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Clinical findings and immunophenotypic profile of plasma cell leukemia: a retrospective study in a comprehensive cancer center in Turkey

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Abstract

Plasma cell leukemia (PCL) is a rare (1-2 %) aggressive plasma cell dyscrasia (PCD) with a poor prognosis and characterized by the presence of more than 20% circulating plasma cells (PCs). Multicolor Flowcytometry is used for differentiating PCs in PCL. In this study, we aimed to compare the clinical, laboratory findings and immunophenotypic profiles of patients with PCL. A total of 512 patients were investigated for PCD in our hospital between 2011-2019. The immunophenotypic profile (including, CD27, CD28, CD38, CD138, CD45, CD117, CD19, CD20, CD56, CD33, CD81, and ckappa /clambda,) were evaluated retrospectively. The clinical findings, pathology reports, immunofixation electrophoresis and MFC results of three patients with PCL were reevaluated using 6 color multiparametric flow cytometry (MFC). Only three patients were diagnosed as PCL. PCs population defined by the co-expression of CD38 and CD138 was observed as 50%, 53%, 60%, respectively. Expression of each CD56, CD117, CD20, CD45 was seen in individual cases. CD19 was negative in all cases. Cytoplasmic kappa light chain expression was detected only in one case. Two of the cases were primary and the other was secondary PCL. Bortezomib-based treatment was initiated. Overall survival of primary PCL cases were 24 months and 6 months, whereas secondary PCL was four months. Co-expression of CD38 and CD138 in identifying PCs with MFC may be considered the best combination and leads to early diagnosis within hours and appropriate treatment in patients with PCL.

Keywords: Plasma cell leukemia, Immunophenotype, CD38, CD138, CD56, CD117

Introduction

TPlasma cell leukemia (PCL) is characterized by the presence of more than 20% PCs or an absolute PCs count ≥ 2x109 / L in peripheral blood (PB). It constitutes 1-2% of all plasma cell dyscrasias (PCD), as a rare aggressive variant of myeloma and has poor prognosis with fulminant course [1]. PCL is defined as 'primary' when peripheral plasmacytosis is detected at the time of diagnosis, and 'secondary' when leukemic transformation is present in a patient with predetermined myeloma. Approximately 60% of patients with PCL are of the primary type [2].In a comprehensive study, the median survival in PCL was reported to be only four months [3], but median survival was prolonged with the use of new drugs such as bortezomib [4].

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In the diagnosis of PCL, morphological evaluation of PB and bone marrow (BM) smears and determination of immunophenotypic properties is very important. However, there is a lack of literature on the immunophenotypic spectrum of PCL because it is a rare disease. Compared with studies on multiple myeloma (MM), it is seen that studies providing immunophenotypic information are limited in both diagnosis and minimal residual disease in PCL [5].

In this study, we aimed to contribute to the literature by comparing the clinical, laboratory findings and immunophenotypic profile of rare PCL cases.

Material and Methods

The study was conducted between 2011-2019 in Prof. Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital. Ethical approval was obtained from the Research Ethics Committee (approval reference number 2019-05/303). 512 patients with PCD were reviewed and 3 patients who had

≥20% plasma cells (PCs) in flowcytometric analysis of PB sample were included. The clinical and other laboratory findings and the immunophenotypic profile (including 10 parameters) of the cases using 6 color multiparametric flow cytometry (MFC) was retrospectively analyzed.

For the morphological evaluation of the cases, May Grunwald-Giemsa stained PB (PB) and blood marrow (BM) aspiration samples were available. For MFC immunophenotyping, in accordance with the sample preparation protocol provided by the manufacturer, the cells were harvested using the whole blood lysed washing technique within 2-4 hours after sampling. CD38, CD138, CD19, CD56, CD45, CD117, ckappa /clambda, CD20, CD33 and CD81 (BectonDickinson, San Jose, CA (BD) were used for immunophenotyping of the samples. The prepared samples were analyzed for at least 10.000 events in BD with BD FACSDiva software on flow cytometry in 6-color BD FACS CANTO II (BectonDickinson, San Jose, CA) as shown in Fig. 1A; forward scattering (FSC) / side scattering (SSC), SSC / CD38, SSC / 138, CD38 / CD138 were plotted and analyzed by sequential gating. Furthermore, protein / immunofixation electrophoresis, pathology reports and biochemical parameters were reviewed (Table 1).

