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Pregledni članak
Review article

CELLULAR DIAGNOSTICS
A Challenge for Laboratory Medicine

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Summary: Investigating cells for genetic features, malignant transformation, surface characteristics, metabolic functions and signalling will be future key elements for diagnosis of diseases. Integrating this new area into Laboratory Medicine will add new competence and responsibility to Clinical Biochemistry and Laboratory Medicine. Cellular genetics will be a main area for the diagnostic laboratory to investigate risk and prognosis for diseases having also impact on a more individualised medication. With the introduction of flow cytometry a more objective way of cell identification by immunophenotyping was added to classical microscopy allowing accurate classification of leukaemias. In addition the functional status and the origin of blood cells or cells in other body fluids (liquor, ascites) can now easily be detected. Cell mediated immunity plays an important role in infectious diseases and in transplantation medicine. The CD4/CD8 ratio of T-lymphocytes is already a routine test for differentiation between viral infection and rejection crises and for monitoring of these conditions. In cellular coagulation platelet function tests will add value to the established plasma tests. The dosage of drugs is now monitored by measuring the blood levels of drugs or their metabolites. In addition investigating the direct effect of drugs on targeted cell functions might be a more specific way.

Key words: genetics, haematology, coagulation, drug monitoring, flow-cytometry

Introduction

The development of a medical discipline is not even but rather cascade-like with peaks and plateaus. The basis for further progress is always combined with new scientific knowledge and advancement in biomedical technology. Although with the identification of the human genome we know the structure of genes, more about their function and products have to be elucidated. Molecular biology techniques, tissue culture techniques, flow cytometry using monoclonal antibodies, mass-spectrometry and other technologies allow measurements in small scale. Even after the introduction of the high-technology instruments of great sensitivity routine diagnostics in Laboratory Medicine using mainly serum as source for the examinations do not bring any additional new information in diagnostics and have thus not changed the patient care. However, today the clinician would also like to identify the tissue or the cell of origin from which a serum component originates. This is even more important in cases with mildly pathological values.

Good laboratory practice is defined as »doing the right thing right at the right time« based on medical knowledge and evidence. Therefore now the most advanced techniques should be used for diagnosis of diseases by investigating in addition to body fluids cells, a new target for diagnosis. Cell functions triggered and regulated by numerous proteins and mediators, influenced by biochemical and physical stimuli will become key elements for pathological physiology and diagnosis of diseases and will open new perspectives in diagnosis and treatment.

Cellular diagnostics in Laboratory Medicine will enter genetics, haematology, haemostaseology, biochemistry, immunology, and drug monitoring. Thus new diagnostic concepts for risk assessment, diagnosis of health, disease, and follow-up of patients, will be developed. In the following review perspectives of cellular diagnostics are given and some examples are described.

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Cellular Genetics

Cellular genetics will be a main area for the diagnostic laboratory to investigate risk and prognosis for diseases having also impact on a more individualised medication. One can envisage the following main areas:

- Diagnosis of monogenic (metabolic) diseases;
- Risk estimation of multigenic conditions of diseases, e.g. arthritis, cardiovascular diseases, hypercoagulability, dementia;
- Effect of genetic fingerprints on individual drug design.

Non-invasive prenatal diagnosis during pregnancy can be achieved by separation of fetal cells from maternal blood by immmunomagnetic capturing followed by investigation with PCR-technology. Thus fetal trophoblasts, lymphocytes, granulocytes, stem cells and nucleated red blood corpuscles all having specific surface antigens can be isolated. These cells are useful for early detection of metabolic disorders. The same approach can be used for the isolation and investigation of malignant cells or their genetic products from blood in cancer patients. Tumour specific genetic material in blood, far from the tumour site, might be a useful diagnostic tool for diagnosis and prognosis. In breast cancer patients a mean concentrations of 211 ng/mL of plasma DNA were measured whereas in healthy controls only 21 ng/mL were detected (1). In patients exhibiting p53 mutations in their primary tumour 65 % also showed p53 mutations in their plasma DNA. Patients with tumour and plasma DNA p53 mutations had the worst prognosis with respect to recurrence and distant metastasis.

