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Focus
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Immunophenotyping: the method and its application in pathology

Flow cytometry immunophenotyping (FCI) provides rapid analysis of any suspended cellular preparation, primarily the blood, using immunofluorescence.

The antigens expressed by a cell ("CD" or *Cluster of Differentiation*) allow the cell to be identified through the use of antibodies labelled with fluorochromes. Certain cellular structures are directly detectable without the use of antibodies.

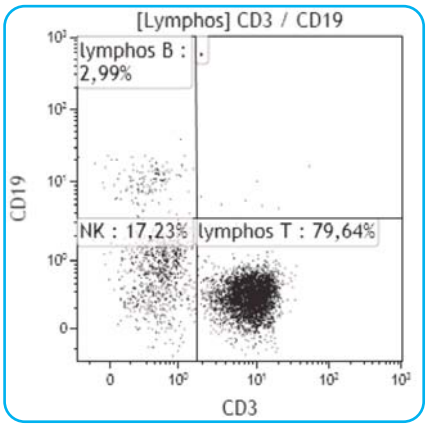
Technological aspects

Immunophenotyping is usually performed on a blood sample, however, it can also be used on bone marrow samples, various bodily fluids, CSF, and any cellular suspension, as long as the cell storage restrictions in various media are respected.

The sample is exposed to a panel of fluorochrome-labelled antibodies that are specific for the disease being screened for. Different red blood cell lysis and permeabilisation stages can be used. Cytometry uses a fluid system that enables the cells to the lined-up in a tube; they are passed in front of lasers and fluorescence detectors (photomultiplier tubes) one-by-one. Often, tens or hundreds of thousands of cells are analysed in each sample.

Today's routine cytometers can detect up to 10 different fluorescent signals or "colours", for which it deciphers the size and the structure of each cell analysed.

Software packages then exploit this extensive data under the form of a chart or cytogram that represents the fluorescent intensity of the cells for each parameter. The expression profiles then enable the cellular sub-populations of interest to be identified.



An example of a conditioned cytogram on lymphocytes:
B-cells are CD19+ and CD3-; T-cells are CD3+ and CD19-; NK cells are CD19- and CD3-.

Applications in immunology

The quantification of the lymphocyte sub-populations in the blood allow for the investigation of cell immunity through a count of B-cells (CD19+), T (CD3+) and NK cells (CD16+ and/or CD56+).
From within the T-cell population, T helpers CD4+ (especially important in the monitoring of HIV seropositive patients) and cytotoxic T-cells CD8+ can be differentiated.

Flow cytometry can also be used in allergy studies to investigate the activation of polynuclear basophiles in the presence of a given allergen. Other highly specialised applications exist in immunology (regulatory T-cells and monocyte activation etc.).

Applications in malignant haematology

Immunophenotyping is a key player in the diagnosis of malignant blood diseases, especially for lymphoproliferative syndromes and acute leukaemias. This technique is part of a multi-disciplinary step involving cytology, cytogenetics, molecular biology and histology and cytology.

Diagnostic applications

B-cell lymphoproliferative syndromes:

The clonality of the B-cells is highlighted by an imbalance in the expression ratio of the immunoglobulin light chains kappa and lambda (this ratio is normally 2/3 kappa for 1/2 lambda).

Certain specific expression results orientate the diagnosis: CD10+ in follicular lymphomas, CD5+ in chronic lymphoid leukaemia (CLL) and mantle cell lymphoma, triple expression of CD103, CD11c and CD25 in hairy cell leukaemia etc.

The diagnosis of CLL relies on the Matutes or Moreau score calculation:

1 point	0 point
Low or negative light chain intensity	Normal light chain intensity
CD5+	CD5-
CD23+	CD23-
FCM7-	FMC7+
CD79a (Moreau) or CD22 (Matutes): - or low	CD79a+ or CD22+

A raised score (4/5 or 5/5) corresponds to CLL, the other lymphoproliferative syndromes have a score that is lower than or equal to 3/5.

T-cell and NK cell lymphoproliferative syndromes:

The diagnosis of these pathologies is more complex and requires a multi-disciplinary effort. The clonality of T-cells can be observed by flow cytometry through the TCR V-beta repertoire study, which detects the selective use of a sub-family. The loss of expression of a pan-T cell antigen is also an indication of malignancy.

