Flow cytometry technology and clinical projects



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Lecture time: November 28,2025

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1 The principles of flow cytometry

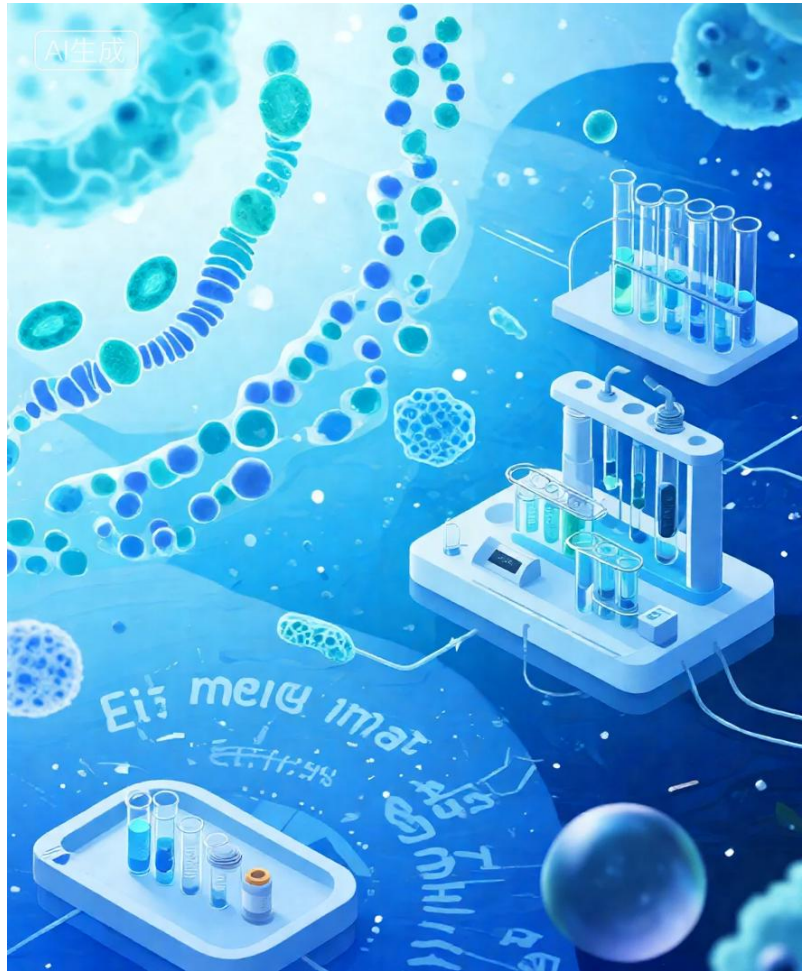
2 Components of flow cytometry

3 Advantages of flow cytometry

4 Types and requirements of specimens

5 Operating process

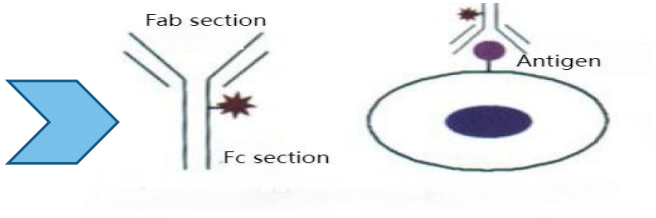
6 Clinical projects



1 The principles of flow cytometry

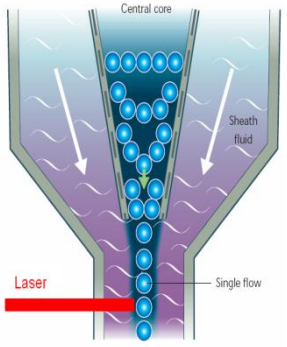
we need to prepare of single cell suspension



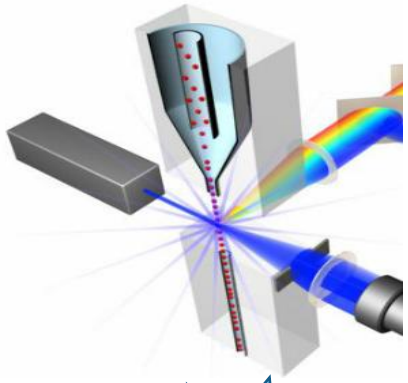


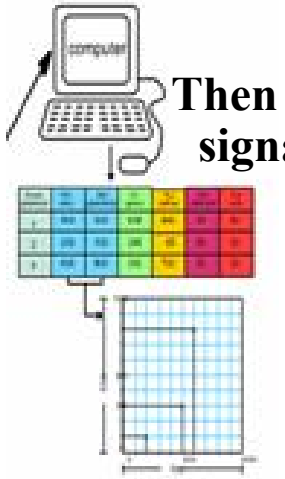
We use fluorescein-labeled antibodies to bind antigens on the surface of the cell

The cells are irradiated one by one by laser



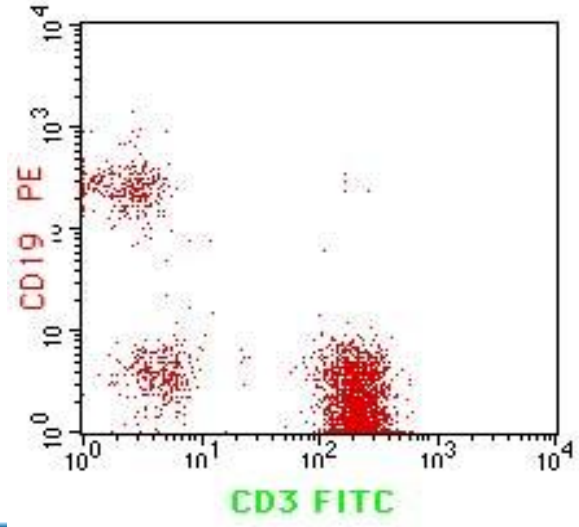
Then we collect the optical signal





Then convert it into digital signal for result analysis.