Cellular Haematology

In haematology cellular diagnostics has always been a main area of interest. With the introduction of flow cytometry a more objective way of cell identification by immuno phenotyping was added to classical microscopy allowing accurate classification of blood malignancies. For identification of white blood cells fluorescence labelled monoclonal antibodies are used. Well defined monoclonal antibodies are used for the identification of the normal white blood cell classes (*Table I*). Malignant or premature white blood cells exhibit a variety of additional abnormal epitopes, which are used for specific identification of cell lineages. As an example the step-by-step diagnosis of a follicular B-cell lymphoma exhibiting a B-cell clone is described in some detail (*Table II, Figure 1*).

Table I Clusters of differentiation in flow cytometry

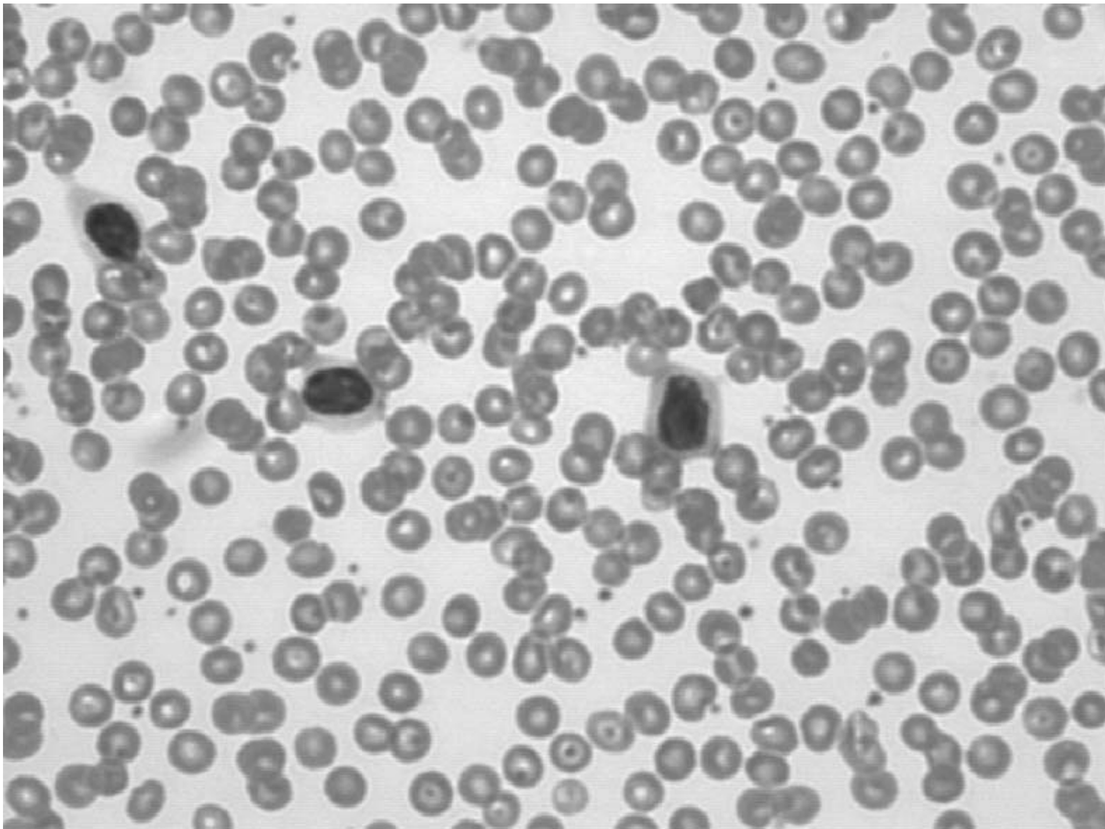
White Blood Cells	Monoclonal Antibodies
Leucocytes	CD 45
Granulocytes	CD 15
Monocytes	CD 14
T-lymphocytes	CD 3
Helper-T-lymphocytes	CD 3, CD 4
Cytotoxic-T-lymphocytes	CD 3, CD 8
B-lymphocytes	CD 19
NK-cells	CD 16/CD 56

Table II Follicular B-cell lymphoma.
Blood count and flow cytometry – 54 years old female of unknown history.

