeloma (MM) presented to South Egypt cancer institute (SECI), Assiut University from January 2015 to September 2017. Also, 20 age and sex matched healthy controls were included in this study. The study was reviewed and approved by the Institutional Review Board of the SECI, Assuit University (approval No 371). An informed written consent was taken from all cases and controls. All patients and controls were subjected to thorough history taking and physical examination.

Complete blood count (CPC) was done using an automated hematology analyzer, Ruby Cell Dyn (American, Serial number: 36026BG). Serum urea, creatinine, total protein, albumin and LDH were performed using Cobas Integra 400 Plus Chemistry Analyzer (Roche Diagnostics GmbH, Mannheim, Germany, S.N.: 500558). Serum protein electrophoresis and immunofixation was performed using a fully automated electrophoresis instrument, Pretty Interlab (Italy, serial number 38405301). Assessment of T, NK, and NKT lymphocytes and cPCs was done using FACSCalibur flow cytometry (BD Biosciences, USA). Skeletal survey and bone marrow aspirate and biopsy were also performed for the patients.

Sample (preparation and storage)

5 ml of peripheral blood were collected via a direct venous puncture with complete aseptic precautions. Two ml were put in a vacutainer tube containing K3 EDTA for CBC and flow cytometric analysis, and 3 ml were placed in a gel vacutainer tube without any anticoagulant for all other chemical tests including serum protein electrophoresis and immunofixation. They were incubated at 37°C for 20 min then centrifuged at 2000g for 10 min for separation of serum. The separated serum was divided into 2 aliquots; one used immediately for chemical tests and the other stored at -20°C until used in protein electrophoresis and immuunofixation.

Serum protein electrophoresis and immunofixation

Serum protein electrophoresis and immunofixation was performed using a fully automated electrophoresis instrument, Pretty Interlab (Italy, serial number 38405301) according to the manufacturer' instructions. After thawing the stored serum, 30 µl of each sample was dispensed in the wells on a disposable sample plate. A control serum was included in each gel.

The Elfolab software program was used to start the process, the instrument automatically performs all the steps of the analytical procedure including application of the samples on the agarose gel plate, electrophoretic migration, gel denaturation, gel staining using Acid Blue stain solution and de-staining using citric acid in aqueous solution, and finally gel drying and densitometric reading. Scanning the gel was done by the scan system. Immunofixation was done for cases that have monoclonal gammopathy, using Pretty Interlab to identify the type of monoclonal band.

Flow cytometric detection of T, NK, and NKT lymphocytes

For detection of T, NK and NKT lymphocytes, 50 µl of blood sample was stained with 5 µl of allophycocyanin (APC)--conjugated CD4, peridinium-chlorophyll-protein (Per-CP)-conjugated CD8 (both from Becton Dickinson (BD) Biosciences, San Jose, CA, USA), phycoerythrin (PE)-conjugated CD16/56 and FITC-conjugated CD3 (Exbio Praha, a.s, Central Bohemian Region, Czech Republic). After incubation for 15 minutes at room temperature, in the dark, red blood cells lysis was done. After one wash, the cells were resuspended in phosphate buffer saline (PBS). Flow cytometric analysis was done by FACSCalibur flow cytometry with Cell Quest software (BD Biosciences, USA). Anti-human IgG was used as an isotype-matched negative control for each sample. Forward and side scatter histogram was used to define the lymphocyte population. Then, the percentages of CD3+ (T-lymphocytes), CD4+ (T-helper cells), CD8+ (T-cytotoxic), CD3-CD16+/56+ (NK) and CD3+CD16+/56+ (NK-T) were assessed within the lymphocytes population as shown in figure 1 (A,B,C). Both relative and absolute count of each lymphocyte subsets were reported.

