Infiltration of CNS by acute leukaemia:

Analysis of fresh and TransFix® stabilised CSF

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Introduction

Flow cytometry (FC) is more sensitive than cytomorphology for detecting haematological malignancies in cerebrospinal fluid (CSF). It is increasingly used to detect leptomeningeal disease in acute leukaemia since it is of prognostic importance even when malignant cells are present at low levels (1).

CSF cells deteriorate within hours after lumbar puncture (2) and require analysis as soon as possible after sampling.
The use of stabilising medium may therefore be useful.

Transfix is a fixative solution that preserves peripheral blood samples (3). It is also used for preserving cells in CSF, and works well for evaluating leptomeningeal B cell Non-Hodgkin's Lymphomas (4-6).
However, it is unclear whether acute leukaemia blasts present in CSF can be successfully stabilised by transfix.

Transfix-induced light scatter changes do not impair cell identification

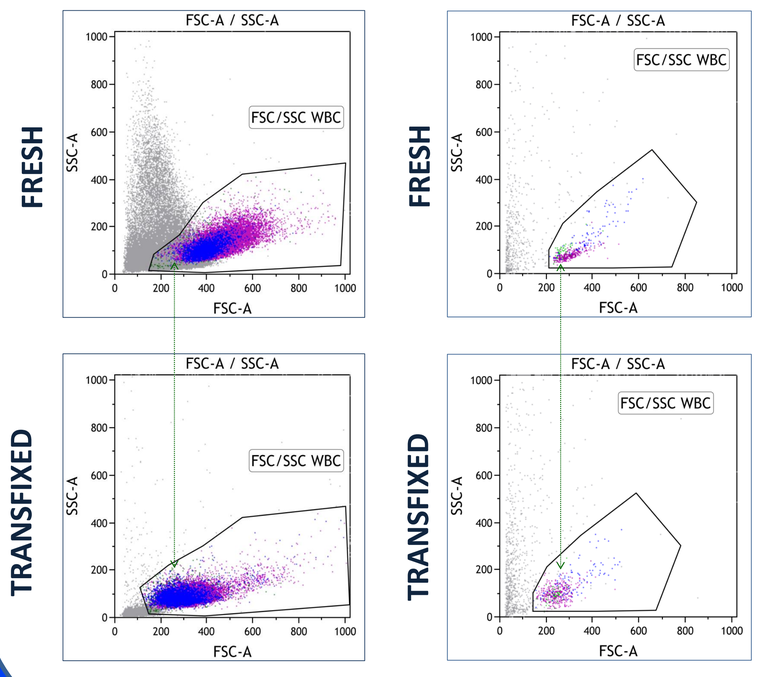

Figure 1. Transfix-treated cells had reduced FSC compared to fresh cells, for all cell populations and samples examined (n=23).

All cases of B-Acute Lymphocytic Leukaemia (B-ALL) (n=4) showed an increased SSC signal after transfix treatment.

No sample or cell population were difficult to gate or recognise due to transfix induced light scatter changes.

Methods

CSF samples were analysed within 2-6 hours of sampling or after 72 hours of storage at +4°C in transfix.

Cells were concentrated by centrifugation, labelled with antisera, washed and acquired within 30 minutes on a Canto II equipped with standard laser, filter and PMT setup. The full sample volume was always acquired.

Data were analysed using Kaluza. Forward and Side light scatter (FSC and SSC), cell yield, the median fluorescence intensity and the coefficient of variation (CV) were recorded for each gated population.