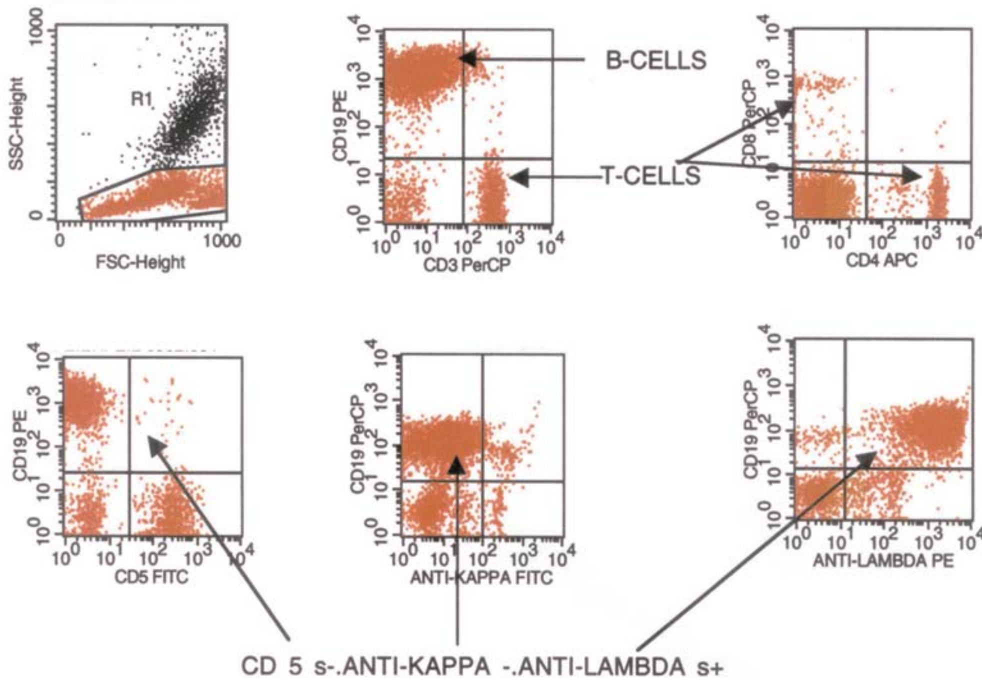
Blood Cells	Patient	Reference Ranges
Blood Count		
Leucocytes (/μL)	12.540	4.300–10.800
Lymphocytes (/μL)	4.264	1.100–4.320
Lymphocytes (%)	34	25–40
Differential blood count	Atypical lymphocytes	
Flow Cytometry		
T-lymphocytes (/μL)	2.480	660–3.655
T-lymphocytes (%)	47	60–85
B-lymphocytes (/μL)	**1.766**	10–989
B-lymphocytes (%)	**34**	7–23
$CD19^+/CD5^+$ – Cells (%)	0	0–20
$CD19^+/kappa^+$ – Cells (%)	5	0
$CD19^+/lambda^+$ – Cells (%)	**100**	0
$CD19^+/CD38^+$ – Cells (%)	**0**	60–80

In addition the functional status and the origin of cells in other body fluids (liquor, ascites) can now easily be detected by means of flow-cytometry in combing fluorescence conjugated monoclonal antibodies recognising specific surface cell markers with cell-cycle analysis after intracellular staining of DNA with probidium-iodide. With this approach an inflammatory leucocytosis in the spinal fluid can be accurately distinguished from a meningial carcinomatosis. The cells investigated in the liquor cerebrospinalis showed two features characteristic for malignant cells: surface expression pattern of the epithelial cells' CAM 5.2 epitope and 76 % of hyperploid DNA concomitant with an increased synthesis rate of 13 %.

Cell mediated immunity plays an important role in infectious diseases, in transplantation medicine for the detection of the immune status of patients, and in immunodeficiencies. The determination of the CD4/CD8 ratio of T-lymphocytes by flow- cytometry is already a routine test for differentiation between these clinical conditions and in the follow-up of patients with severe viral infections and rejection crises. In uncomplicated post-transplantation course a ratio of 1.46 ± 0.96 is measured. Due to increase of CD4+ T-helper cells the ratio increases to 2.34 ± 0.72 approximately one week before the clinical symptoms of a rejection



1a. Microscopy – The B-cell clone, haematoxxylin-eosin staining.



1b. Flow cytometry scattergrams showing CD-markers expressed. The lambda-positive B-cell clone is specific for this B-cell lymphoma. Cell Quest software (Becton Dickinson, San Jose, CA, USA) was used.

