

Immunophenotypic heterogeneity of normal plasma cells: comparison with minimal residual plasma cell myeloma

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ABSTRACT

Plasma cell myeloma (PCM) exhibits immunophenotypic aberrancies that can be used for minimal residual disease (MRD) detection after therapy. The authors sought to determine whether non-neoplastic plasma cells, especially in the bone marrow (BM) post various therapies, would exhibit immunophenotypic variations interfering PCM MRD detection. The authors studied the flow cytometric immunophenotypes of non-neoplastic plasma cells from 50 BM specimens, including 12 untreated BM and 38 BM specimens from patients with non-plasmacytic haematological malignancies undergoing various therapies, and compared with 59 BM specimens positive for PCM MRD. Non-neoplastic plasma cells showed heterogeneous expressions of CD45 (78% (41–100)) and CD19 (80% (52–97)), and were negative for CD20 and CD117. CD56 was observed in a small subset (6% (0–37)) and CD28 in a larger subset (15% (0–59)) of non-neoplastic plasma cells, with the latter more frequently expressed in post-treatment BMs ($p=0.01$). However, despite a partial immunophenotypic overlap, PCM cells could be reliably discriminated from non-neoplastic plasma cells by the presence of a higher number of aberrancies (3 (1–6) vs 0 (0–2)) and stronger intensity and uniformity of aberrant expression ($p<0.001$ in each marker using a cut-off value). In addition, simultaneous assessment of cytoplasmic κ/λ with surface markers detected light chain restriction in all 59 PCM cases. In conclusion, non-neoplastic plasma cells in BM are more immunophenotypically heterogeneous than previously understood; however, these immunophenotypic variations differ from those of PCM. With advances in multicolour flow cytometry and application of recently validated markers, PCM MRD may still be reliably distinguished from non-neoplastic plasma cells.

INTRODUCTION

In recent years, flow cytometric immunophenotyping (FCI) has been utilised as an adjunct to traditional morphological examination and immunohistochemistry in the diagnosis and monitoring of plasma cell myeloma (PCM) in bone marrow (BM).^{1–2} Minimal residual disease (MRD) detected by FCI at various intervals post-chemotherapy and/or autologous stem cell transplantation has been shown to provide additional information in predicting clinical outcome in PCM patients.^{3–6}

Detection of PCM at the level of MRD after therapy relies on distinguishing PCM cells from background non-neoplastic plasma cells; the latter can constitute up to 99% of total plasma cells in an individual BM specimen.⁷ PCM cells are known to have immunophenotypes that differ from non-neoplastic plasma cells, such as an aberrant expression of CD28, CD56 and/or CD117, and frequent loss of CD19 and CD45.^{2, 3, 7–11} Immunophenotypic assessment of immunoglobulin light chains is also helpful, but shows limited sensitivity. Cytoplasmic κ/λ restriction is generally apparent only when PCM cells represent 30% or more of the total BM plasma cells.¹ Therefore, the current consensus of the European Myeloma Network (Euroflow) for PCM MRD testing focuses on the detection of surface marker aberrancies^{1, 2, 4} only. The markers of recommendation include CD38/CD138/CD45 for gating and CD19/CD20/CD27/CD28/CD56/CD117 for PCM identification.

Recently, several studies have shown that myeloma cells after therapy may exhibit changes in the expression of CD19, CD20, CD45 or CD56 in approximately a third of patients,^{7, 12, 13} and these changes can complicate detection of MRD by FCI analysis. It also has been shown that non-neoplastic plasma cells can exhibit immunophenotypic heterogeneity overlapping with myeloma cells.^{14–17} In aggregate, these data raise questions about the reliability of MRD detection by FCI in PCM without light chain assessment. It is noteworthy that most data about non-neoplastic plasma cells were generated from a relatively small number of healthy donors and that markers recommended by the European Myeloma Network for detecting MRD of PCM have not been fully examined as a panel.

In this study, we assessed the immunophenotype of plasma cells obtained from untreated BM specimens as well as plasma cells derived from BM after various cancer therapies. The latter group included patients who received a wide spectrum of therapies, even beyond the current regimen for PCM treatment, in order to fully examine the reactive plasma cell immunophenotype under various conditions. With this information, we compare the immunophenotypes of non-neoplastic plasma cells with PCM cells in patients positive for MRD. Our goal is to examine if cancer treatment would increase immunophenotypic variation of normal plasma cells and if FCI could effectively distinguish myeloma cells from normal plasma cells, despite these variations.

