

# Rapid and accurate identification of acute promyelocytic leukemia with a novel multiparametric flow cytometric scoring system

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<https://doi.org/10.3324/haematol.2025.289104>

Received: September 14, 2025.

Accepted: November 24, 2025.

Early view: December 4, 2025.

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**SUPPLEMENTARY FIGURES and TABLES**

<b>A</b>	CD99% median (range)	CD99 MFI median (range)	CD9% median (range)	CD9 MFI median (range)	CD34% median (range)	CD2% median (range)	CD11b% median (range)	HLA-DR% median (range)	CD33% median (range)	CD33 MFI median (range)	CD13% median (range)	CD13 MFI median (range)	CD56% median (range)	CD38% median (range)	CD117% median (range)	CD133% median (range)	MPO% median (range)	CD4% median (range)	CD16% median (range)	CD15% median (range)
<b>Total</b> N = 255	100 (0-100)	63 (2-392)	100 (0-100)	189 (2-1622)	4 (0-100)	0 (0-100)	0 (0-100)	2 (0-100)	100 (0-100)	271 (5-2641)	100 (0-100)	276 (15-1800)	0 (0-100)	100 (63-100)	100 (0-100)	0 (0-100)	100 (0-100)	0 (0-100)	0 (0-19)	0 (0-100)
<b>APLs</b> N = 158	100 (97-100)	100 (42-392)	100 (88-100)	357 (51-1622)	1 (0-100)	0 (0-100)	0 (0-0)	0 (0-42)	100 (68-100)	382 (38-2641)	100 (68-100)	288 (33-1270)	0 (0-100)	100 (88-100)	100 (0-100)	0 (0-100)	100 (100-100)	0 (0-100)	0 (0-0)	0 (0-64)
<b>Non-APL AMLs</b> N = 97	100 (0-100)	39 (2-392)	25 (0-100)	26 (2-380)	55 (0-100)	0 (0-100)	0 (0-100)	100 (0-100)	100 (0-100)	155 (5-750)	100 (0-100)	235 (15-1800)	0 (0-100)	100 (63-100)	100 (1-100)	100 (0-100)	48 (0-100)	84 (0-100)	0 (0-19)	0 (0-100)
<b>P Value</b>	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.032*	< 0.001*	< 0.001*	0.54	< 0.001*	0.90	0.47	< 0.001*	< 0.001*	< 0.001*	0.20	< 0.001*

<b>B</b>	CD99 n (%)	CD9 n (%)	C34 n (%)	CD2 n (%)	CD11b n (%)	HLA-DR n (%)	CD33 n (%)	CD13 n (%)	CD56 n (%)	CD38 n (%)	CD117 n (%)	CD133 n (%)	CD65 n (%)	CD14 n (%)	MPO n (%)	CD7 n (%)	CD4 n (%)	CD16 n (%)	CD15 n (%)	
<b>Total</b> N = 255																				
<b>neg</b>	36 (23%)	45 (18%)	143 (56%)	208 (82%)	233 (92%)	168 (66%)	2 (1%)	4 (2%)	223 (88%)	0 (0%)	12 (5%)	174 (79%)	220 (98%)	228 (91%)	19 (7%)	228 (89%)	133 (61%)	252 (100%)	225 (89%)	
<b>pos</b>	122 (77%)	210 (82%)	112 (44%)	45 (18%)	19 (8%)	87 (34%)	253 (99%)	251 (98%)	30 (12%)	242 (100%)	240 (95%)	45 (21%)	5 (2%)	23 (9%)	236 (93%)	27 (11%)	86 (39%)	0 (0%)	27 (11%)	
<b>Unknown</b>	97	0	0	2	3	0	0	0	2	13	3	36	30	4	0	0	36	3	3	
<b>APLs</b> N = 158																				
<b>neg</b>	0 (0%)	0 (0%)	110 (70%)	116 (73%)	158 (100%)	151 (96%)	0 (0%)	0 (0%)	145 (93%)	0 (0%)	3 (2%)	153 (99%)	147 (100%)	158 (100%)	0 (0%)	156 (99%)	110 (89%)	158 (100%)	154 (97%)	
<b>pos</b>	61 (100%)	158 (100%)	48 (30%)	42 (27%)	0 (0%)	7 (4%)	158 (100%)	158 (100%)	11 (7%)	145 (100%)	152 (98%)	1 (1%)	0 (0%)	0 (0%)	158 (100%)	2 (1%)	13 (11%)	0 (0%)	4 (3%)	
<b>Unknown</b>	97	0	0	0	0	0	0	0	2	13	3	4	11	0	0	0	35	0	0	
<b>Non-APL AMLs</b> N = 97																				
<b>neg</b>	36 (37%)	45 (46%)	33 (34%)	92 (97%)	75 (80%)	17 (18%)	2 (2%)	4 (4%)	78 (80%)	0 (0%)	9 (9%)	21 (32%)	73 (94%)	70 (75%)	19 (20%)	72 (74%)	23 (24%)	94 (100%)	71 (76%)	
<b>pos</b>	61 (63%)	52 (54%)	64 (66%)	3 (3%)	19 (20%)	80 (82%)	95 (98%)	93 (96%)	19 (20%)	97 (100%)	88 (91%)	44 (68%)	5 (6%)	23 (25%)	78 (80%)	25 (26%)	73 (76%)	0 (0%)	23 (24%)	
<b>Unknown</b>	0	0	0	2	3	0	0	0	0	13	0	32	19	4	0	0	1	3	3	
<b>P Value</b>	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.28	0.040*	0.005*	> 0.95	0.079	< 0.001*	0.035*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	> 0.95	< 0.001*	