Interpretation of result



How does flow cytometry quantify cells?

The physical and chemical properties of cells or particulate matter (surface, cytoplasm or nucleus) are detected by detecting fluorescence signals and quantified by these properties.

Preparing reagents: fluorescein-labled antibodies

↓

The antibody binds specifically to the corresponding antigen on the cell

↓

The cells expressing the antigen are labeled with fluorescence;

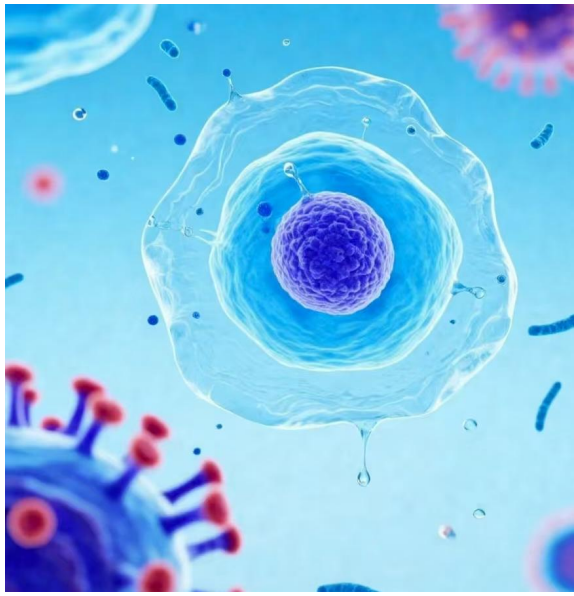
Cells with high antigen expression have strong fluorescence intensity

→

Finally, we can obtain the following information:

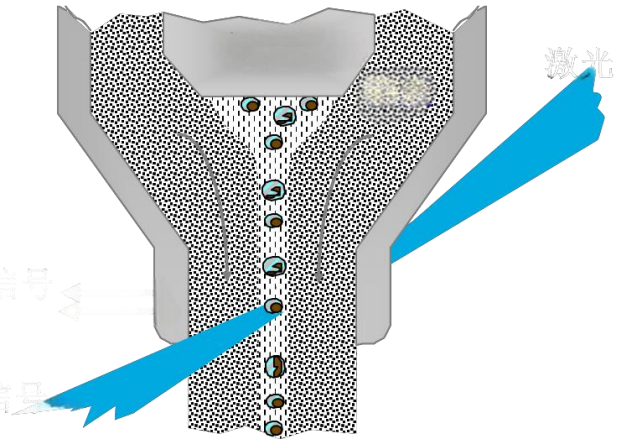
Positive cell concentration;

Target protein concentration



2 To better understand the principle of the flow cytometry, we also need to understand **the composition of the flow cytometry.**

Flow path

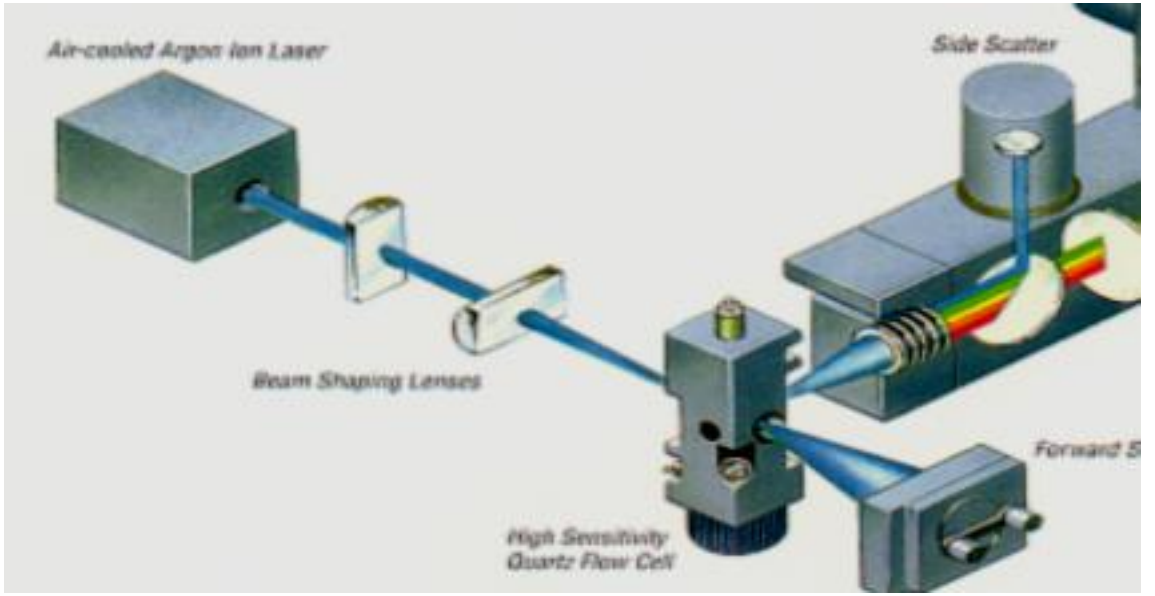


Due to the action of the sheath fluid, the target cells are confined to the axial flow line.

Light path

The optical path system of a flow cytometry functions like its "eyes," enabling us to observe cellular characteristics.

First, the laser source acts as a bright light source emitting specific-colored beams. These high-intensity lasers excite fluorescent substances within cells. The optical lens system serves as a precision sight, focusing the laser beam into a narrow, straight beam that accurately targets cells. The aperture restricts the beam's propagation range for clearer detection. Filters act as color filters, allowing only specific wavelengths to pass through, enabling separate detection of different fluorescence signals.



Circuit

Perform signal detection and analysis

The fluorescent signal is captured by a photomultiplier tube (PMT), which converts it into an electrical signal. This enables analysis of the voltage pulse's height, area, and width.