Figure 1. Follicular B-cell lymphoma. 54 years old female, laboratory data described in *Table II.*

crises in kidney transplant recipients. Viral infections are characterised by a decrease of $CD4^+$ T-helper cells and a relative increase of $CD8^+$ cytotoxic T-cells show a ratio < 0.9 (2, 3). The ratio has been demonstrated also a useful tool in the follow-up, staging of cancer patients (4) and in monitoring antiviral therapy in patients with HIV infections (5, 6). Flow cytometry enables also to measure specific functions of cells. Monocytes/macrophages are important in cell-mediated response in infection and rejection crises in the post-transplantation course. The HLA-DR expression of blood monocytes are markers of their functional status and useful for the prognosis. Increased HLA-DR expression on monocytes are characteristic for graft rejections; in contrast decreased expression are found during viral and bacterial infections (7).

Cellular Haemostaseology

In cellular coagulation platelets functions are the target for diagnostic tests used in addition to the established plasma tests investigating coagulation factors. Conventional platelet counts performed in routine haematology only allow discrimination between thrombopenia and thrombocytosis that might be related with disturbances in blood coagulation. Collagen and ADP platelet activation tests have been established for monitoring platelet functions and platelet inhibitors. Recently a platelet function analyser has been marketed and promoted for assessing effectiveness of platelet antagonists and platelet transfusions (8, 9).

The ADP platelet activation test is based on the fact that the expressions of membrane integrins (GPIIb/IIIa) are increased when ADP is bound to the purine receptor. Monoclonal antibodies conjugated with fluorescence dyes had been raised for these integrins and are used for flow cytometric determination of platelet activation. In *Figure II* the ADP-concentration dependent CD-41 (GPIIb) and PAC-1 (GPIIB/IIIa) expression is shown. Reference ranges for CD 41 and PAC-1 expression on freshly isolated blood thrombocytes using stimulation with 10 µmol/L ADP for 15 minutes at 37 °C are summarised in *Table III*. With this approach and the appropriate stimulant the compliance of patients suffering from thromboembolism and receiving platelet activation inhibitors like aspirin and plavix can be monitored. In addition in syndromes related to kidney and liver diseases concomitant with hemorrhage or thrombophilia platelet function testing might add useful information to targeted therapy.

Cellular Drug Monitoring

Measuring the blood levels of drugs or their metabolites now monitors the dosage of drugs. Neither the biochemical, pharmacological effects of prescribed drugs nor their effects on targeted cell functions are so far measured in clinical laboratories, in spite of the fact that this would be much more specific. There

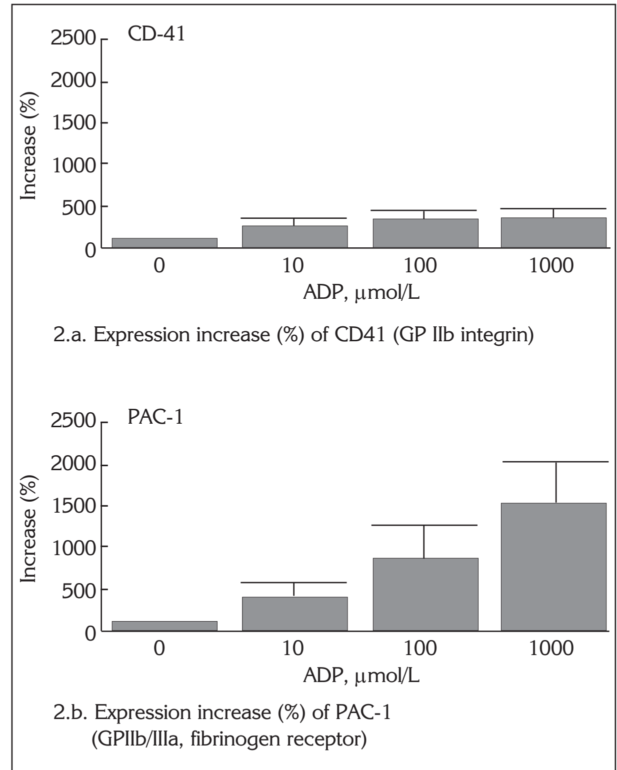


Figure 2. *In vitro* activation of isolated platelets by ADP. Conditions: 15 minutes incubation at 37 °C, expression measured by flow cytometry

Table III Reference ranges of CD41 (GP IIb) and PAC-1 (GPIIb/IIIa) expression in isolated platelets. Conditions: incubation with 10 µmol/L ADP, 15 minutes, 37 °C; measurement by flow cytometry.

