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Objective

The expression pattern of common antigens including cytoplasmic kappa/lambda ratio (CyKLR) was evaluated by multiparametric flowcytometry (MFC) to explore their relevance in discriminating NPC from APC across the spectrum of plasma cell proliferative disorders (PCPDs).

A total of 900 patients with diagnosis of PCPD were evaluated (MM: 724, PCL: 15, SMM: 12, MGUS: 42, Plasmacytoma: 54, Amyloidosis: 31, POEMS: 22). Bone marrow aspirate from cases (n=900) and controls (n=30) were processed as per standard operating procedure (SOPs) followed in our lab derived from consensus recommendations from the Euro-flow protocol. An aberrant PC cluster was defined by >30 plasma cells with light chain expression beyond the range of control samples (0.26 to 3.08) with/without aberrant immunophenotypes. Flow cytometric dot plots below shows the analysis strategy for MRD evaluation (Figure 1).

Figure 2 displays flow cytometry plots used for the identification of plasma cell compartments. The plots show the relationship between various parameters, including FS INT, FS PEAK, SS Area, 38 AF700, 138 PB, 117 APC, 19 PCS, 81 AF700, 45 ECD, 56 PC7, and 27 KO.

Panel A: FS INT vs FS PEAK plot. The linear regression line shows a strong positive correlation with $R: 1.90, 99.51\%$.

Panel B: [R 1] SS Area vs FS INT plot. The plot shows two distinct populations, R 1 (orange) and R 2 (black).

Panel C: [R 2] 38 AF700 vs 138 PB plot. The plot shows the Plasma Cell compartment (green) and other populations (black).

Panel D: [Plasma Cell compartment] 117 APC vs 19 PCS plot. The plot shows Normal PC (green) and APC (purple) populations.

Panel E: [Plasma Cell compartment] 81 AF700 / 45 ECD plot.

Panel F: [Plasma Cell compartment] 19 PCS / 56 PC7 plot.

Panel G: [Plasma Cell compartment] 56 PC7 / 27 KO plot.

Panel H: [Plasma Cell compartment] 27 KO / 19 PCS plot.

Amongst the entire cohort, 20% (n=181) samples displayed only APC, 16% (n=145) only NPC and 64% (n=574) showed coexistence of NPC and APC. The PC compartment was solely characterized by light chain expression and IPT aberrancy in 36% (n=326) and 22% cases (n=197), respectively (p<0.001). Using pattern-based recognition for three common patterns i.e. CD19vsCD56 (57%), CD19vsD27 (49%) and CD27vs 56 (449%), APC was separable from NPC in 90% cases and in remaining 10% of cases, APC-NPC differentiation achieved using gating markers.