Table 1. Clinico-hematological profile of three patients with plasma cell leukemia

	Case1	Case2	Case3
PCL Type	Primary	Primary	Secondary
Sex	Female	Female	Male
Age (Years)	80	76	55
Organomegaly/ Extraosseous Plasmacytoma,	Splenomegaly	-	Testicular Plasmacytoma
Bone Lesions	Osteopenia	Lytic Lesions	Multiple Lytic Lesions
Ca, Total(mmol/L)	2.27	2.08	2.23
Creatinine (µmol/L)	87	70	89
LDH (U/L)	295	564	162
M Protein Type %	11% Ig A Kappa	52 % Ig G Lambda	10% Ig A Lambda
B2-M (mg/L)	8.14	13.4	4.56
WBC (109/L)	25,2	20	11
Hg (g/L)	54	60	133
PLT (109/L)	79	112	289
PCs in BM (%)	70 CD138+, MUM1+, PAX5+, CD20+, BCL1+, CD56 +	80 CD138+ MUM1+, PAX5+, CD20-, CD56+	70 CD138+ CD20-
PCs in PB (%)	50	53	60
Overall Survival, Months	8	24	4

PCL, plasma cell leukemia; Ca, calcium; N, normal; LDH, lactate dehydrogenase; β2-M, beta 2-microglobulin; WBC, white blood cells; Hg, hemoglobin; Plt, platelet; PCs, plasma cells.

Results

CASE 1

An 80-year-old woman was admitted to the hematology outpatient clinic with a complaint of fatigue. Splenomegaly, deep anemia, and leukocytosis were detected. Laboratory investigations were as follows: White blood cells (WBC) : 25.2x10^9 /L(4-10 x10^9), Hemoglobin (Hg): 54 g /L(110-160 g/L), Mean corpuscular volume (MCV): 103 fL(80-100 fL), Platelet (Plt): 79x109 /L(100-300 x109), Total Protein (TP): 63g /L(66-83 g/L), ALB: 37 g /L(35-52 g/L), Creatinine(Crea): 87 µmol/L(48- 110 µmol/L), Lactate dehydrogenase(LDH): 295 (0- 247 U/L), Calcium(Ca): 2.27mmol/L(2.15- 2.65 mmol/L) and Beta 2-microglobulin (β2-M): 8.14 mg/L (0.8-2.4 mg/L). BM aspiration revealed 70% PCs and PB 50% PCs. In protein electrophoresis, 11% M protein was present, and immunofixation electrophoresis (IFE) showed a band compatible with IgA Kappa. MFC analysis of the patient from PB is shown in Table-2. Based on the findings, the patient was diagnosed as Primary PCL and VCD (bortezomib-cyclophosphamide-dexamethasone) chemotherapy was initiated. The patient did not come for regular follow-up and died 6 months after diagnosis.

