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ORIGINAL ARTICLE

Flow Cytometric Evaluation of Traditional and Novel Surface Markers for the Diagnosis of Plasma Cell Dyscrasias

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Abstract Increasing interest has been expressed for flow cytometric immunophenotyping for diagnosis and monitoring in plasma cell dyscrasias over the last decades. The aim of this investigation was to compare the expression strength of various cell surface markers used traditionally or currently under investigation on normal and abnormal PC populations. We enrolled 295 consecutive patients undergoing bone marrow aspiration in the workup of monoclonal gammopathies, selecting 54 normal and 241 abnormal PC populations via flow cytometry to characterize the expression of CD45, CD38, CD138, CD19, CD56, CD20, CD27, CD28, CD81, CD117 and CD200 on the cell surface of PCs. We observed significant differences in the expression strength of all assessed markers between normal and abnormal PC populations in all markers except for CD20. While none of them was conclusive on its own, the combination of CD81 positivity and CD117 negativity was present in 98.1% of normal PC populations tested. In contrast, particularly CD117 positivity, but also CD81 negativity was indicative of an abnormal PC phenotype. Our results highlight the descriptive value of CD81 and CD117 for the allocation of bone marrow PCs to a normal or abnormal phenotype.

Keywords Multiple myeloma · Plasma cell dyscrasias · Plasma cells · FACS · Flow cytometry

Abbreviations

mAbs	Monoclonal antibodies
MGUS	Monoclonal gammopathy of undetermined significance
PC	Plasma cell
NK cell	Natural killer cell

Introduction

Multiple myeloma is a clonal plasma cell (PC) malignancy leading to anemia, skeletal lesions, renal failure and hypercalcemia [1–4]. It accounts for approximately 13% of neoplastic hematologic disorders and for 1% of all malignant diseases [5]. A multistep development is assumed [3–7]. A "monoclonal gammopathy of undetermined significance" (MGUS) is present in over 3% of the population above 50 years of age and in some cases develops further into a smoldering multiple myeloma which ultimately leads to the development of a symptomatic multiple myeloma or a PC leukemia [2, 4, 6, 7]. All these disorders are traditionally referred to as PC dyscrasias [8].

The diagnosis of multiple myeloma is based on laboratory-chemical and radiological parameters [2]. However, over the last decades a lot of research has been undertaken to reveal the diagnostic and prognostic value of flow

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cytometric analysis in monoclonal gammopathies [4, 9]. Notably, flow cytometry has been reported to be more sensitive than histology in detecting small monoclonal PC populations, particularly if these coexist with polyclonal populations [10]. A combined staining for cluster of differentiation (CD) 38, CD138 and CD45 for clear identification of PCs has been suggested by the European Myeloma Network [11]. Phenotypically normal PCs have been described to have weak or moderate expression of CD19, but not CD56 on their cell surface (CD19+/−, CD56−) [12–14]. In contrast, malignant PCs lack expression of CD19, whereas expression of CD56 can be detected in the majority of multiple myeloma cases (CD19−/CD56+). On the basis of these findings, it could be shown that in the bone marrow of MGUS patients both, normal and malignant PCs are present [13, 15]. A marker usually missing on normal PCs, but found in distinct subpopulations of myeloma cells is CD20 [15–17]. Referring to existing literature, expression of CD27, CD28, and CD117 is heterogeneous in monoclonal gammopathies [13, 18–24]. CD81 and CD200 which are of interest for the detection of minimal residual disease were included in this investigation as well [25, 26]. Flow cytometry is nowadays considered a powerful tool for the evaluation of therapy responsiveness and monitoring of minimal residual disease in PC dyscrasias [27].

The aim of this study was to better characterize the expression of traditional (CD45, CD38, CD138, CD19, and CD56) and novel surface markers (CD20, CD27, CD28, CD81, CD117 and CD200) on normal and abnormal PCs for their descriptive value in PC dyscrasias.

Materials and Methods

Patient Recruitment and Inclusion Criteria

The sole inclusion criterion for this study was a bone marrow aspiration for flow cytometric immunophenotyping in the workup of monoclonal gammopathies (patients in whom a monoclonal gammopathy was suspected the first time), or suspected progression of diagnosed multiple myeloma. A total of 295 consecutive patients admitted to the Department of Medicine and Medical Oncology, Wilhelminen Hospital, Vienna, Austria, or the Department of Internal Medicine, Hospital of The Order of the Brothers Hospitallers, Vienna, Austria, meeting this criterion were recruited between September 2009 and July 2013. All patients gave their informed consent for use of those specimens for scientific investigations. In these cases the need for an ethics vote was waived by the local ethics committee (Ethikkomission der Stadt Wien).

