

COMPARING ANTIBODY EXPRESSION AND STAINING INTENSITY BETWEEN BD FACSCANTO II AND SYSMEX XF-1600 IN FOLLICULAR LYMPHOMA PROGRESSING TO B-LYMPHOBLASTIC LEUKAEMIA/LYMPHOMA

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Introduction

Follicular lymphoma (FL) is the most common form of indolent lymphoma in the western world, accounting for approximately 25% of malignant lymphomas (Ciabanu et al 2013). Disease progression of FL is often unpredictable with 25% - 30% of FL cases progressing; most commonly to diffuse large B cell lymphoma, Hodgkin lymphoma, Burkitt lymphoma and rarely B-lymphoblastic leukaemia/lymphoma (B-ALL) (Swerdlow et al, 2017). This phenomenon carries a very poor prognosis, with a life expectancy of approximately 10 months post diagnosis (Morley et al, 2008). Therefore, detection of disease progression is essential. Flow cytometry can be used to detect progression of FL to B-ALL. This relies on the ability of the flow cytometer to enable differentiation of the two malignant cell populations. Here we assess the antibody expression pattern and staining intensity (SI) between two flow cytometers; the BD FACSCanto II and the Sysmex XF-1600, in a case of FL progressing to B-ALL.

Methods

A patient with known FL re-presented with a lymph node mass in the right groin and right axillae. The patient's bone marrow aspirate was examined and found to have a population of abnormal lymphocytes and blast cells as shown in figure 1. Subsequently, immunophenotyping was performed using the following antibodies: CD3, CD7, CD10, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD34, CD38, CD45, CD117 and HLADR. Cytoplasmic antigen expression was also determined for the following antigens: CD3 CD22, CD79a, MPO and TDT. The data acquisition was performed on a BD FACSCanto II and the Sysmex XF-1600 with 20,000 cells acquired for each tube. Data analysis was performed on BD FACSDiva software and Sysmex XF-1600 software. The populations of interest (lymphocytes and blast cells) were gated around based on their SSC and CD45 characteristics allowing for their antigen expression pattern and SI to be determined. Additionally, the CD20 and CD10 dual positive population was gated around in order to identify the FL cells. The gated FL cells were displayed on the following plots: SSCvCD45, CD10vCD20, CD10vCD19 and CD20vCD19. The results from both flow cytometers were then compared.

Figure 1: Bone marrow aspirate morphology

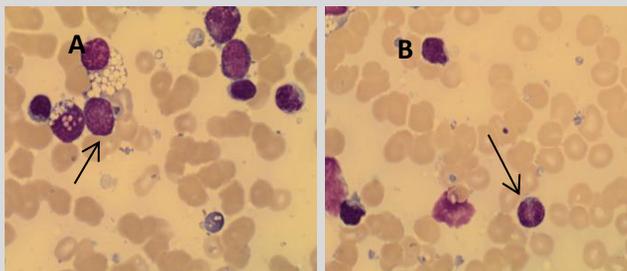


Figure 1A) Numerous heavily vacuolated blast cells suggestive of acute leukaemia. **B)** Small lymphocytes with cleaved nucleus, consistent with known FL.

References:

- Ciabanu, A., Stanca, O., Triantafyllidis, I. and Lupu, A., 2013. Indolent Lymphoma: Diagnosis and Prognosis in Medical Practice. *Maedica*, 8(4), pp.338-342.
- Morley, N.J., Evans, L.S., Goepel, J., and Hancock, B.W., (2008) Transformed follicular lymphoma: The 25-year experience of a UK provincial lymphoma treatment centre. *Oncology reports* https://doi.org/10.3892/or_00000095 953-956.
- Swerdlow, S., Campo, E., Harris, N., Jaffe, E., Pileri, S., Stein, H., Theile, J., Arbor, D., Hasserjian, R., Le Beau, M., Orazi, A. and Siebert, R., 2017. *WHO Classification Of Tumours Of Haematopoietic And Lymphoid Tissues*. 4th ed. Lyon: IARC, pp.266-268.

Results

Immunophenotyping analysis on both flow cytometers allowed identification of a population of blast cells expressing the antigens CD19, HLADR, TdT (moderate SI), CD10 (bright SI) and CD45 (dim SI). However, these cells lacked CD20 expression. This immunophenotype is consistent with the World Health Organisation (WHO) criteria for B-ALL (Swerdlow et al, 2017). Additionally both flow cytometers allowed simultaneous identification of a population of mature B-lymphocytes which expressed CD20, CD19, CD10 (moderate SI) and CD45 (bright SI), but lacked expression of TdT; an immunophenotype consistent with the previously diagnosed FL. The dot plots from the Sysmex XF-1600 and the BD FACSCanto II are displayed in Figures 2A and 2B respectively.

Figure 2: The dot plots for both the Sysmex XF-1600 and the BD FACSCanto II

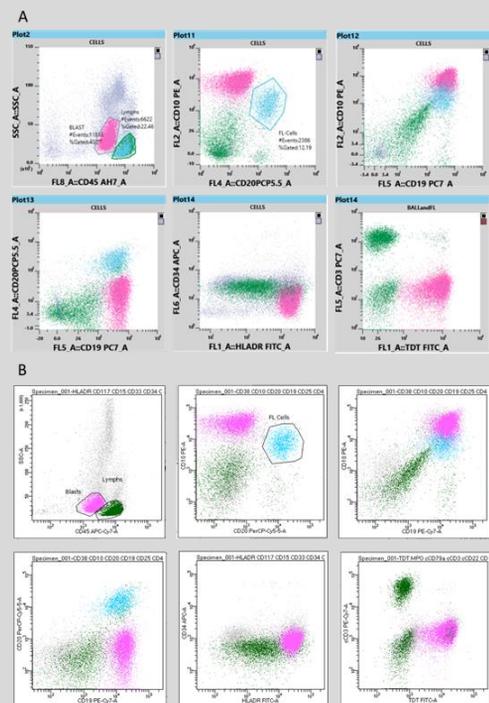


Figure 2A/B) The dot plots used to show the antigen expression pattern and staining intensity for the antigens CD19, HLADR, TdT, CD10, CD20 and CD45 on the Sysmex XF-1600 (A) and the BD FACSCanto II (B). Blast cells are shown in pink, lymphocytes shown in green and FL cells show in turquoise on the plots : SSCvCD45, CD10vCD20, CD10vCD19 and CD20vCD19.

Conclusion

This case highlights the benefits of flow cytometry and the importance of including complex cases when assessing new instrumentation to ensure accurate detection of multiple populations. Both flow cytometers allowed detection of the two malignant cell populations: the mature B-cell population of the previously diagnosed FL and the newly presenting blast cell population of the B-ALL. The immunophenotyping results produced by both the BD FACSCanto II and the Sysmex XF-1600 were comparable with regards to the antigen expression pattern and staining intensity.