The electrical pulse signal is converted into a digital signal through A/D conversion.

Digital signals are transmitted to the computer for storage, drawing, and statistical analysis

The instrument shown in this photo is the flow cytometry I use in my work.





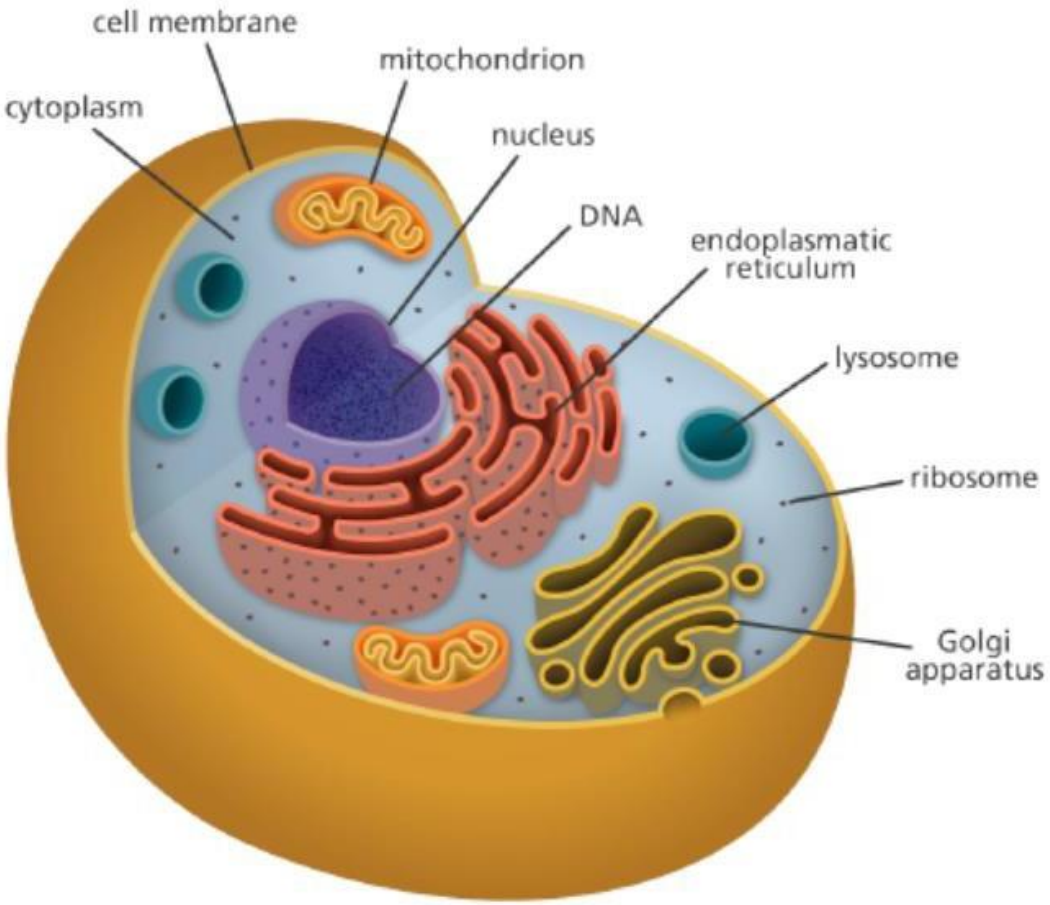
3 What are the advantages of flow cytometry technology?

1.Quick quantitative analysis of cells

2.Multi-parameter cell analysis

3.Automate cell sorting

Flow cytometry is used to rapidly quantify cells in order to sort a particular cell subset from a cell population for further study.

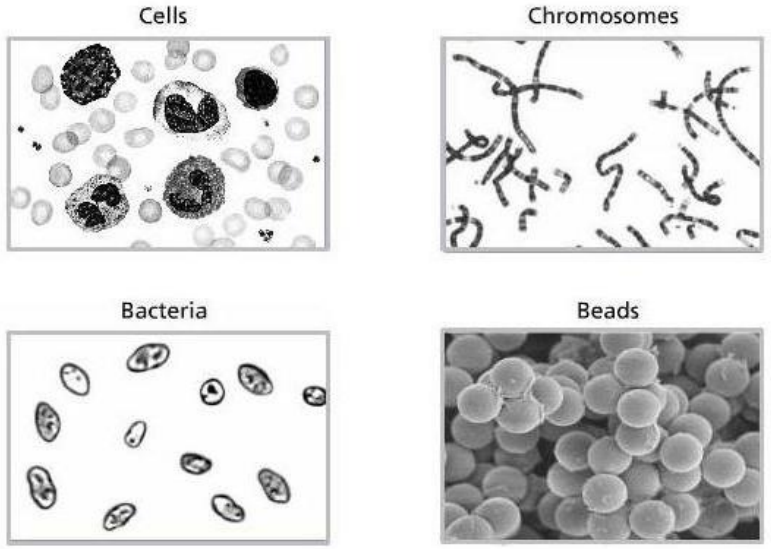


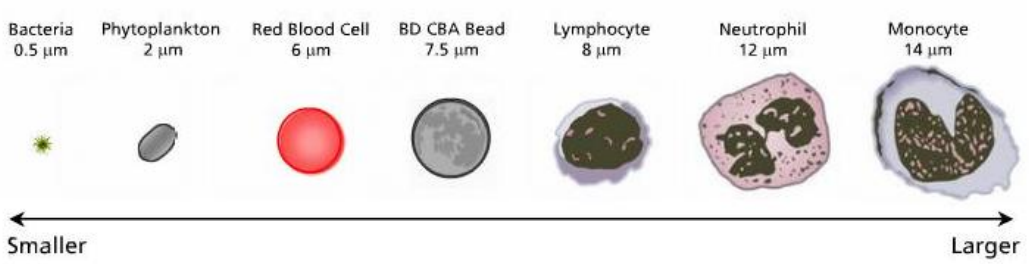
What information can flow cytometry tell us

Bioinformatics of cell surface and cell interior.

