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Immunophenotyping of lymphoproliferative disorders: state of the art

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Summary

Immunophenotyping was introduced into diagnostic pathology over 30 years ago to assist in the diagnosis and classification of lymphoproliferative disorders. Today the role of immunophenotyping has been expanded beyond this to include the detection of markers of prognosis, determination of disease phenotypes associated with specific chromosomal abnormalities, detection of targets for immunotherapy and to monitor residual disease. Immunoperoxidase detection methods remain the most popular in histopathology, whilst flow cytometry is most commonly applied for haematological samples. The range of monoclonal antibodies available, including those which work in routinely performed tissue specimens, continues to increase. This is in part a result of gene expression studies identifying precise genetic signatures for certain lymphoproliferative disorders and the generation of new protein markers to gene products of upregulated genes. This review summarises the current status and applications of immunophenotyping in the assessment of many of the lymphoid malignancies.

Key words: Phenotype, immunohistochemistry, flow cytometry, lymphoproliferative, lymphoma.

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INTRODUCTION

Immunophenotyping characterises cellular antigen expression by their ability to bind antibodies. This has an essential role in the diagnosis and classification of lymphoproliferative disorders. Although immunophenotyping has been available since the 1970s, it is still an evolving technique. Both new antibodies, which are being added on an almost weekly basis, and new applications are appearing. The recent development of gene expression profiling, or microarray studies, has shown that some disorders have specific genetic signatures. Monoclonal antibodies are now being developed to the products of those genes that are upregulated in neoplastic disorders, and novel immunophenotypical markers are thereby being created. These new antibodies will enable further refinement of the classification of lymphoproliferative disorders and help us gain important prognostic information by identifying biologically distinct subgroups. In addition, new therapeutic targets may be identified.

The applications of immunophenotyping therefore have expanded beyond diagnosis and disease classification to predicting prognosis, detecting therapeutic targets and disease monitoring. Phenotypical profiles of neoplastic lymphoid cells are increasingly being used as surrogate markers of genetic abnormalities related to malignant transformation. Phenotyping is also being applied to assess whether a neoplastic cell expresses a specific target antigen for immunotherapy (i.e., monoclonal antibodies bound to cytotoxic agents). During and after treatment of a lymphoid malignancy, minimal residual disease activity may be assessed by following the expression of a particular abnormal immunophenotypical profile. These novel applications highlight the increasing importance of immunophenotyping in the analysis of lymphoproliferative disorders.

Immunophenotyping of lymphoproliferative disorders, be it by flow cytometry or immunohistochemistry, therefore has a number of roles including:

1. Determining the B- or T-cell lineage of an abnormal lymphoid population.
2. Determining the clonality of lymphoid proliferations, especially those of B-cell origin.
3. Determining whether lymphoid cells have a phenotype associated with a specific disorder or a particular chromosomal abnormality.
4. Establishing the phenotype of neoplastic lymphoid cells which may be useful for monitoring residual disease.
5. Assessing the expression of specific molecules which may be targets for immunotherapy (e.g., CD20).
6. Assessing cell proliferation and turnover rate.
7. Detection of markers which may assist in determining prognosis.

In this review, some general comments will be made on immunophenotyping methods, followed by a more extensive discussion of those lymphoproliferative disorders with distinctive phenotypical features. We have not set out to give an exhaustive list of data on the immunophenotype of all lymphoproliferative disorders or all methods available for detecting antigen expression. Data are presented on some of the newer antibodies that have been shown, at least in initial studies, to have prognostic significance or to be useful targets for immunotherapy. We accept that, in this rapidly evolving field, some conflicting data are emerging, especially in regard to the prognostic significance of some novel immunophenotypical profiles.

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IMMUNOPHENOTYPING METHODS

Immunophenotyping was first described in 1941 by Albert Coons, who demonstrated the use of a fluorescence labelled antibody to localise cellular antigens in tissue sections. This immunofluorescence technique began to be used in leukaemia diagnosis in the 1970s, but was not universally applicable to routine diagnostic laboratories as they required fluorescence microscopy or large flow cytometers.[1] These drawbacks were largely overcome by the development of immunoenzymatic techniques using a number of different enzyme labels, such as acid phosphatase, horseradish peroxidase and alkaline phosphatase.[2,3] Immunoperoxidase techniques were most applicable to tissue samples, but due to the high levels of endogenous peroxidase in haemopoietic cells (especially erythroid and myeloid cells) they were not as suitable for blood and bone marrow samples. Immunoalkaline phosphatase techniques were developed and found to be preferable for use with these peroxidase-rich samples.[4] Immunoperoxidase procedures became widely used on tissue samples and remain the method of choice for immunohistology. With further developments in flow cytometry and the availability of bench-top flow cytometers applicable to routine laboratories, fluorescence labelling has largely replaced immunoalkaline phosphatase for the analysis of haematological samples.