Flow Cytometric Immunophenotyping

The staining protocol and gating strategy used in this study is well established at our department and routinely used for the workup of suspected plasma cell dyscrasias as well as for follow up of diagnosed multiple myeloma. A total number of 80,000 events was collected on the flow cytometer for each test tube.

Two different flow cytometry antibody panels were used for bone marrow aspirates according to whether multiple myeloma was suspected initially or the course of disease was documented. Six test tubes were used in all follow-up examinations of MGUS and multiple myeloma (Table 1A and Fig. 1). Another 3 test tubes served to rule out the presence of abnormal B cells in bone marrow samples of those patients for whom a monoclonal gammopathy was suspected first-time. In these cases, phycoerythrin (PE)-labelled monoclonal antibodies (mAbs) for CD5 (Beckman Coulter, Brea, U.S.), fluorescein isothiocyanate (FITC)-labelled mAbs for CD10 (Beckman Coulter, Brea, U.S.) and FITC-labelled polyclonal rabbit anti-human Kappa and Lamda mAbs (both Dako, Santa Clara, U.S.) were used in addition.

Sample Preparation

Samples were analysed within 24 h. Lysis of erythrocytes and erythrocyte precursors in 3 ml bone marrow was performed by adding 50 ml ammonium chloride and incubation for 12 min at room temperature. Cells were washed twice with phosphate buffered saline (PBS) and then resuspended in 1.5 ml PBS-bovine serum albumin (BSA) with 200 µl new-born calf serum.

Staining Procedure

A volume of 50 µl of antibody cocktail was added to each of the 6 test tubes consisting of FITC-labelled mAb for CD27, CD28, CD38 and CD81 (all BD Biosciences), PE-labelled mAb for CD20, CD56, CD117, CD138, CD200 (all BD Biosciences, New Jersey, U.S.). Peridininchlorphyll protein (PerCP)-labelled mAb was used for CD45 (BD Biosciences, New Jersey, U.S.) and allophycocyanin (APC)-labelled mAb for CD19 and CD38 (both BD Biosciences, New Jersey, U.S.). 50 µl of bone marrow cell suspension were then added to each test tube and samples incubated for 30 min at 4 °C before cells were washed and resuspended in 0.5 ml PBS with 1% paraformaldehyde. Unstained controls were used in order to determine autofluorescence of cells, as well as isotype control antibodies to avoid falsification of results due to unspecific binding via the Fc-region.

Table 1 Antibody panels in individual tubes and classification of expression strength for each channel used in this investigation

Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
(A) Tubes 2–6 were used to stain bone marrow aspirates. Tube 1 of our protocol (not shown) was utilized to stain whole blood samples and is not part of this investigation					
CD45 PerCP	CD45 PerCP	CD45 PerCP	CD45 PerCP	CD45 PerCP	CD45 PerCP
CD38 APC	CD38 APC	CD38 APC	CD38 APC	CD38 APC	CD38 FITC
FITC isotype	CD19 FITC	CD28 FITC	CD27 FITC	CD81 FITC	CD19 APC
PE isotype	CD56 PE	CD117 PE	CD20 PE	CD138 PE	CD200 PE

Expression strength		Location of population center			
		PerCP	APC	FITC[a]	PE[a]
(B) Expression strength was assessed based on the location of the population's center in the according flow plot					
Negative	–	$< 10^3$	$< 10^3$	$< 10^3$–10^4	$< 10^3$
Weak	−/+	10^3	10^3	10^3–10^4	10^3
Moderate	+	10^3–10^4	10^3–10^4	10^4	10^3–10^4
Strong	+/++	10^4	10^4	10^4–10^5	10^4
Very strong	++	10^4–10^5	10^4–10^5	10^5	10^4–10^5

[a]Borders between negative and weak expression were individually determined based on the isotype control

Data acquisition and analysis was performed on a BD FACSCalibur™ or a FACSCanto II™ flow cytometer (both BD Biosciences, New Jersey, U.S.) using CellQuest and FACS Diva v8.0.1 Software (BD Bioscience, New Jersey, U.S.), respectively. The staining and analysis procedure was identical for all enrolled patient.