Flow cytometric detection of circulating plasma cells

The mononuclear cells were isolated by density gradient centrifugation on Ficoll (Biochrom GmbH, Germany) for 15 minutes at 300g and stained with FITC-conjugated fluorescence-labeled CD138, PE-conjugated CD38, Per-CP-conjugated CD19 and APC-conjugated CD45 (all from BD Biosciences, San Jose, CA). After incubation for 15 minutes at room temperature in the dark and one wash with PBS, the cells were resuspended in PBS. Flow cytometric analysis was done by FACSCalibur flow cytometry with Cell Quest software. Fifty thousand events were analyzed for each patient, and the number of plasma cells detected among these 50,000 events was recorded. The number of cPCs was measured by gating on $CD45^-$ $CD19^-$ cells. Then the expression of CD38 and CD138 on the $CD45^-$ $CD19^-$ cells was detected as shown in figure 2 (A, B, C). The cPCs were identified as the cells that are $CD138^+CD38^+CD45^-CD19^-$.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism version 7.0 b software (GraphPad Software Inc., San Diego, CA, USA). Qualitative data is expressed as frequency and percentage, while quantitative data is expressed by mean± standard error (SEM). Kruskal –Wallis and Mann-Whitney analysis was used to detect the statistical significance differences between groups. Spearman's correlation was used to correlate the studied parameters. *P* value < 0.05 was considered significant. The Kaplan-Meier method for generation of actuarial survival curves was used to assess overall survival (OS).

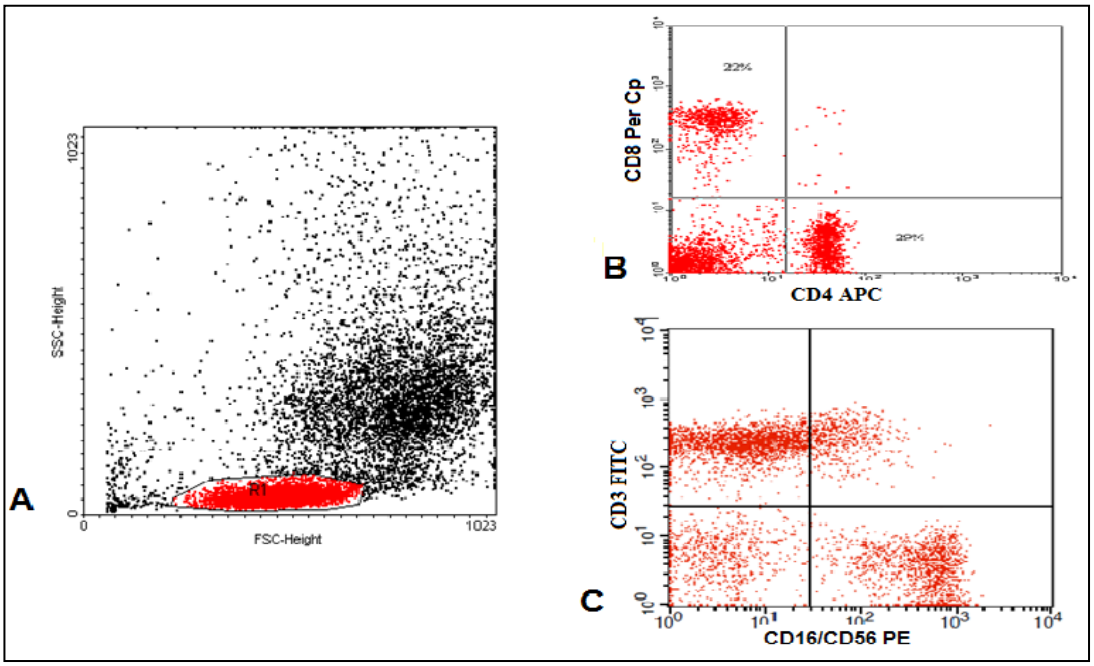

Figure 1. Flow cytometric analysis of lymphocyte subsets. **A:** Forward and side scatter histogram was used to define the lymphocytes population (R1). **B, C:** The expression of CD16/56, CD3, CD4 and CD8 were assessed in the lymphocytes population to detect lymphocytes subsets.

