Automated Detection of GPI-deficiency in Paroxysmal Nocturnal Hemoglobinuria (PNH)

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Background

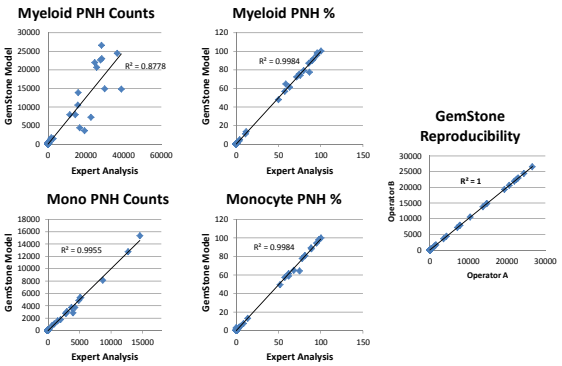
Flow Cytometry is used for the detection of glycosylphosphatidylinositol (GPI) deficient clones in paroxysmal nocturnal hemoglobinuria (PNH) and other bone marrow failure disorders. Although the Clinical Cytometry Society published guidelines for the performance of PNH assays, data analysis has not been standardized. Current analysis requires trained analysts using gates to quantify PNH cells. Automated analysis could improve objectivity, standardization, and speed.

In this study, we develop an automated analysis of PNH samples using GemStone™ (Verity Software House). We compare the results with traditional analysis of PNH samples performed by a trained expert.

Methods

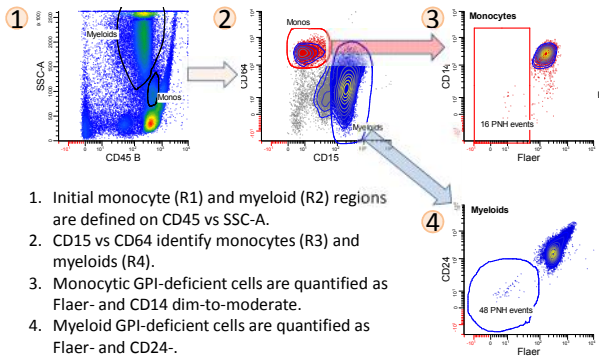
One hundred eighty (180) peripheral blood samples submitted for PNH analysis were assayed in a clinical laboratory using BD Canto II flow cytometers. The white blood cells were prepared using a stain-lyse-wash technique. The data were not censored for data quality, the presence of a hematologic malignancy, or other factors.

Results



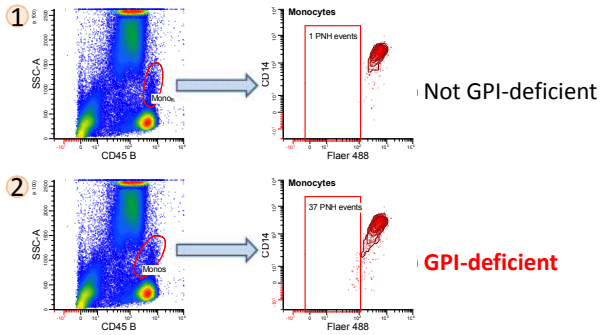
By human analysis, 24 of the 180 samples were reported as showing the presence of a GPI-deficient clone. Automated analysis by GemStone identified 25 samples with a GPI-deficient clone, including the 24 identified by the human analysis. The single case of disagreement was found to be dependent on how gates were drawn in the human analysis. Expert and GemStone percentages of GPI-deficient cells were highly correlated. GemStone results produced by 2 operators were identical (r^2=1.00).

Gate-based Method



1. Initial monocyte (R1) and myeloid (R2) regions are defined on CD45 vs SSC-A.
2. CD15 vs CD64 identify monocytes (R3) and myeloids (R4).
3. Monocytic GPI-deficient cells are quantified as Flaer- and CD14 dim-to-moderate.
4. Myeloid GPI-deficient cells are quantified as Flaer- and CD24-.

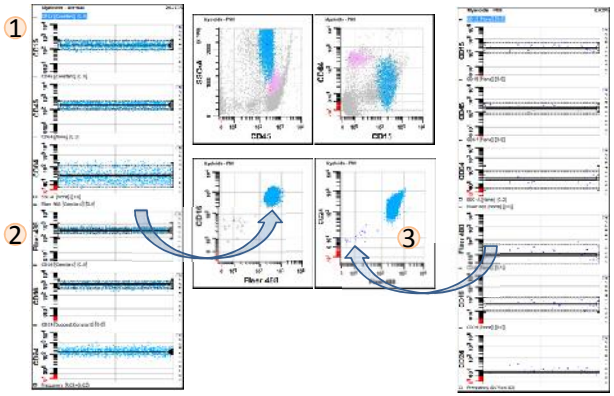
Gate Subjectivity



The one discrepant case between gating and modeling was due to a subjective gating decision that reclassified the sample.

1. With the original monocyte gate, one GPI-deficient monocytic cell is detected.
2. A modest change in the CD45 vs. SSC-A monocyte gating region produces 37 GPI-deficient cells - a diagnostic difference in the results.

GemStone Method



1. CD15, CD45, CD64, and SSC select myeloids.
2. Normal Myeloid cells are positive for Flaer, CD16, and CD24.
3. Myeloid PNH cells are dim to negative for Flaer, CD16 and CD24.

A similar strategy was used for monocytes. No gates or user-interventions are used in this method. All decisions are based on probabilities defined by the model.

Conclusions

- GS results were reproducible (r^2=1) between operators. Automation of GS required no case-by-case operator decisions.
- The average GS analysis time was 35 seconds/case, compared to 300 seconds/case for human analysis. 180 cases required less than 2 hours for GS and over 15 hours for human analysis.
- The results suggest that PNH analysis may require fewer markers than currently recommended. For monocytes, CD14 was not necessary for the analysis; in some cases it hindered the PSM. Only FLAER and selection markers were required. For modeling myeloids, CD16 and CD24 were approximately equivalent - and therefore redundant - in resolving the target populations. FLAER was the strongest marker in PNH detection.
- We found that automated analysis of PNH by GemStone produces results that correlate strongly with expert analyst. The probability-based approach provides higher objectivity, speed, and reproducibility.