Determination of Expression Strength

Strength of marker expression was divided into negative, weak, moderate, strong and very strong based on where the center of the population was located in the flow plot (Table 1B). Negative expression was defined as a signal strength that did not exceed that of the isotype control. Weak was chosen when the population center sat right where the isotype control ended. Expression strength detected in the first decade over the isotype control was described as moderate, and between the first and second decade (with reference to the isotype control) as strong. Populations appearing even higher (within the second decade from the isotype control) were defined as very strong.

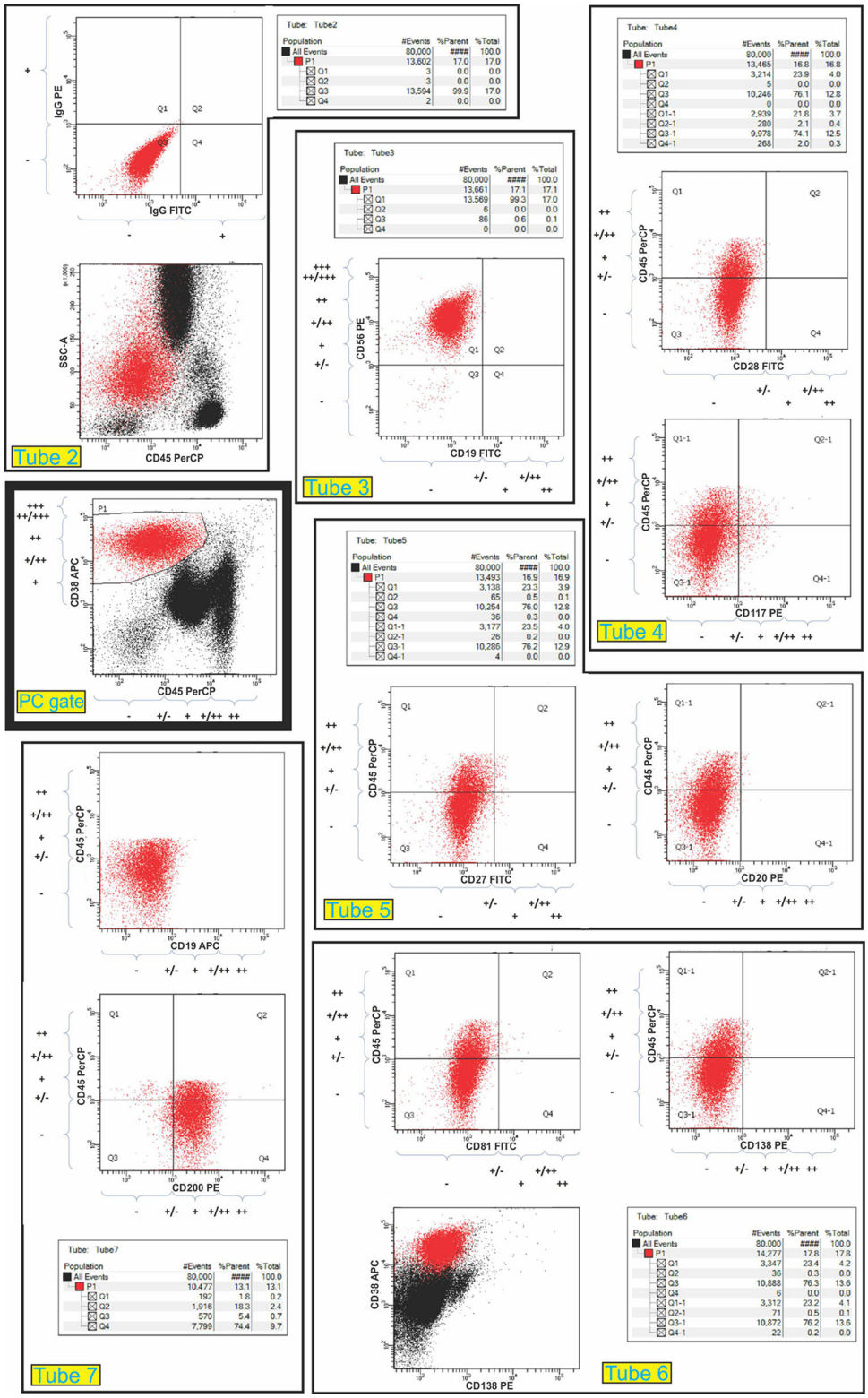
Gating Strategy

As suggested in literature combined staining for CD45, CD38, CD19 and CD56 was used for the identification and characterisation of PCs [12–14]. PCs were defined by their dim CD45 expression and CD38 positivity and considered normal if showing weak or moderate CD19 expression and negative or weak CD56 expression. PCs were classified abnormal by CD19 downregulation or upregulation of CD56. Debris was excluded from the analysis in the FSC/SSC plot and hematogenous were discriminated from PCs by their higher CD19 expression. PCs were further distinguished from similarly CD38 positive NK cells either by negative or dim expression of CD45 or the PC-specific marker CD138 in case they were also positive for CD45 [9, 28].

Based on the described expression pattern we divided the study population in a normal group, where only normal PCs were described and an abnormal group, in which the abnormal PC population was characterized. After identification of normal or abnormal PC populations expression strength of CD20, CD27, CD28, CD81, CD117, CD138 and CD200 was determined (Fig. 1). These markers were plotted against CD45 to allow distinction of expression strength in the normal and abnormal population in cases these coexisted in the bone marrow aspirate (Fig. 2).

Statistics

Statistical analysis was performed using the GENMOD procedure in SAS 9.4 to fit a cumulative logit model to the ordinal expression data. The likelihood ratio test was used for comparison of the two groups (normal vs abnormal).


Tube 2
IgG PE
IgG FITC
SSC-A
CD45 PerCP
Tube 3
CD56 PE
CD19 FITC
Tube 4
CD45 PerCP
CD28 FITC
CD117 PE
PC gate
CD38 APC
CD45 PerCP
Tube 5
CD27 FITC
CD20 PE
Tube 7
CD19 APC
CD200 PE
Tube 6
CD81 FITC
CD138 PE
CD38 APC
CD138 PE

◀**Fig. 1** Gating strategy used in this investigation. A total number of 80,000 events was collected on the flow cytometer for each test tube. The actual number of PCs analyzed was dependent on the disease status of the individual patient. *PC gate* Plasma cells (PCs) (red) were identified by their dim expression of CD45 and CD38 positivity in all tubes presented. *Tube 2* Bone marrow aspirates were stained with CD45-PerCP, CD38-APC and two isotype controls for the FITC and PE channels. The typical localisation