TO THE EDITOR:

Minor PNH clones do not distinguish inherited bone marrow failure syndromes from immune-mediated aplastic anemia

Atsushi Narita,[1] Shunsuke Miwata,[1] Masayuki Imaya,[1] Yusuke Tsumura,[1] Ayako Yamamori,[1] Manabu Wakamatsu,[1] Motoharu Hamada,[1] Rieko Taniguchi,[1] Yusuke Okuno,[2] Hideki Muramatsu,[1] and Yoshiyuki Takahashi[1]

[1]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; and [2]Department of Virology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

An article by Shah et al[1] discusses the predictive value of paroxysmal nocturnal hemoglobinuria (PNH) clones in diagnosing bone marrow failure (BMF). The authors investigated 454 patients with aplastic anemia (AA), inherited BMF syndromes (IBMFSs), or other hematologic diseases and concluded that PNH clones could be used to distinguish AA from IBMFSs. However, very small PNH clones (about 22 per million cells on average) can also be detected in the granulocytes of healthy individuals,[2] and the authors based their conclusions on the observation that the PNH clone was not detected in a small number of 22 patients with IBMFSs and other hematologic diseases.

We recently performed a retrospective analysis of 133 patients with BMF, including 107 with AA and 26 with IBMFSs, who were genetically diagnosed by next-generation sequencing.[3] To achieve definitive diagnosis, a combination of clinical information, laboratory results, and genetic testing was used. In 112 of these patients, PNH-type granulocytes and red blood cells were also evaluated by flow cytometry. The cutoff values for determining the presence of minor PNH clones were >0.020% for $CD11b^+ CD55^- CD59^-$ granulocytes and >0.037% for glycophorin $A^+ CD55^- CD59^-$ erythrocytes on the basis of means plus 2 standard deviations for 31 healthy controls. None of the healthy controls tested positive for PNH clones using these cutoff values. A patient with more than 1% PNH-type granulocytes and/or erythrocytes was judged as having major PNH clones. This study was approved by the ethics committee of the Nagoya University Graduate School of Medicine and was performed in accordance with the Declaration of Helsinki.

In patients with AA and IBMFS who had an identified PNH clone, the median percentages of PNH-type granulocytes were 0.016% (range, 0.002%-1.336%) and 0.012% (range, 0.002%-0.231%), respectively (supplemental Figure 1A) and that of PNH-type erythrocytes were 0.010% (range, 0.001%-18.586%) and 0.009% (range, 0.001%-0.033%), respectively (supplemental Figure 1B). Despite not being clinically diagnosed as having PNH, 2 patients with AA had major PNH clones. Among the patients whose samples were subjected to flow cytometry, 32 of 91 patients with AA and 9 of 21 patients with IBMFSs were positive for PNH clones. Our results demonstrated that minor PNH clones provided a 78% positive predictive value (PPV), 17% negative predictive value (NPV), 57% specificity, and 35% sensitivity for AA diagnosis and exclusion of IBMFSs. We have summarized the characteristics of the 9 patients with IBMFSs identified as having PNH clones in Table 1. Using a higher threshold of 0.1% for minor PNH clones based on previous reports,[4] minor PNH clones were demonstrated to have a PPV of 91%, NPV of 20%, specificity of 95%, and sensitivity of 11% for AA diagnosis and exclusion of IBMFSs.

The selection and expansion of PNH clones, which are frequently detected in patients with AA, is the result of an escape mechanism directed against immunologic attack on hematopoietic stem cells.[5] However, in our cohort study, minor PNH clones were detected in some patients with IBMFSs, even though the appearance of PNH clones is unlikely in those patients because the pathogenesis of BMF is not thought to be mediated by immunologic attack. Several earlier studies indicated that PNH clones were not detected in patients with IBMFSs. Keller et al[6] studied 26 patients with Shwachman-Diamond

The full-text version of this article contains a data supplement.

Table 1. Clinical characteristics of patients with IBMFS who had PNH clones

Diagnosis	Age (y)	Sex	Family history	Physical anomaly	PNH clone (%) Granulocytes†	Erythrocytes‡	Chromosome fragility test	TL (SD)	Gene	Nucleotide change	Amino acid change	Zygosity
FA	10	M	–	–	0.065	0.007	+	−1.84	FANCA	c.2546delC	p.S849fs*40	Homo
FA	13	M	–	+	0.039	0.010	+	0.83	FANCA	c.2470T>C	p.C824R	Hetero
									FANCA	c.1418T>C	p.L473P	Hetero
FA	6	F	+	+	0.041	0.033	+	−0.26	FANCA	c.2470T>C	p.C824R	Hemi
			+	+					FANCA	Deletion	–	
FA	5	F	+	+	0.025	0.004	+	−3.58	FABCG	c.1066C>T	p.Q356X	Hetero
									FABCG	c.194delC	p.65fs*7	Hetero
FA	2	M	–	–	0.032	0.009	+	2.05	FANCG	c.307 + 1G>C	–	Homo
DC	2	F	–	+	0.099	0.008	–	0.83	TINF2	c.845G>A	p.R282H	Hetero
DC	1	F	–	+	0.231	0.014	–	−5.73	TINF2	c.845G>A	p.R282H	Hetero
DC	11	F	–	+	0.061	0.002	ND	−3.55	TINF2	c.845G>A	p.R282H	Hetero
SDS	6	M	–	+	0.020	0.015	–	−1.99	SBDS	c.184A>T	p.K62X	Hetero
									SBDS	c.258 + 2T>C	–	Hetero

