Validating the use of TransFix® to stabilise cerebrospinal fluid (CSF) for flow cytometry immunophenotyping of haematological malignancies

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Introduction

Flow cytometry immunophenotyping is invaluable for the diagnostic and prognostic work-up of haematological malignancies. Over the last decade, multi-colour flow cytometry analysis has been used increasingly to investigate leptomeningeal disease; several studies show the utility and sensitivity of this technique for both B cell Non-Hodgkin's Lymphoma and Acute Leukaemia diagnostics [1-6]. Cells within CSF samples are typically low in number and degrade quickly [7]. Therefore, the recommendation is to use cell stabilisation reagents for CSF samples that require flow cytometry analysis [8].

Previous studies have shown that TransFix stabilised CSF samples have a cell yield equal to or better than that of fresh CSF samples and so may be used for immunophenotypic analysis after 18 hours of storage [9]. Some cell characteristics may change after TransFix treatment: for example, light scatter properties of peripheral blood and CSF leukocytes may be altered [9, 10] and the fluorochrome signal intensity of certain bound antisera may differ, although this does not necessarily hamper analysis [9].

Objectives

This study aimed to validate TransFix as a means of stabilising leukocytes in CSF for up to 72 hours following lumbar puncture. Also, to validate antisera panels for TransFix treated CSF samples.

Fresh CSF samples were split into two aliquots and analysed fresh or stabilised with TransFix/EDTA.

TransFix-induced light scatter changes do not impair cell identification

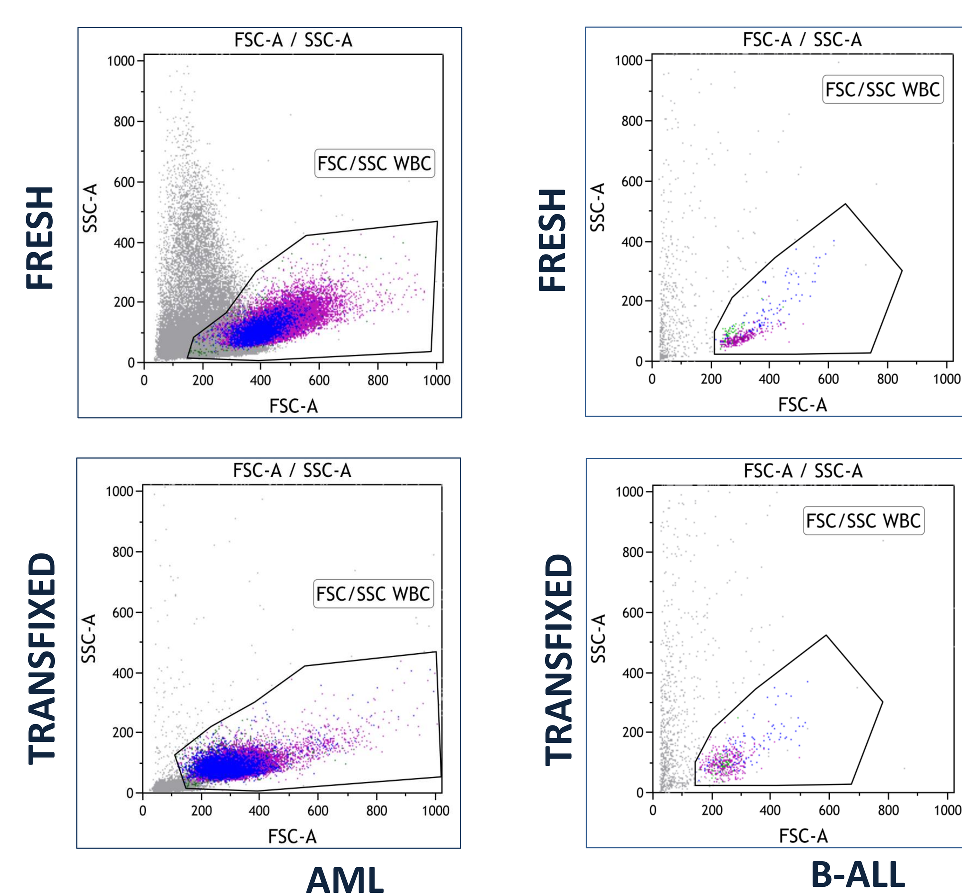

Figure 1. TransFix-treated cells had reduced FSC compared to fresh cells, for all cell populations and samples examined (n=23).