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MATERIALS AND METHODS

Study groups

All patients were diagnosed and treated at The University of Texas MD Anderson Cancer Center (MDACC). The study was conducted in accordance with protocols approved by the Institutional Review Board at the MDACC. The cases in this study were divided into two groups.

Group 1 included BM aspirate specimens collected from patients with no evidence of plasma cell neoplasms. These specimens were further subdivided into two subsets: Group 1A specimens were obtained from patients with lymphoma who underwent BM staging prior to initiation of any type of cancer therapy. These BM specimens were proven negative for lymphoma involvement by morphological examination and FCI. Group 1B specimens were obtained from patients who had a non-plasma cell haematological malignancy and had received various anticancer treatments prior to BM aspiration and biopsy. These cases were obtained from a consecutive series of patients except that the BM specimens with insufficient plasma cells obtained for FCI were excluded. Group 2 included BM aspirate specimens obtained from patients with an established diagnosis of PCM and had been treated with one or more anticancer therapies including chemotherapy, lenalidomide and autologous stem cell transplant. According to the modified European Bone Marrow Transplant criteria,¹⁸ a complete response was defined by a negative immu-

nofixation test in serum and urine, and <5% plasma cells in the BM. A near complete remission was defined by the absence of M protein on serum protein electrophoresis, but positive immunofixation and <5% plasma cells in BM. In this study, we chose cases with <5% plasma cells in BM by differential counts on aspirate smears and confirmed by CD138 immunohistochemistry on paraffin sections. The final cases included all had proven positive residual diseases either by in situ hybridisation performed on paraffin sections or serum immunofixation.

Immunohistochemistry and in situ hybridisation

Routinely fixed, paraffin-embedded sections of BM aspirate clot specimens (without decalcification process) were assessed by immunohistochemistry and in situ hybridisation. Immunohistochemical analysis for CD138 was performed following heat-induced epitope retrieval. In situ hybridisation analysis for κ/λ was performed using a fluorescein-tagged oligo-probe (Ventana Medical Systems, Tucson, Arizona, USA). These studies were performed in an automated immunostainer (Ventana Medical Systems) according to the manufacturer's instructions with the appropriate positive and negative controls.

Flow cytometry immunophenotyping

BM aspirate specimens were collected in EDTA-anticoagulant and processed within 24 h of collection. After incubation with

