Increased sensitivity of flow cytometry for detection of minimal residual disease and circulating tumor cells in B Non-Hodgkin Lymphoma

Christiane Siewert[1], Karin Hartmann[2], and Thomas Nebe[2]*
[1] Miltenyi Biotec GmbH, Bergisch Gladbach, Germany | [2] Hematology-Laboratory Mannheim, Germany | * Corresponding author

Introduction

Minimal residual disease (MRD) assessment is of increasing importance in the context of patient monitoring in various hematologic malignancies. MRD has been shown to be an independent predictor of poor clinical outcome. However, the detection of residual tumor burden requires highly sensitive methods. Flow cytometry is an attractive method for fast and reliable detection of rare circulating cells, provided that an aberrant immunophenotype exists and sufficient numbers of total cells are acquired. We have established an immunomagnetic pre-enrichment protocol which has the potential to increase the sensitivity of flow cytometry analysis for the detection of MRD or small amounts of circulating tumor cells at the time of diagnosis.

Methods

Leftover material from 94 patient samples submitted for routine diagnostics was used for the detection of clonal B cells by flow cytometric immunophenotyping. The patient cohort included different types of low grade B cell lymphoma and a few myeloma samples for feasibility. Samples were tested in parallel and equal amounts of patient material were subjected to immunomagnetic pre-enrichment of B cells and bulk lysis, respectively.

CD19$^+$ B cells were immunomagnetically enriched from 1–2.5 mL of whole blood. In brief, EDTA whole blood was incubated with StraightFrom® Whole Blood CD19 MicroBeads and the magnetically labeled blood sample was directly applied to a Whole Blood Column placed in a MACS® Separator. After washing, the column was removed from the MACS Separator and magnetically labeled cells were eluted by flushing the column (fig. 1).

This immunomagnetic pre-enrichment method was compared to a bulk lysis procedure. The corresponding EDTA blood volume was mixed and incubated with ammonium chloride–based red blood cell lysis solution. Cells were then centrifuged and washed.

CD19$^+$ magnetically pre-enriched samples and cells after bulk lysis were collected for fluorescent staining with 7- or 8-color antibody panels containing relevant antibody combinations according to the initial diagnosis (table 1). Stained cells were analyzed by flow cytometry (MACSQuant® Analyzer 10, Miltenyi Biotec). Results obtained from both enrichment procedures were compared to established routine diagnostic flow cytometry measurements (Navios, Beckman Coulter) starting from 50–100 µL of blood without enrichment.

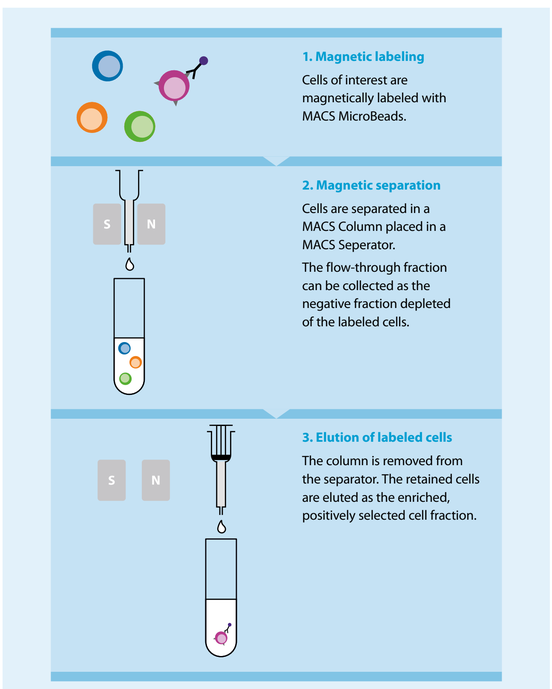

Figure 1

Fluorochrome	B-NHL panel	HCL panel
VioBlue®	CD20	CD20
VioGreen™	CD45	CD45
FITC	Anti-kappa	Anti-kappa
PE	CD10	CD11c
PerCP-Vio® 700	-	CD25
PE-Vio® 770	CD38	CD103
APC	Anti-lambda	Anti-lambda
APC-Vio® 770	CD5	CD5