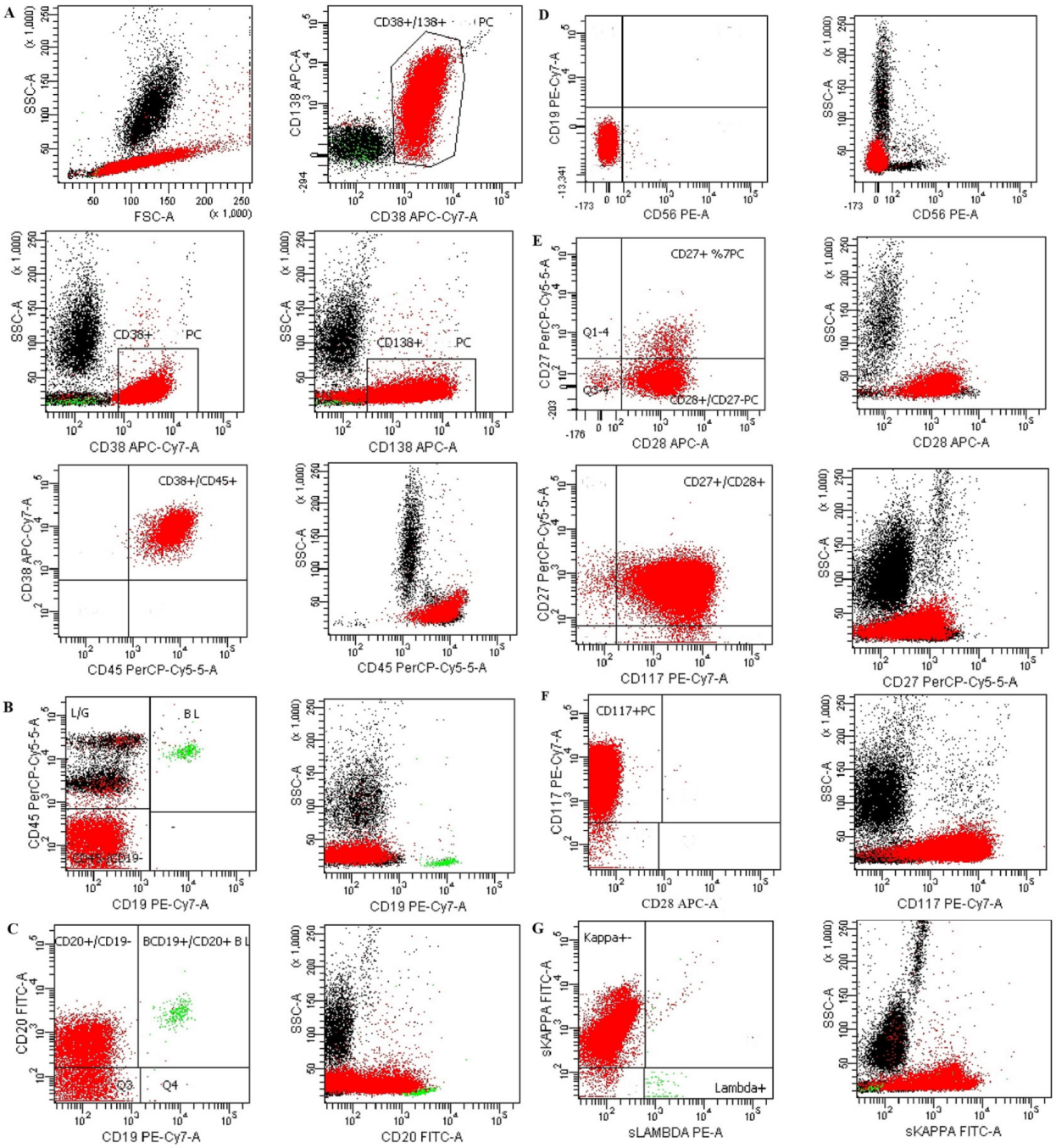

Figure 1. Antigen expressions of PCs.

A. PCs (red) were determined by sequential gating FSC/SSC, SSC/CD38, SSC/ CD138, CD38/ CD138 dot plots. The best distinction was found in CD38/ CD138 dot plot. CD45 expression was observed in one case. Because of CD45 expression on neoplastic PCs, CD45 was not used for gating.

B. Display of all events on the CD45/CD19 dot plot; PCs(red) CD45-/CD19-, B lymphocytes (BL, green) CD19+/CD45+, T lymphocytes/NK cells (L, black) CD19-/CD45 bright+ and neutrophils/monocytes (G, black) CD19-/CD45+. In addition, on the right CD19+ BL was checked to see if there was CD19 expression on the PCs. CD19 was negative in all three cases.

C. On the left, dot plot of the case with CD20 expression, the PCs are red, BL are green. The positivity of CD20 expression on PCs was checked using the SSC/CD20+ BL on the right.