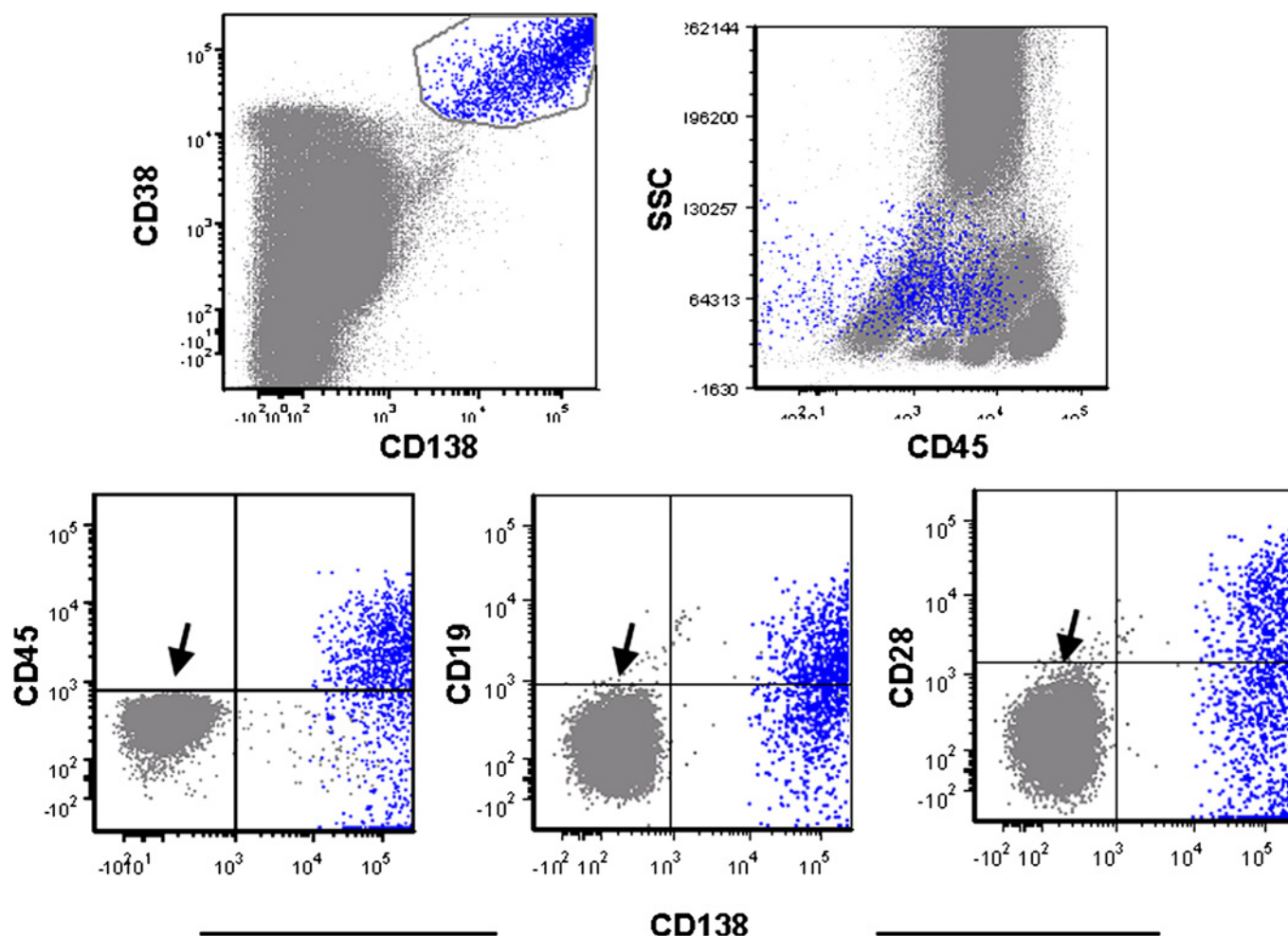


Figure 1 Flow cytometry analysis: upper row: plasma cells are primarily identified by CD38/CD138 gate and displayed on the SSC/CD45 plot for comparison with total mononuclear cells. Lower row: Erythroid precursors (grey cell population indicated by arrows) served as internal negative controls, and the expression levels of CD45, CD19 and CD28 were assessed on plasma cells.

monoclonal antibodies for 15 min at 4°C, erythrocytes were lysed with ammonium chloride (PharmLyse, BD Biosciences, San Diego, California, USA) at room temperature for 10 min using a standard lyse/wash technique. Samples were acquired on FACSCanto II instruments (BD Biosciences). For the detection of cytoplasmic antigens, cells were fixed and permeabilised using 4% formaldehyde and 0.25% Saponin. The antibody combinations used were: Tube 1: CD38-FITC; CD28-PE; CD138-APC; CD117- PE-Cy7; CD19-PerCP-Cy5.5; CD56-V450; CD45-V500; and Tube 2: cytoKappa-FITC; cytoLambda-PE; CD138-APC; CD38-PerCP-Cy5.5; CD20-V450; CD45-V500. All antibodies were purchased from BD Biosciences. At least 100 000 total events were acquired. In many cases with a low number of plasma cells, up to 500 000 events were required to obtain at least 100 cells in the plasma cell gate in all tubes. Cases in which too few plasma cells collected were excluded from this study. The total plasma cells acquired for flow cytometry analysis were comparable among Groups 1, 2 and 3.

Data were analysed using FCS Express software (De Novo Software, Los Angeles, California, USA). Non-viable cells, debris and aggregates were excluded based on forward scatter-height/forward scatter-area (FSC-H/FSC-A). Plasma cell populations were identified by bright CD38/CD138 expression (figure 1), further refined by FSC-H/FSC-A gates to exclude non-specific binding and displayed on CD45/SSC plots (figure 1). Internal controls were utilised consistently in all cases and erythroid precursors served as negative internal controls (figure 1). Erythroid precursors were identified by SSC/CD45, with no reactivity to CD38/CD138. For the tube with cytoplasmic κ/λ stains, light chain expression patterns were assessed on the entire plasma cell population as well as the CD45dim/negative plasma cell population (figure 2). κ/λ Expression was also simultaneously assessed on CD20+ CD45 bright+ lymphocytes to determine mature B cell clonality, and cases of B cell lymphoma/leukaemia with monotypic plasma cells were excluded.

