

Detection of PNH cells in the bone marrow

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Introduction

Peripheral blood is considered the specimen of choice for detecting cells with a paroxysmal nocturnal hemoglobinuria (PNH) phenotype by flow cytometry because normal immature precursors with under-developed glycosylphosphatidylinositol (GPI) and GPI-anchored proteins will cause false positive results. Appropriate use of ancillary antibodies and gating strategies will allow detection of PNH cells in bone marrow without false positive results.

Methods

Initial maturation study

In the first section, bone marrow samples were utilized to evaluate expression of the GPI anchor using FLAER and other GPI linked antigens on granulocytes as they matured from precursors to neutrophils. A number of combinations were used in this portion of the study.

- CD13 /CD55 or CD59/CD45/CD34/CD16
- FLAER /CD13 /CD45 /CD15 /CD16
- FLAER/CD55 or CD59/CD34/CD15/CD45
- FLAER/CD55 or CD59/CD45/CD15/CD16 ECD

Evaluation of bone marrow for PNH cells

PNH evaluations of bone marrow and concomitant peripheral blood samples were evaluated using the combination FLAER Alexa/ CD55+CD59 PE/ CD45 ECD/ CD15 PC5/ CD33 PC7 for evaluation of granulocytes. The most mature granulocytes were selected for evaluation of CD55, CD59 and FLAER by first gating on granulocytes with the greatest side and forward angle light scatter followed by secondary gating on the subset expressing the brightest CD15 and CD45.

- FLAER/CD55+CD59/CD45/CD15/CD33

Spiked samples

In the third section, a cell suspension from normal bone marrow sample was spiked using serial dilutions of cell suspension from a highly positive PNH sample. These spiked cell suspensions were evaluated using the same combination used in the bone marrow study section above.

- FLAER/CD55+CD59/CD45/CD15/CD33

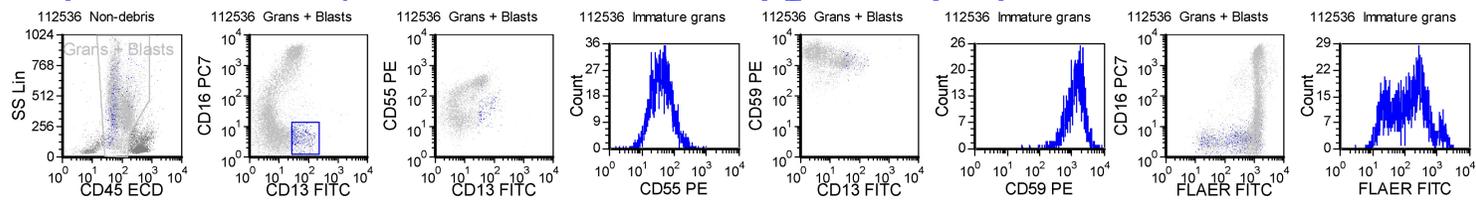
Results

Initial maturation study

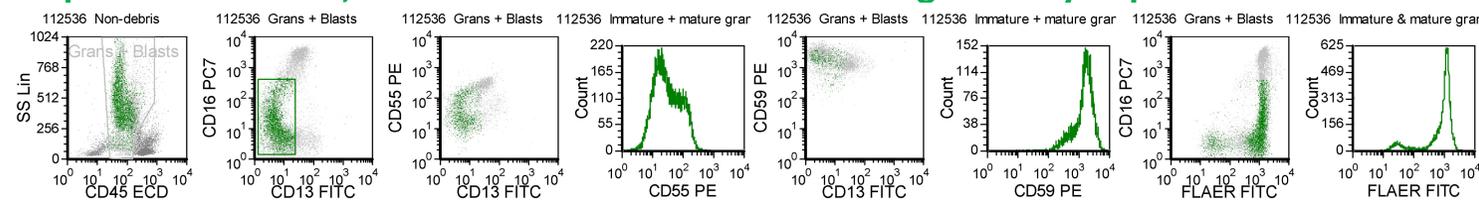
- CD13/CD55 or CD59/CD45/CD34/CD16 and FLAER /CD13 /CD45 /CD15 /CD16

CD13 and CD16 were combined with CD55, CD59 or FLAER to evaluate expression of the two GPI linked antigens and GPI anchor as granulocytes matured from blasts to neutrophils in 5 bone marrow specimens. Phenotypic blasts expressing CD34 showed dim to moderate intensity staining for CD55. The expression of CD55 then decreased in immature granulocytic precursors expressing CD13 without CD16 (CD13+/CD16-) and was steadily acquired as the granulocytes matured into neutrophils expressing both CD13 and CD16 (CD13+/CD16+). The pattern of GPI anchor expression based on FLAER staining looked very similar to CD55. However, CD59 showed a different pattern and was expressed with bright intensity at all levels of maturation from the CD34 positive blasts and CD13+/CD16- precursors to CD13+/CD16+ mature neutrophils.

