VALIDATION OF A SINGLE 10-COLORS TUBE FACILITATING DISCRIMINATION OF MONOCLONAL FROM POLYCLONAL PLASMA CELLS IN MGUS AND INDOLENT MYELOMA

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INTRODUCTION

Monoclonal gammopathy of uncertain significance (MGUS) and smoldering multiple myeloma (SMM) are plasma cell disorders with a risk of progression to myeloma of approximately 1% and 10% per year. Smoldering multiple myeloma (SMM) is a heterogeneous disease. The percentage (%) of clonal Plasma Cells (PCs) in Bone Marrow PC population determining by MFC (Multiparameter Flow Cytometry), when >95%, is a progression risk-factor of both MGUS and SMM [1]. The goal of present work is to validate a 10-Colors tube to discriminate monoclonal from polyclonal PCs to calculate this %.

METHODS

Bone marrow aspirate samples from 8 patients with PC disorder (5 MGUS, 1 SMM and 2 MM) and from 3 patients with reactive plasmocytosis were analyzed on Navios® (Beckman Coulter-BC), using a novel 10-markers tube: CD138-PE.CF594, CD38-BV421 (BD Horizon), CD20-PC5.5, CD27-PC7, CD56-APC, CD19-AA700, CD117-AA750, CD45-KO (BC) and cytoplasmic κ-FITC and λ-PE (Dako) (Figure 1). The effectiveness of this tube to discriminate PCs populations is assessed by their ratios κ/λ and the absence of fluorescence interference (signal distortion). The comparison of PCs levels as well as the % expression and the normalized MFI (Median Fluorescence Intensity) of common markers (CD138, CD38, CD19, CD56, CD117, CD45) with our routine screening tube is determined. Occurrence of aberrant phenotype is evaluated according to the literature description.

Fluorescence channel/ Fluorochrome	FL1/ FITC	FL2/ PE	FL3/ ECD or PE-CF594	FL4/ PE.Cy5.5	FL5/ PE.Cy7	FL6/ APC	FL7/ AA700	FL8/ AA750	FL9/ BV421	FL10/ KO
Screening tube Blasts/Plasma cells	CD138	CD13	HLA - ECD	CD33	CD34	CD56	CD19	CD117	CD38	CD45
Plasmocyte clonality tube	cKappa	cLambda	CD138 – PE.CF594	CD20	CD27	CD56	CD19	CD117	CD38	CD45

Table 1. 10-Colors MFC panels used for detection and differenciation of reactive vs monoclonal Plasma Cells

RESULTS

In 5 MGUS patients with mixing PCs populations, a back-gating strategy on CD138+ CD38++ cells using MFC based on CD19/CD56/CD20/CD27/CD117 allowed to discriminate monoclonal PCs with the κ/λ ratios >10 or <0.1 from polyclonal PCs (Figure 1). Fluorescence interference study didn't show contamination of CD138-PE.CF594 and CD38-BV421 (strongly expressed on PCs) on CD19-AA700 (frequently negative on monoclonal PCs).

The novel tube show an excellent correlation (R = 0.997) and a significant proportional bias in the % PCs (CD138+CD38++ cells) comparing with the Screening tube (Passing-Bablok comparison Y = 0.7237X + 0.0265), due to the permeabilization step inducing cellular loss.

Comparisons of percentages and MFI of other common PC markers show no statistically significant or clinically difference (p-value>0.05) between screening and new tubes.

Phenotype analysis of our 8 monoclonal PCs cases show concordance with literature data [2]: 100% of cases with loss of CD19, 50% CD56+, 12,5% CD117+, 50% CD27dim or neg, 62,5% CD45 very dim or neg, 12,5% CD20+ and 62,5% CD38dim (Table 2). No phenotypic aberration was observed with reactive of residual polyclonal PCs.

Abnormal phenotype on PCs	% of cases presenting the abnormal phenotype	Theorical % [2]
CD19neg	100%	96%
CD56+	50% (4 cases/8)	60-75%
CD117+	12,5% (1 cases/8)	30-32%
CD27dim or neg	50% (4 cases/8)	40-68%
CD45 very dim or neg	62,5% (5 cases/8)	80%
CD20+	12,5% (1 cases/8)	17-30%
CD38dim	62,5% (5 cases/8)	80%

Table 2. % of aberrant phenotype observed on clonal PCs compared with the theorical percentage.