D. On the left, CD19/CD56 dot plot, the PCs are shown in CD19-/CD56- red color and CD56 expression was not observed in two cases with extramedullary involvement. On the right, expression of CD56 on PCs was checked using SSC/CD56 dot plot . CD56 is positive on NK cells.

E. In secondary PCL case (with negative CD56 expression but positive CD28 expression) on the upper left CD27/CD28 dot plot, PCs are shown as red (CD27-/CD28+). Note the decrease in CD27 expression. The positivity of CD28 expression on PCs was checked using SSC/CD28+ T lymphocytes on the upper right. In the primary PCL(with negative CD28 expression), on the left CD27 / CD117 dot plot, the PCs are shown as red (CD27 +/CD117+). The positivity of CD27 expression on PCs was checked using the SSC/CD27+ lymphocytes on the right.

F. On the left CD117 / CD28 dot plot of the primary PCL case, PCs were shown in red color (CD117 +/CD28-). On the right, SSC/CD117 dot plot shows the positivity of CD117 expression on PCs (red) with reference to CD117 negativity in granulocytes.

G. On the left, cKappa / cLambda dot plot shows clonality with kappa light chain restriction. On the right, SSC /cKappa dot plot was used to check the ckappa positivity in PCs (red).

CASE 2

A 76-year-old woman was admitted to the hematology clinic with complaints of weight loss, night sweats, back pain and a history of hypertension, osteoporosis, and dementia. Laboratory investigations were as follows: WBC: 20x109/L(4-10 x10^9), Hg: 60 g /L(110-160 g/L), MCV: 112 fL(80-100 fL), Plt: 112x10^9 /L(100-300 x10^9), TP: 104 g /L(66-83 g/L), ALB: 27 g /L(35-52 g/L), Crea: 70 µmol/L(48- 110 µmol/L), LDH: 564 (0- 247 U/L), Ca: 2.08 mmol/L(2.15- 2.65 mmol/L) and β2-M: 13.4 mg/L (0.8-2.4 mg/L). 53% and 80% PCs were detected in PB and BM; respectively.

MFC immunophenotyping of the PB is summarized in Table-2. 52% M protein was present and an IgG lambda biclonal band was detected in serum IFE. Physical examination of the patient was normal. Bone scan revealed significant L1, L5 and minimal L2, L3 compression, and diffuse osteoporosis. VCD chemotherapy was started with the diagnosis of primary PCL. M protein regressed to 6.9% . BM biopsy showed PCs infiltration of 8-10%, and the findings were consistent with a very good partial response. The patient died after 24 months of diagnosis.

CASE 3

A 55-year-old male patient admitted to the neurosurgery clinic with complaints of low back pain and a mass was detected in the thoracic vertebra. He was admitted to the hematology clinic due to the presence of plasmacytoma. Laboratory investigations were as follows: WBC: 11x10^9/L(4-10 x10^9), Hg: 133 g /L(110-160 g/L), MCV: 92 fL(80-100 fL), Plt: 289x10^9 /L(100-300 x10^9), TP: 84 g /L(66-83 g/L), ALB: 42 g /L(35-52 g/L), Crea: 89 µmol/L(48- 110 µmol/L), LDH: 162 (0- 247 U/L), Ca: 2.23 mmol/L(2.15- 2.65 mmol/L) and β2-M: 13.4 mg/L (0.8-2.4 mg/L). 70% PCs were detected in BM aspiration. Protein electrophoresis showed 10% M protein and serum IFE was found to be compatible with IgA Lambda. The patient was diagnosed with MM.

The patient was given 2 cycles of high dose dexamethasone and 2 cycles of VTD (bortezomib-thalidomide-dexamethasone) treatment and autologous stem cell transplantation was performed. Post-transplant response was assessed as consistent with complete remission. Recurrence occurred 6 months after transplantation and lenalidomide-dexamethasone treatment was initiated. Remission was followed up with Lenalidomide treatment, however progression was observed in the 20th month of treatment. MFC analysis from the patient's PB showed 60% CD38 +, CD138 +, CD45 +, CD19-, CD56-, CD20-, CD27-, CD28 +, CD33-, CD117-, CD81 +, sLambda + monoclonal PCs (Table 2). The patient was diagnosed with secondary PCL. He died 4 months after diagnosis.

