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Development of Hemolytic Paroxysmal Nocturnal Hemoglobinuria without graft loss following Hematopoietic Stem Cell Transplantation for Acquired Aplastic Anemia

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Introduction

Paroxysmal Nocturnal Hemoglobinuria (PNH) is the most common clonal hematopoietic disorder arising in patients with acquired aplastic anemia (aAA)^{1, 2}. PNH is caused by mutations in *PIGA*, a gene that encodes the catalytic subunit of an enzyme involved in the biosynthesis of Glycosylphosphatidylinositol (GPI) anchors, transmembrane glycolipids required for cell surface expression of many proteins³. PNH clones likely arise as immune escape mechanisms in aAA by preventing CD1D-restricted T cell recognition of GPI anchors and GPI-linked auto-antigens^{4, 5}. Though many patients with aAA treated with immune suppression therapy (IST) will develop subclinical PNH clones, only a subset will develop PNH disease, characterized by increased thrombosis, intravascular hemolysis, and potential for severe organ dysfunction. In contrast to IST, allogeneic hematopoietic stem cell transplantation (HSCT) for patients with aAA is thought to cure bone marrow aplasia and prevent hematopoietic clonal evolution to PNH⁶. Herein, we present a phenomenon of host-derived PNH disease arising in a patient with aAA many years following matched sibling donor bone marrow transplantation (MSD-BMT), highlighting the importance of monitoring for this clonal disease in aAA patients with stable mixed donor/recipient chimerism after

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Author Contributions:

JHO developed and performed the functional biology studies and prepared the manuscript. N.S. assisted with PNH clone sequencing and analysis. Y.H. J.L.D. and D.S.M. performed immunogenetic and chimerism studies. D.B. assisted with analysis and manuscript revision. T.S.O. contributed to case report and oversaw experiment and manuscript development.

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HSCT. We also provide a literature review for similar occurrences of PNH arising after HSCT.

Case

A 15 year-old male with Diabetes Mellitus Type I was diagnosed with acquired aplastic anemia (aAA) based on 20–30% bone marrow cellularity and a pancytopenia with White Blood Cell Count 2,900, Absolute Neutrophil Count 900/ μ L, Hemoglobin 8.6 g/dL, Mean Corpuscular Volume 107 fL and Platelets 18×10^9 /L. PNH and chromosomal stress testing to rule-out Fanconi anemia were negative at diagnosis. Five months after diagnosis (January 2003), he underwent MSD-BMT with thymoglobulin and cyclophosphamide (200mg/kg) conditioning. Prior to transplant, repeat PNH testing revealed asymptomatic PNH clonal expansion with 8.1% GPI negative red blood cells (RBC) and 10.1% GPI negative neutrophils. He achieved complete trilinear engraftment, and immune suppression prophylaxis was discontinued within the first year post-BMT. In the fourteen years since BMT, he has maintained greater than 90% total donor chimerism in peripheral blood.

In 2007 (approximately four years post-BMT), he developed moderate isolated thrombocytopenia (platelet range: $50 - 100 \times 10^9$ /L) in the setting of 98% donor chimerism. Bone marrow biopsy showed decreased cellularity and markedly decreased megakaryocytes. Cyclosporine was restarted, which maintained platelet counts between $75 - 100 \times 10^9$ /L. Repeat bone marrow biopsy 4 months post cyclosporine initiation was normocellular, though still with decreased megakaryocytes. He was transitioned to tacrolimus secondary to medication tolerability. His platelets again fell to $30 - 65 \times 10^9$ /L and repeat marrow in 2008 (five months later after previous marrow) was hypocellular with severely decreased megakaryocytes. At this time (five years post-BMT) he was given more aggressive therapy consisting of anti-thymocyte globulin ATG, prednisone and cyclosporine. This intervention led to improvement, but not resolution of cytopenias. In 2011 (eight years after BMT), PNH testing was done for the first time since BMT and demonstrated a minor 0.2% GPI negative clone in the granulocyte population.

He remained cyclosporine-dependent over the ten years after ATG administration, though he was successfully weaned to low doses while maintaining excellent blood counts, including Hgb >11 g/dL, ANC >1000 / μ L, and platelets $>80 \times 10^9$ /L. However, he exhibited steady growth of a PNH clone, encompassing 17.5% erythrocytes, 4.49% granulocytes and 33.65% monocytes (Figure 1a). In addition, he developed episodes of hemoglobinuria, and an elevated LDH, consistent with symptomatic hemolysis. The role of eculizumab infusions versus second HSCT were under discussion at the time of manuscript preparation. Peripheral blood engraftment studies at time of the above PNH screening revealed 90% total and 91% T cell donor chimerism. This chimerism has been relatively unchanged over the past 5 years (Figure 1b) despite the growth of his PNH clone, raising the question of whether this PNH clone was host- or donor-derived. Recent bone marrow analysis however, demonstrated total donor chimerism of only 77%. When BM cells were sorted based on presence of GPI anchors by FLAER staining, PNH cells were 97% host-derived. The residual 3% of cells in this sorted PNH fraction were donor-derived; however, due to limitations of PNH cell purity based on the FACS gating strategy, this small fraction of donor-derived cells may not

have been true PNH cells. BM cells with normal FLAER signal (indicating absence of PNH) were 99% donor derived (Figure 1c). We speculate that the PNH cells are likely less stable in peripheral circulation compared to normal cells. Sequencing analysis indicated that all PNH cells were derived from a single clone with a 14 base pair duplication in exon 2 of *PIGA* (Figure 1d). These data collectively indicate that the vast majority of residual host-derived bone marrow cells in this patient are part of a single PNH clone.

