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The IVDR ebook will:

- introduce readers to the IVDR, outlining the differences between the IVDR and the IVDD, explaining the regulatory impact of the new regulation.
- shows how the IVDR will change things for in vitro diagnostics labs, particularly those using laboratory-developed tests,
- illustrate the problems and solutions using a case study.

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Letter to the Editor

CD8 Antigen Expression in Chronic Lymphocytic Leukemia: Does it Have any Relevant Meaning?

We have read with special interest the article of Jain et al. (1), published at volume 94, July 2018 of *Cytometry: Part B—Clinical Cytometry*. There, the authors report a single patient with the diagnosis of chronic lymphocytic leukemia (CLL) characterized by an aberrant CD8 antigen expression on CLL B-cells. Based on the three large series of cases hitherto published, namely, those of Mulligan et al. (2) Carulli et al. (3) and Kern et al. (4), and also taking into account the very small series of cases cited by them, Jain et al. are probably correct when they assert that only approximately 120 cases have been described in the literature until now. Considering that from the 1980s flow cytometry ceased to be used almost exclusively in the research setting and entered in the field of laboratorial medicine (5), undoubtedly the diagnosis of those more or less 120 patients, over almost 40 years of clinical application of flow cytometry around the world, is what one can definitely call an extremely rare clinical finding.

In our own experience, during a period of 7 years (2009–2015) performing systematic flow cytometry assays, we found only 1 patient (0.4%) with the diagnosis of CLL which B-cells exhibited CD8 positivity, out of a total of 218 patients with a B-cell chronic lymphoproliferative disease diagnosed in our center at the northeast of Brazil.

The patient was a 72-year-old woman who presented with a CBC showing a hemoglobin concentration of 14.2 g/dL; white blood cell count of 15.4×10^9/L (lymphocytes count = 9.8×10^9/L) and platelet count of 265.0×10^9/L. Beyond the typical CLL immunophenotype (CD5+, CD10–, CD20 + ^{weak}, CD23+, CD79b–, CD103–, FMC7–, kappa+^{weak}), the CLL peripheral blood B-cells were characterized by the presence of CD8 antigen (CD8 positivity in 67% of CLL B-cells, moderate intensity of expression), CD25 positivity and weak expression of CD45 (Fig. 1). CD3, CD4, and CD38 were absent.

We ponder that there are two main possibilities, not mutually exclusive, which could explain the rarity of patients with the diagnosis of CD8+ CLL: the first and most obvious is that the CD8 antigen be expressed very infrequently in cases of CLL. However, another hypothesis should be considered: it concerns the diagnostic approach used to study samples with the suspicion of a chronic lymphoproliferative disease. Namely, although international guidelines (6) for flow cytometry diagnosis of hematological diseases recommend the use of a lymphocyte screening tube containing markers for B-lymphocytes (CD19, CD20, kappa, lambda), T-lymphocytes (CD3, CD4, CD5, CD8, CD38, TCRγδ) and NK-lymphocytes (CD56), for reasons directly related to financial costs and time savings, some centers around the world, especially those located in countries of low income, do not start the immunophenotypical study using the lymphocyte screening tube. Rather, they resort to the old fashioned, but yet effective and cheap, combination of an automatic complete blood count plus peripheral blood smear review with the aim to decide the initial antibody panel to be used (7). In this scenario, based on the well-known much higher prevalence of mature B-cell neoplasms (over 90% worldwide) when compared to mature T and NK-cell neoplasms (8), the presence of lymphocytosis rationally points to the initial use of a panel with specificity for the diagnosis of a mature B-cell lineage disease and which, usually, does not include monoclonal antibodies with specificity for the CD8 antigen. Only in the event where the primarily suspected diagnosis is not reached, the panel is then expanded.

Another aspect that should be mentioned, with regard to CD8 antigen, is the participation of autologous CD8+ T-cells and their role in patients with CLL. Particularly, autologous CD8+ T-cells have been shown to be expanded in CLL patients while they are able to recognize tumor associated antigens (9). More recently, exhaustion of CD8+ T-cells, which is an immunological state of T-cells characterized by dysfunctions in key T-cell properties, as proliferation and cytotoxicity, has been reported in CLL patients. Characteristically, these exhausted CD8+ T-cells have a very specific immunogenic profile, namely, positivity for the antigens CD160 (BY55), CD244 (2B4), CD279 (PD-1), and T-cell immunoglobulin domain and mucin 3 (Tim-3). More important, exhausted CD8+ T-cells are probably associated with more severe clinical stages of CLL (Rai II, III, and IV) which suggest some role of these cells on the prognosis of CLL (10,11).

But, after all, why to consider the expression of CD8 antigen on CLL? Essentially, it is important to remember that new immunological markers have shown to be of prognostic value in patients with CLL as, for example, CD1d (12,13) and CD49d (14,15).