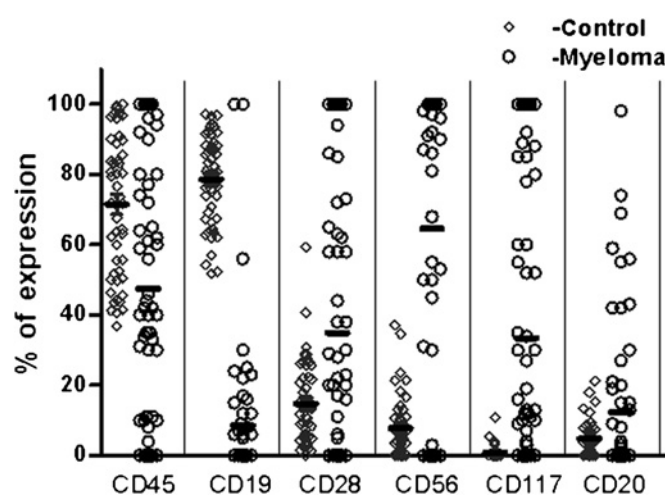


Figure 2 Upper row: the non-neoplastic plasma cells in a Group 1B patient show decreased CD19, as well as increased CD56 and CD28 expression. The patterns are continuous and heterogeneous not forming discrete populations. Middle row: Myeloma cells (arrows) show strong and uniform expression of aberrant CD56, CD28 and CD117, along with uniform loss of CD19. The aberrancies are observed together in the same cell population. Lower row: In a Group 2 patient, cytoplasmic κ/λ light chain expression assessed on the entire plasma cell population yields an inconclusive ratio of 3:1; when assessed on a plasma cell population with decreased CD45 κ -light chain restriction is evident.

Statistics

The Fisher's exact test was used for comparison of categorical variables and the t test was applied for numerical comparisons. A p value of <0.05 was considered as statistically significant. The comparisons were conducted between Group 1A and Group 1B as well as between Group 1 and Group 2.

RESULTS

Study groups

The study included 50 patient samples with non-neoplastic plasma cells in Group 1, consisting of 12 untreated patients in Group 1A and 38 post-therapy patients in Group 1B. The study also included 59 patients with PCM MRD in Group 2. A total of 13 additional cases were excluded either due to insufficient plasma cells obtained for FCI analysis (Group 1A: n=1; Group 1B: n=4; and Group 2: n=4) or incidental clonal plasma cells (n=3) or clonal B cells detected in Group 1B (n=1). The latter group had no clinical symptoms, or suspicion for a plasma cell neoplasm or lymphoma, and clonality was identified incidentally in this study by FCI assessment. Patient demographic information as well as the plasma cells counted on BM aspirate smears is shown in table 1.

Group 1A included four patients with a newly diagnosed classical Hodgkin's lymphoma and eight patients with non-Hodgkin's lymphoma. All BM specimens were negative for lymphoma involvement. Group 1B consisted of 38 patients' status postchemotherapy, hypomethylating agents, tyrosine kinase inhibitors and/or stem cell transplant for underlying haematopoietic malignancies, namely, acute myeloid leukaemia (n=15), precursor B lymphoblastic leukaemia (n=1), myelodysplastic syndromes (n=7), chronic myelogenous leukaemia (n=4), Philadelphia chromosome-negative myeloproliferative neoplasms (n=4) and lymphoma restaging BM after chemotherapy (n=7).

Group 2 patients included 59 patients with PCM who were status post various therapies and had BM positive for residual disease as defined in Materials and methods section, and who had sufficient plasma cells obtained for FCI analysis. In each case, BM aspirate and biopsy were performed and <5% plasma cells were present by morphological assessment and confirmed by CD138 immunohistochemical stains. In situ hybridisation for κ/λ light chains was performed in 29 cases, and demonstrated monoclonal plasma cells in 21 (72%) cases. In some cases, plasma cell clonality was identified only in a subset of plasma cells or was focally present admixed with background polyclonal plasma cells. Serum paraprotein (M protein) levels were measured in 50 patients around the time of FCI studies and confirmed by immunofixation, and were positive in 40 cases, with a median level of 0.60 (0–3) g/dL.