Acute leukaemias:

Flow cytometry confirms the blastic nature of the cells through the expression of immaturity markers and by determining their cell line: B-lymphocytes, T-lymphocytes or myeloid cells will determine the therapeutic action to be taken. It is also a crucial technique for the diagnosis of certain rare diseases (dendritic cell leukaemia, biphenotypic leukaemia or acute myeloblastic M1 leukaemia etc.)

	Main markers
B lymphocyte cell line	CD19, CD20, CD22, heavy and light chains
T lymphocyte cell line (and NK cells)	CD3, CD5, CD2, CD7, CD4/CD8, TCR (CD16, CD56)
Myeloid cell lines	MPO, CD13, CD33, CD117
Markers of immaturity	CD34, low CD45, CD38, HLA-DR

Other blood diseases:

Dyshematopoiesis can also be detected via the presence of expression results that differ from normal cells. Immunophenotyping is in full development within the area of myelodysplasic syndromes, with a boom in multi-parametric analysis and development of IT innovations that enable complex data to be analysed. Within the context of myloma, flow cytometry can identify pathological plasma cells (their proportion is a differential diagnostic element between myloma and MGUS (monoclonal gammopathy of undetermined significance)) and their clonality.

Please also note that flow cytometry is not a useful diagnostic tool for myeloproliferative neoplasias.

Follow-up and evaluation of the response to treatment

Within the context of lymphoid acute leukaemias, the persistence of malignant cells after treatment (residual disease or MRD: *minimal residual disease*) is a relapse risk factor. The sensitivity of flow cytometry is generally equal to that of molecular biology, up to 10^{-5} cells. These applications are, nevertheless, reserved for clinical protocols and require immediate technical handling of the sample.

Within the context of lymphoproliferative diseases, the sensitivity for the monitoring patients is between 0.1% and 1% (10^{-3} to 10^{-2}) and this test must be offered in accordance with the particularities of each disease.

Other applications in blood diseases

- Assessment of the thienopyridine anti-platelet drug (anti-aggregants) response (clopidogrel and ticlopidine) using the VASP protein phosphorylation test.
- Diagnosis of hereditary spherocytosis or Minkowski-Chauffard disease: EMA (eosin-5-maleimide) provides rapid screening that is both sensitive and specific for patients that are upstream of tests such as ektacytometry.
- Screening for "PNH" (paroxysmal nocturnal haemoglobinuria): loss of antigenic expression that is dependant on an GPI anchor (glycosyl-phosphatidylinositol), such as CD55 and CD59 and other markers found on red blood cells and/or leukocytes.
- Diagnosis of a foetal-maternal haemorrhage: in flow cytometry: there are equivalents of the Kleihauer test (screening of foetal blood cells in maternal blood).

In practice...

Test	Price	Markers tested for
T4 T8	B80	CD45, CD3, CD4, CD8
B-lymphocytes CD19+	HN	CD45, CD19
NK lymphocytes	HN	CD45, CD16, CD56
Phenotype screening of a malignant blood disease	B300	By default: a complete "lymphoid" profile: an investigation of the B, T and NK sub-populations, B-cell clonality (kappa/lambda), and screening for atypical T-cell phenotypes (CD4/CD8, phenotypic gaps) In cases with particular indications: Screening and characterisation of blast cells for acute leukaemias. Characterisation of plasma cells within the context of myeloma (in bone marrow only)

Please provide the following with the sample:

- **In all cases, the last full blood count result:**
  For the absolute value calculation for CD4 and other lymphocyte sub-populations for the interpretation and quantification of a clone for a malignant blood disease.

- **For complete immunophenotyping:**
  - **Clinical details:** immune disorder context? Known blood disease? Under treatment? Tysabri* or Rituximab* treatment? Etc.
  - **Suspected diagnosis:** can help us to choose the most appropriate markers.
  - **Unstained slides for cytology examinations,** , often essential for a diagnosis.

Immunophenotyping on fresh cells, the sample must be sent as quickly as possible at room temperature.

NB : There is a specific request form for blood diseases available at **www.biomnis.com ›test menu›test request forms-consent forms-other documents.**

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