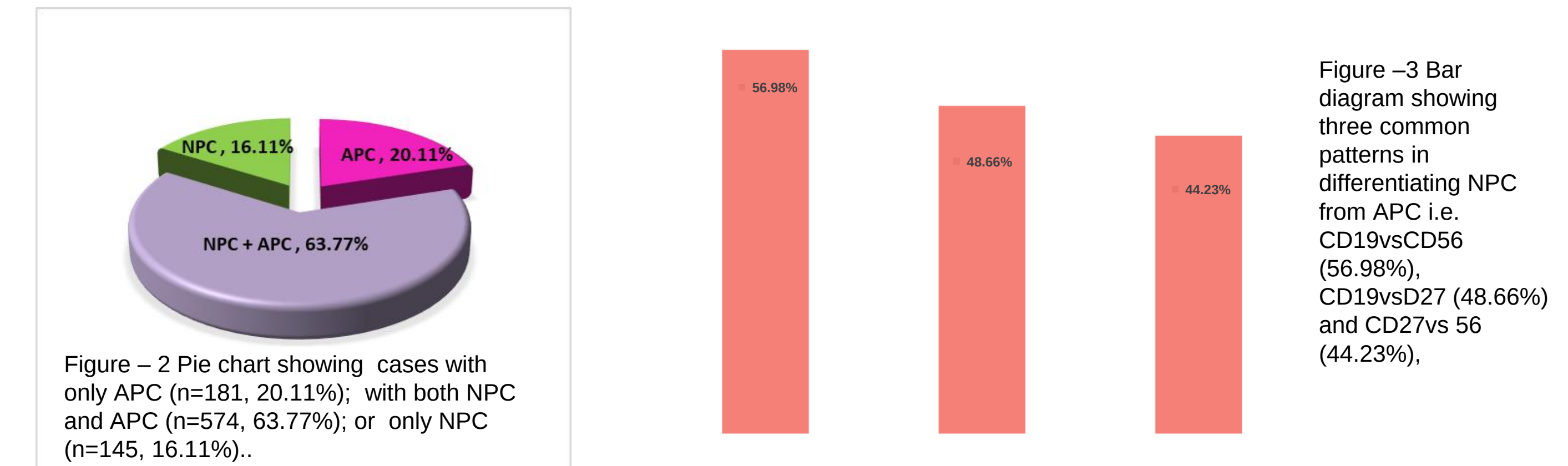


TABLE – 1 Frequency of aberrations across spectrum of PCPD

	MM_Dx (n=378)	MM_FUP (n=237)	MGUS (n=42)	SMM (n=12)	PCL (n=15)	Amyloidosis (n=31)	Plasmacytom a (n = 54)
CD117	21 (5.55%)	20 (8.43%)	3 (8.57%)	3 (25%)	0	1 (4%)	1 (1.85%)
CD138	17 (4.49%)	20 (8.43%)	0	1 (8.33%)	0	1 (4%)	2 (3.7%)
CD19	297 (78.57%)	185 (78.05%)	30 (85.71%)	9 (75%)	8 (88.88%)	15 (60%)	29 (53.7%)
CD27	253 (66.93%)	160 (67.51%)	13 (37.14%)	7 (58.33%)	6 (66.66%)	13 (52%)	15 (27.77%)
CD38	169 (44.70%)	124 (52.32%)	20 (57.14%)	8 (66.66%)	5 (55.55%)	9 (36%)	16 (29.62%)
CD45	221 (58.46%)	151 (63.71%)	28 (80%)	8 (66.66%)	6(66.66%)	13 (52%)	26 (48.14%)
CD56	192 (50.79%)	151 (63.71%)	22 (62.85%)	8 (66.66%)	6(66.66%)	14 (56%)	17 (31.48%)
CD81	117 (30.95%)	83 (35.02%)	9 (25.71%)	5 (41.66%)	6(66.66%)	3 (12%)	5 (9.25%)

This study shows that In all instances with all aberrant or all non-neoplastic PC, clonality assessment for intracytoplasmic immunoglobulin light chains is the ultimate proof for discriminating NPC vs APC population. Nonetheless, pattern-based recognition is one of the most helpful parameters in most cases with co-existing NPC and APC. Thus, the combined assessment of clonality and other immunophenotypic markers is required for reliable and precise assessment of NPC and APC compartments in PCPDs either at diagnosis or during follow up.

1. Flow cytometric immunophenotyping and minimal residual disease analysis in multiple myeloma. *Am J Clin Pathol.* 2009
2. Immunophenotyping in multiple myeloma and related plasma cell disorders. ***Best Pract Res Clin Haematol.*2010**
3. An approach for diagnosing plasma cell myeloma by three-color flow cytometry based on kappa/lambda ratios of CD38-gated CD138+ cells. ***Diagn Pathol.*2012**

- Multiparametric Flow cytometry (MFC) has become a valuable tool in diagnosis and response assesment across the spectrum of PCPDs.
- With the current level of advancements in the domain of flow cytometric immunophenotyping (FCMI), the most crucial step in the plasma cell compartment for the immunophenotypic (IPT) evaluation is the selection of the IPT markers that can help in discriminating aberrant clonal PCs (APC) from the non-neoplastic polyclonal PCs (NPC)

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