

Hemodilution is the Primary Cause of Discordance Between Morphologic and Flow Cytometric Myeloid Blast Estimates in Bone Marrow Aspirate Samples.



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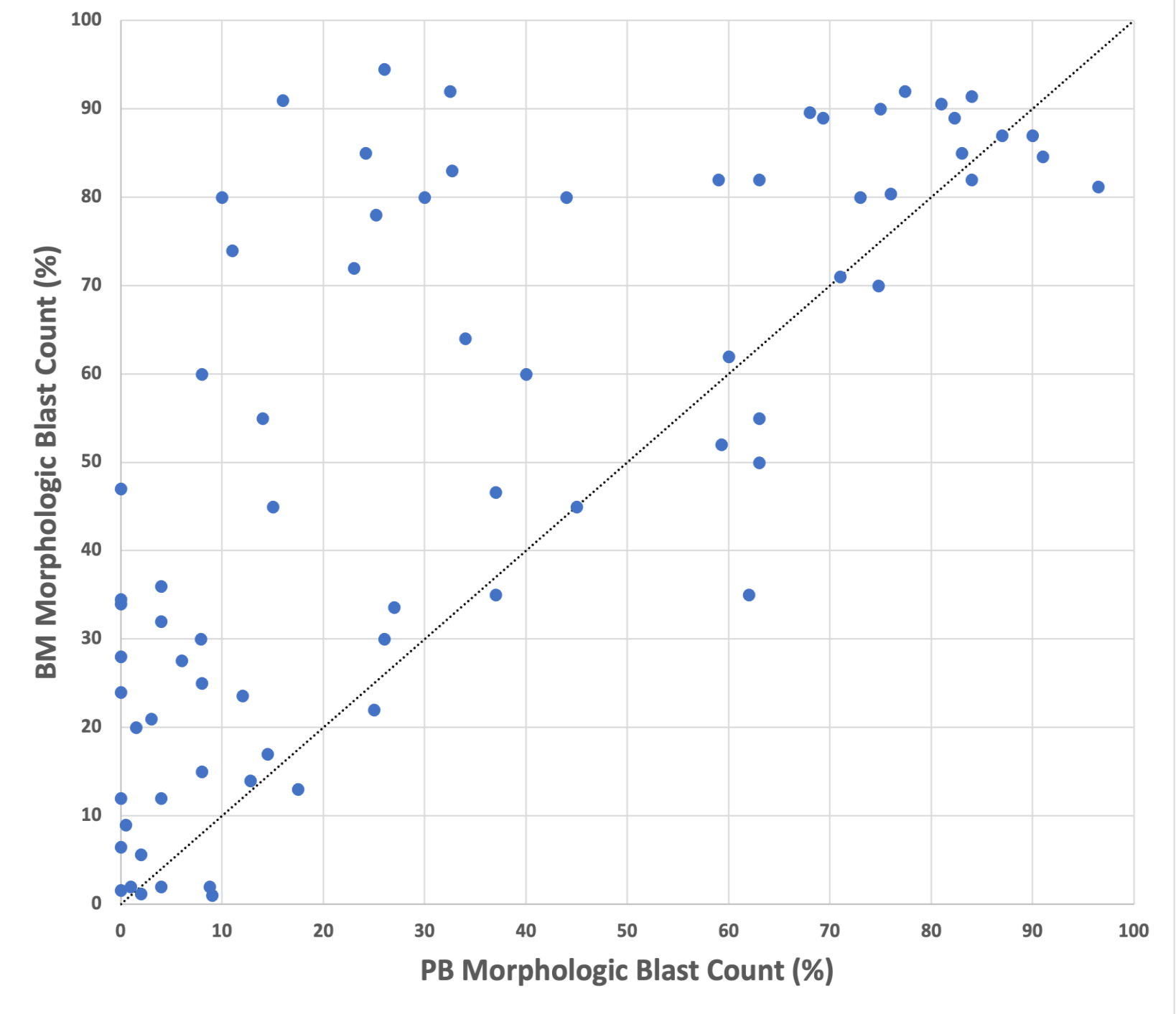
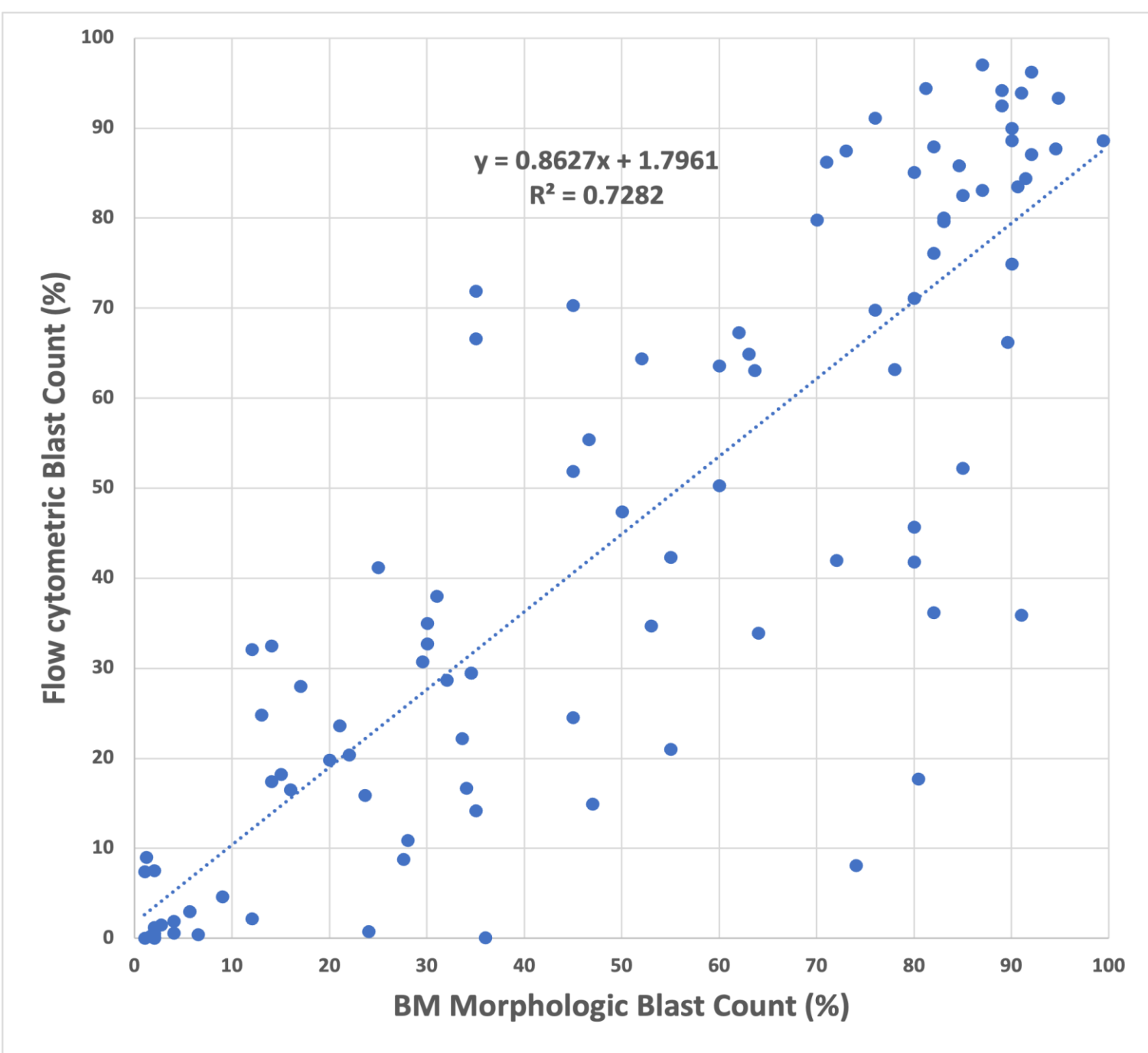
Introduction