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

Stabilised CSF aliquots were stored at 4-8°C for 72 hours prior to analysis. Cells were concentrated by centrifugation, labelled, washed and acquired within 30 minutes on a FACS Canto II flow cytometer. For all antibodies used, cells in fresh and stabilised samples were compared in terms of forward and side scatter (FSC, SSC), median fluorescence intensity (MFI), and coefficient of variation. Data were analysed using Kaluza or DIVA software.

Gating strategy: For all samples, data was first checked in a fluidics versus CD45 plot to confirm fluidics stability and, if required, a gate was set to exclude any electronic noise. A gate was set in a FSC-A versus SSC-A plot around cells deemed to have viable scatter properties. Coincidence was monitored in a FSC-A versus FSC-H plot and, where required, excluded. Events with overt non-specific antisera binding were monitored normally in, for example, APC-H7 versus PE-Cy7 plot or by using an implausible combination such as CD34 versus CD16. There was no binding (data not shown). Further gating varied depending on the screen used.

TransFix maintains key antigen expression

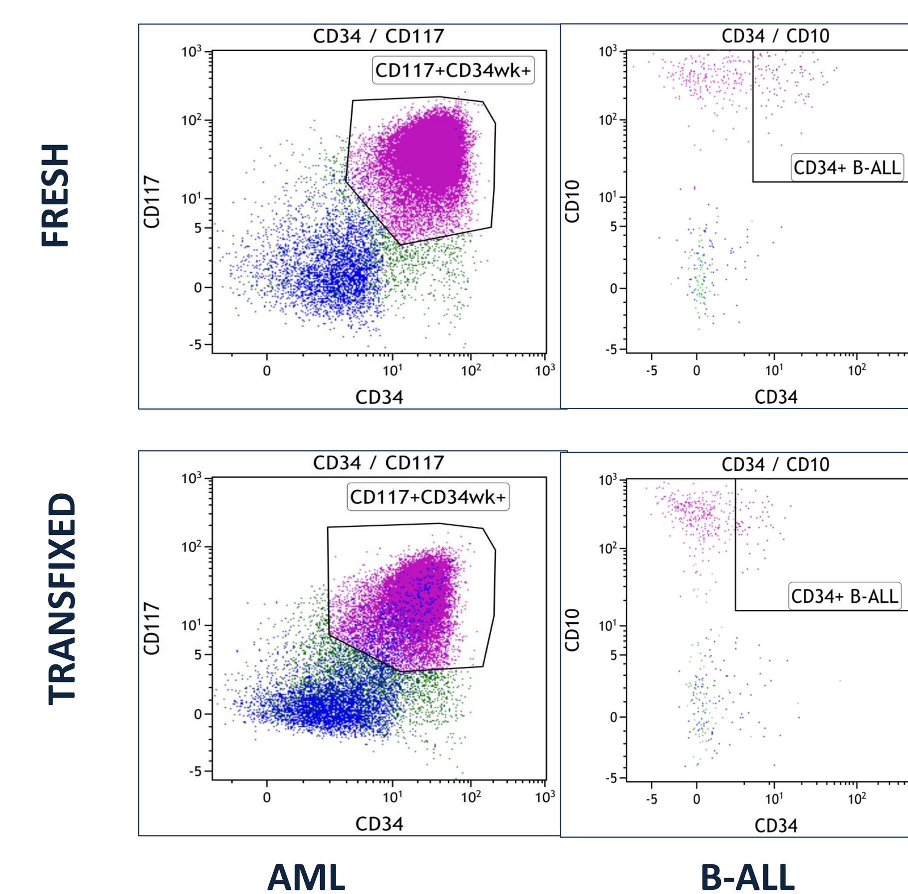

Figure 2. Examples of CD34-PerCp-Cy5.5 expression on a presentation sample positive for CD34+ AML and a post-treatment sample that showed persistent presence of the diagnosed CD34+/- B-ALL.

