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Detection of paroxysmal nocturnal hemoglobinuria clones to exclude inherited bone marrow failure syndromes

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Abstract

Background—Inherited bone marrow failure syndromes (IBMFS) and acquired aplastic anemia (AA) are life-threatening marrow failure disorders. These entities can be difficult to distinguish because they present similarly. Correct diagnosis is imperative for proper therapy.

Design and methods—This is a retrospective, single-center study of patients <40 yr of age, evaluated for bone marrow failure, and assayed for the presence of a PNH clone in the pediatric or adult hematology/oncology clinics from 2001 to present. Patients were also evaluated for IBMFS.

Results—We present results from 156 patients with marrow failure, 20 of whom have IBMFS. None of the IBMSF patients had paroxysmal nocturnal hemoglobinuria (PNH) clones.

Conclusions—Although further studies are needed, our results suggest that the detection of a PNH clone can be a useful diagnostic tool to exclude the diagnosis of IBMFS and focus the work-up and treatment on an acquired form of marrow failure.

Keywords

paroxysmal nocturnal hemoglobinuria; bone marrow failure; aplastic anemia; inherited marrow failure; pancytopenia

Inherited bone marrow failure syndromes (IBMFS) and acquired aplastic anemia (AA) are life-threatening bone marrow failure (BMF) disorders. These entities can be difficult to distinguish because they both present with pancytopenia and a hypocellular marrow (1, 2).

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Author's contribution

AD designed the study, analyzed the data, and wrote the paper. RB designed the study, analyzed the data, and wrote the paper. HJ, LR, MB, and MA contributed to the data analysis and edited the paper.

IBMFS can be caused by germline mutations that result in impaired DNA repair (e.g., Fanconi anemia), abnormally short telomeres (e.g., dyskeratosis congenita), ribosomopathies (e.g., Schwachman– Bodian–Diamond syndrome), abnormalities in the thrombopoietin gene (amegakaryocytic thrombocytopenia), or other causes (3). However, the majority of AA is acquired and mediated by immune destruction of hematopoietic progenitors. In contrast to IBMFS, acquired AA is often responsive to immunosuppressive therapy (4). It is therefore critical to distinguish between these two etiologies, both for diagnosis and appropriate treatment.

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired bone marrow failure disorder characterized by clonal populations of hematopoietic progenitors with somatic mutations in *PIGA*. The *PIGA* gene product is necessary for glycosylphosphatidylinositol anchor protein (GPI-AP) biosynthesis. Up to 70% of patients with acquired AA have a small PNH clone at diagnosis (5, 6). The deficiency of GPI-AP on the surface of these mutated stem cells renders them relatively resistant to immune attack; thus, the cells do not have an inherent proliferative advantage but rather have a conditional advantage because they escape immune destruction (6). This is believed to be the basis for the high incidence of PNH clones in AA. Based on this finding, we hypothesized that the presence of a PNH clone could distinguish immune vs. genetic causes of AA.

Patients and methods

The protocol for review of these patients was approved by the Institutional Review Board of The Johns Hopkins University School of Medicine. This was a retrospective study performed via chart review of electronic records in accor dance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice.

Subjects

Subjects were eligible for the study if they were <40 yr of age, evaluated for bone marrow failure, and assayed for the presence of a PNH clone in the pediatric or adult hematology/ oncology clinics from 2001 to present. We defined marrow failure as cytopenias persisting >3 months and marrow cellularity that was low for age. Severity of AA was categorized by established criteria (7). Stata10 IC (College Station, TX, USA) was used for statistical analysis.

Genetic diagnoses

IBMFS cases were definitively diagnosed by test(s) specific for their disease. Telomere syndrome patients had telomere lengths measured by flow cytometry and FISH (8). Telomere syndromes was defined by TERC, TERT, DKC1 mutations, or classic features of dyskeratosis congenita or related disorders (8). Fanconi anemia was determined by positive diepoxybutane test and gene testing. Diamond–Blackfan anemia and Shwachman–Diamond–Bodian Syndrome were defined by their gene mutations, RPS19 and SBDS, respectively. Congenital amegakaryocytic thrombocytopenia was diagnosed with c-*MPL* mutations.