Discussion

Recurrence or development of new PNH clones following HSCT for aAA has been infrequently reported. This underreporting is likely because PNH screening is not part of standard post-HSCT surveillance guidelines in patients with aAA who do not have symptomatic PNH disease pre-HSCT, despite the fact that up to 50% of patients with aAA have detectable PNH clones⁷. In one series of 33 patients who received HSCT for PNH clones (both clinical and subclinical clones either associated with aAA or *de novo* disease), 2/25 with available follow-up (both with previous aAA) had re-emergence of a PNH clone⁸. One patient had a transient PNH clone at 17 months post-HSCT which regressed by 24 months post-HSCT. The second patient had emergence of a small but stable PNH clone that persisted through donor stem cell boost and 85% donor chimerism. Another report of PNH occurring after HSCT for aAA details a small donor-derived PNH clone after a second HSCT for late graft failure⁹. For *de novo* PNH without concurrent aAA, there is one report of relapse after BMT from a monozygotic twin with no conditioning regimen¹⁰. In another report, PNH relapse occurred after syngeneic PBSC transplant conditioned with high dose cyclophosphamide alone (200mg/kg total dose), leading the authors to postulate that more intensive conditioning regimens may be necessary to myeloablate established PNH clones and eliminate host immune cells that select for expansion of PNH cells¹¹.

We presented an unusual case of aAA treated with MSD-BMT with subsequent relapse and development of a large PNH clone over 10 years after initial therapy (figure 1e). The initial aAA relapse manifested as cytopenias, as well as the subsequent expansion of PNH many years later are events both likely attributable to continued selective immune pressure from residual host T cells post-BMT. The very late PNH clonal expansion relative to the timing of initial cytopenia recurrence may have been due to the institution of immune suppression therapy in response to cytopenia recurrence, thus temporarily limiting the selective immune pressure that ultimately led to PNH clone expansion once immune suppression was weaned. Another feature that is particularly unique about this case is the dichotomy between the host-derived PNH clone which sequentially increased in size, compared to the stable peripheral chimerism demonstrated over that same period. Interestingly chimerism in bone marrow was considerably lower than in peripheral blood, correlating with a larger proportion of PNH cells in BM than in peripheral blood. Thus, what was initially thought to be a donor derived PNH clone based on size of the PNH clone in erythrocytes and monocytes in peripheral blood was actually host-derived. While there are reports of peripheral blood chimerism overestimating donor chimerism compared to bone marrow studies¹² which could be applicable to this case, an alternative explanation is that the shorter life-span of host derived PNH cells in peripheral circulation may also drive higher donor chimerism in peripheral blood versus bone marrow in the setting of PNH clones. The clinical implications of these

findings suggest that despite stable mixed donor chimerism, host-derived PNH can relapse after HSCT for aAA. Based on this report, PNH screening should be considered in patients that received HSCT for aAA who have persistent mixed hematopoietic chimerism after HSCT, particularly for patients who may have symptoms of PNH, including elevated LDH, renal dysfunction, and history of unexplained thrombosis.

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Abbreviations

PNH	Paroxysmal Nocturnal Hemoglobinuria
aAA	Acquired Aplastic Anemia
GPI	Glycosylphosphatidylinositol
IST	Immune Suppression Therapy
HSCT	Hematopoietic Stem Cell Transplantation
MSD	Matched Sibling Donor
BMT	Bone Marrow Transplant

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Figure 1:

A small PNH clone was present in our patient immediately prior to BMT. PNH clone was again present 7 years post-BMT when it was checked again due to cytopenias and this clone has since exhibited continuous growth (a). Stable peripheral engraftment above 90% for 14 years post-BMT in unenriched, T cell and Myeloid cell lines (b) Sorted PNH+ and normal cells from bone marrow aspirate using FLAER. PNH+ cells were found to be 3% donor while PNH- cells were 99% donor. Unenriched marrow was 77% donor derived (c). Sanger sequencing of DNA derived from the PNH cells showed a single duplication of 14 base pairs in exon 2 of *PIGA* suggesting all PNH cells likely arose from a single clone. Highlighted sequence delineates site of the acquired duplication within the *PIGA* gene (d). Brief clinical timeline indicating important points in disease progression and therapeutic intervention (e).