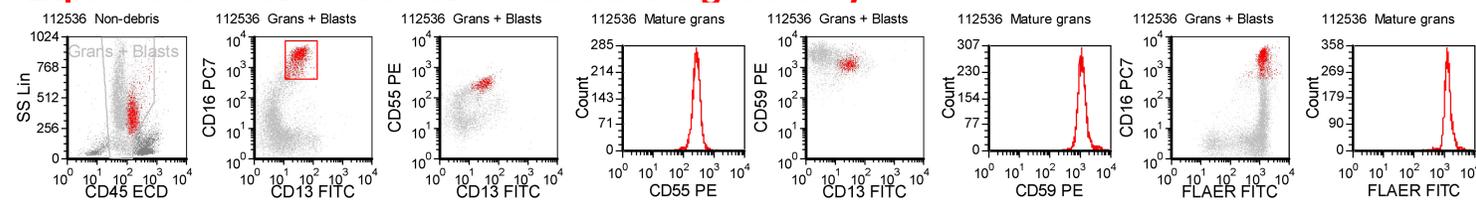
Expression of CD55, CD59 and FLAER on early granulocytic precursors



Expression of CD55, CD59 and FLAER on intermediate granulocytic precursors



Expression of CD55 and CD59 on mature granulocytes

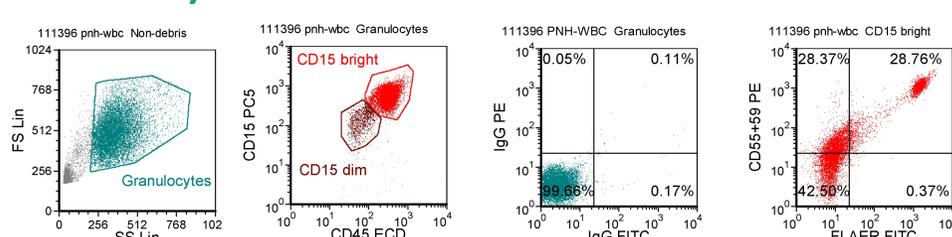


Evaluation of bone marrows for PNH cells

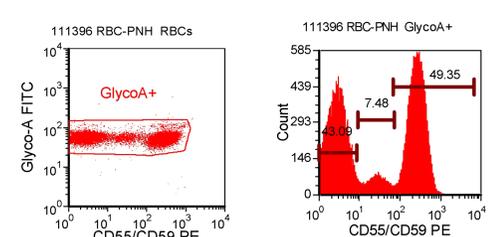
- FLAER/CD55+CD59/CD45/CD15/CD33

Of 68 bone marrows tested for the presence of PNH cells, 8 were positive using CD55, CD59 and FLAER together with CD45 and CD15 to select the most mature granulocytes (FLAER ALEXA/CD55+CD59 PE/CD45 ECD/CD15 PC5/CD33 PC7). Red blood cells deficient in CD55 and CD59 were also detected in all the positive samples. Of the 8 positive bone marrow samples, 5 were confirmed in the peripheral blood using the same combination of antibodies and gating strategy.

Granulocytes



Red blood cells

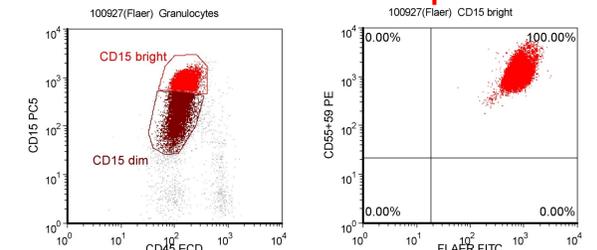


Spiked samples

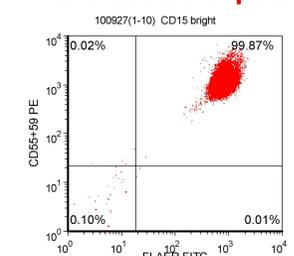
- FLAER/CD55+CD59/CD45/CD15/CD33

Serially diluted PNH granulocytes spiked into a normal marrow sample were detected down to ~0.1% of the cells analyzed.

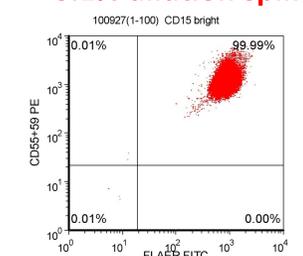
Normal marrow no spike



~1% dilution spike



~0.1% dilution spike



Conclusion

Bone marrow can be utilized for detecting PNH cells by using adjunct antibodies and gating strategies to select only the most mature granulocytes, avoiding immature precursors that will cause false positive results. Only 2 cases of refractory anemia with excess blasts (RAEB) and 1 acute myeloid leukemia were evaluated for PNH cells in this study. Though they were negative for PNH cells by the methodology used, caution is recommended when evaluating any sample with increased blasts and precursors at the levels of RAEB or greater. This is a topic for future studies.

References

1. Brodsky R, Mukina G, Li S et al. Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. Am J Clin Pathol. 2000;114:459-466.
2. Borowitz M, Craig F, DiGiuseppe J et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. Cytometry Part B. 2010;00:000-000 (Online in advance of print)