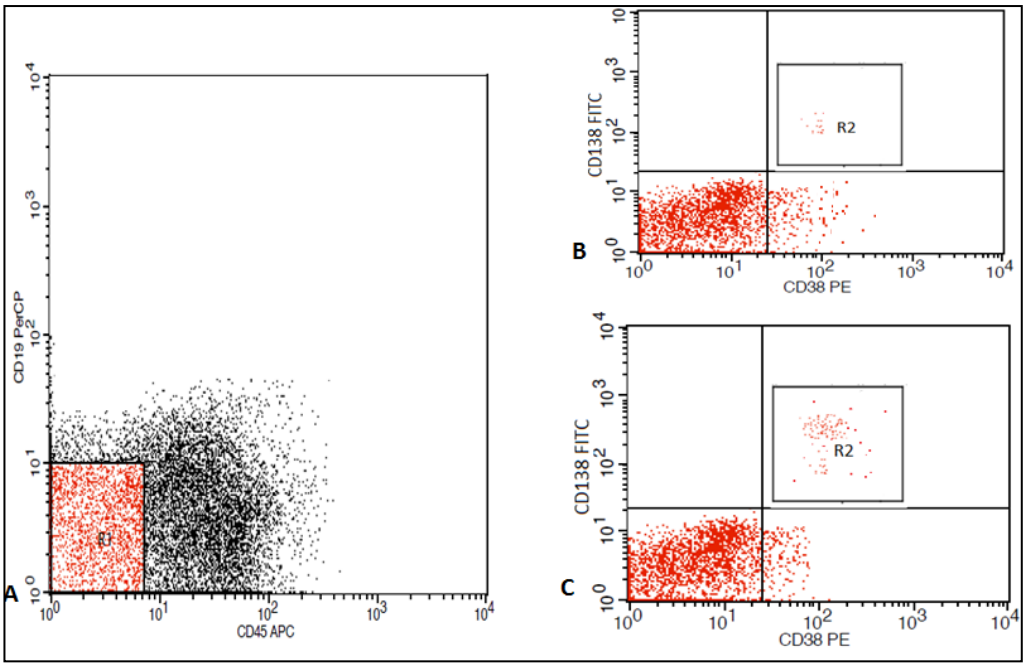

Figure 2. Flow cytometric detection of circulating plasma cells. A: The number of circulating plasma cells (cPCs) was measured by gating on CD45- CD19- cells (R1). Then the expression of CD38 and CD138 on the $CD45^-$ $CD19^-$ cells was detected. The cPCs were the cells that are $CD138^+CD38^+CD45^-CD19^-$ cells (R2). B, C: Represent two patients with a few and many circulating myeloma cells respectively.

Results

Demographic data

The demographic and clinical characteristics of the studied groups were summarized in Table 1. Two groups were enrolled in this study; the first group included a total of 40 newly diagnosed MM patients with median age 61 years with 55% (n= 22) male and 45% (n= 18) female while the second group included 20 controls with 60% (n= 12) male and 40% (n=8) female.

Table 1. General and clinical characterization of multiple myeloma patients and the control group.

	MM patients (n=40)	Control group (n=20)	*P*-value
Age (year)	61 (38-71)	58 (36-69)	NS
Sex (male/ female)	22/1n8	12/8	NS
Hemoglobin (gm/dl)	9.81±1.98	11.7±2.17	0.045
Total protein (gm/dl)	8.92±1.95	7.3±1.4	0.034
Albumin(gm/dl)	3.12±0.81	4.2±0.68	0.003
β2 microglobulin mg/L	5.09±1.51	2.76 ±0.57	0.001
Calcium (mg/dl)	11.23±2.68	8.1±0.9	NS
Creatinine (mg/dl)	1.33±0.34	0.9 ±0.15	0.001
LDH (U/L)	750.86±67.32	261±52	0.032
M protein (gm/dl)	4.64±0.21	na	n
BM plasma cells %	30.2±2.9	na	na
Circulating Plasma cells /50000 WBCs	Mean=63.18±16.49 Median = 8 (0-277)	na	na
ISS score (%)			
1	6 (15%)	na	na
2	7 (17.5%)		
3	27 (65%)		
DS stage number (%)			
I	8 (20%)	na	Na
II	10 (25%)		
III	22 (55%)		

Mann Whitney U Test, Data represented as means ± SEM, Number and (%); *P*≥0.05 is not significant (NS);
LDH: lactate dehydrogenase; ISS: International Staging System; DS: Durie Salmon; No number; %: percentage; na: not applied.

According to Durie Salmon (DS) staging system [29], MM patients have been classified into 22 patients (55%), had stage III disease, 10 patients (25%) had stage II and 8 patients (20%) had stage I disease. Whereas, according to the International Scoring System (ISS)[30], there were 27 (67.5%) had stage 3 , 7 had stage 2 (17.5) and 6 (15%) had stage 1. Radiotherapy was administered for 9 patients (60%) for osteolytic bony lesions.

There was a significant decrease in hemoglobin and serum albumin levels in MM patients compared to the control group (P= 0.045), (P= 0.003), respectively. While, there was a significant increase in β2 microglobulin, creatinine and lactate dehydrogenase (LDH) in MM patients compared to the control group (Table 1).