Flow cytometry

Flow cytometry is widely used to assist in the diagnosis, classification, detection and monitoring of minimal residual disease in the majority of lymphoproliferative disorders. This method allows the rapid and simultaneous analysis of multiple cell parameters, including cell size, complexity, and both surface membrane and intracellular antigens, on large numbers of fresh viable cells (Table 1). The major advantage of flow cytometry is that a number of cellular antigens can be analysed simultaneously on a cell population using multiple monoclonal antibodies labelled with fluorochromes, each of which emits light at a different wavelength. The large range of antibodies available makes flow cytometry the method of choice for establishing the phenotype of lymphoid malignancies and detecting clonality of B-cell disorders.

Flow cytometry requires cells to be in suspension and can be performed on a wide range of specimens including peripheral blood, bone marrow aspirate, fine needle aspirate (FNA) and fluid samples. Cell suspensions can also be prepared from cells extracted from solid tissue biopsies (e.g., lymph nodes) and analysed by flow cytometry. The mechanical dissociation of cells is done by teasing out cells from fresh unfixed tissue using a scalpel and forceps, needle and syringe and wire mesh, or using an automated device (e.g., Medimachine, BD Biosciences, USA; DakoCytomation, Denmark). Enzymatic digestion using a proteolytic enzyme (e.g., pepsin or trypsin) can be used to assist with separation of cells from fibrotic samples. Red cell lysis can be used to remove contaminating red cells that may interfere with the flow cytometric analysis; this does not denature or destroy cellular antigens. Density gradient centrifugation (e.g., Ficoll-Hypaque) can also be used to remove red cells and cell debris from specimens. This method also concentrates the cells of interest. As flow cytometers are now readily available, are simple to use and can be used to analyse all sample types, flow cytometry is becoming the method of choice for the phenotypical analysis of lymphoproliferative disorders.

However, there remains a major drawback of flow cytometry: the inability to directly assess cellular morphology and to correlate this with antigen detection. Therefore, it is critical that the cell sample to be analysed by flow cytometry is assessed morphologically (i.e., cell smear or cytocentrifuge preparation of the extracted cells). This not only ensures that it morphologically resembles the initial specimen and that there are sufficient intact cells, but also guides antibody selection. Typically, many nodal large cell lymphomas, fibrotic lesions (e.g., mediastinal large B-cell lymphoma) and Hodgkin lymphomas do not yield diagnostic samples suitable for flow cytometry due to the relatively low numbers of viable malignant cells extracted compared with the reactive surrounding cells.

As morphology cannot be used to 'isolate' the cell of interest, other parameters must be used. Identification of the cell population of interest by flow cytometry is

TABLE 1 Comparison of immunohistochemistry and flow cytometry

	Immunohistochemistry	Flow cytometry
Morphological correlation	Direct assessment of morphology with antigen expression	No visual correlation, depends on other parameters for recognition of cell types
Automation	No equipment is needed; automation is available	Flow cytometer
Specimen types	Paraffin and frozen sections; smears of blood, bone marrow; body fluids	Fresh unfixed cell samples: blood; bone marrow; body fluids; fine needle aspirates; fresh solid tissues (requires tissue disaggregation)
Antibodies	Limited (for paraffin sections) but expanding	Unlimited
Turn-around time	Slow; only performed after tissue processing and histology reviewed	Hours, i.e., same day
Permanent record	Yes	Listmode analysis for re-analysing data
Simultaneous detection of multiple antigens	Difficult	Routine – enables aberrant phenotypes to be detected
T/B cell clonality	Difficult	Routine (for B cells)
Interpretation	Subjective	Objective; quantitative. Depends on gating correct cell population
Minimal residual disease assessment and rare cell analysis	Difficult	Routine, especially if the neoplastic cells have a specific phenotype