What can a flow cytometry detect?

- **Peripheral blood：anticoagulant whole blood**
- **Bone marrow：anticoagulant bone marrow blood**
- **Hydrothorax and ascites**
- **Organizational entity：distribute into cell suspension**
- **Else：Artificial microspheres**





4 Specimen Requirements

01 The appropriate cell concentration is required:

Suggestion 10^5-10^6/ml

02 Blood sample:

EDTA, heparin, or sodium citrate anticoagulant whole blood



03 Hemolytic specimens should be discarded

What are the requirements for specimens when performing flow cytometry testing?

4 Specimen Requirements

04

‧Erythrocyte specimen: Dilute 1000 times with physiological saline or PBS

05

Platelet sample: Avoid using heparin anticoagulant; dilute 20-fold with PBS or normal saline

06

WBC sample: Lysis is required to remove red blood cells

5 operating process

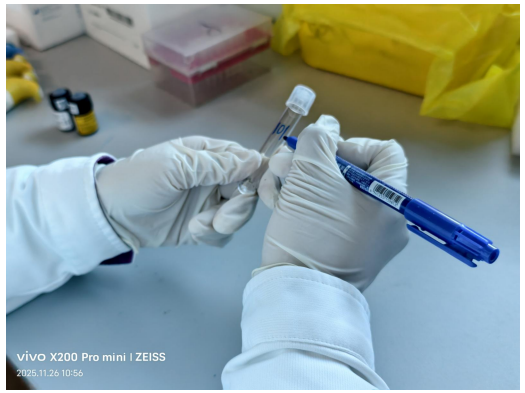
2ml of whole blood anticoagulant specimen sent for testing



Add 20μl of the mixed antibody containing CD45-FITC/CD4-RD1/CD8ECD/CD3-PC5/CD56-PC7 to the flow cytometry tube.



Start numbering the test tube



Number qualified specimens, register them, charge through the LIS system



5 operating process

Invert the specimen and mix thoroughly. Add 50μl of whole blood to the flow cytometry tube.

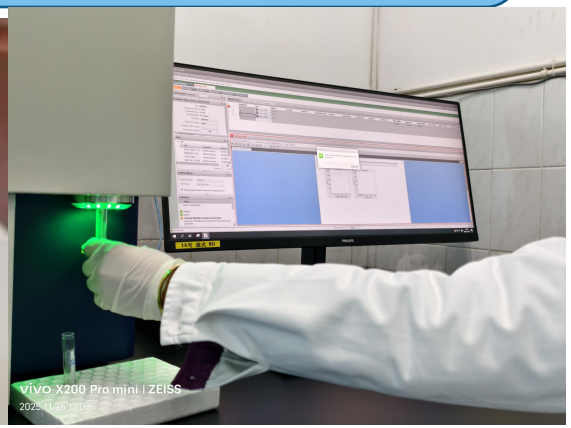


Gently mix and store at room temperature in the dark for 15 minutes. Add 500μl hemolysin and let it stand for 8 minutes.

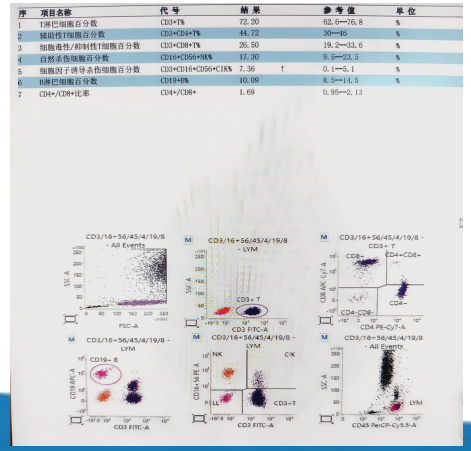


After complete hemolysis and complete clearance of the liquid, perform flow cytometry on the machine.





Export the detection results and verify them



6 Clinical projects

Immunology

- ✓ Lymphocyte subset analysis
- ✓ HIV immunotyping
- ✓ Ankylosing Spondylitis Disease Testing
- ✓ Infectious disease testing
- ✓ Monitoring of immune reconstruction after transplantation

Hematology

- ✓ Leukemia immunotyping
- ✓ Leukemia minimal residual detection
- ✓ Platelet and related disease detection
- ✓ Hematopoietic stem cell count
- ✓ Reticulocyte count
- ✓ PNH check

Oncology

- ✓ Lymphocyte subset analysis
- ✓ Cell cycle and ploidy analysis
- ✓ Immune status monitoring
- ✓ Evaluation of cellular immunotherapy
- ✓ Direction on using the medicine