of PCs in the CD45/SSC plot with negative or weak expression of CD45 and SSC medium is illustrated. The isotype controls were used to distinguish between negative and positive expression in the FITC and PE channels. *Tube 3* Bone marrow aspirates were stained with CD45-PerCP, CD38-APC, CD19-FITC and CD56-PE fluorescent antibodies. PCs were then characterized for their expression of CD19 (FITC) and CD56 (PE). In this example the negative (−) expression of CD19 and strong (+/++) expression of CD56 indicate an abnormal PC population. *Tube 4* Bone marrow aspirates were stained with CD45-PerCP, CD38-APC, CD28-FITC and CD117-PE fluorescent antibodies. PCs were then characterized for their expression of CD28 (FITC) and CD117 (PE). Both markers were plotted against CD45 to compare their expression strength on normal (usually CD45+) and abnormal (usually CD45− or −/+) PC populations in cases these coexisted. In this example both CD28 and CD117 expression are negative (−). *Tube 5* Bone marrow aspirates were stained with CD45-PerCP, CD38-APC CD27-FITC and CD20-PE fluorescent antibodies. PCs were then characterized for their expression of CD27 (FITC) and CD20 (PE). Both markers were plotted against CD45 to compare their expression strength on normal (usually CD45+) and abnormal (usually CD45− or −/+) PC populations in cases these coexisted. In this example both CD27 and CD20 expression are negative (−). *Tube 6* Bone marrow aspirates were stained with CD45-PerCP, CD38-APC CD81-FITC and CD138-PE fluorescent antibodies. PCs were then characterized for their expression of CD81 (FITC) and CD138 (PE). Both markers were plotted against CD45 to compare their expression strength on normal (usually CD45+) and abnormal (usually CD45− or −/+) PC populations in cases these coexisted. In this example both CD81 and CD138 expression are negative (−). The stronger expression of CD138 on CD38-positive cells (red) relative to the main CD38-negative population confirms the correct localisation of the PC gate (P1) and rules out the presence of other CD38 positive cells (such as stem cells) in this gate. *Tube 7* Bone marrow aspirates were stained with CD45-PerCP, CD38-FITC CD19-APC and CD200-PE fluorescent antibodies. CD19 expression had already been assessed in tube 2 in the FITC channel. However, since PCs are strongly auto-fluorescent we validated CD19 expression in the APC channel in which auto-fluorescence is less prominent than in the FITC channel. Negative (−) CD19 expression as described in tube 2 was here confirmed. PCs were then characterized for their expression of CD200 (PE). It was plotted against CD45 to compare their expression strength on normal (CD45+) and abnormal (CD45− or −/+) PC populations in cases these coexisted. In this example CD200 is moderately expressed (+). "Tube 1" is not displayed, as this was used for staining of whole blood samples, and was not part of the bone marrow analysis. The flow cytometry dot plots have been generated with BD FACSDiva v8.0.1 (color figure online)