Distribution of $CD3^+$, $CD4^+$ and $CD8^+$ T-lymphocytes

We found a no difference in the number of total lymphocytes and T lymphocytes between MM patients and the control group. However, the frequency of $CD8^+$ T lymphocytes was significantly increased in MM patients. In contrast, the frequency $CD4^+$ T lymphocytes were significantly decreased in MM patients compared to controls. The $CD4^+$/ $CD8^+$ cells ratio was significantly decreased in MM patients as shown in Table 2.

There was a significant difference in T lymphocyte subsets among different patient's groups according to the Durie-Salmon staging as shown in Figure 3 (a, b, c).

Table 2. Comparison between lymphocytes subsets in multiple myeloma patients and the controls.

	MM patients (n=40)	Control healthy(n=20)	*P*-value
Total lymphocytes (10^9/L)	3.52±0.153	4.35±0.168	NS
T Lymphocytes ($CD3^+$) (%)	66.61±2.21	65.67±2.19	NS
T Lymphocytes ($CD3^+$) (10^9/L) count	1.84±0.48	2.05±0.62	NS
$CD4^+$ %	39.29±1.7	45.13±0.82	0.017
$CD4^+$(10^9/L)	1.15±0.34	1.35±0.33	0.01
$CD8^+$ %	28.53±1.16	24.71±0.91	0.026
$CD8^+$(10^9/L)	0.86±0.32	0.75±0.17	0.035
$CD4^+$/$CD8^+$ ratio	1.46 ±0.12	1.76±0.22	0.045
Natural killer cells (NK)%	9.21±1.83	11.98±1.92	0.001
Natural killer cells (NK) (10^9/L)	0.19±0.08	0.37±0.11	0.0001
Natural killer T cells (NK-T)%	4.79± 1.1	7.08±1.8	0.001
Natural killer T cells (NK-T) (10^9/L)	0.13±0.05	0.21±0.08	0.0001

Mann-Whitney U Test; Data represented as means ± SEM. P≥0.05 is not significant (NS).

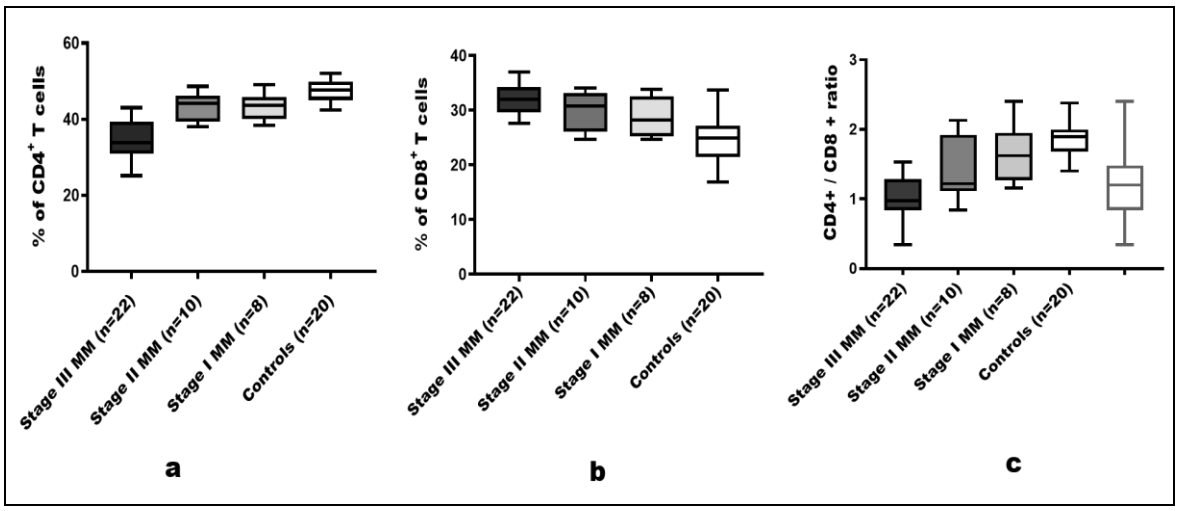

Figure 3. Difference in the percentage of different T-cells subsets among different patient's groups characterized by Durie Salmon staging including; MM with stage III (n=22), MM with stage II (n=10) , MM patients with stage I (n=8) and the control group (n=20) . There was a highly significant difference in the percentage of (a) $CD4^+$T-helper cells (*P*= 0.0001), (b) $CD8^+$T-cytotoxic cells (*P*= 0.0001), (c) $CD4^+$/ $CD8^+$ ratio (*P*=0.0001) between different patient's groups. (Kruskal –Wallis test, *P*-value when < 0.05 is significant; CD: cluster of differentiation; %: percentage; NK: Natural Killer, NKT:Natual Killer T cell).