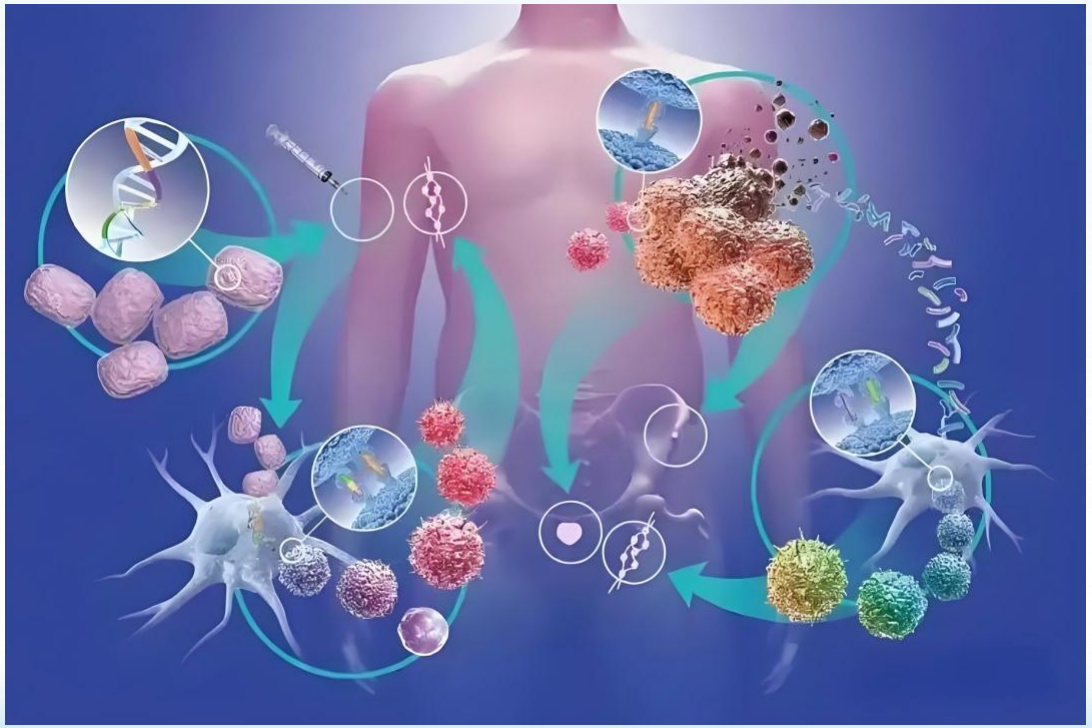


What projects have we carried out in clinical practice?

- Analysis of lymphocyte subsets
- Stem/progenitor cell count
- Detection of cytokine
- Detection of platelet function
- Detection of Human leukocyte antigen B27
- Immunophenotyping of leukemia
- Diagnosis of MRD
- Diagnosis of PNH

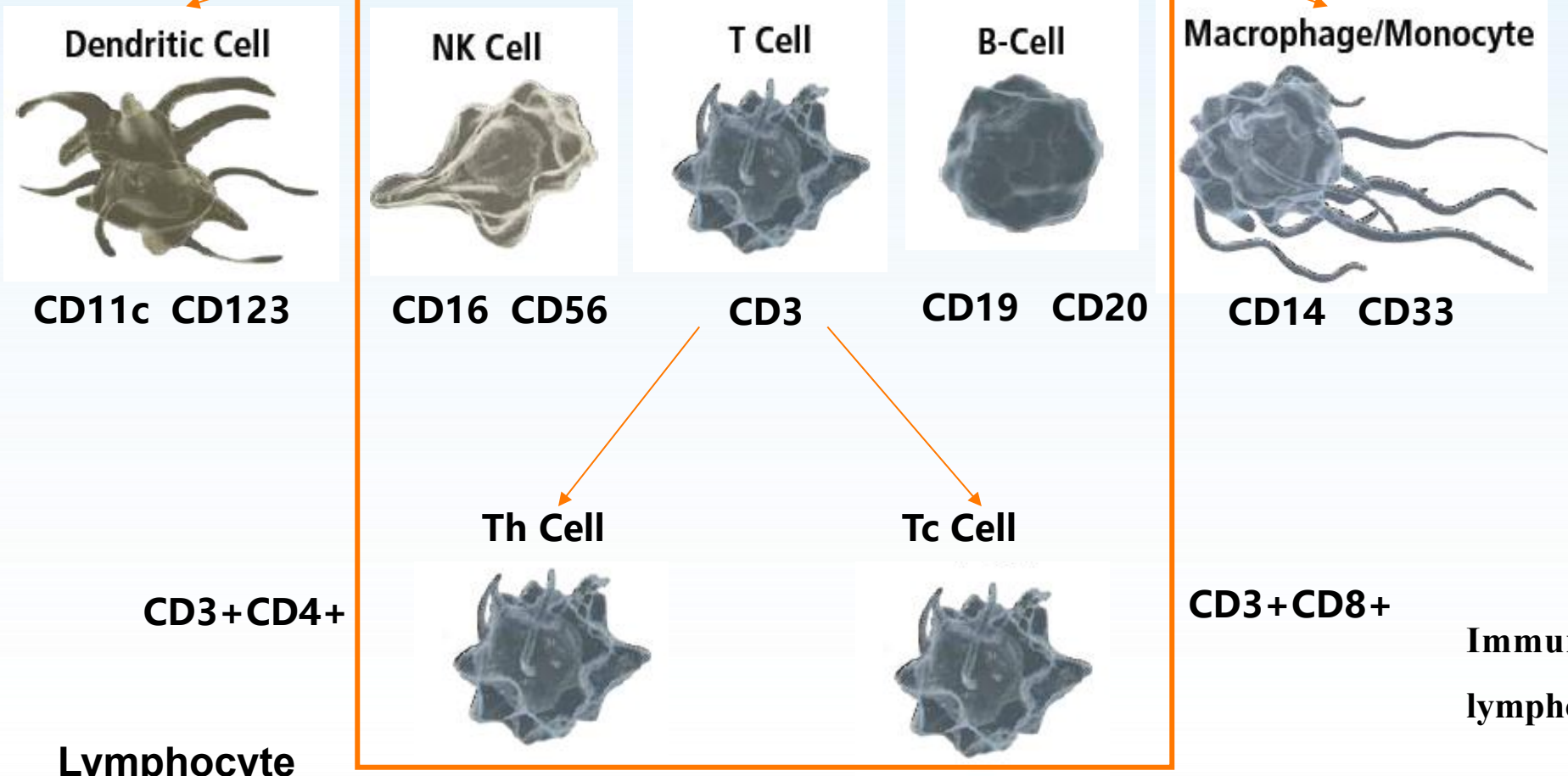
（1） Analysis of lymphocyte subsets

Immune cells are the cellular groups in Homo sapiens responsible for immune defense functions. Like loyal guardians, they constantly protect our bodies, defending against the invasion of external pathogens and the rebellion.