Results

Demographic Data

Out of the 295 bone marrow aspirates analyzed in 54 a normal PC population was described, and in 241 an abnormal population. The demographic information and diagnoses are displayed in Table 2.

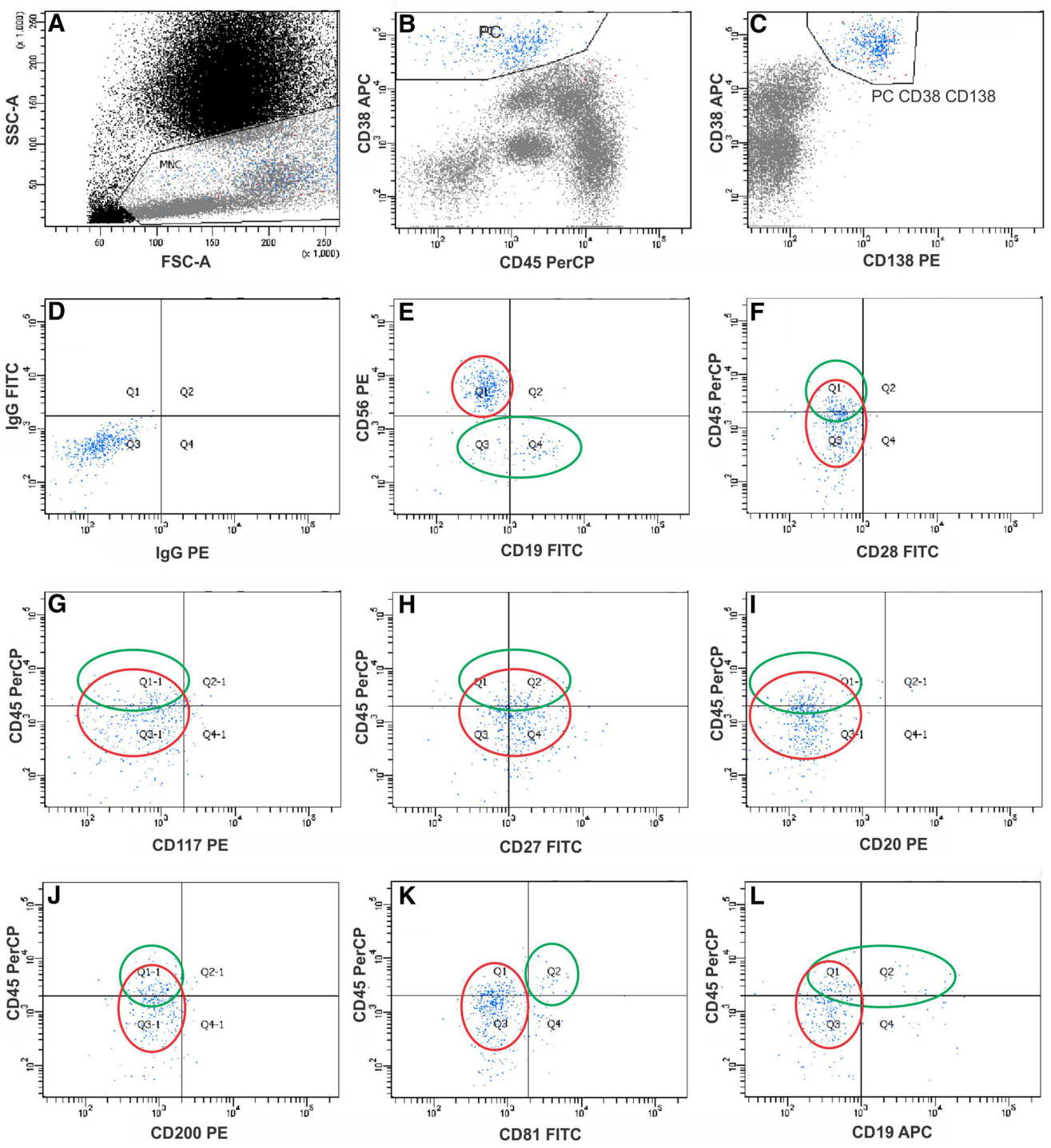
Marker Expression

The individual marker expression was determined in normal and abnormal PC populations and the proportional distribution across expression strength categories evaluated. We found a highly significant difference between groups in all of the assessed markers, whereby CD56, CD138, CD20, CD28, CD117 and CD200 were upregulated, and CD19, CD38, CD45, CD27 and CD81 were downregulated in abnormal PC populations. A summary of the obtained results is provided in Table 3. The combination of CD81 positivity and CD117 negativity was present in almost all (98.1%) normal PC populations tested but also in 17% of abnormal PC populations (Online Resource 1). CD81 negativity or CD117 positivity, or both, was present in a large percentage (83.0%) of abnormal PC populations, but in $< 2\%$ of normal PC populations. Importantly, after removing 13 patients (3 in the normal and 10 in the abnormal group) with unknown final diagnosis (Table 2) the obtained results well resemble results including these patients (Online Resource 2 + 3).

Discussion