Table 2. Immunophenotypic profile of PCL cases

Case no	CD38	CD138	CD45	CD19	CD56	CD20	CD27	CD28	CD33	CD117	CD81	cKappa+ /cLambda
1	+	+	-	-	-	+	+	-	-	+	+	**+Kappa**
2	+	+	-	-	+	-	-	-	-	-	+	**+Lambda**
3	+	+	+	-	-	-	-	+	-	-	+	**+Lambda**

cKappa/cLambda, cytoplasmic kappa/ cytoplasmic lambda

Discussion

PCL is an aggressive course and a rare sub-variant of Multiple Myeloma (MM). In a study of 49.000 MM patients, 291 (0.6%) PCL cases were detected. PCL was detected in three of the 512 patients (0.6%) who were referred for MM panel to our laboratory. PCL is defined as 'primary' when peripheral plasmacytosis is detected at the time of diagnosis, and 'secondary' when leukemic transformation occurs in a patient with pre-diagnosed MM, and 60-70% of the cases are diagnosed as primary PCL [2, 3]. In our study, two cases with ages 76 and 80 years were primary PCL. It was reported that 63.6% of the patients diagnosed with PCL were shown to be over 60 years of age, and primary PCL was observed to be approximately one decade earlier than secondary PCL [1, 3, 5]. However secondary PCL case (55 yo) was younger than the primary PCL cases in our study. Although there are studies in the literature that report PCL to be more frequent in men, our two cases were women [1]. However, our patient number is too small to make a conclusion about gender or age of the PCL cases.

The clinical data observed in our series (Table 1) are consistent with previous reports that primary PCL patients generally have more extramedullary disease, anemia, thrombocytopenia, elevated LDH and β2-microglobulin serum levels [6, 7].

In a retrospective study, the clinical characteristics of patients presenting with PCL was reported as follows: median WBC: 18.6x109/L, median Hb: 92 g/L, median Plt: 75x10^9/L, osteolytic lesions 44%, extramedullary plasmacytoma 14%, hepatomegaly 21% and splenomegaly 13%. Splenomegaly and thrombocytopenia were present in one of our primary PCL cases, extramedullary plasmacytoma (testis plasmacytoma) in secondary PCL and leukocytosis, anemia and were detected in both primary PCL cases (Table 1) [8].

PCL is characterized by the presence of >20% PCs in the blood or an absolute PCs count >2x10^9/L. In our study, the PCs percentage of 3 cases was found to be 50%, 53% and 60%. However in recent studies, these criteria is argued to be too high and use of additional techniques such as MFC to detect PCL and the percentage of PCs included in the diagnostic criteria to be lowered to >5% or >0.5x109/L. It is emphasized that changing the diagnostic crieria can help to make the diagnosis of patients earlier and have improve the prognosis. In addition, it has been reported that PCs in PB can

independently predict survival in MM patients when it does not meet the PCL criteria (percentage and / or number of PCs) [1, 9-11].

An accurate gating strategy should be used for a reproducible and sensitive immunophenotypic analysis of PCs. For this purpose, various approaches based on the expression of CD38, CD138 and / or CD45 are used. European Myeloma Network has recommended the use of CD38, CD138 and CD45 with light scattering (FSC / SSC) [12, 13]. In this study, immunophenotypic properties of 3 PCL cases were studied and evaluated in accordance with the proposed panels. We used the FSC/SSC properties with the coexpression of CD38 and CD138 to identify PCs. The identification of a neoplastic PCs population should never be based on a single parameter. The distinction between normal PCs and neoplastic PCs should be made by evaluating multiple parameters. CD38 expression of neoplastic PCs is weaker than that of normal PCs, but CD45 expression is negative or weak [12]. It has been shown that gating using CD38 positive and CD45 negative or weak expression alone cannot identify CD45 (+) neoplastic PCs. However, CD38 is a nonspecific marker that can be detected on hematopoietic stem cells, monocytes, and lymphocytes [14, 15]. Thus, the best combination would be obtained by the addition of CD138 monoclonal antibody specific for PCs for immunophenotyping. However, it should be noted that neoplastic PCs with weak positive or negative expression of CD38 and CD138 have been identified and CD138 loss has been associated with poor prognosis [34]. In our study, gating was performed by evaluating CD38, CD138 coexpression in all 3 cases and CD45 expression was observed in one case (Fig. 1A). CD38, CD138 co-expression was found to be 50%, 53%, 60%.