Immune cell



Immune cell are classified into DC, lymphocyte, monocyte.

The immune cells collaborate and coordinate with each other, enabling us to maintain health in complex and ever-changing environments and resist the invasion of various diseases.

When the immune system becomes overly "aggressive" and immune cells are hyperactive, they may mistakenly identify the body's own normal tissue cells as foreign pathogens and launch attacks, triggering autoimmune diseases.

Conversely, if the immune system is too "sluggish" and immune cell function is impaired, it becomes unable to effectively defend against pathogen invasion. A typical example is AIDS (Acquired Immunodeficiency Syndrome), where the human immunodeficiency virus (HIV) primarily attacks the CD4+ T lymphocytes in the human body. As the number of these critical immune cells progressively declines, the immune system gradually collapses, leading to death from various opportunistic infections and cancers.

To maintain immune cell function and balance the immune system, we should adopt several lifestyle measures.

A balanced diet is fundamental, ensuring sufficient intake of proteins, vitamins, and minerals to provide essential nutrients for immune cell production and maintenance.

Moderate exercise is equally vital, as it enhances blood circulation, boosts immune cell activity, and strengthens immunity.

Adequate sleep is equally important, as the body repairs itself and the immune system regenerates during rest.

Maintaining a positive mindset also positively impacts the immune system.

By adopting these healthy habits, we can better nurture immune cells, keeping them in peak condition to safeguard our health.

1 **Quantitative analysis of T cell subsets**

- Total T cells,
- T4/T8 ratio,
- percentage /absolute counts of T4 and T8 cells

2 **Quantitative analysis of lymphocyte subsets:**

- Total T cells,
- T4, T8, B cells,
- NK cells (both percentage and absolute counts),
- T4/T8 ratio.

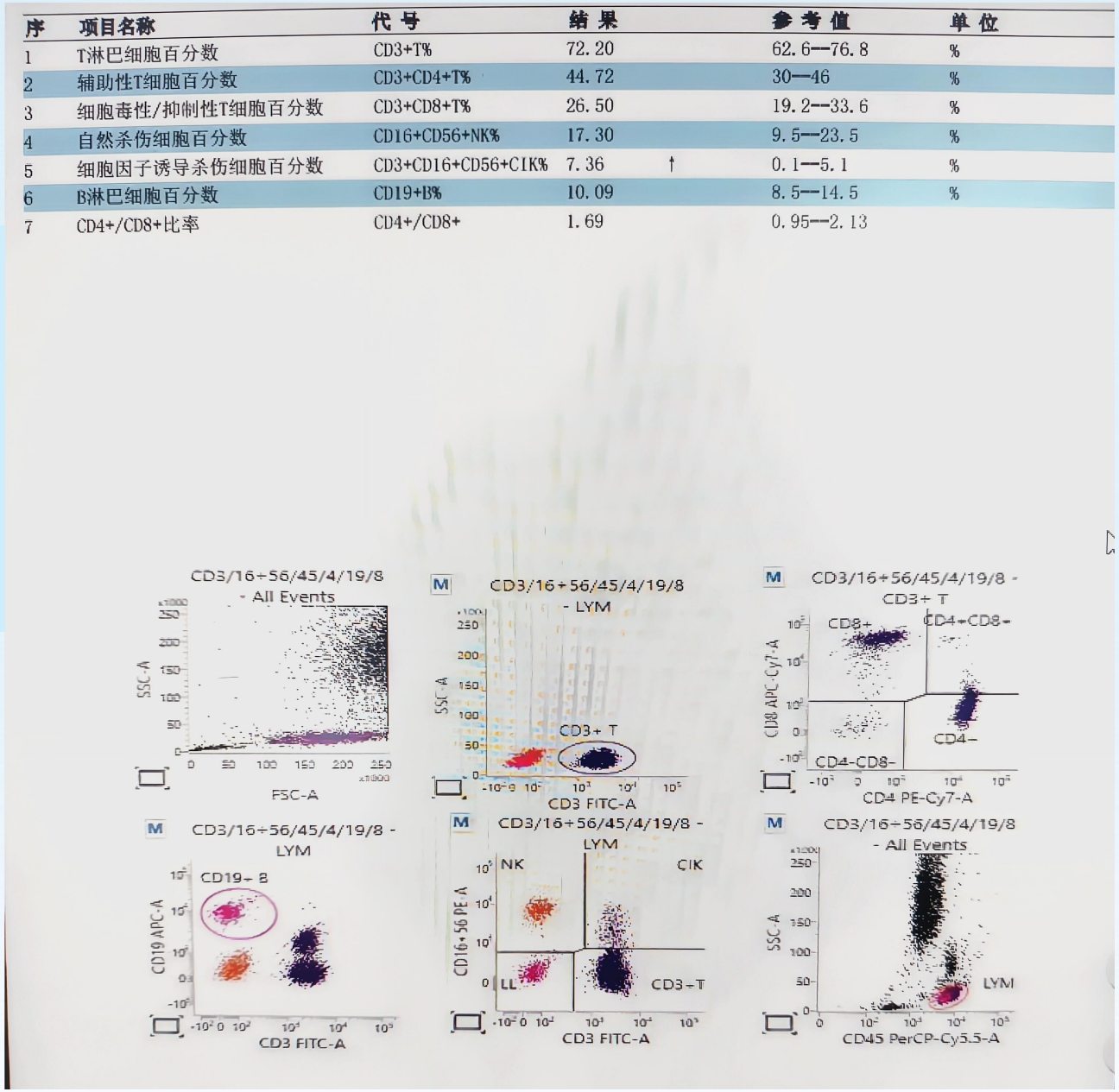
3 **Lymphocyte activation assay:**

- HLA-DR-CD3-negative resting T cells,
- HLA-DR+CD3+ activated T cells,
- HLA-DR-CD8-negative resting Th cells,
- HLA-DR+CD8+ activated Th cells

Understanding the immune function state of the body is of great significance for the auxiliary diagnosis, efficacy evaluation and prognosis judgment of diseases, as well as the diagnosis of sub-health state and the risk assessment of related diseases.