Table 1

Results

1 Increased number of B cells for immunophenotyping after CD19$^+$ pre-enrichment from whole blood

The clonality of B cells in a sample submitted for follicular lymphoma without enrichment (fig. 2A, upper row) or after magnetic CD19$^+$ pre-enrichment (fig. 2A, lower row) was analyzed by flow cytometry for the ratio of kappa to lambda light chain expression within the rare CD20$^+$CD10$^+$ subset. In another case, the malignancy of B cells in a submitted hairy cell leukemia (HCL) sample without enrichment (fig. 2B, upper row) or after magnetic CD19$^+$ pre-enrichment (fig. 2B, lower row) was analyzed for co-expression of CD11c and CD103 by flow cytometry. In both cases, magnetic CD19$^+$ pre-enrichment lead to an increased number of B cells available for flow cytometric immunophenotyping of B cell clonality.

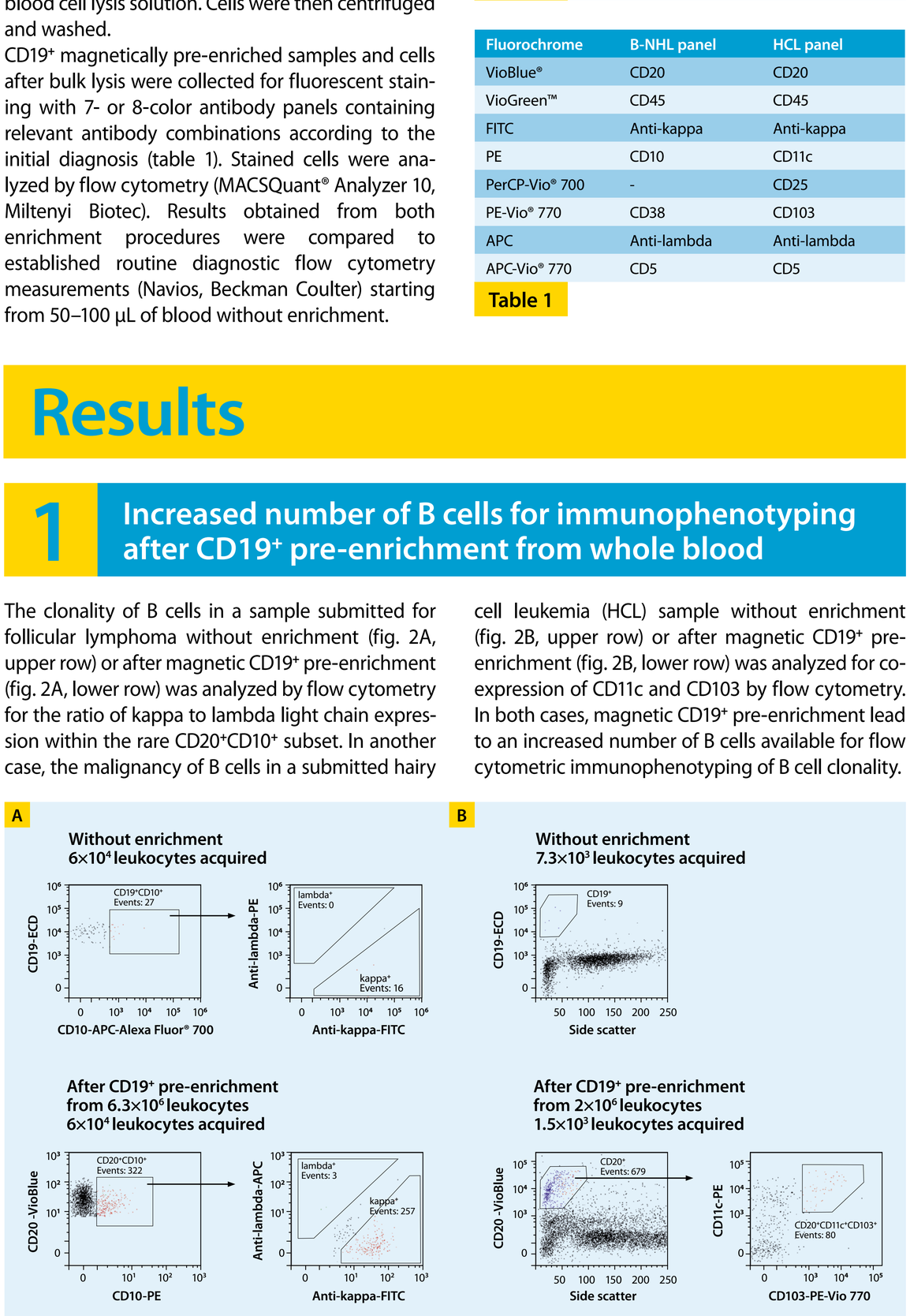

Figure 2

2 Time-efficient analysis of pre-enriched CD19$^+$ B cells compared to bulk lysis

As a representative example, the flow cytometry analysis of one submitted HCL sample is shown in figure 3 without enrichment (A), after bulk lysis (B), and after magnetic CD19$^+$ pre-enrichment (C). Malignant B cells were identified based on aberrant expression of CD11c and CD103 among CD19$^+$CD20$^+$ cells. Both, magnetic CD19$^+$ pre-enrichment and bulk lysis resulted in a comparable number of B cells available for immunophenotyping. However, the analysis after magnetic CD19$^+$ pre-enrichment was more efficient with an acquisition time of only 1 min/sample as compared to >5 min/sample acquisition time after bulk lysis.