Bone marrow morphology and flow cytometry (FC) are routinely used to determine the blast percentage (BP) in patients with acute myeloid leukemia (AML). We evaluated the concordance of the myeloid blast percentage between the two methods to assess the contributions of hemodilution and viability to discordance.

Methods

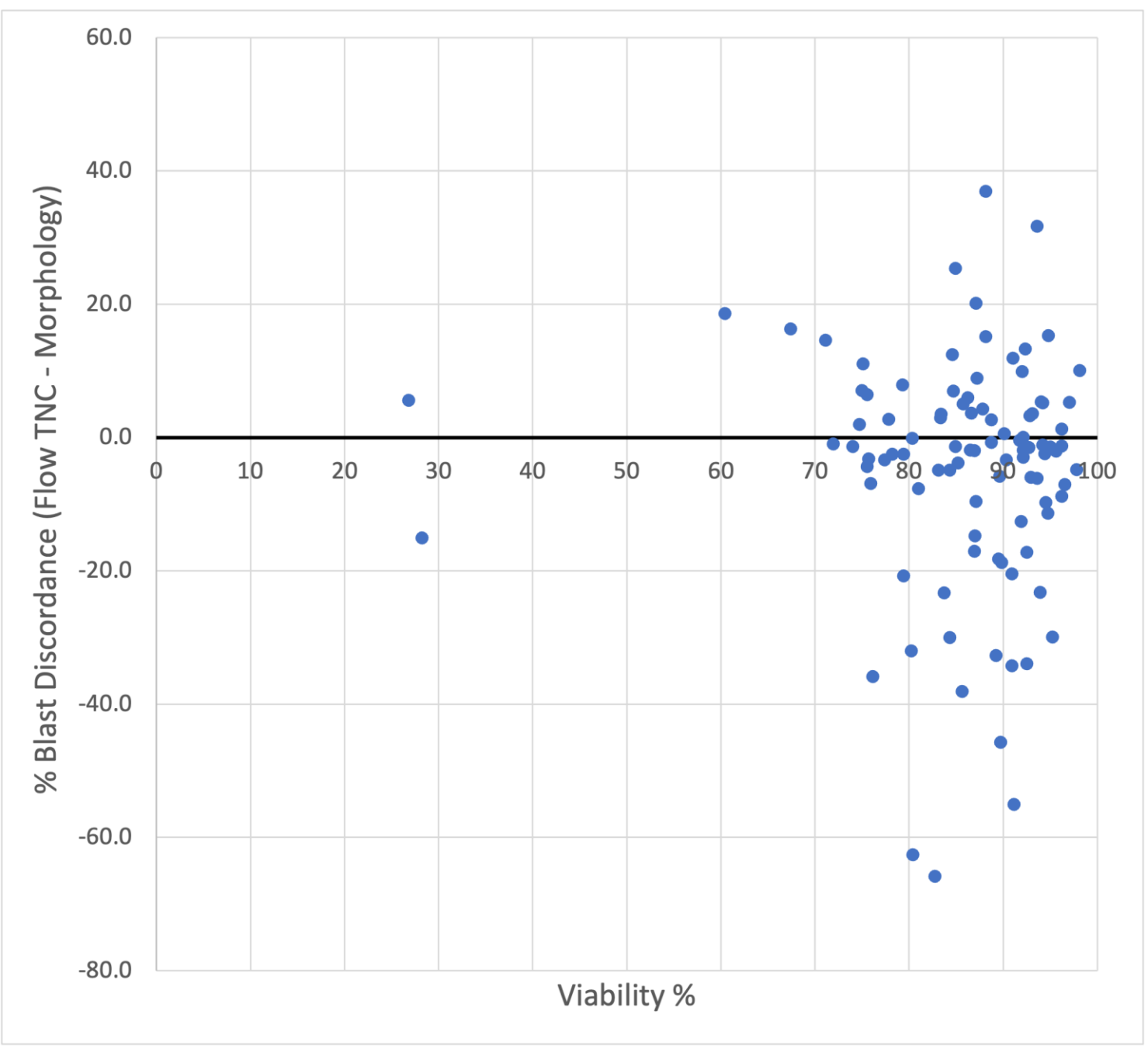
- Bone marrow aspirate samples (N=100) at pre-enrollment to the NCI MyeloMATCH clinical trial for myeloid neoplasms had blast counts determined by morphology and blast equivalents enumerated by flow cytometry.
- The flow cytometric blast percentage was determined using both total white cell (TWC) and total nucleated cell (TNC) denominators.
- All samples were also assessed for viability and hemodilution by flow cytometry. Viability was determined as the fraction of nucleated white cells (CD45+ and SYTO16+) having normal light scatter characteristics. Hemodilution was determined as the fraction of high SS neutrophilic lineage cells expressing high CD16.
- Samples with a difference in the blast count between morphology and flow cytometry exceeding 20% were classified as discordant.