DC, dyskeratosis congenita; F, female; FA, Fanconi anemia; Hemi, hemizygous; Hetero, heterozygous; Homo, homozygous; M, male; ND, not described; SD, standard deviation; SDS, Shwachman-Diamond syndrome; TL, telomere length.
†CD11b$^+$CD55$^-$CD39$^+$.
‡Glycophorin A+ CD55$^-$CD59$^-$.

syndrome to determine the presence of PNH clones, and none of these patients had detectable PNH clones. Similarly, DeZern et al[4] reported the absence of PNH clones in 20 patients with IBMFSs. Furthermore, no *PIGA* gene mutations were detected in any of the 110 patients with Shwachman-Diamond syndrome in a 55-gene panel analysis study.[7] One possible explanation for the discrepancy between previous studies and our observations is the detection sensitivity of PNH blood cells. As shown in Table 2, the cutoff values for PNH positivity differed among the studies. In our cohort, PNH clones in granulocytes were above the cutoff (>0.020%) in 9 patients with IBMFSs, of which 5 were excluded using the criteria of Shah et al[1] (>0.05%) and 8 were excluded by using the criteria of DeZern et al[4] (>0.1%). When we set a higher threshold of 0.1% for minor PNH clones, we observed a decrease in sensitivity (11%) but a substantial improvement in specificity (95%), PPV (91%), and NPV (20%) compared with the original lower threshold for AA diagnosis and exclusion of IBMFSs. A higher threshold for PNH clones may be useful for differentiating IBMFS.

To summarize, the findings suggest that the assessment of minor PNH clones with a low cutoff value might not be able to completely distinguish acquired AA from IBMFS, but using a higher cutoff value (eg, ≥0.1%) may be useful in the differential diagnosis. Therefore, further studies with larger patient cohorts are required to investigate the appropriate cutoff values optimized for the purpose of differential diagnosis between acquired AA and IBMFS.

Table 2. Summary of the frequency of PNH clones detection in patients with IBMFS according to previous studies

Study	Disease (n)	Patients with IBMFS who have a PNH clone	Cutoff value	Year
Keller et al[6]	SDS (3), SDS likely (16), SDS possible (7)	0/26	Red cells >1.0%	
			Neutrophils >1.0%	
DeZern et al[4]	DC (9), FA (4), DBA (2), SDS (3), c-MPL (2)	0/20	0.1%	2000-2008
			0.01%	2009-2014
Shah et al[1]	DC (3), FA (2), DBA (1), Others (3)	0/9	Granulocytes >1.0%	2010-2018
			Erythrocytes >1.0%	2010-2018
			Granulocytes >0.05%	2018-2020
			Monocytes >0.3%	2018-2020
			Erythrocytes >0.01%	2018-2020
Our data	DC (8), FA (9), DBA (3), SDS (1)	9/21	Granulocytes >0.020%	
			Erythrocytes >0.037%	

DBA, Diamond–Blackfan anemia.

ORCID profiles: A.N., 0000-0001-6518-4726; M.I., 0000-0002-2970-1543; Y.T., 0000-0002-9604-9655; Y.O., 0000-0003-3139-9272.

Correspondence: Yoshiyuki Takahashi, Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8650, Japan; e-mail: ytakaha@med.nagoya-u.ac.jp.

References

1. Shah YB, Priore SF, Li Y, et al. The predictive value of PNH clones, 6p CN-LOH, and clonal TCR gene rearrangement for aplastic anemia diagnosis. *Blood Adv.* 2021;5(16):3216-3226.

2. Araten DJ, Nafa K, Pakdeesuwan K, Luzzatto L. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. *Proc Natl Acad Sci USA.* 1999;96(9):5209-5214.

3. Miwata S, Narita A, Okuno Y, et al. Clinical diagnostic value of telomere length measurement in inherited bone marrow failure syndromes. *Haematologica.* 2021;106(9):2511-2515.

4. DeZern AE, Symons HJ, Resar LS, Borowitz MJ, Armanios MY, Brodsky RA. Detection of paroxysmal nocturnal hemoglobinuria clones to exclude inherited bone marrow failure syndromes. *Eur J Haematol.* 2014;92(6):467-470.

5. Young NS. Aplastic anemia. *N Engl J Med.* 2018;379(17):1643-1656.

6. Keller P, Debaun MR, Rothbaum RJ, Bessler M. Bone marrow failure in Shwachman-Diamond syndrome does not select for clonal haematopoiesis of the paroxysmal nocturnal haemoglobinuria phenotype. *Br J Haematol.* 2002;119(3):830-832.

7. Kennedy AL, Myers KC, Bowman J, et al. Distinct genetic pathways define pre-malignant versus compensatory clonal hematopoiesis in Shwachman-Diamond syndrome. *Nat Commun.* 2021;12(1):1334.