Immunophenotypic and molecular features of cuplike morphology in AML

BHS GAM 2020

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Background

- Careful evaluation of leukemic blast morphology supplemented by immunophenotyping may provide clues to a specific category of the WHO 2016 classification of AML.
- Nuclear invaginations, referred to as 'fishmouth' or 'cuplike' nuclei (Figure 1) have been associated with a lack of CD34 and HLA-DR expression, a normal karyotype and mutations in NPM1 and FLT3. According to literature these findings may help in rapid recognition of the entity 'AML with mutated NPM1'.

Objectives

- To evaluate the association between cuplike blasts (CLB) and specific immunophenotypic and molecular patterns based on:
  - a literature study.
  - the evaluation of de novo AMLs at our institution.

Methods

- A literature study was based on 7 publications.
- Bone marrow slides of de novo AMLs at our institution between January 2018 and May 2019 (n=41) were reviewed for % of CLB (as % of total blasts), immunophenotype and mutational status of NPM1 and FLT3 by 1 analyst blinded for all results.

Results

- Inconsistent results were found in literature as studies differed in definition of CLB and in inclusion criteria. In most studies, the definition of cuplike AML is restricted to cases with ≥10% CLB. A summary is shown in Table 1.
- At our institution, 5/41 (12%) de novo AMLs had ≥10% CLB (Table 2), all demonstrating mutated FLT3. HLADR expression was absent in only 1/5 cases, whereas CD34 expression was absent in 3/5 cases. These 3 cases all showed mutated NPM1.
- In 5 additional cases with 5-9% CLB (Table 3), 3 cases demonstrated NPM1 mutation, of which 2 with concomitant FLT3 mutation. These 3 cases all lacked CD34 and HLADR expression.
- Not all cases with FLT3 (n=13/41, 32%) and/or NPM1 (n=10/41, 24%) mutation demonstrated CLB. In total, 7/13 (54%) FLT3 mutated cases and 6/10 (60%) NPM1 mutated cases showed the presence of ≥5% CLB.

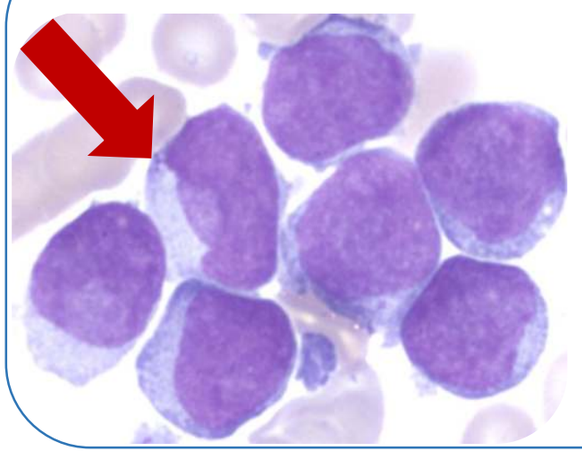
Conclusions

- At our institution, 24% of de novo AMLs showed ≥5% CLB and 12% showed ≥10% CLB.
- All cases (5) with ≥10% CLB showed FLT3 mutation (100% PPV), irrespective of absence of CD34 or HLADR expression.
- All cases (6) with ≥5% or ≥10% CLB in combination with absence of CD34 expression showed NPM1 mutation (100% PPV).
- A follow-up of a larger series of cases will be required to confirm these findings.

[1] Kussick SJ, Stirewalt DL, Yi HS, et al. A distinctive nuclear morphology in acute myeloid leukemia is strongly associated with loss of HLA-DR expression and FLT3 internal tandem duplication. *Leukemia*. 2004 Oct;18(10):1591–8.
[2] Chen W, Rassidakis GZ, Li J, et al. High frequency of NPM1 gene mutations in acute myeloid leukemia with prominent nuclear invaginations ('cuplike' nuclei). *Blood*. 2006 Sep 1;108(5):1783–4.
[3] Kroschinsky FP, Schäkel U, Fischer R, et al. Cup-like acute myeloid leukemia: new disease or artificial phenomenon? *Haematologica*. 2008 Feb;93(2):283–6.
[4] Bennett JM, Pryor J, Laughlin TS, Rothberg PG, Burack WR. Is the association of 'cup-like' nuclei with mutation of the NPM1 gene in acute myeloid leukemia clinically useful? *Am J Clin Pathol*. 2010 Oct;134(4):648–52.
[5] Rakheja D, Konoplev S, Su M, Wheeler D, Muzny DM, Ruvolo VR, et al. High incidence of IDH mutations in acute myeloid leukaemia with cuplike nuclei. *Br J Haematol*. 2011 Oct;155(1):125–8.
[6] Carluccio P, Mestice A, Pastore D, et al. Immunophenotypic and molecular features of 'cuplike' acute myeloid leukemias. *Eur J Haematol*. 2014 Feb;92(2):121–6.
[7] Park BG, Chi H-S, Jang S, et al. Association of cup-like nuclei in blasts with FLT3 and NPM1 mutations in acute myeloid leukemia. *Ann Hematol*. 2013 Apr;92(4):451–7.