PNH clones

The presence of a PNH clone was assayed by peripheral blood flow cytometry on granulocytes, monocytes, and erythrocytes (9, 10). We used 4-color flow cytometry with a sensitivity of detection of 0.1% (2000–2008) and a 6-color flow cytometry with a sensitivity of .01% (2009 to present).

Results

A total of 156 subjects fulfilled the criteria for BMF. The characteristics of the population are summarized in Table 1. Of these, twenty cases (12.8%) were identified as having an IBMFS by mutational analysis and with documentation of cytopenias prior to age 5 yr (Table 2). Four patients were outliers after the marrow evaluations; while they were hypo-cellular for age (80% cellular when age 1–5), their cellularity was not within the aplastic range (<25%) and considered MDS. These patients were ultimately found to have an IBMFS and assessed with this group. These patients were excluded from the AA cohort, however, as they would not meet criteria for aplasia.

The remaining 132 patients were characterized as acquired AA due to documented normal blood counts within 5 yr before diagnosis. Of these acquired patients, 27 patients met criteria for moderate AA, 82 met criteria for severe AA, and 23 met criteria for very severe AA (ANC <200) at the time of the presentation of their disease. Three acquired AA patients had monosomy 7 and two had trisomy 8 at presentation to our center. Of these acquired AA patients, 61 (46%) had a PNH clone present by FLAER assay (9). For those with PNH clones present, the median laboratory values (with ranges) include erythrocyte clone size 0.06% (0.02–37) [including Type II and Type III cells], granulocyte clone size 0.4% (0.01–94), and monocyte clone size 0.8% (0.1–91). None of the 20 patients with IBMFS patients had a PNH clone at 0.01% level of detection by peripheral blood flow cytometry (9). Strikingly, the identification of a PNH clone had a 100% positive predictive value for identifying an acquired vs. an inherited marrow ($P = 0.0001$, chi squared), although the negative predictive value was only 54%.

Separating the patients into age cohort by decade revealed a difference in PNH clones by age (Fig. 1). In the group of patients aged 0–9 yr, only 5.9% (one patient) had a PNH clone; patients aged 10–19 yr, 40% had PNH clones; patients aged 20–29 yr, 64.3% had PNH clones; and patients aged 30–39 yr, 48.7% had PNH clones ($P = 0.001$).

Discussion

These data illustrate the utility of evaluation for a PNH clone as an early step in the diagnostic algorithm for marrow failure. Based on our findings, we recommend that clinicians check a FLAER-based multicolor flow cytometry assay for PNH in patients who present with marrow failure to ensure reasonable tests are performed going forward. This test should be performed early in the diagnostic algorithm of marrow failure because we found that the presence of a PNH clone helps to exclude the diagnosis of IBMFS and focuses the work-up to acquired forms of bone marrow failure. A caveat to this is that PNH is rare in younger children and IBMFS often present in childhood in the first decade of life.

We found an increasing prevalence of PNH clones by age cohort. Thus, the likelihood of finding a PNH clone is higher in older children and young adults than it is for children in the first decade of life; however, finding PNH cells in any age group appeared to exclude IBMFS.

There is a biologic rationale for PNH clones in patients with acquired AA and their absence in patients with IBMFS. Acquired AA typically results from autoimmune destruction of hematopoietic stem cells (HSC) (11), whereas inherited conditions have a genetic predisposition to marrow failure due to the particular mechanism of the underlying disease (3). Immunosuppressive therapy (IST) with antithymocyte globulin and cyclosporine (ATG/CSA) is the standard treatment for patients for these patients; most IBMFS patients do not response to IST. The leading hypothesis to explain the relationship between AA and PNH is that the autoimmune attack of AA preferentially targets normal HSC over PNH HSC (12). Up to 40% of PNH evolves from AA. In addition, up to 70% of patients with acquired, but not inherited, AA have small, clonal, populations of PNH cells at diagnosis (5, 6, 13). These small PNH populations may be clinically silent for many years, and later expand, particularly at the time of relapse. An autoimmune attack does not underlie IBMFS. The IBMFS result from stem cell exhaustion due to short telomeres, DNA repair defects, ribosomopathies, or other intrinsic cell defects; thus, there is no selective pressure from the immune system to favor the outgrowth of a PNH clone. These data are in agreement with Keller *et al*. (14) who studied 25 SDS patients for the presence of a PNH clone using a flow cytometry. The authors concluded that bone marrow failure in SDS does not select for the outgrowth of PNH because none of these patients had a detectable PNH clone.