TransFix maintains key antigen expression

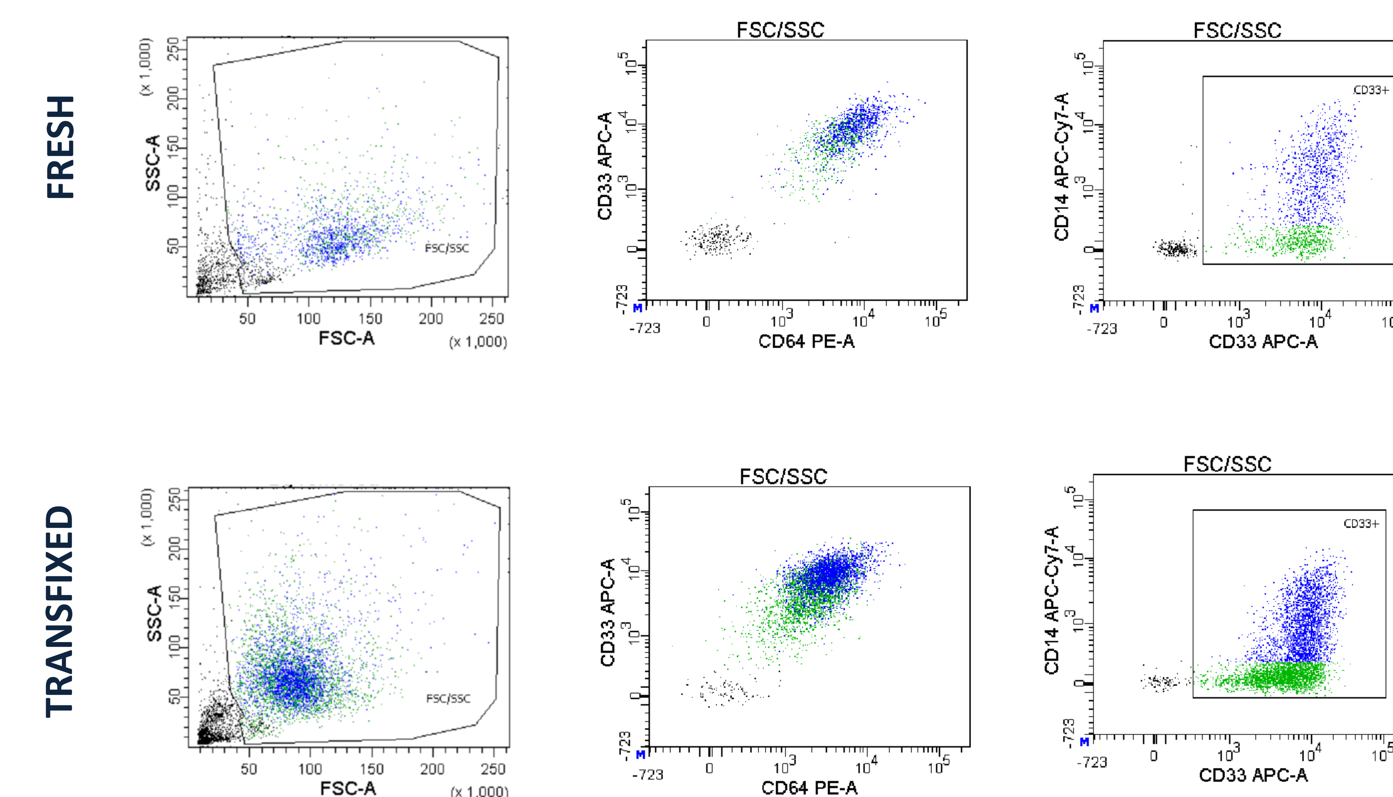

Figure 3. Example of a presentation sample from a patient with monocytic/monoblastic AML, detecting leptomeningeal disease. The FSC/SSC gate for the fresh sample includes monocytic cells with low FSC, these may be deemed as dead/dying cells, however are included since all antigen labelling showed equal distribution in all plots to those monocytes with 'viable' light scatter properties. The monocytic cells in the TransFix treated sample have more homogeneous scatter properties, and cell yield was higher in the treated sample. Expression of CD64, CD33 and CD14 was weaker on the treated cells compared to fresh sample; however this did not affect data interpretation.

Conclusions

- ❖ TransFix preserved light scatter and key antigen expression patterns to allow for analysis of diagnostic and follow-up CSF specimens for patients with CNS infiltration.
- ❖ The signal from stabilised cells was weaker compared to fresh samples and gates required adjusting, but this did not affect relative population sizes or qualitative description of abnormal populations.
- ❖ Critically, data generated from this study validated the use of TransFix stabilised CSF for 72 hours as the clinical interpretation between fresh and stabilised samples was the same.

References

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