Distribution of NK cells and NK-T cells in peripheral blood of multiple myeloma patients and healthy controls

NK cells were significantly decreased in MM patients in comparison to the control group. There was a significant difference in the percentage of NK cells between different patient's groups characterized by Durie Salmone stage as shown in Figure 4(a). Also, the percentage of NK-T cells were significantly decreased in MM patients in comparison to the control group. Also, there was a significant difference in the percentage of NK-T cells between different patient's groups characterized by Durie Salmone staging as shown in Figure 4 (b).

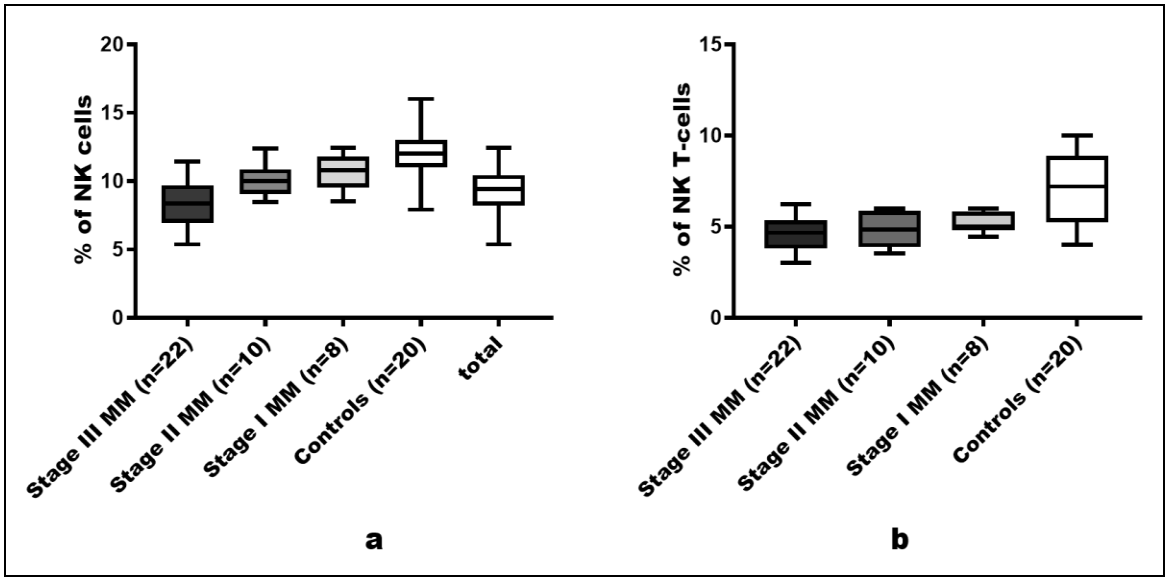

Figure 4. Comparison of the percentage of NK, and NK-T cells among different patient's groups characterized by Durie Salmone staging including; MM with stage III (n=22), MM with stage II (n=10) , MM patients with stage I (n=8) and the control group (n=20). There was a highly significant difference in the percentage of (a) NK cells (P= 0.0001) and (b) NK-T cells (*P*= 0.0003) between different patient's groups characterized by Durie Salmon staging. (Kruskal –Wallis test, *P*-value when < 0.05 is significant.)

Circulating (PCs)

Circulating plasma cells were detected in 32 patients (80%). The median number of cPCs was 8 (range, 0 - 277) per 50,000 events. In 8 patients (20%), no cPCs was seen; 12 patients (30%) had 1 to 5 circulating PCs, 9 patients (22.5%) had 6 to 12 cPCs and the 11 remaining patients (27.5%) had more than 12 cPCs as shown in Table 1 and Figure 5.