Leukaemic blasts with cup-shaped morphology

Figure 1. Cuplike nuclei



Cuplike AML are characterized by 10% or more blasts with prominent, cup-like nuclear invagination spanning at least 25% of the nuclear diameter.

Definition by Kussick et al, 2004 [1]

Literature study

Table 1. Summary of literature study

Author	Study population	Number of cuplike AML	Comparison group	FAB type: N (%)	Lack of CD34	Lack of HLADR	NPM1 mutated	FLT3 mutated
Kussick et al, 2004 [1]	5-year survey of nonAPL/ nonmonocytic AML.	**N=19**	**Cuplike negative AML N=24**	**M1/M2?**	68%* (95% with partial loss)	68%* (100% with partial loss)	ND	84%*
Chen et al, 2006 [2]	Review of AMLs at the institution (no further specification)	**N=24**	**Control group N=20**	**M1**	71%*	50%*	64%*	88%**
Kroschinsky et al, 2008 [3]	**266** randomly selected patients from AML96 protocol (DSIL). Exclusion of APL.	**N=55 (all FAB types)**	**Cuplike negative AML N=211**	NS (**M1** and **M2** 62% of cuplike AML)	93%**	33%**	60%*	73%**
		N=35 (non-monocytic)			84%**	76%**	64%*	80%*
Bennett et al, 2010 [4]	**17** AML with mutated NPM1, 3-year interval.	**N=6**	**Cuplike negative N=11**	ND	4/6	2/6	6/6	3/6
Rakheja et al, 2011 [5]	**12** cuplike AML with sufficient amplifiable DNA, 8-year interval.	**N=12**	**Control group N=23**	**M1:** 12 (100%)	100%*	67%*	100%*	80%**
Carluccio et al, 2013 [6]	**68** de novo AML (M0/M1/ M2), 3-year interval.	**N=15**	**Cuplike negative N=53**	**M0:**1 (7%) **M1:**2 (13%) **M2:** 12 (80%)	53%*	40%*	50%**	64%* ITD 7%**, TKD
Park et al, 2013 [7]	**208** patients diagnosed with AML (excl. APL) with available NPM1 and FLT3 mutation status.	**N= 44**	**Cuplike negative N=164**	**M0:** 0 (0%) **M1:** 23 (38%) **M2:**11 (19%) **M4:** 5 (13%) **M5:** 1 (11%) **M7/M8:** 0(0%) **MRC:** 4 (14%)	72%*	-	71%*	48%*,ITD 11%*, TKD

*statistically significant; **statistically insignificant
ND, Not determined; NS, not specified
APL, acute promyelocytic leukemia; MRC, myelodysplasia-related change
ITD, internal tandem duplication; TKD tyrosine kinase domain

CLB in 41 de novo AMLs at our institution

Table 2. Cases with ≥ 10% CLB (n=5/41)

Case	% CLB	FAB type	Lack of CD34	Lack of HLADR	NPM1 mutated	FLT3 mutated
1	17	M0/M1	Y	N	Y	Y
2	16	M1	N	N	N	Y
3	13	M5/M4	Y	N	Y	Y
4	11	M5	N	N	N	Y
5	30	M0/M1	Y	Y	Y	Y

Table 3. Cases with 5-9% CLB (n=5/41)

Case	% CLB	FAB type	Lack of CD34	Lack of HLADR	NPM1 mutated	FLT3 mutated
6	5	M4eo	ND	ND	N	N
7	6	M2	Y	Y	Y	Y
8	7	M0/M1	ND	ND	N	N
9	5	M1	Y	Y	Y	N
10	8	M1	Y	Y	Y	Y

ND, Not determined
Y, Yes (Present); N, No (not present)