While many studies have been performed to reveal ideal antibody panels for the discrimination between normal and malignant PCs, the comparison of results is limited due to differences in methodology, such as number and selection of evaluated markers, antibody clones and fluorochromes, staining protocols and gating strategies as well as instrument configurations [27, 29, 30].

We used CD45, CD38, and CD138 to identify PCs and CD19 and CD56 to distinguish between normal and abnormal PCs according to existing literature [4, 13, 15, 17, 20, 24, 31–33]. Categorizing cells via their immune phenotype allowed us to perform group allocation of PCs to "normal" or "abnormal" despite the hemodilution frequently observed in bone marrow aspirates, with PCs often not accounting for more than 2% of cells. Given the specificity of this marker combination [12–14] and the robustness of the immune phenotype also for very small PC populations, we refrained from an additional cytoplasmic stain for kappa and lambda light chains. We nevertheless acknowledge the lack of cytoplasmic light chains for the determination of clonality as a limitation of this investigation. To ensure optimal sample quality all specimens were processed within 24 h and stored and transported at 4 °C until processing. The expression of further markers was then assessed, and results are discussed here.


A
SSC-A
FSC-A
MNC
B
PC
CD38 APC
CD45 PerCP
C
PC CD38 CD138
CD38 APC
CD138 PE
D
IgG FITC
IgG PE
Q1
Q2
Q3
Q4
E
CD56 PE
CD19 FITC
F
CD45 PerCP
CD28 FITC
G
CD45 PerCP
CD117 PE
Q1-1
Q2-1
Q3-1
Q4-1
H
CD45 PerCP
CD27 FITC
I
CD45 PerCP
CD20 PE
J
CD45 PerCP
CD200 PE
K
CD45 PerCP
CD81 FITC
L
CD45 PerCP
CD19 APC