Immunophenotypic comparisons of non-neoplastic plasma cells: with or without cancer treatment

The non-neoplastic plasma cells constituted only a small proportion of total BM nucleated cells, assessed either by manual counting on BM aspirate smears or by FC analysis. The numbers of plasma cells were not different between Groups 1A and 1B. Immunophenotypically, the plasma cells were mostly positive for CD45 and CD19, but the expression of these markers was heterogeneous. CD45 was expressed by a median of 73% cells (range, 37%–100%) (table 1) and CD19 was expressed by a median of 79% cells (range, 52%–97%) (table 1) (one example: figure 1, lower row). The plasma cells were consistently negative for CD117. CD56 expression was observed in small subsets of plasma cells (median, 6% and up to 37%). CD20

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Table 1 Immunophenotypic comparisons of non-neoplastic plasma cells (obtained from treated and untreated bone marrows) with residual myeloma cells

	Group 1A (n = 12)	Group 1B (n = 38)	Group 2 (n = 59)	p Value*
Age (median, range)	58 (19–76)	57 (16–89)	63 (38–79)	p1=NS/p2=0.016
Gender (men:women)	8:4	23:15	32:27	NS
Plasma cells by counting (%; median, range)	1 (0–2)	1 (0–2)	2 (0–4)	p1=NS/p2<0.001
Plasma cells by FC (%; median, range)	0.05 (0.01–0.68)	0.07 (0.01–0.39)	0.43 (0.10–2.30)	p1=NS/p2<0.001
Plasma cell immunophenotype				
% Positive, median and range; positive cases using cut-off values				
CD45 (%)	63 (37–98)	78 (41–100)	42 (0–100)	p1=0.200/p2=0.001
Aberrant: ≤40% (aberrant cases (%))	1 (8%)	0 (0%)	26 (44%)	p3=0.000
CD19 (%)	86 (64–94)	79 (52–97)	0 (0–100)	p1=0.192/p2=0.000
Aberrant: ≤50% (aberrant cases (%))	0 (0%)	0 (0%)	54 (92%)	p3=0.000
CD56 (%)	6 (0–37)	6 (0–24)	84 (0–100)	p1=0.314/p2=0.000
Aberrant: ≥25% (aberrant cases (%))	2 (16%)	0 (0%)	43 (73%)	p3=0.000
CD28 (%)	4 (0–26)	15 (0–59)	20 (0–100)	p1=0.010/p2=0.396
Aberrant: ≥40% (aberrant cases (%))	0 (0%)	2 (5%)	21 (36%)	p3=0.000
CD117 (%)	0 (0–1)	0 (0–11)	12 (0–100)	p1=0.320/p2=0.000
Aberrant: ≥20% (aberrant cases (%))	0 (0%)	0 (0%)	25 (42%)	p3=0.000
CD20 (%)	4 (0–9)	4 (0–21)	0 (0–98)	p1=0.726/p2=0.049
Aberrant: ≥20% (aberrant cases (%))	0 (0%)	1 (3%)	13 (22%)	p3=0.000
Total number of aberrant markers				p1=0.141
0 (0–1)	1 (0–3)	3 (1–5)		p2=0.000
0	9	35	0	
1	3	3	4	
2	0	0	17	
3	0	0	26	
4	0	0	8	
5	0	0	4	
Myeloma cells as of total plasma cells	NA	NA	82 (20–100)%	

Group 1A: negative first lymphoma staging bone marrows, untreated; Group 1B: patients diagnosed with a non-plasma cell haematological malignancy who recently received various anticancer treatments; and Group 2: myeloma bone marrows, positive for minimal residual disease.

*P1: Group 1A versus Group 1B, Student t test; P2: Group 1 versus Group 2, Student t test; P3: Group 1 versus Group 2, Fisher's exact test using cut-offs based on receiver operator curve results to define 'positive' and 'negative' expression levels.

FC, flow cytometry.

expression was infrequent. Overall, these markers showed no differences in expression by non-neoplastic plasma cells between untreated (Group 1A) and treated (Group 1B) BM specimens. CD28 was expressed by plasma cells in a subset of cases (median, 11%; range 0%–59%) and was significantly higher in BMs of patients with various haematological malignancies status postcancer treatment (table 1) (4% in Group 1A vs 15% in Group 1B, $p=0.01$).

Immunophenotypic comparison of non-neoplastic plasma cells versus residual myeloma cells

The number of plasma cells was significantly higher in PCM patients even with minimal involvement by PCM (Group 2) than in Group 1 (Group 1A + Group 1B), either by microscopic counting ($p<0.001$) or by FCI enumeration ($p<0.001$) (table 1). The PCM cells represented a median of 82% (range, 20%–100%) of total plasma cells. Overall, when the percentage of the plasma cell population positive for each marker was assessed as numerical variables, the PCM cells showed differential expressions in CD45, CD19, CD117, CD20 and CD56 (table 1) (figure 3), but not CD28 ($p=0.396$), as compared with non-neoplastic plasma cells.