Transfix maintains key B-ALL antigen expression

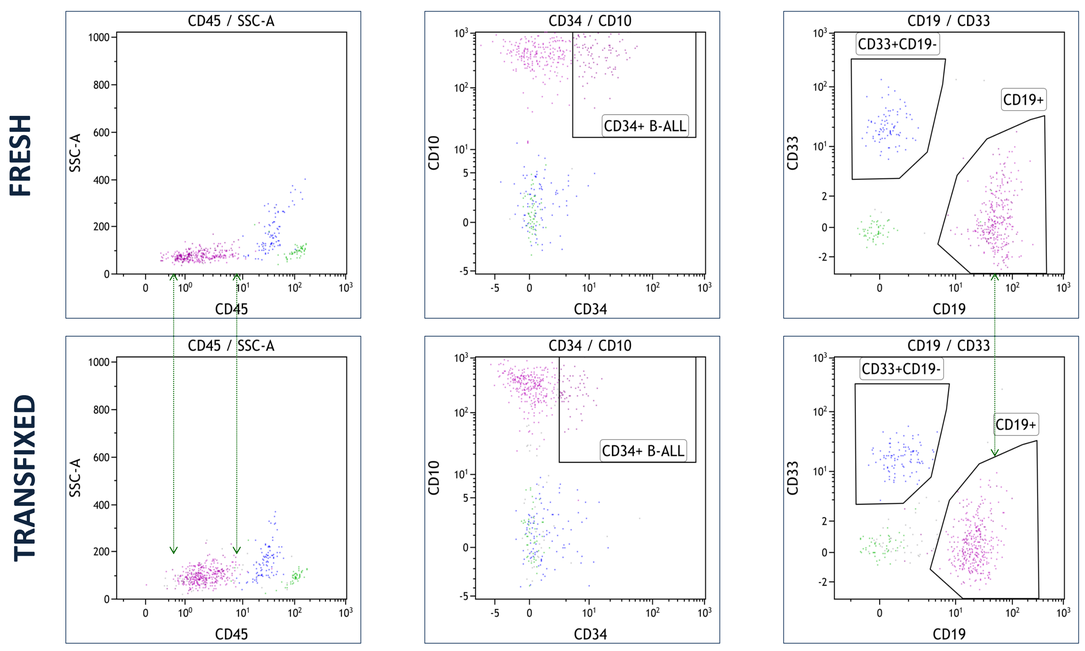

Figure 2. Example of a post-treatment, follow-up CSF sample from a B-ALL patient. CD19, CD34 and CD10 have reduced signal on transfix treated cells; however this did not hamper cell recognition or gating.

Transfix maintains key myeloid antigen expression

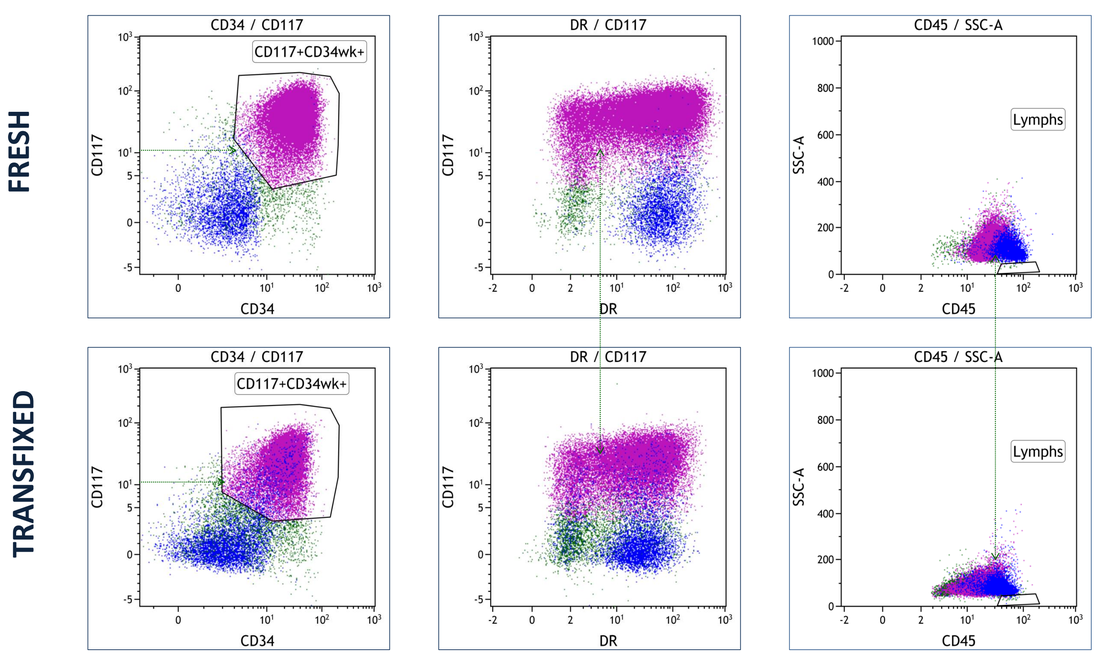

Figure 2. Example of a presentation CSF sample from a patient with central nervous system (CNS) infiltration of acute myeloid leukaemia. Expression of CD45, HLA-DR, CD34 and CD117 was maintained to allow cell identification and gating.

Conclusions

- Transfix preserved light scatter and key antigen expression patterns to allow for analysis of diagnostic and follow up CSF specimens for patients with CSN infiltration.

- Full evaluation of the antibody panels to be used on transfixed samples is required since some antigens were expressed too dimly for analysis.

References

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