◀**Fig. 2** Distinction of marker expression between coexisting normal and abnormal PC populations. This figure illustrates an example in which a normal (green) and an abnormal (red) PC population coexist. The presented bone marrow aspirate has been processed as demonstrated in Fig. 1 and selected flow plots are displayed **a** Mononuclear cells (MNC) were identified within the FSC/SSC plot. **b** Within the MNC population, PCs were identified in the CD38/CD45 plot. **c** PC identification was further confirmed in the CD38/CD138 plot. **d** Isotype control for FITC and PE. **e** Within the PC population, a CD19+/−/CD56− normal (green) and a CD19−/CD56+ abnormal (red) population was found. **f** In this example CD28 expression was negative (−) in both, CD45+ normal and CD45+/− abnormal cells. **g** CD117 expression was negative (−) in both, CD45+ normal and CD45+/− abnormal cells. **h** CD27 expression was weak (+/−) in both, CD45+ normal and CD45+/− abnormal cells. **i** CD20 expression was negative (−) in both, CD45+ normal and CD45+/− abnormal cells. **j** CD200 expression was negative (−) in both, CD45+ normal and CD45+/− abnormal cells. **k** CD81 expression was moderate (+) in CD45+ normal, but negative (−) in CD45+/− abnormal cells. **l** Negative (−) CD19 expression in CD45+/− abnormal PCs and weak (+/−) CD19 in CD45+ normal PCs was here confirmed in the APC channel. The flow cytometry dot plots have been generated with BD FACSDiva v8.0.1 (color figure online)

Normal PCs showed high expression of CD27, and in MGUS CD27 expression is still significantly higher, than in MM, referring to previous studies [18, 19]. Our data confirm CD27 expression in the large majority (98.2%) of normal PC populations, whereas expression in abnormal populations was negative in 49.8%. Loss of CD27 was found to be associated with disease progression or relapse, and heterogeneity of CD27 expression in MM might be due to stage-dependent strength of expression [18, 19, 34].

In the normal populations of this study all PCs were negative for CD28. Lacking expression of CD28 in normal PCs has been reported before [13, 20, 21]. Pellat-Deceunynck et al. [20] described CD28 expression in 63% of patients in the accelerated phase of multiple myeloma, but none in patients in the chronic phase. In our abnormal populations 5.8% showed moderate and 5.4% weak expression of CD28.

Referring to previously performed studies, CD117 cannot be detected on normal PCs [13, 22, 23, 35]. Accordingly, all normal populations in our study displayed negative CD117 expression on PCs. Hence, positive expression of CD117 was clearly associated with an abnormal phenotype and was present in a little < 50% of aberrant populations. Other research groups found

Table 2 Characteristics and diagnoses of the study population

Patient characteristics and diagnoses	
Patients undergoing routine bone marrow aspiration in the workup of monoclonal gammopathies or suspected progression of diagnosed multiple myeloma (n = 295)	
Patients for which a normal PC population was described with flow cytometry (n = 54)	
Male (n = 32)	Female (n = 22)
Mean age: 67.8 years	Mean age: 65.6 years
MGUS: 30	
MM:8	
MM, complete remission: 1	
Excluded MM: 3	
AML: 1	
CLL, IgM paraproteinemia: 1	
NHL: 1	
Bone marrow carcinosis: 1	
Osteomyelitis: 1	
Paraproteinemia IgM kappa: 1	
Hepatitis, polyclonal IgG expansion: 1	
Reactive bone marrow: 2	
Final diagnosis unknown: 3	
Patients for which an abnormal PC population was described with flow cytometry (n = 241)	
Male (n = 120)	Female (n = 121)
Mean age: 66.5 years	Mean age: 68 years
MGUS: 63	
Smoldering MM: 6	
MM: 158	
PC leukemia: 2	
Amyloidosis (incidental abnormal PC population): 1	
B-NHL(incidental abnormal PC population): 1	
Final diagnosis unknown: 10	

AML Acute myeloid leukemia, *CLL* chromic lymphatic leukemia, *MGUS* monoclonal gammopathy of undetermined significance, *MM* multiple myeloma, *NHL* non-Hodgkin lymphoma

We observed negative CD20 expression in all normal, and also the majority of abnormal populations. However, in abnormal populations 5% show weak to moderate expression. Those findings correspond approximately with that of previously performed studies [15–17, 24].

Table 3 Expression strength of investigated markers on plasma cells (PCs) in normal and abnormal PC populations obtained from bone marrow aspirates (n = 295)