The numerical cut-off for each marker was determined by receiver operator curve (ROC) analysis based on the data from Groups 1 and 2, with the goal of achieving the best sensitivity and specificity for separating PCM cells from non-neoplastic plasma cells. These numbers were rounded up for practical use. For example, optimal cut-offs based on the ROC curves for CD45, CD19, CD56 and CD28 were 38% (rounded up to ≤40%), 52% (rounded up to ≤50%), 27% (rounded up to ≥25%) and 42%

(rounded up to ≥40%), respectively. The optimal cut-offs for CD20 and CD117 were 4% and 6%, respectively. In these instances, a conventional 20% cut-off was used to avoid over-calling a marker positive. Overall, if 'positive' was defined by the above cut-offs for each marker, PCM cells in Group 2 patients showed significantly altered expression of all markers assessed, compared with Group 1 ($p<0.001$ for each marker). PCM cells showed a median of three aberrancies (range, 1–5) whereas non-neoplastic plasma cells had a median of 0 aberrancies (range 0–1) (table 1). Among the 'aberrancies' in non-neoplastic plasma cells, lower expressions of CD45 and CD19, followed by increased CD28 expression, were the most frequently observed changes. When analysed by patterns, aberrant expression levels (either negative or positive) of CD20, CD28, CD56, CD117, CD19 and CD45 were rather uniform in PCM cells in a given case; all aberrancies were observed together in the same population of cells (figure 2, middle row). In contrast, the expression levels of 'aberrant markers' in non-neoplastic plasma cells were more heterogeneous (figure 2, upper row). For example, the CD56+ subset of non-neoplastic plasma cells was composed of CD19+ and CD19- cells, with CD19+CD56+ plasma cells outnumbering CD19-CD56+ plasma cells (a median of 68% and 32% in CD56+ subsets, respectively). Overall, the PCM cells in all cases showed an immunophenotypic pattern that effectively distinguished these cells from background polytypic plasma cells.

Cytoplasmic light chain assessment

Cytoplasmic κ/λ light chains were coanalysed with the surface markers CD45, CD38, CD138 and CD20. Both plasma cells and