Reference Ranges	CD41 basal	CD41 15 min ADP	PAC-1 basal	PAC-1 15 min ADP
% Increase of MF	100	247–477	100	1061–2057
Mean fluorescence	493–802	1677–2875	3.0–9.0	53–152

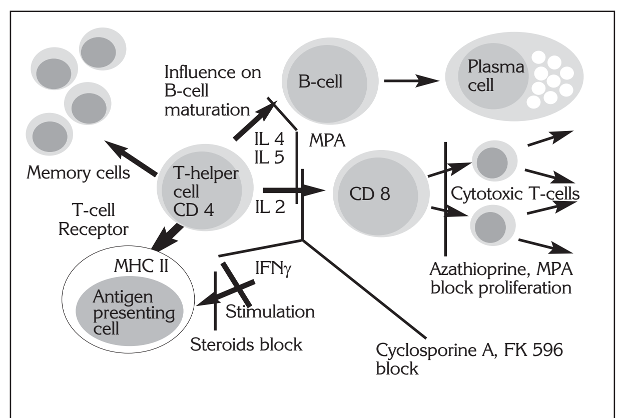


Figure 3. Cellular targets of immunosuppressive drugs

are some approaches towards monitoring biological effects of immunosuppression in organ transplantation. The cellular targets of the most common immunosuppressive drugs are shown in *Figure 3*. Cyclosporine A, the most commonly used drug in transplantation medicine, is a cytokine suppressor by inhibiting intracellular IL-2 gene transcription and encoding of IL-3, IL-4, and IFNγ in $CD4^+$ T-helper cells thus inhibiting the immunological cellular cascade. In patients receiving cyclosporine A the percentage of $CD4^+$ T cells producing IL-2 compared with healthy controls was significantly reduced and correlated well with the blood levels of the drug (10). The antimetabolite mycophenolic acid, a competitive inhibitor of IMP-dehydrogenase (11) and thus an inhibitor of guanine nucleotide synthesis, blocks *in vitro* and *in vivo* lymphocyte proliferation concomitant with reduction of IL-2 receptor expression on B- and T-lymphocytes (12–14). These investigations demonstrates that functional effects on the cellular immune system in transplant recipients can be used for clinical monitoring being much more representative for the individual condition than the usual trough-levels of immunosuppressive drugs.

In summary, investigating cells for genetic features, malignant transformation, surface characteristics, metabolic functions and signalling will be future key elements for diagnosis of diseases. Integrating this new area into Laboratory Medicine will add new competence and responsibility to Clinical Biochemistry and Laboratory Medicine.

CELULARNA DIJAGNOSTIKA
Izazov za laboratorijsku medicinu

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Kratak sadržaj: Izučavanje genetskih osobina, malignih transformacija, površinskih karakteristika, metaboličkih funkcija i signalnih komunikacija ćelija će u budućnosti biti ključni elementi za dijagnostikovanje oboljenja. Integracija ovih novi oblasti u laboratorijsku medicinu će zahtevati nove kompetentnosti i odgovornosti kliničke biohemije i laboratorijske medicine. Ćelijska gentika će biti glavna oblast u dijagnostičkim laboratorijama za ispitivanje rizika i prognoze oboljenja, a što je povezano i sa zahtevima za individualizacijom terapije. Uvođenjem floucitometrije mnogo se objektivnije identifikuju ćelije imunofenotipizacijom a dodavanjem ove tehnike klasičnoj mikroskopiji mnogo se tačnije klasifikuju leukemije. Osim funkcionalnog statusa i porekla ćelije krvi ili ćelije u drugim telesnim tečnostima (likvor, asciti) se sada mogu otkriti mnogo jednostavnije. ćelijski posredovani imunitet ima veoma značajnu ulogu u infektivnim oboljenjima i u transplatacionoj medicini. CD4/CD8 odnos T-limfocita je već rutinski test za diferencijaciju između virusne infekcije i krize odbavicanja i praćenje ovih stanja. Osim dobro poznatih plazma testova danas se pridodaju ćelijski koagulacioni trombocitni funkcionalni testovu. Doziranje lekova je moguće pratiti merenjem njihovog nivoa u krvi ili njihovih metabolita. Osim toga ispituju se i efekti lekova na ciljne ćelijske funkcije, što će u budućnosti biti još specifičnije.

Ključne reči: genetika, hematologija, koagulacija, praćenje lekova, floucitometrija

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