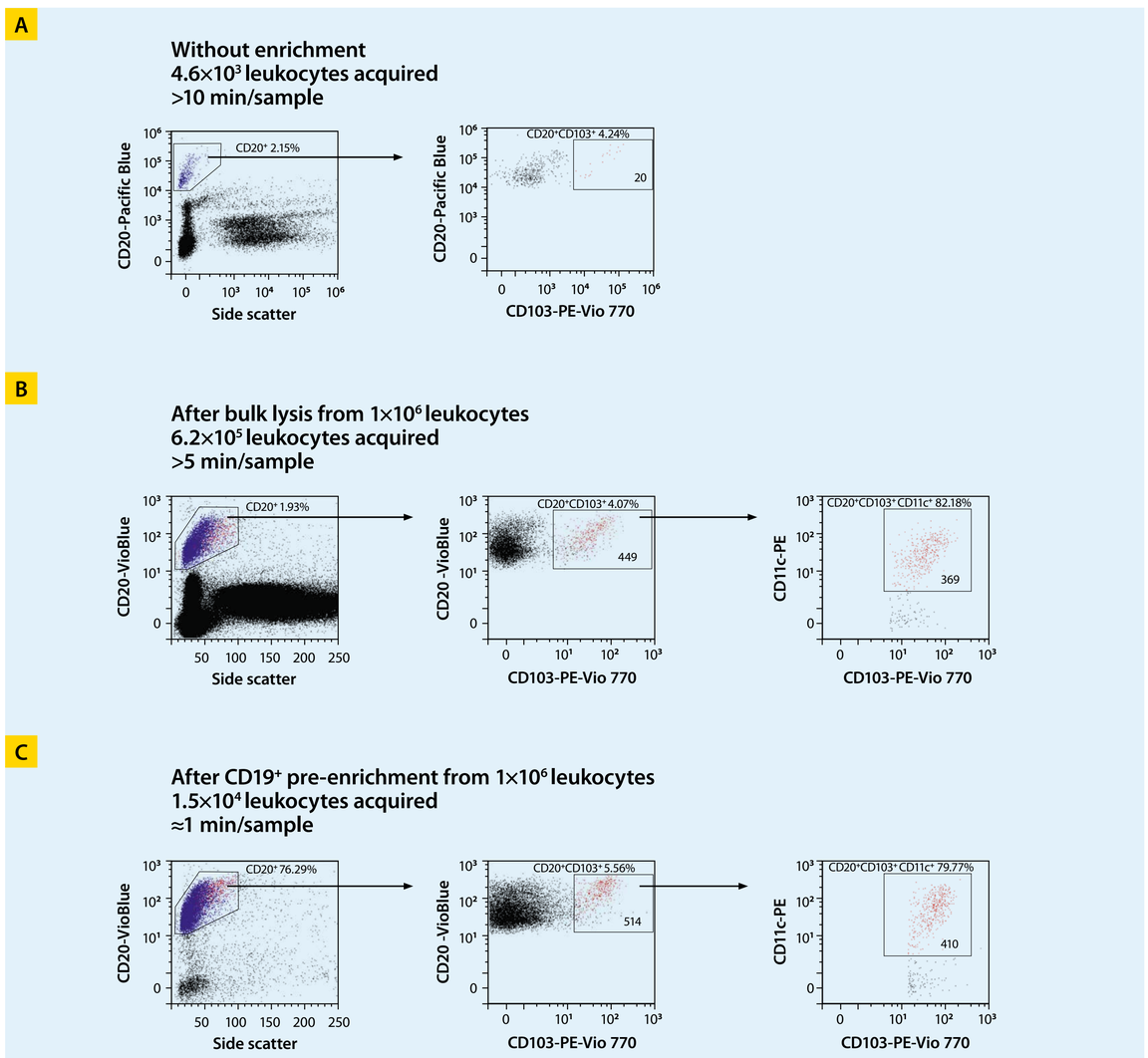

Figure 3

3 Highly sensitive analysis of rare aberrant B cells after pre-enrichment directly from whole blood

Another HCL sample submitted for MRD analysis was analyzed by flow cytometry after magnetic CD19$^+$ pre-enrichment from 1 mL of whole blood containing 3.1×10^6 leukocytes (fig. 4). After pre-enrichment, 1.4×10^4 leukocytes were acquired and analyzed for the presence of malignant cells.

Combined gating for aberrant expression of CD11c and CD103 identified a small but distinct cluster of 21 cells clonal for lambda light chain expression. This corresponds to a theoretical sensitivity of 0.000677% (21 clonal cells among 3.1×10^6 leukocytes).