In summary, PNH screening should be part of a routine diagnostic algorithm in patients who present with bone marrow failure of uncertain etiology unless an IBMFS is obvious from the family history or patient's phenotype. For example, a patient born to parents with one child with FA would not benefit from an evaluation for a PNH clone. Although the absence of a PNH clone does not exclude an acquired form of bone marrow failure, the presence of GPI anchor-deficient cells in at least two lineages, especially in patients with no family history of bone marrow failure or other stigmata of IBMFS, virtually excludes the diagnosis of IBMFS thereby eliminating the need for additional costly tests and delays in initiating definitive therapy. Moreover, this could reduce the need to refer patients for genetic consultation and testing as well as alleviate patient and family anxiety associated with genetic diseases. Larger cohorts of patients with bone marrow failure in which IBMFS are considered should be tested to validate the findings of our retrospective series.

Acknowledgments

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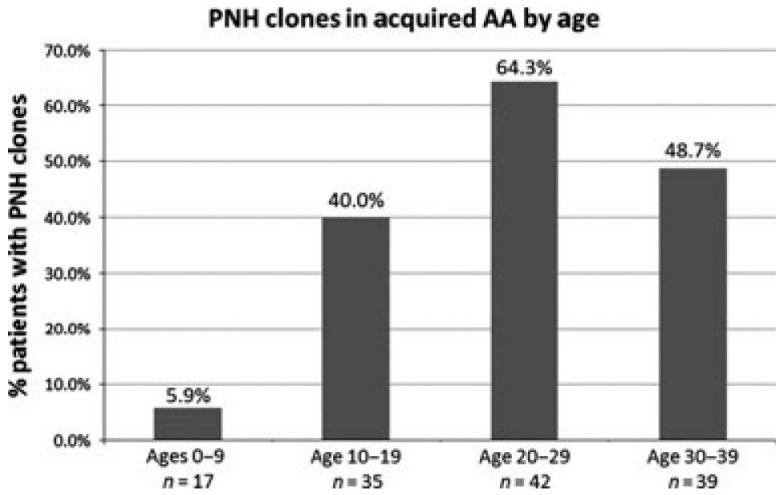

Figure 1.
PNH clones in Acquired AA by age.

Table 1

Characteristics of all bone marrow failure patients

Demographics	
Median age	21 yr (range 0–40)
% male	48%
Caucasian	63%
African American	18%
Asian	10%
Other	9%
Laboratory values	
Median absolute reticulocyte count	3.3 K/mm^3 (2.5–132)
Median absolute neutrophil count	0.21 K/mm^3 (0–6.36)
Median hemoglobin	10 g/dL (2.9–14.5) [71% of the cohort was transfused at the time of presentation]
Median platelet count	Platelets 19 K/mm^3 (1–174)
Median lactate dehydrogenase	139 U/L (91–945)
Median bone marrow cellularity	10% (range 0–80%)

Table 2

Characteristics of IBMFS patients without PNH clones

Median age (range)	16.5 yr (1–38)
Gender	50% male
Family history present	30%
Physical abnormalities	40%
Moderate AA	60%
Severe AA	15%
MDS	25%
Diagnosis	
Telomere syndromes (<1st percentile length)	DKC1
	n = 1
	TERT
	n = 2
	TERC
	n = 1
	Classical DKC
	n = 5
Fanconi anemia	FANCA
	n = 2
	FANCC
	n = 2
Diamond-Blackfan anemia	RPS19
	n = 2
Schwachman-Bodian-Diamond syndrome	*SBDS*
	n = 3
Congenital amegakaryocytic thrombocytopenia	*c-MPL*
	n = 2