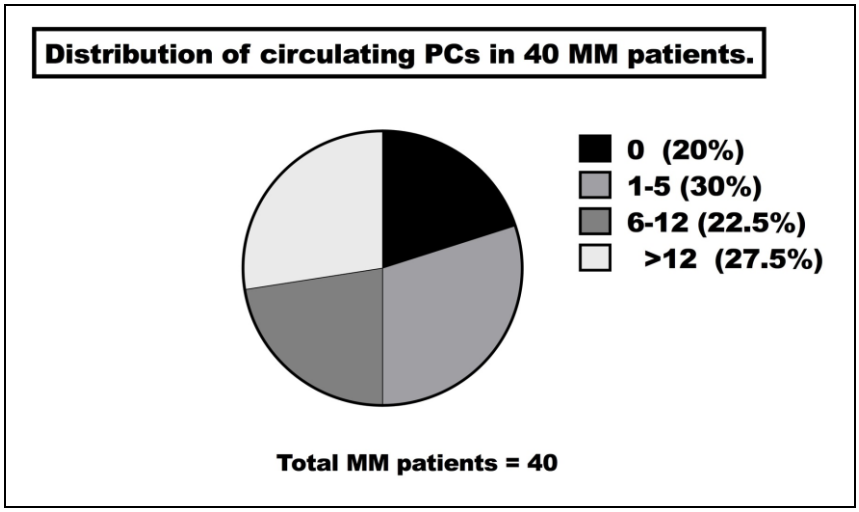

Figure 5. Distribution of circulating plasma cells (PCs) in the 40 MM patients. Patients were divided into 4 groups based on the number of PCs.

The effect of cPCs and lymphocyte subsets on overall survival (OS) in MM patients

Using ROC analysis, the optimum cutoff for cPCs, predicting for the highest risk of 2-year mortality was around 8 cPCs per 50,000 events. The median OS for those with <8 cPCs was 22.5 months compared with 18 months for patients with ≥8 cPCs (P<0.002 (as shown in Figure 6. By univariate analysis, none of the studied lymphocyte subsets had a significant effect as shown in Table 3.

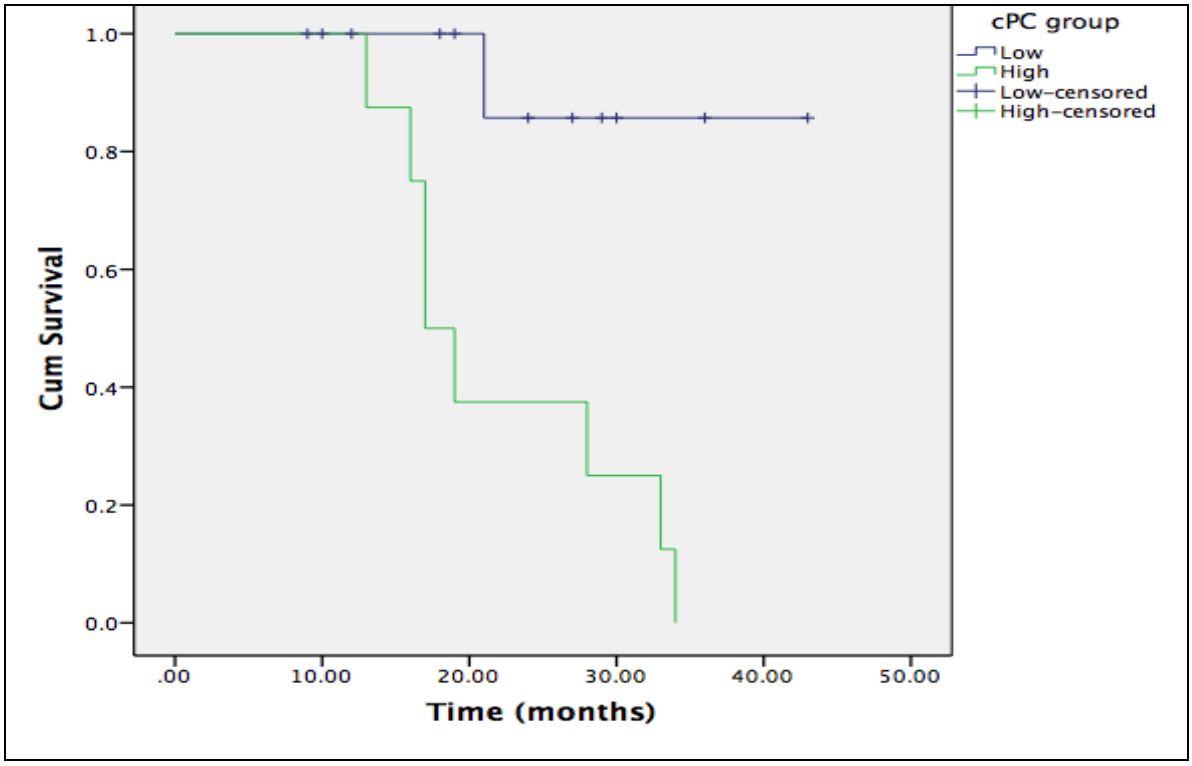

Figure 6. shows the Kaplan-Meier Curve for overall survival (OS) in MM patients based on the presence of cPCs

