The Utility of Flow Cytometry in the Diagnosis of Acute Monocytic and Monoblastic Leukaemias

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INTRODUCTION

Immunophenotyping remains an important tool for diagnosing and classifying acute leukaemias. However, stages of monocytic maturation can be difficult to delineate by both morphology and immunophenotyping. This is of particular relevance for detecting early monocytic blast equivalents (monoblasts and promonocytes) in the diagnosis of acute myelomonocytic, acute monocytic and acute monoblastic leukaemias. Currently, CD14 and CD64, are most often utilised within this setting. Monoblasts typically show a CD64+, CD14- phenotype but in our experience the expression of CD14 by promonocytes is heterogenous (CD64+, CD14+/-).

AIMS

This study aimed to identify a flow cytometry gating strategy for monocytic maturation, particularly identifying promonocytes in an acute monocytic and monoblastic leukaemia cohort. A secondary aim was to determine if CD14 conjugated to different fluorophores, targeting different CD14 epitopes (CD14 FITC Mo2 epitope; Beckman coulter CD14 FITC clone 116 and CD14 APC; Becton Dickinson CD14 APC-Cy7 clone MϕP9) and if this would improve the identification of promonocytes.

METHOD

Eight patients with acute monocytic or monoblastic leukaemia were assessed. Mature monocyte populations were referenced from a control group for gating comparison. Monoblast and mature monocyte populations were gated and compared to a manual morphological differential of monocyte populations. The proposed promonocyte region between monoblast and mature monocyte gate was analysed (example, Figure 1) and percentage difference between manual differential made.

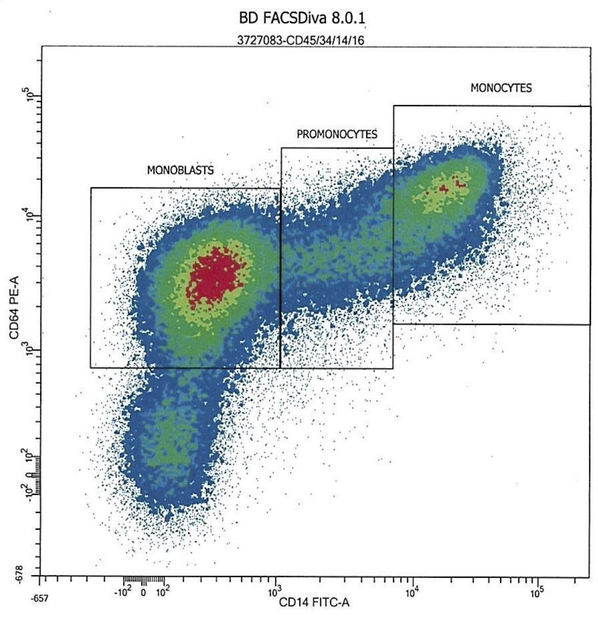

Figure 1. Flow cytometry dot plot example CD14 APC-Cy7 clone MϕP9 antibody against CD64, with proposed promonocyte region gated for analysis.

RESULTS

In all patients, promonocyte populations were under quantified using either antibody compared to morphology (Table 1). The mean percentage difference from morphological promonocyte differential between FITC Mo2 epitope was 58.4% (SD 21.22%, p=0.01) and CD14 APC 58% (SD 23.12%, p=0.006). No discernable difference between either epitope was noted; both antibodies demonstrate promonocyte CD14 expression similar to that seen in mature monocytes.

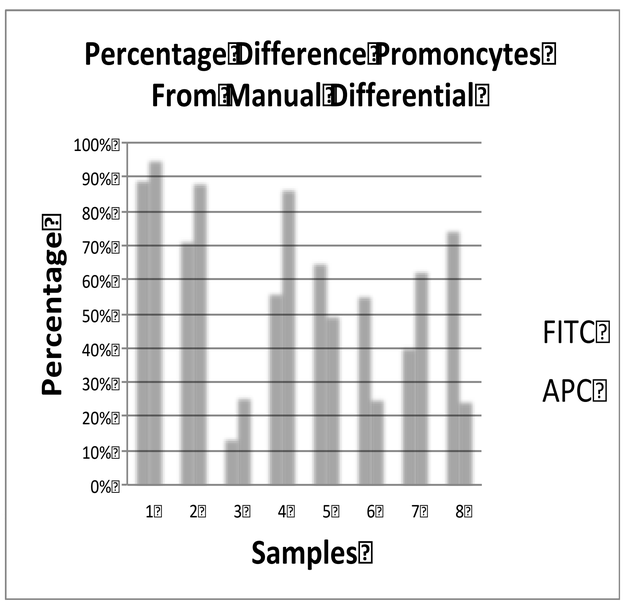

Table 1. Demonstrates percentage difference of promoncytes by manual differential compared to CD14 FITC Mo2 epitope (Beckman coulter CD14 FITC clone 116) and CD14 APC (Becton Dickinson CD14 APC-Cy7 clone MϕP9).

CONCLUSIONS

In summary, promonocyte populations cannot be easily gated and quantified using standard immunophenotyping methods to help ascertain blast equivalents, and secondly, the FITC Mo2 epitope for CD14 is no more accurate than CD14 APC for discriminating different monocyte populations. This reinforces the importance of morphological assessment of monocyte populations compared to immunophenotyping alone in defining monocyte lineage acute leukaemias.

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DISCLOSURE OF INTEREST

None Declared