The five-item lymphocyte immunological analysis report on the right is issued by our hospital.

序	项目名称	代 号	结 果		参 考 值	单 位
1	T淋巴细胞百分数	CD3+T%	72.20		62.6—76.8	%
2	辅助性T细胞百分数	CD3+CD4+T%	44.72		30—46	%
3	细胞毒性/抑制性T细胞百分数	CD3+CD8+T%	26.50		19.2—33.6	%
4	自然杀伤细胞百分数	CD16+CD56+NK%	17.30		9.5—23.5	%
5	细胞因子诱导杀伤细胞百分数	CD3+CD16+CD56+CIK%	7.36	↑	0.1—5.1	%
6	B淋巴细胞百分数	CD19+B%	10.09		8.5—14.5	%
7	CD4+/CD8+比率	CD4+/CD8+	1.69		0.95—2.13	



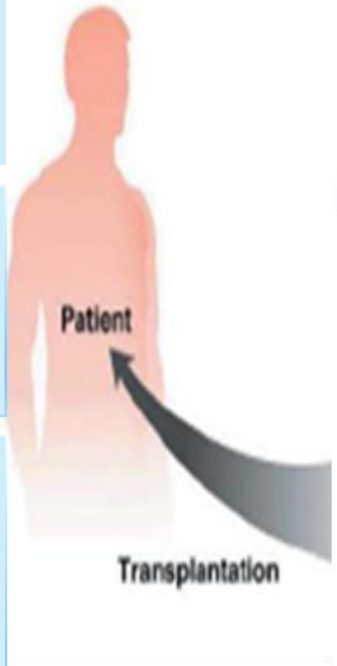
(2) Stem/progenitor cell count

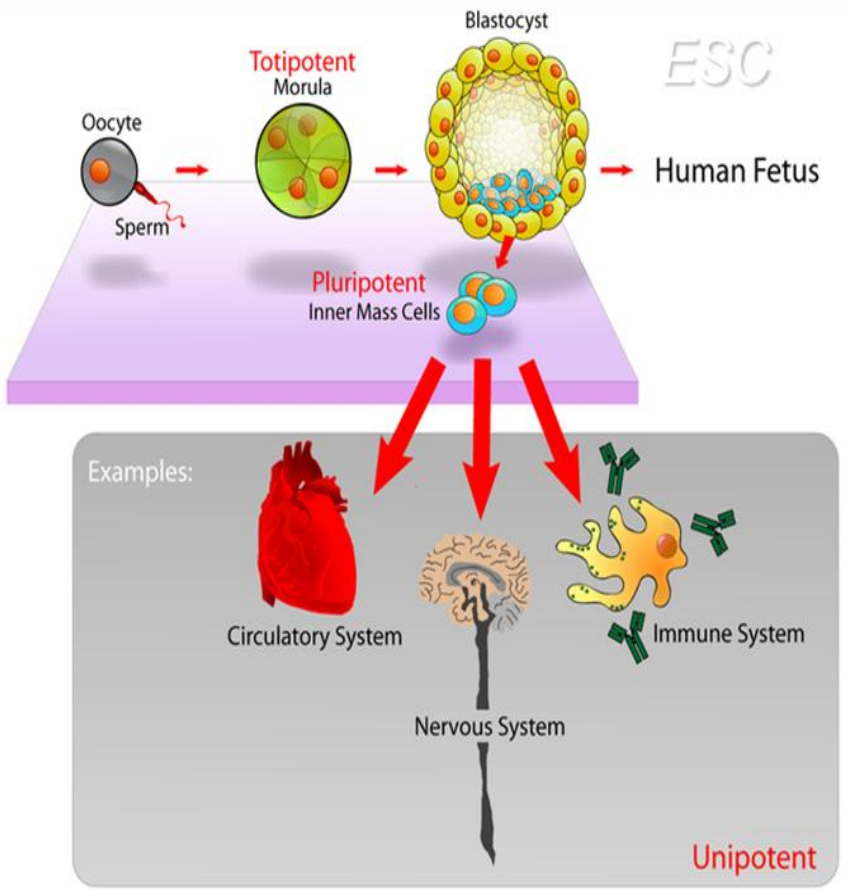
1 Stem cells, also known as 'universal cells' in biology, possess self-renewal capacity and multi-directional differentiation potential, enabling the regeneration of various tissues, organs, and even the human body.

2 Stem cell therapy is to replace and repair the damaged cells of patients through stem cell transplantation, so as to restore the function of cell tissue and treat diseases.

3 Chemotherapy Intensity Management: The rate of blood cell recovery after chemotherapy depends on the extent of damage to hematopoietic stem/progenitor cells. CD34+ cell analysis helps determine the appropriate chemotherapy intensity.