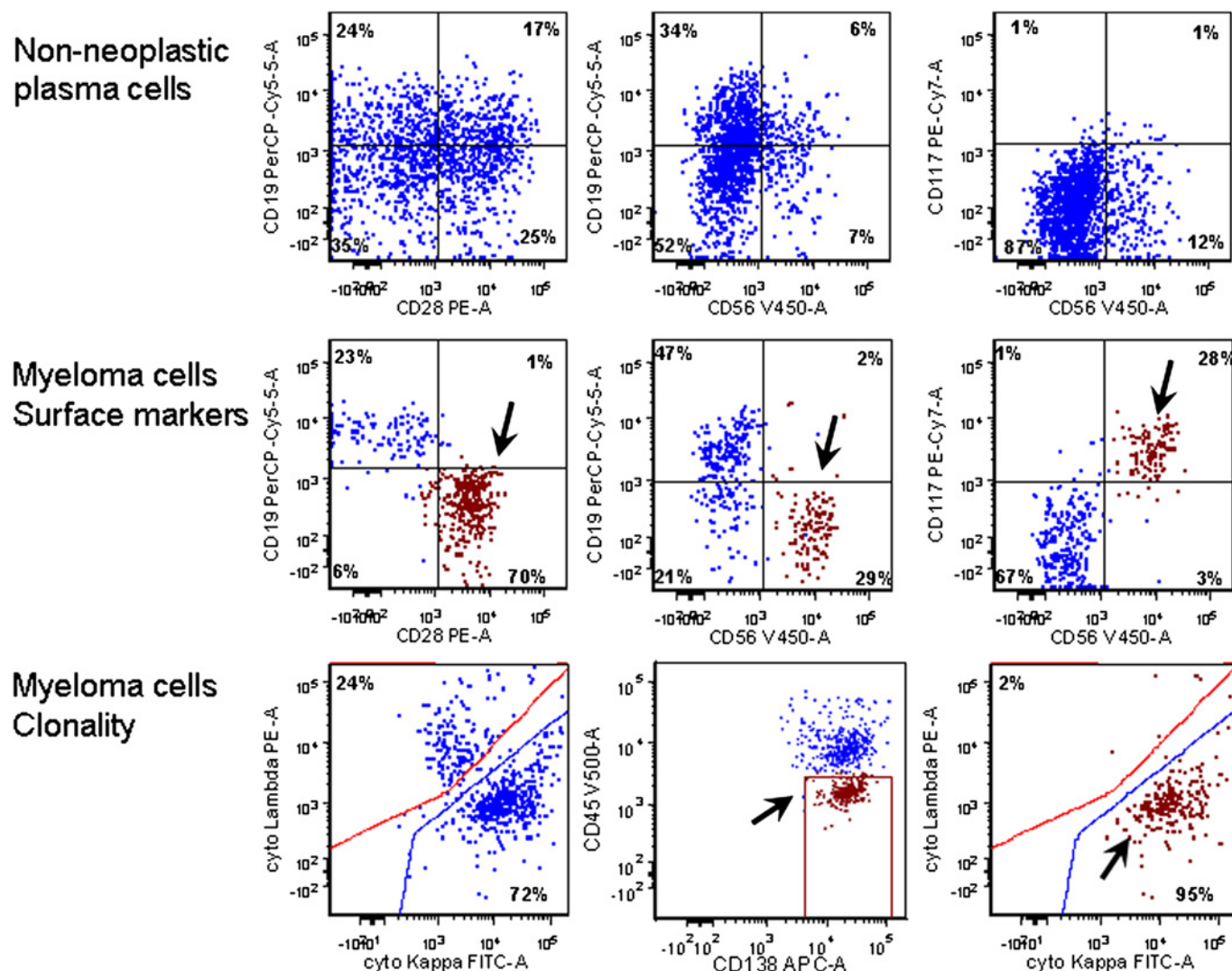


Figure 3 Immunophenotypic comparisons of non-neoplastic plasma cells (control, Group 1) and residual myeloma cells (myeloma, Group 2). Each symbol represents a case, and is expressed as % of plasma cells with positive expression compared with the negative controls.

lymphocytes were assessed. When plasma cells were analysed in total, PCM cells demonstrated cytoplasmic light chain restriction or excess in 51 cases. In 8 (14%) additional cases, clonality was only revealed when gating on CD45 dim/negative plasma cells, instead of the entire plasma cell population (figure 2, lower row).

DISCUSSION

In this study, we show that immunophenotypic heterogeneity exists within non-neoplastic plasma cells and that these variations seem to be more pronounced in the postcancer therapy setting. However, these aberrancies in non-neoplastic plasma cells differ from those of PCM cells.

In non-neoplastic plasma cells from lymphoma staging BM specimens, that is, 'normal' plasma cells, CD19 and CD45 expression levels are heterogeneous, with variable intensity. However, although a proportion of the plasma cells fell into the 'negative' quadrant, the expression patterns showed a spectrum rather than forming a discrete subset distinct from the main plasma cell population. The variations in CD19 and CD45 expression were observed similarly in non-neoplastic plasma cells derived from BM specimens obtained from patients with various non-plasma cell malignancies post-therapy, indicating

that the heterogeneity of CD45 and CD19 expression is intrinsic rather than a 'reactive' or 'acquired' change in normal plasma cells. In contrast, CD117 and CD20 were consistently negative in non-neoplastic plasma cells (Group 1A), and did not seem altered by treatment (Group 1B). CD56 can be observed in a subset of non-neoplastic plasma cells, up to 37%, in our series. Unlike CD56 expression on BM myeloid and monocytic cells that often increases after chemotherapy or administration of growth factors,¹⁹ the expression of CD56 in non-neoplastic plasma cells does not appear to be different between treated (Group 1B) or untreated patients (Group 1A). CD28 is an important antigen for T cell activation via its ligand CD86 by aiding in B and T cell interaction. CD28 was initially found to be positive in several PCM cell lines,²⁰ and later reported to be expressed in 15%–45% PCM cases.^{1, 21} Expression of CD28 by PCM cells is associated with a high tumour proliferation index, disease relapse and advanced stage,²² and therefore is considered as a poor prognostic marker.¹ CD28 was described as absent in normal plasma cells, based on an earlier study conducted in eight healthy donor BMs and two tonsil specimens.²³ CD28 expression, however, could be induced during B cell differentiation into plasma cells.²⁴ In our series in which a large number of non-neoplastic plasma cells were assessed, expression of CD28 was

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variably observed in non-neoplastic plasma cells, and expression seems to be induced or increased following cancer therapy. Interestingly, a recent study showed that the expression of CD28 on PCM cells and CD80/CD86 on dendritic cells is important for the interaction between PCM cells and the BM microenvironment and that these interactions may closely mirror interactions that support the survival of normal long-lived normal plasma cells in the BM.²⁵

