blood advances

**TO THE EDITOR:**

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

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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

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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† | PNH clone (%) 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.

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**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.

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