\*Statistically significant differences.

**Supplementary Table S1.** Overall MFC antigen expression and mean fluorescence intensity (MFI) in all AML cases. **A)** Median expression percentage and median MFI in all analyzed AML cases and comparison between APLs and Non-APL AMLs. **B)** Antigen positivity rates and comparison between APLs and Non-APL AMLs.

Antigen Positivity on Blast-Cells (Cut-off $\geq$ 20%)	Univariate Analysis			
	APL Cases (%) (N=61)	Non-APL AML Cases (%) (N=97)	OR (95% CI)	p Value
CD99	61/61 (100.0)	61/97 (62.9)	73.00 (9.95-9309.48)	< 0.001
CD9	61/61 (100.0)	52/97 (53.6)	106.60 (14.59-13585.72)	< 0.001
CD34 (Cut-off $\geq$ 10%)	21/61 (34.4)	64/97 (66.0)	0.26 (0.13-0.50)	< 0.001
CD2	18/61 (29.5)	3/97 (3.1)	11.24 (3.77-44.36)	< 0.001
HLA-DR	1/61 (1.6)	80/97 (82.5)	0.01 (0.00-0.02)	< 0.001
CD13	61/61 (100.0)	93/97 (95.9)	5.92 (0.61-789.79)	0.14
CD33	61/61 (100.0)	95/97 (97.9)	3.22 (0.26-447.05)	0.40
CD11b	0/61 (0.0)	19/97 (19.6)	0.03 (0.0-0.24)	< 0.001
CD15	0/61 (0.0)	23/97 (23.7)	0.02 (0.00-0.18)	< 0.001
CD56	4/61 (6.6)	19/97 (19.6)	0.32 (0.09-0.86)	<b>0.023</b>
CD133	1/61 (1.6)	44/97 (45.4)	0.01 (0.00-0.05)	< 0.001
CD4	13/61 (21.3)	73/97 (75.3)	0.09 (0.04-0.19)	< 0.001
CD7	2/61 (3.3)	25/97 (25.8)	0.12 (0.02-0.39)	< 0.001
CD117	61/61 (100.0)	88/97 (90.7)	13.20 (1.62-1713.79)	<b>0.010</b>
CD65	0/61 (0.0)	5/97 (5.2)	0.34 (0.03-1.78)	0.22
CD14	0/61 (0.0)	23/97 (23.7)	0.02 (0.00-0.18)	< 0.001
MPO (Cut-off $\geq$ 10%)	61/61 (100.0)	78/97 (80.4)	32.59 (4.21-2912.55)	< 0.001

**Supplementary Table 2.** Univariate analysis for MFC antigens association with the differential diagnosis of APL. The p values in bold are statistically significant.

A	Orfao et al.'s Score (Points)	Total (N=158) n (%)	APLs (N=61) n (%)	Non-APL AMLs (N=97) n (%)	P-Value	B	APLY Score (Points)	Total (N=158) n (%)	APLs (N=61) n (%)	Non-APL AMLs (N=97) n (%)	P-Value
	0	6 (3.8)	0 (0.0)	6 (6.2)	< 0.001		0	55 (34.9)	0 (0.0)	55 (56.7)	< 0.001
1	57 (36.0)	8 (13.1)	49 (50.5)	1		0 (0.0)	0 (0.0)	0 (0.0)			
2	48 (30.4)	15 (24.6)	33 (34.0)	2		31 (19.6)	0 (0.0)	31 (32.0)			
3	47 (29.7)	38 (62.3)	9 (9.3)	3		4 (2.5)	1 (1.6)	3 (3.1)			
				4	52 (32.9)	44 (72.2)	8 (8.2)				
				5	16 (10.1)	16 (26.2)	0				

**Supplementary Table 3.** Overall distribution of AML cases according to diagnostic scoring systems. **A)** Distribution of all AML samples based on their Orfao et al.'s Score, with a comparative analysis between APL and non-APL AML cases. **B)** Distribution of all AML samples based on their APLY Scores, with a comparative analysis between APL and non-APL AML cases.