Abnormal laboratory results

Cell markers

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Summary

Cell markers serve as a monogram to help identify and classify cells. The majority are molecules or antigens within the plasma membrane of cells. Specific combinations of markers are unique to different cell types. These molecules are not merely markers, but also have important functional roles. Knowing which molecules are present can help in the diagnosis of disease or in directing treatment.

Key words: flow cytometry, immunocytochemistry, immunophenotyping.

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Introduction

Most cell markers are molecules in the cell membrane which can be used to identify cell types. They are classified by their clusters of differentiation (CD) which are recognised by specific antibodies.

How does the laboratory analyse cell surface markers?

There are two common immunophenotyping methods used to analyse cell markers. These are flow cytometry, which is performed on fresh, unfixed cell suspensions, and immunohistochemistry which is performed on fixed specimens. These tests can be performed on blood, bone marrow, lymph nodes and other tissues.

An understanding of these tests and the necessary specimen preparation is important for practitioners collecting fine needle aspirates and surgical biopsies, to ensure optimal processing and interpretation.

Flow cytometry

Flow cytometry uses a laser light source to analyse the size, complexity and physical properties of fresh viable cells in suspension after labelling with fluorescent monoclonal antibodies. One to two thousand cells can be analysed per second.

The advantages of flow cytometry include the ability to rapidly and simultaneously analyse multiple cell parameters. The disadvantage is the inability to directly assess the cellular morphology of the cell population under analysis. A smear of the specimen must be stained and reviewed microscopically in correlation with flow cytometry to ensure analysis of the correct cell population, to assess cell viability and to guide the selection of antibodies to be tested. Flow cytometric analysis may be severely compromised if the samples contain insufficient material or too many dead cells.

Although the acquisition of data can be automated, the interpretation of the results and their clinical significance requires substantial input and critical judgement from trained haematologists or pathologists. Results should be analysed in conjunction with the clinical presentation, cellular morphology and cytogenetics when appropriate.

Immunohistochemistry

Immunohistochemistry is the phenotyping method of choice for tissue biopsies and is an integral component of routine diagnostic histopathology. It allows direct visualisation of labelled cell surface antigens and cellular morphology via light microscopy. The selection of antibodies available for use on paraffin section is more limited and the turnaround time is slower than for flow cytometry. Results may be severely compromised if the samples are too small or inadequately fixed.

When are these tests useful?

To assess abnormal cell populations

Generally this analysis is requested by haematologists or pathologists to further investigate aberrant cell populations found during microscopy of blood, marrow, lymph nodes or other tissues. Flow cytometry is now an essential tool in the diagnosis of haematological malignancies such as leukaemia and lymphoma.

For example, immunophenotyping may be recommended to investigate persistent peripheral blood lymphocytosis. Lymphocytosis may be due to a reactive state such as resolving viral infection, prior splenectomy or due to an underlying lymphoproliferative disorder such as chronic lymphocytic leukaemia. CD8 T lymphocytes predominate in reactive lymphocytosis whereas B-chronic lymphocytic leukaemia has a distinctive immunophenotype characterised by the expression of mature B cell markers (CD19, CD20 and CD23),

weak expression of monoclonal surface immunoglobulin and co-expression of the T cell marker, CD5. Recent studies suggest that expression of other markers such as CD38, ZAP70 and p53 correlates with a poor prognosis. The routine use of these assays requires further study and standardisation.[1]

Flow cytometry is not useful in the diagnosis of Hodgkin's lymphoma and other fibrotic tumours. This is because there are a low number of viable malignant cells in the sample compared to the numerous surrounding reactive cells.

To monitor for minimal residual disease

Flow cytometry is one of several methods used to detect minimal residual disease in patients with no clinical or morphological evidence of disease. In patients with a known haematological malignancy such as acute lymphoblastic leukaemia, flow cytometry may be useful to detect low levels of persistent disease following therapy.

To quantify cell populations

Clinicians may also request the analysis of specific markers to help guide therapy, for example using flow cytometry to measure CD4 lymphocyte counts in immunosuppressed or HIV positive patients. Patients with low CD4 counts are at greater risk of opportunistic infections. This is particularly true when the CD4 lymphocyte count in peripheral blood falls below 200 cells/microlitre or 0.2 x 10^9/L.

To assess cell proliferation

Ki-67 (MIB1) is an important marker of cell proliferation which can be assessed by immunohistochemistry or flow cytometry to assist diagnosis and guide therapy.[1] For example, Burkitt's lymphoma is characterised by a very high growth fraction with nearly 100% of cells positive for Ki-67. This is much higher than seen in other lymphomas. Because of this high proliferative index, Burkitt's lymphoma can frequently be cured with intensive chemotherapy.

To identify disease-specific targets for therapy

Rituximab, an antibody specific to CD20, is an important advance in the treatment of non-Hodgkin's lymphoma. Similarly, trastuzumab, which targets the human epidermal growth factor receptor 2 protein (HER2), is a new therapy for breast cancer. Testing appropriate patient specimens for these antigens helps to determine whether patients may benefit from the use of these targeted therapies. CD20 may be found on B lymphocytes by either immunophenotyping or immunohistochemistry. HER2 is found by immunohistochemistry or by the DNA-based technique fluorescent *in situ* hybridisation.

To identify foreign cell populations

In some laboratories the Kleihauer assay, used to detect fetomaternal haemorrhage, is now performed by flow cytometry. Similar methodologies have been developed to detect blood doping in athletes by identifying homologous blood cell antigens.[2]

To detect paroxysmal nocturnal haemoglobinuria

Paroxysmal nocturnal haemoglobinuria is a rare haematological disorder characterised by marrow aplasia, intravascular haemolysis and an increased risk of venous thrombosis. It is due to an acquired inability to produce a molecule which anchors certain cell membrane proteins. This leads to a deficiency in specific membrane proteins. Flow cytometric analysis can detect clonal populations of blood cells deficient in these proteins, greatly simplifying the diagnosis.

Conclusion

Analysis of blood and tissue for cell surface markers is a widely accepted and useful tool. It assists clinicians in diagnosing and managing a variety of conditions, particularly haematological malignancies.

References

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2. Nelson M, Popp H, Sharpe K, Ashenden M. Proof of homologous blood transfusion through quantification of blood group antigens. Haematologica 2003;88:1284-95.

Further reading

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Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 135)

5. Flow cytometry is a useful technique for diagnosing Hodgkin's disease.
6. Immunosuppression in patients with HIV can be assessed by flow cytometry.