Normal PCs usually express CD19, but CD20 is often negative. CD19 was observed to be negative in all three cases and CD20 expression was observed positive in one case (1 B, C). In addition, although CD20 expression is reported as a poor prognostic factor [7, 16], survival was observed as 24 months in the patient with CD20 expression. However, more comprehensive studies are needed to reach a definite conclusion.

CD56, which is thought to play an important role in the attachment of PCs to the stroma in BM, is not expressed by normal PCs. However, most of the myeloma cells express CD56 abnormally so that normal PCs and neoplastic PCs can be separated [17]. It has also been reported that PCL PCs lose CD56 expression and this can be used to differentiate between PCL and MM immunophenotypically [18]. In addition, lack of CD56 expression has been shown to be associated with more aggressive disease and extramedullary spread and CD56 expression is associated with good prognosis [19]. However, M. Hundemer et al. reported that there was no significant difference between CD56 + and CD56− group in terms of overall survival after melphalan-based high-dose chemotherapy, and that high-dose chemotherapy could overcome the negative effect of CD56 -negative MM [20]. In our cases, CD56 expression was not observed in two cases with extramedullary involvement (Figure. 1D).

N Robillard et al. reported that CD28 expression was observed in patients with progressed disease and associated with poor response to the therapy. They observed that CD28 was positive in MM patients without CD56 expression and concluded CD28 expression was associated with tumor expansion [21]. In our study, CD56 expression was not present, but CD28 expression was observed in the secondary PCL case, (Figure 1E) and the patient had a testis plasmacytoma with a survival of 4 months.

Although CD117 was reported to be expressed in approximately one third of MM patients [22], Pan Ying et al. showed that the absence of both CD56 and CD117 expression in new diagnosed MM patients was associated with the worst prognosis [23]. In our case of secondary PCL, CD56 and CD117 expression was not observed and survival was 4 months. It has also been reported that CD117 is limited to the neoplastic population, and therefore can be considered as a 'tumor-associated marker' for monitoring minimal residual disease and may be a valuable marker for the use of tyrosine kinase selective inhibitors [24]. Mateo G. et al showed that prognosis was better in MM patients with CD28 negative CD117 expression, in one of our primary PCL cases CD28 negative CD117 expression was observed and survival was 24 months (Figure 1F). CD117 should be added to the immunophenotyping panels for PCL / MM cases as it has a place in the prognosis and treatment options in PCL cases.

CD27 is strongly expressed in PCss in the BM of healthy individuals [25]. However, it has been reported that CD27 is heterogeneously expressed in MM neoplastic PCs and loss of CD27 expression may have a poor prognostic value in MM [22]. Compared with MM, primary PCL was found to over-express CD27 antigen homogeneously [26]. However, functionality and signal transduction characteristics of CD27 on PCs of healthy individuals and primary PCL patients are unknown. Loss of CD27 expression in MM cells suggests that CD27 expression is regulated differently in MM and primary PCL. In our study, CD27 expression was observed in primary PCL, whereas loss of CD27 expression was observed in secondary PCL (Figure 1E). Guikema et al. investigated the functional role of CD27 expression on primary PCL cells and demonstrated that CD27 expression protects PCL cells from dexamethasone-induced apoptosis [26]. Increased expression of CD27 in PCL has been described as an antiapoptotic pathway to the survival of neoplastic PCs causing activation of nuclear factor κB (NF-κB) and has been reported to be suppressed by other novel proteasome inhibitors such as bortezomib [19].