Recently, Peceliunas and colleagues studied 11 BM specimens obtained from patients with Hodgkin's lymphoma and observed similar immunophenotypic variations in plasma cells.¹⁷ They concluded that CD19, CD56, CD38, CD45 and CD20 expression characteristics were of little value in discriminating PCM cells from immunophenotypically atypical non-clonal plasma cells, challenging the approach of using surface marker immunophenotyping without assessing κ/λ clonality in detection of PCM MRD. In our study, we tested all the markers recommended by the European Myeloma Network¹ except for CD27, since the loss of CD27 expression almost always is coupled with CD19-negativity in PCM.²¹ Among these, CD28 and CD117 were valuable additional markers which were not tested by Peceliunas and colleagues.¹⁷ In addition, 8-colour rather than 6-colour flow cytometry used in our study allowed us to better assess these markers in the same plasma cell population. In order to show the quantitative difference, we applied ROC in assessing each marker, and we showed differential expression between non-neoplastic plasma cells and PCM cells in all the markers assessed, including CD28. It is noteworthy that, in this study, the ROC cut-offs were calculated in order to demonstrate the quantitative differences between PCM and reactive plasma cells. If of interest in clinical use, they need to be validated and tailored for each laboratory. Nevertheless, we show that despite the presence of immunophenotypic overlaps, FC immunophenotyping can reliably distinguish PCM from background polytypic plasma cells. The differences between PCM cells versus non-neoplastic plasma cells with immunophenotypic variations can be summarised as follows: (1) Aberrant expression of these markers is often more intense and more uniform in all the PCM cells, in contrast with a heterogeneous spectrum of expression in non-neoplastic plasma cells; (2) PCM cells exhibit a higher number of aberrant markers, with a median of 3, in contrast with non-neoplastic plasma cells with a median of 0; and (3) Aberrant markers are coexpressed in the same cell population in PCM, in contrast with non-neoplastic plasma cells in which the immunophenotypic variations are not consistent in the same subsets of cells. For example, as we confirmed that CD56 could express in a subset of non-neoplastic plasma cells in some cases as described by others,^{14–17} we also found that CD56+ subset of non-neoplastic plasma cells was often composed of CD19+CD56+ and CD19-CD56+ cells, similar to that described by others.¹⁷ It is noteworthy that we detected κ/λ clonality in all 59 cases, by gating on all plasma cells as well as CD45-dim/negative plasma cells. The technical advances of 8-colour flow cytometry allow simultaneous detection of surface immunophenotype and cytoplasmic light chains, and this approach increases the sensitivity of clonality assessment and provides greater assurance in detection of MRD. The usefulness of clonality assessment by multicolour flow cytometry, however, will need to be further tested in MRD-negative cases, as well as cases with a much lower per cent of neoplastic plasma cells.

In summary, in this study, we compared minimal residual neoplastic plasma cells with background normal plasma cells, and extended this comparison with a broader spectrum of reactive conditions (various cancer treatments). We show that

Take-home messages

Immunophenotypic variations exist in non-neoplastic plasma cells which partially overlap the aberrant phenotype of plasma cell myeloma (PCM), but these altered expression patterns differ from PCM cells in intensity, uniformity and number of alterations per case. Using most of the markers recommended by the European Myeloma Network and taking advantage of multicolour (8-colour) flow cytometry, PCM cells can be consistently and reliably discriminated from background polytypic plasma cells. Simultaneously assessing κ/λ light chain in conjunction with surface markers can further increase diagnostic accuracy.

immunophenotypic variations exist in non-neoplastic plasma cells which partially overlap the aberrant phenotype of PCM, but these altered expression patterns differ from PCM cells in intensity, uniformity and number of alterations per case. Using most of the markers recommended by the European Myeloma Network and taking the advantage of multicolour (8-colour) flow cytometry, PCM cells can be consistently and reliably discriminated from background polytypic plasma cells in our MRD positive cases. Simultaneously assessing κ/λ light chain in conjunction with surface markers can further increase diagnostic accuracy.

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Contributors DL: performed the experiment and was involved in manuscript writing; LJM, LJL: design of the study and proofreading the manuscript; YH, YZ, GT, LP: participated in the experiment, sample collection and data analysis; SAW: study design, manuscript writing and data analysis and responsible for submission.

Competing interests None.

Patient consent Obtained.

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