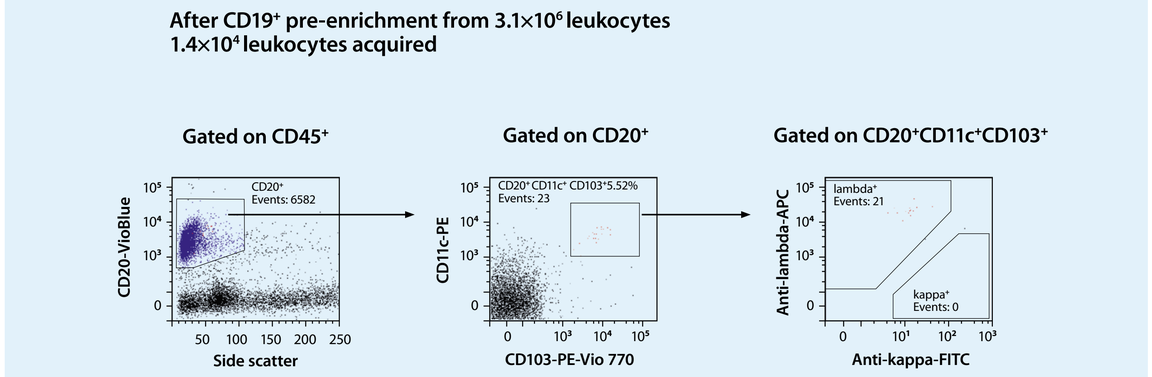

Figure 4

Conclusion

Using the enrichment methods presented here, flow cytometry reaches the sensitivity known from competing MRD methods like PCR or next generation sequencing. Immunomagnetic pre-enrichment may pave the way to achieve even higher sensitivities. The magnetic column used here, allows processing of up to 15 mL of whole blood for direct enrichment of B cells. This potential to start from higher total numbers of leukocytes provides the opportunity to push the current limits of sensitivity even further and to make the combination of magnetic pre-enrichment and flow cytometry the first method of choice for detection of MRD.