With extensive immunophenotyping with MFC, it is possible to accurately identify the disease in a short time by demonstrating abnormal immunophenotypic expression in PCs (CD20+, CD56+, CD28+, CD117+) and limited expression of light chains cKappa / cLambda (Figure 1G.)

CD81 plays a role in regulating motility and intracellular signaling. Paiva B et al. reported that CD81 expression may play an important role in the pathogenesis of MM as an independent prognostic factor for patients with symptomatic MM and a marker for the risk of progression in smoldering MM [27]. Immunophenotypic studies of CD81 expression in patients with PCL are insufficient and the potential prognostic value is unknown. In our study, CD81 expression was positive in all three cases.

CD33 is usually expressed on immature myeloid cells, as well as monocytes, but in some studies, it has been reported in 5-35% of MM cases [28]. It has been reported that plasmacytomas with CD33 expression have a worse prognosis than those without CD33

expression [29]. However, there is not enough data about CD33 expression in PCL [30]. CD33 expression was not observed in our cases. Further studies are needed to determine CD33- PCL association.

Since PCL is a rare aggressive disease with poor prognosis, new therapeutic strategies are needed to improve the prognosis of PCL patients. Combinations of alkylating agents, especially melphalan, with glucocorticoids are insufficient in treatment. Studies with small series have shown that thalidomide has a potential role in PCL. However, severe heart and lung toxicity has been reported. The use of lenalidomide with melphalan and glucocorticoids; produced transient partial response (PR) [1]. Recently, new drugs have been produced that target not only PCs but also their microenvironment. Bortezomib is one of these agents and is the first proteasome inhibitor used in the clinic. This compound has been reported to affect MM survival by several mechanisms such as NF κ B blockage, inhibition of angiogenesis, DNA repair. Several clinical studies have demonstrated the efficacy of Bortezomib in patients with recurrent and refractory myeloma. Because of the promising clinical outcomes of Bortezomib in MM patients and the relationship between MM and PCL, the in vitro and in vivo efficacy of this compound on PCL was investigated. In vitro studies have shown that Bortezomib can induce cell death in PCL. In addition, Bortezomib has been reported to significantly reduce circulating PCs in a patient with PCL. It has been suggested that Bortezomib should be used alone or in combination with other drugs (dexamethasone) in patients with PCL [31-33]. In our study, bortezomib-based treatment regimen was applied. Given the poor prognosis of PCL, autologous stem cell transplantation (OSCT) is recommended after high-dose treatment. However, two of our cases were not eligible for OSCT, and the patient with secondary PCL could not have OSCT because he had an OSCT before and relapse has been occurred in the 6th month after transplantation.

Although our study was limited to small sample size due to its retrospective nature and PCL being a rare disease, immunophenotyping with MFC demonstrated that it is possible to distinguish PCL from non-neoplastic reactive PD as well as other chronic lymphoproliferative diseases with plasmacytoid morphology. Co-expression of CD38 and CD138 is the best way to identify PCs with MFC. However, although prospective studies are needed to differentiate MM and PCL by immunophenotyping, PCs in PCL show a partially different pattern compared to MM. In PCL, stronger CD20, CD27 and CD28 expression as well as loss of CD117 and CD56 are observed more frequently. CD56 antigen expression was also associated with a better prognosis and CD20 antigen expression was associated with shorter survival. From this point of view, it can be thought that immunophenotypic features may help to explain the differences in survival of cases.

In conclusion, in order to detect PCL early, to detect abnormal expressions for the follow-up of minimal residual disease, and to classify risk in terms of prognosis; increasing the use of MFC with standard panels incorporating antibodies against antigens such as CD19, CD20, CD56, CD33, CD81, CD27, CD28 and CD117, and prospective multicenter studies in new diagnosed patients should be performed.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was conducted between 2011-2019 in Prof. Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital. Ethical approval was obtained from the Research Ethics Committee (approval reference number 2019-05/303).

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