Therefore, if the CD8 antigen expressed on CLL B-cells is just a kind of immunophenotypical curiosity, an epiphenomenon perhaps related to the process of neoplastic transformation without any clinical significance or, alternatively, if it is an useful prognostic marker, is a question that can hardly receive a definitive answer with the paucity of patients with CD8+ CLL so far reported. The largest study published (4), a cohort of 5523 CLL patients where 61 of them exhibited CD8 expression, showed that by multivariate analysis CD8 positivity was independently related to a shorter time to treatment. This suggests that CD8 expression may have prognostic value in patients with CLL, but it is fundamentally necessary that this initial

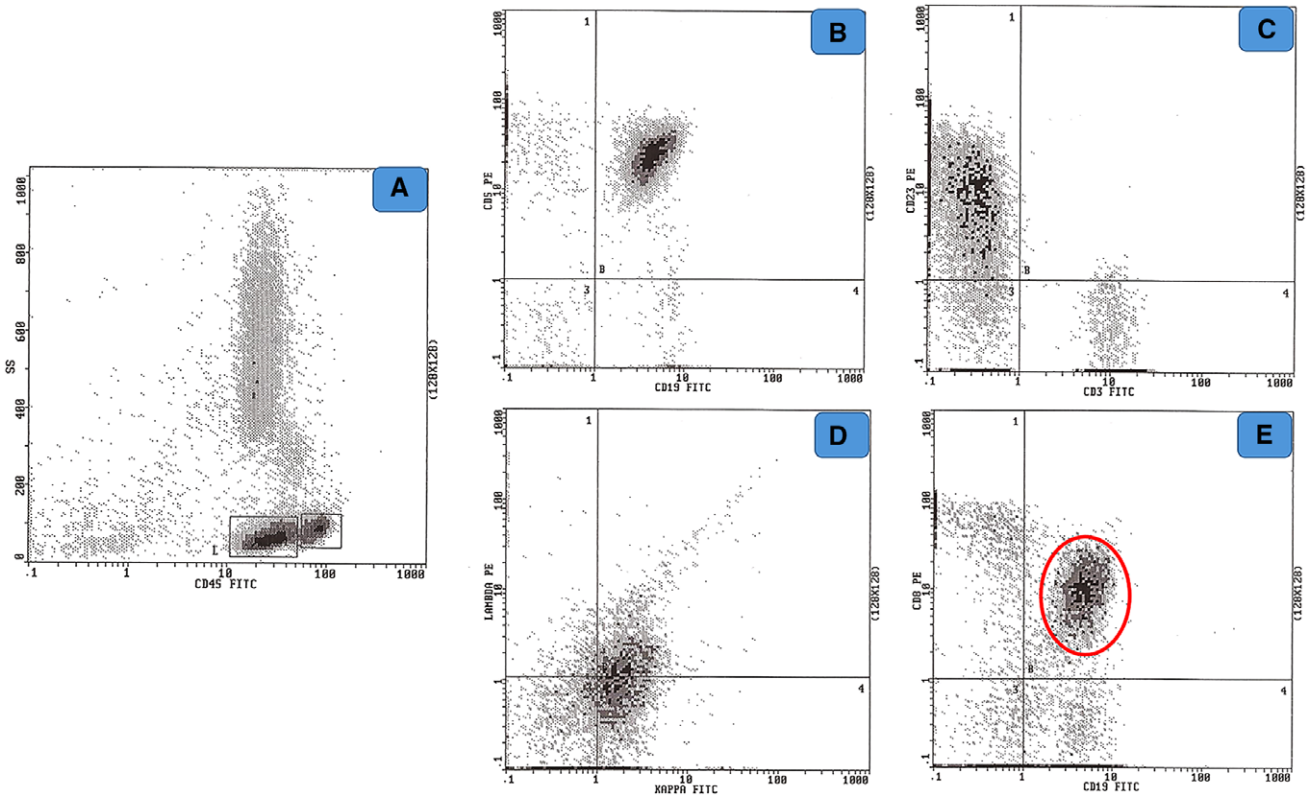

FIG. 1. Flow cytometric immunophenotyping of peripheral blood sample. Dot-plot **A**: CD45 × Side Scater (SS) analysis shows two populations of lymphoid cells: inside gate L, the cells are characterized by weak CD45 expression. Dot-plot **B**: CD19 × CD5. The cells show coexpression of CD19 and CD5. Dot-plot **C**: CD3 × CD23. The cells show expression of CD23 and CD3 negativity. Dot-plot **D**: kappa × lambda. The cells show weak expression of kappa light-chain. Dot-plot **E**: CD19 × CD8. The cells show coexpression of CD19 and CD8 (red ellipse). Notice that the abnormal population of CD8+ CLL B-cells present a moderate intensity of CD8 expression, inferior when compared with the intensity of expression viewed on the residual CD8-lymphocytes (upper left quadrant). [Color figure can be viewed at wileyonlinelibrary.com]

evidence be validated with a larger number of patients in an independent series, in a manner similar to what was done for CD49d, for example (16).

Therefore, more than ever, here the *cliché* is surely appropriate: the systematic and widespread use of panels that include the CD8 antigen associated with multicenter efforts shall be essential, if we hope to clarify the role of CD8 in those rare cases where this lineage T-cell antigen is abnormally expressed on CLL B-cells.

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DECLARATION OF INTEREST

The author reports no conflict of interest.

AUTHOR CONTRIBUTION

D.M.M performed flow cytometry analysis, reviewed the case, reviewed the literature and wrote all the manuscript.

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