Results

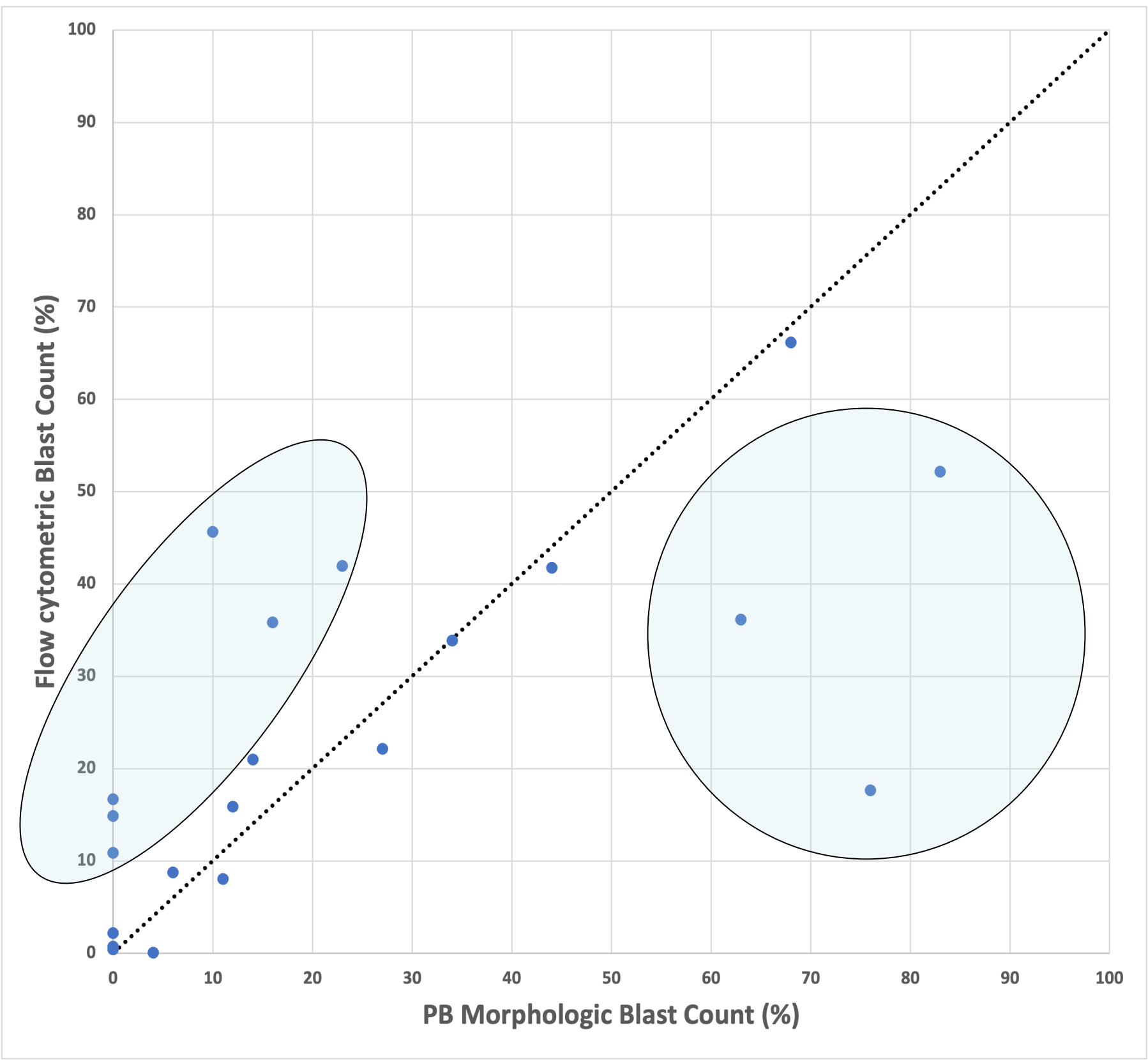


A strong linear correlation was noted between morphologic and flow cytometric blast estimates. 34% of samples showed discordance of greater than 20%, 25% showing underestimation of the blast percentage by flow cytometry and 7% showing overestimation. 2% of samples showed overestimation by flow cytometry using the WBC denominator (TWC) that normalized using the TNC denominator due to the presence of increased nucleated red cells.

A subset of AML show prominent discordance in blast enumeration between peripheral blood and bone marrow aspirate, suggesting differences in AML biology.



Variability in blast discordance was not correlated with viability, although relatively few samples showed poor viability.



A small subset of discordant samples(N=6) show a higher blast % by flow cytometry than seen in blood, consistent with partial hemodilution.

Prominent monocytic differentiation appears to be a cause for discordance in a small subset of samples (N=3) having higher blast % by morphology.

Underestimation was associated principally with increased hemodilution, particularly when differences in the morphologic blast percentages between blood and marrow were present, the flow value being intermediate between the two.

Conclusions

- Good correlation between flow cytometry and morphologic myeloid blast count with a variability of ~20% for good quality samples.
- One-third of samples show underestimation by flow cytometry, primarily due to hemodilution, particularly when differences in blast percentages between peripheral blood and bone marrow are present.
- Prominent monocytic differentiation and increased nucleated red cells may contribute to discordance in a small subset of cases.
- Specimen viability was not significantly associated with blast discordance, perhaps due to generally good quality of samples received on this trial.
- Assessment of hemodilution is important to identify bone marrow samples where enumeration of blast equivalents may be underestimated by flow cytometry.
- Improved flow cytometric methods are needed for accurate enumeration of blast equivalents in AML having monocytic differentiation.

References

Godwin CD, Zhou Y, Othus M, Asmuth MM, Shaw CM, Gardner KM, Wood BL, Walter RB, Estey EH. Acute myeloid leukemia measurable residual disease detection by flow cytometry in peripheral blood vs bone marrow. Blood. 2021 Jan 28;137(4):569-572. doi: 10.1182/blood.2020006219. PMID: 33507294; PMCID: PMC7845008.