Marker	Populations	Expression					*p* value
		Negative	Weak	Moderate	Strong	Very strong	
CD19	Normal		66.7% (36)	33.3% (18)			
	Abnormal	94.6% (228)	5.4% (13)				< 0.0001
CD56	Normal	79.6% (43)	20.4% (11)				
	Abnormal	29.5% (71)	5.4% (13)	18.7% (45)	46.5% (112)		< 0.0001
CD38	Normal				27.8% (15)	72.3% (39)	
	Abnormal			1.7% (4)	58.5% (141)	39.8% (96)	< 0.0001
CD138	Normal	5.6% (3)	42.6% (23)	50% (27)	1.9% (1)		
	Abnormal	8.7% (21)	23.7% (57)	59.8% (144)	7.9% (19)		< 0.0419
CD45	Normal	5.6% (3)	38.9% (21)	48.1% (26)	7.5% (4)		
	Abnormal	66% (159)	22.8% (55)	10% (24)	1.2% (3)		< 0.0001
CD20	Normal	100% (54)					
	Abnormal	95% (229)	2.9% (7)	2.1% (5)			0.0259
CD27	Normal	1.9% (1)	9.3% (5)	88.9% (48)			
	Abnormal	49.8% (120)	28.2% (68)	22% (53)			< 0.0001
CD28	Normal	100% (54)					
	Abnormal	88.8% (214)	5.4% (13)	5.8% (14)			0.0007
CD117	Normal	100% (54)					
	Abnormal	50.6% (122)	22% (53)	26.1% (63)	1.2% (3)		< 0.0001
CD81	Normal	1.9% (1)	3.7% (2)	94.4% (51)			
	Abnormal	70.1% (169)	11.2% (27)	18.3% (44)	0.4% (1)		< 0.0001
CD200	Normal	87% (47)	13% (7)				
	Abnormal	32% (77)	24.5% (59)	41.1% (99)	2.5% (6)		< 0.0001

The expression strength for each marker was compared between normal and abnormal PC populations, based on the allocation to the various expression categories (i.e. the proportions falling into negative, weak, moderate, strong, very strong). Statistical analysis was performed using the GENMOD procedure in SAS 9.4 to fit a cumulative logit model to the ordinal expression data. The likelihood ratio test was used for comparison of the two groups (normal vs abnormal)

comparable results in monoclonal gammopathies. Positive CD117 expression was stated with 50% for MGUS [23], and with 27–35.2% for multiple myeloma cases [13, 22–24]. Notably, CD117 positivity in multiple myeloma seems to be associated with improved overall survival [23].

CD81 seems to be useful as adverse prognostic marker in this disorder [26]. In multiple myeloma CD81 is often underexpressed [36, 37]. Literature suggests positive expression in multiple myeloma in 13–45% of cases [26, 38], and in 20% of MGUS cases [38]. In the abnormal populations of this study CD81 expression was positive only in 29.9%, whereas in the normal populations weak to moderate expression could be observed in almost all patients (98.1%). Our results therefore confirm those of previously performed studies and suggest the downregulation of CD81 to be of diagnostic use in PC dyscrasias. A recent investigation on 59 monoclonal gammopathy patients even demonstrated negative or dim CD81 expression in abnormal PC populations in 95% of cases, whereas in normal PC populations missing or weak expression of CD81 was not detected at all [39].

CD200 is not positively expressed on normal PCs [35, 40]. We found negative expression in 87%, and weak expression in the rest of the normal populations. In literature expression in monoclonal gammopathies is stated with 54.1% in MGUS [41], and with 61.8–78% in multiple myeloma [25, 40–42]. In the aberrant populations of this study 68.1% of patients showed weak, moderate or strong expression on PCs, clearly linking CD200 expression with an abnormal PC phenotype.

In summary, we provide further information obtained from a large patient collective on the heterogeneous observations reported regarding the expression pattern of surface markers on abnormal PCs in PC dyscrasias. We identified PCs in the bone marrow using CD45, CD38, and CD138 and distinguished between normal and abnormal PCs based on their expression of CD19 and CD56 to then study the expression strength of CD20, CD27, CD28, CD81, CD117 and CD200 on the respective normal and abnormal PC populations. Although significant differences

with respect to expression strength was evident in all markers tested, not one of them was conclusive on its own. The combination of CD81 positivity and CD117 negativity was present in virtually all normal PC populations tested. CD81 negativity or CD117 positivity, or both, was present in a large percentage (83.0%) of abnormal PC populations, but in $< 2\%$ of normal PC populations. Hence, these markers provide valuable additional information in cases where other markers do not allow a clear characterization of the phenotype, particularly when CD19 cannot be clearly assigned to a distinct expression category (e.g. if no isotype control is used).

Compliance with Ethical Standards

Conflict of interest The authors declare they have no conflict of interest with respect to the present manuscript.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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