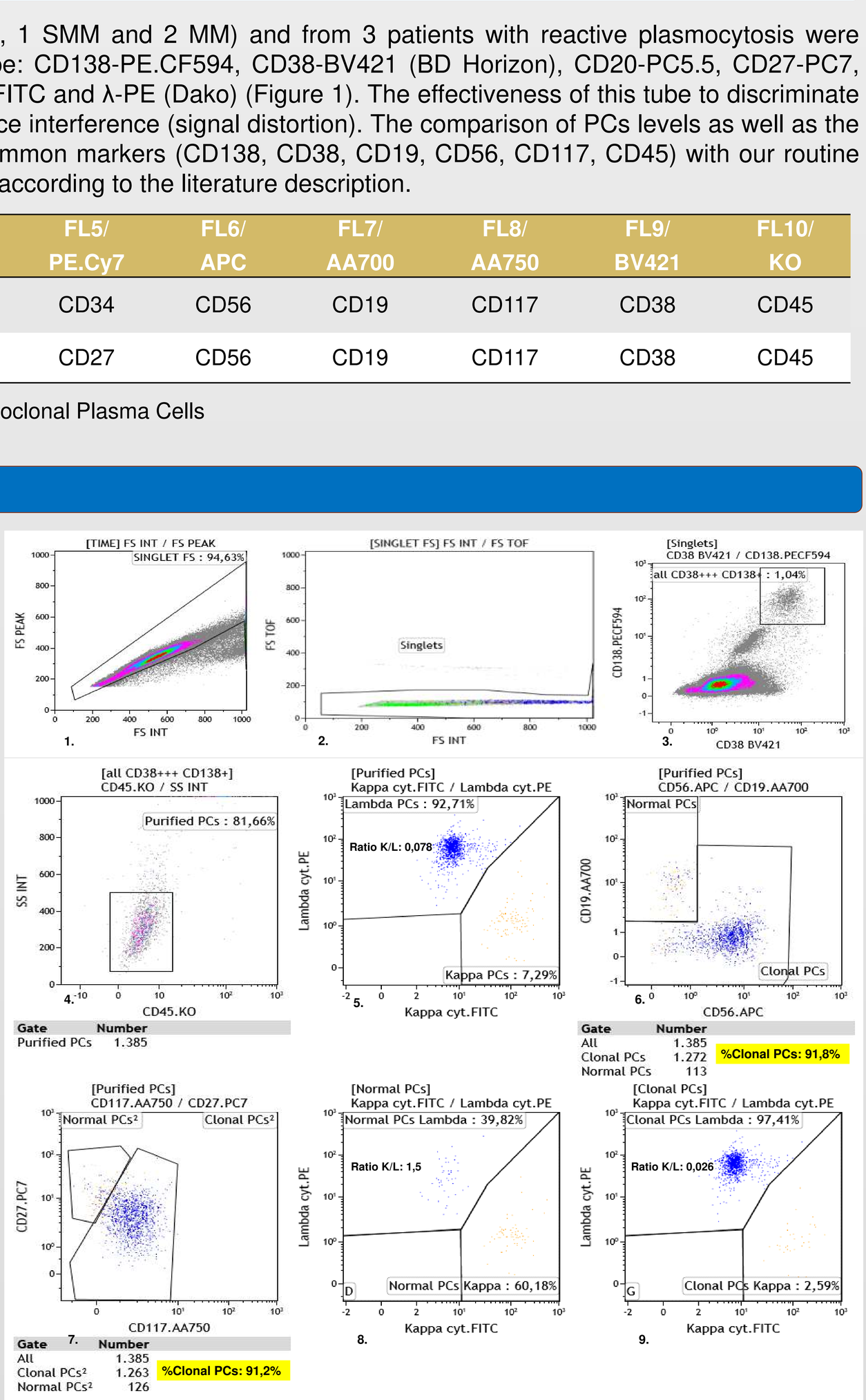

Figure 1. Gating strategy to isolate clonal PCs from the total PCs compartment by MFC. CD138+/CD38++ events are selected on the singlet cell population (bivariate plots 1. 2. and 3.). These events are purified by eliminating high-SSC events (purified PCs) which are eosinophils and neutrophils with aspecific fixation of CD38/CD138 (bivariate plot 4.). Using back-gating strategy on cKappa PCs (orange) and cLambda PCs (blue), the cluster of clonal PCs can be isolated from de polyclonal PCs on complementary bivariate plots such as CD19/CD56 or CD117/CD27 (6. and 7.). In this case, the clonal PCs have the aberrant phenotype: CD19neg, CD56+ CD27dim and CD117+dim. The K/L ratio is used to confirm the polyclonal character (ratio K/L between 1 and 3) or monoclonal character (ratio K/L >10 or <0.1) on each isolated PCs population.

DISCUSSION AND CONCLUSION

This 10-color tube is validated for the routine use to determine the precise % of monoclonal PCs. This work is the basis for our further study to establish a phenotypic score predicting high risk SMM and can also be used in the MRD detection of residual myeloma cells, with the bulk-lysis technique.

1. SV Rajkumar, O Landgren and MV Mateos. Smoldering multiple myeloma. Blood 2015: 125(20).
2. T Jelinek, R Bezdekova, M Zatopkova et al. Current applications of multiparameter flow cytometry in plasma cell disorders. Blood Cancer Journal 2017: 7 e617.