POSITIVE OR NEGATIVE? THAT IS THE QUESTION!

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The cytometrical definition of a positively or negatively stained cell population by (immuno)fluorescence techniques is an issue of utmost importance in the diagnostic assessment of haematological malignancies. Interestingly, the technical criteria for a clearcut and standardized definition of what is 'positive' and what is 'negative' have not been addressed in sufficient detail in the flow cytometric literature.

As a consequence, an exquisitely objective technique like flow cytometric analysis of fluorescence may fall in the realm of subjective interpretation, and the actual diagnostic role of some critical but weakly expressed cell markers can remain controversial (as an example ZAP70).

The analysis and interpretation of fluorescence distribution diagrams was developed with lymphocyte subset studies in the early '80s. Since lymphocyte subpopulations defined by surface monoclonal antibodies usually yield clearcut and well separated fluorescence distribution clusters (i.e. "discrete" or "heterogeneous" distributions), the setting of cutoff limits, quadrants or windows, has been conventionally the most natural and appropriate way to define cell subsets and to calculate their respective percentage over the whole lymphocyte population.

Unfortunately, such an approach was also applied to the rising flow cytometric analysis of haematological malignancies, where homogeneous leukemic cell populations usually stain massively but weakly. A strong 'percent positive' mental imprinting was established among many laboratory haematologists worldwide. The appropriate technical approach to the interpretation of weakly stained haematolymphoid neoplastic cells is however quite different from that used in non-oncological lymphocyte subsetting.

Statistical homogeneity is likely to imply biological homogeneity. This concept is widely applied in other diagnostic assessments of malignant blood diseases, such as cytogenetics and molecular analysis. For example, the demonstration of a translocation or of a gene mutation is simply reported in a qualitative fashion as 'present' or 'absent' with no reference to any cell percentage, and the same occurs in immunocytochemistry / immunohistochemistry analyses.

Percentages are not very informative when performing a complex, multiple-color analysis of leukemic cells. The most common error resides in the attempt to dissect homogeneous fluorescence distributions with a cutoff marker in order to calculate a putative percentage of 'positive' cells. Another deeply stuck error is to use an arbitrarily established 'percentage of positive' cell value (i.e. 20%-30%) to assess whether the entire abnormal cell population can be deemed as positive or negative for a given marker.

This technically inappropriate usance, although just reported as 'customary' in the scientific literature, has been routinely applied by many laboratories, and was also included into influential international guidelines (Bain, 2002). As a consequence, also credited External Quality Assesment (EQA) schemes like UKNEQAS Leukaemia Immunophenotyping (reference) request that participants stick to the recommendations included in the most recent guidelines.

In this lecture a critical review of the technical and statistical approaches used to define as 'positive' or 'negative' a weakly fluorescent leukaemic cell population will be presented.