Table 3 Univariate analysis of the effect of lymphocyte subsets' counts on overall survival (OS) in MM patients

Type of cell	Cutoff level by ROC analysis (x10^9)	2-year OS (%)		*P* value
		High count	Low count	
CD 3 positive	1.98	75.0	55.6	NS
CD 4 positive	1.59	19.0	66.0	NS
CD 8 positive	0.823	56.0	71.4	NS
Natural Killer	0.38	100	61.4	NS
Natural Killer T	0.211	100	61.4	NS

P≥0.05 is not significant (NS).

Correlation between lymphocyte subsets, cPCs and clinical parameters

The correlations between the different type of lymphocytes and cPCs with some studied parameters of the 40 MM patients are summarized in Table 4. The results show a significant positive correlation between frequency of $CD4^+$T cells and hemoglobin. While, significant negative correlations were found between frequency of $CD4^+$T cells with BM plasma cells, β2 microgloblin, M-protein, creatinine and calcium levels. On the other hand, the results show significant positive correlations between frequency of $CD8^+$T cells with β2 microgloblin and M-protein.

In addition, the results show a significant positive correlation between frequency of NK cells and hemoglobin, whereas significant negative correlations were found between the frequency of NK cells with β2 microgloblin, creatinine and calcium levels. No significant correlation was found between frequency of NK-T cells and any of the studied parameters.

Concerning the correlation between cPCs and different T-cell subsets, NK and NKT-cells, the results showed a significant positive correlation between cPCs and number of $CD8^+$ cytotoxic T-cells in MM patients (r = 0.46), (*P*=0.006). On the other hand, a significant negative correlation was found between cPCs and number of NK cells (r = -0.38), (*P*=0.02). No significant correlation was detected between cPCs and number of $CD3^+$, $CD4^+$ and NKT-cells (data are not shown).

Table 4. Correlation between circulating plasma cells, lymphocyte subsets and clinical parameters in the 40 multiple myeloma patients.

		BM plasma cells	β2 microglobulin	M protein	Albumin	Creatinine	Calcium	Hemoglobin
r	cPCs	0.821	0.26	0.680	-0.505	0.275	0.33	0.25
P value		0.0001	0.1	0.001	0.023	0.241	0.003	0.11
r	% CD8+ cells	0.285	0.44	0.560	-0.347	0.226	0.31	-0.29
P value		0.224	0.003	0.010	0.134	0.339	0.2	0.06
r	% CD4+ cells	- 0.766	-0.55	- 0.618	0.063	-0.568	-0.61	0.62
P value		0.0001	0.0002	0.004	0.793	0.0002	0.0001	0.0001
r	CD4+/	- 0.653	-0.58	- 0.725	0.31	- 0.381	- 0.44	0.40
P value	CD8+ ratio	0.002	0.0001	0.000	0.002	0.097	0.005	0.01
r	%NK cell	-0.124	-0.48	-0.121	0.218	-0.466	-0.46	0.45
P value		0.601	0.001	0.610	0.134	0.002	0.002	0.003
r	%NKT cell	-0.226	-0.21	-0.021	0.291	- 0.23	- 0.21	0.21
P value		0.275	0.18	0.931	0.213	0.223	0.2	0.2

r ; Spearman correlation, P≤0.05 is significant; cPCs: Circulating plasma cells; NK: Natural killer cells; NKT: Natural killer T cells;

Discussion

Multiple myeloma is a lymphoproliferative disease that is characterized by the uncontrolled proliferation of plasma cells, which is accompanied by defects in the immune cells. Recent studies are increasingly focusing on the quantitative and functional profiles of NK cells and T-cells, including conventional T-cells, NK-T-cells, due to their importance in the development of MM. In our study we focused our attention on 40 newly diagnosed MM patients and evaluated the frequency of different lymphocytes subset and cPCs and their impact on survival.

First, we analyzed the distribution of different lymphocyte subsets. We found a decrease of $CD4^+$ T helper cells and increase of $CD8^+$ cytotoxic T-cells among MM patients. As a result, there was a decrease in $CD4^+$/ $CD8^+$ T-cell ratio in MM patients compared to the controls. Moreover, $CD4^+$/ $CD8^+$ T-cell ratio has been shown to decrease upon progression of the disease. This may indicate that the cellular immune response in MM patients is cytotoxic response which is a part of the host immune surveillance mechanism to control tumor growth.[31] Recruitment of cytotoxic cells into the tumor microenvironment could reflect a host immune surveillance mechanism to control tumor growth, which would be inefficient in newly-diagnosed symptomatic patients.[32]

However, the changes in T-lymphocytes show a significant difference between patient groups as divided by Durie Salmon staging system. Some previous studies reported that the increase in cytotoxic lymphocytes were correlated with disease burden reflected by β2 microglobulin and M-protein [33] and the clinical outcome in MM patients [34-38]. Also, the decrease in the $CD4^+$ T-cells appeared to be associated with increase in BM plasma cells and serum β2 microglobulin [37, 39] which came in accordance with our results.

There were discordant results regarding the frequency of NK cells in MM; some studies reported an increase in NK cells, [21, 22, 40] others report no changes, [23, 41] or a decrease NK cells [42]. NK cells were first identified owing to their ability to target tumor cells without a need for priming, in contrast to T-cells .We found that in MM patients with a high tumor burden (stage III), the NK cell frequency was decreased which agreed with a previous study [43]. Nevertheless, progress in the understanding of the mechanisms perpetuating this effect have led to new opportunities to recover or augment NK cell function therapeutically in MM [44, 45].

Also, our results indicated a reduction of NK-T cells in peripheral blood of MM patients in comparison to the control group and that the patients with high tumor burden (stage III) and progressive diseases had the most NK-T cell deficiency. Earlier reports had founded that NK-T cell defects may be important in the development of MM [17, 46, 47] and that NK-T cells could be a new target for immune-based treatment of MM patients [48]. Despite constituting a minute proportion of all T-cells, NK-T cells have an important role in tumor immune surveillance and are able to induce strong antitumor responses through the release of cytokines, such as interferon-γ (IFN-γ) [49].

In our study, we demonstrated the presence of cPCs in 80% of newly diagnosed MM patients. This result is in agreement with previous studies which have showed the presence of cPCs across the spectrum of plasma cell disorders such as

MM, [50, 51] smoldering myeloma, [26, 52] and amyloidosis [53].

In addition, the number of cPCs in our study was positively correlated with the percentage of bone marrow plasma cells, the level of serum calcium and M protein, and negatively correlated with serum albumin level. Also, the presence of ≥ 8 cPCs per 50,000 events was associated with poor outcomes for 2-year OS. Such findings could possibly indicate that the presence of cPCs may predict the prognosis of MM, which is reported by previous studies [54, 55].

The earlier study by Witzig *et al.,* (1993), suggested that detection of cPCs was a marker for disease activity in patients with MM [56]. Furthermore, cPCs have also predicted for early relapse after autologus stem cells transplantation in MM patients [50, 57] and appear to correlate well with the response to therapy.

The presence of cPCs in MM patients are thought to reflect a different disease biology. It may be due mobilization of plasma cells from bone marrow to the peripheral blood.[56] Since plasma cells are normally not detected in peripheral blood, the ability to isolate cPCs is highly relevant to MM. Although the biology of cPCs is poorly understood, their detection is associated with increased risk of malignant transformation in MGUS (monoclonal gammopathy of undetermined significance) or smoldering MM, and of poorer outcomes in MM.[58, 59]

The association between the increased number of cPCs in MM patients with the increase in the $CD8^+$ cytotoxic T cells and decrease in NK cells may indicates that the change in the immune cells homeostasis in MM patients could play a role in the release of cPCs in peripheral blood of MM patients.

Two limitations of our study, including a small number of patients was studied, and the cutoff of ≥ 8 cPCs which is based on data from our institution, this needs to be validated across other institutions.

We may conclude that alterations in the immune cells homeostasis in MM patients could play a role in the development of MM and may be associated with the release of plasma cells in the peripheral blood. Also, the quantitative estimation of cPCs in patients with newly diagnosed MM may be used as a predictor of survival.

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