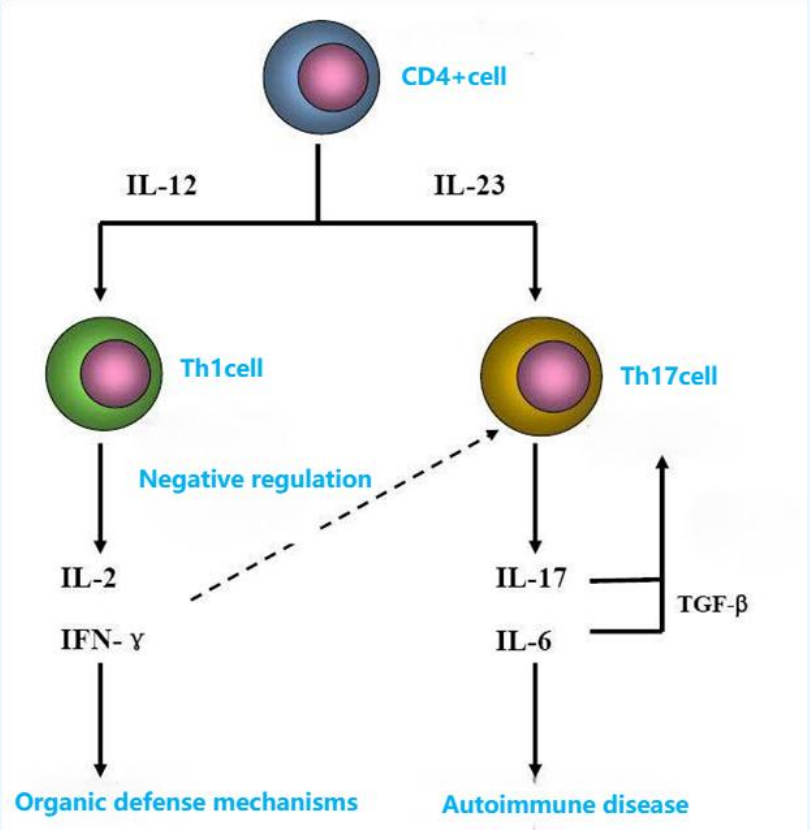
4 Differentiation of anemia types (e.g., aplastic anemia, iron-deficiency anemia, etc.): In aplastic anemia cases where stem cells are affected, CD34+ cell counts may drop significantly (<0.10%). For iron-deficiency anemia, CD34+ cell counts remain within normal ranges.





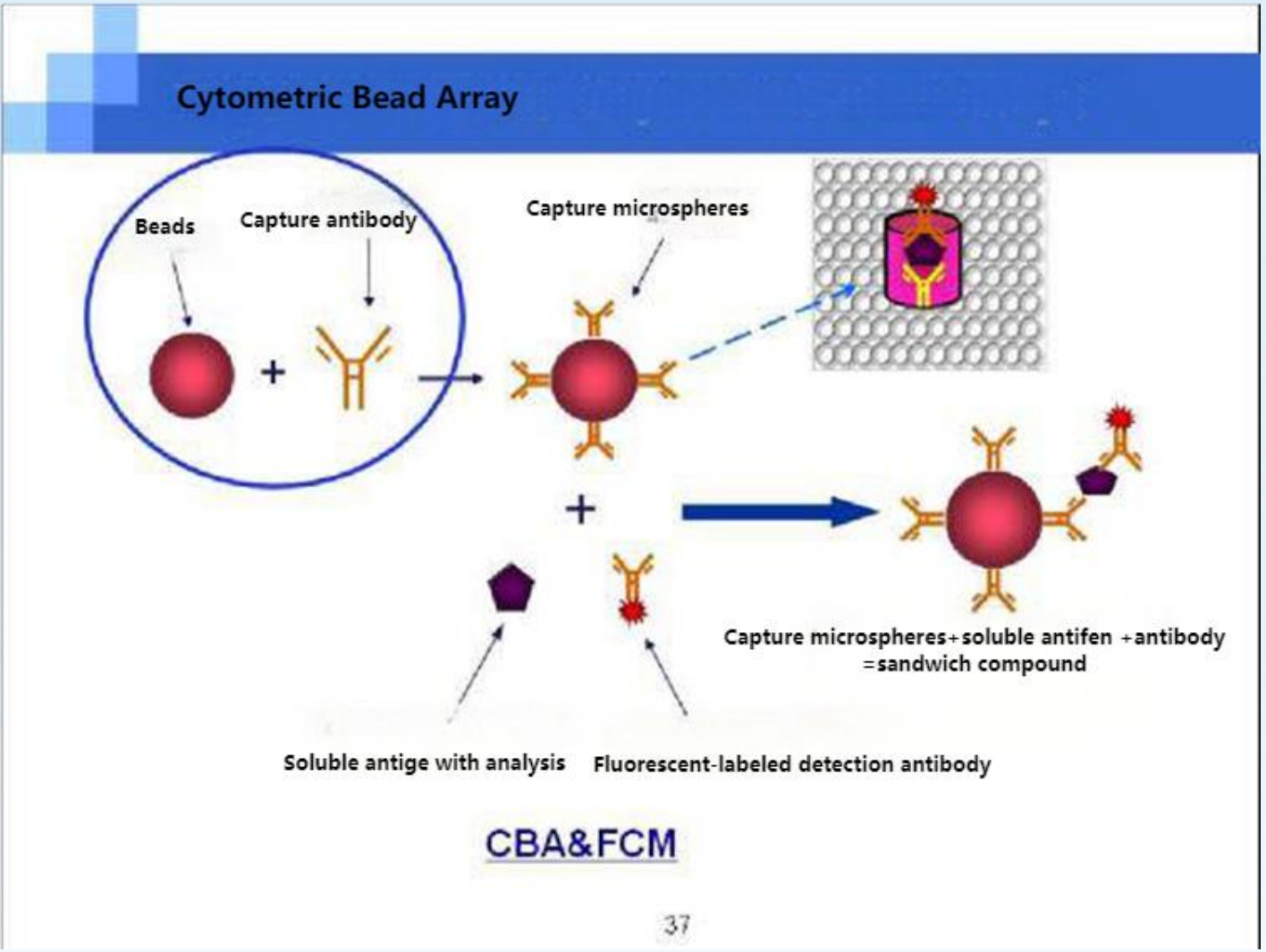
Stem cell transplantation utilizes autologous harvested cells, which are isolated in the laboratory, and then infuses the stem cells into the Homo sapiens body to reconstruct normal hematopoietic function.

(3) Detection of cytokine



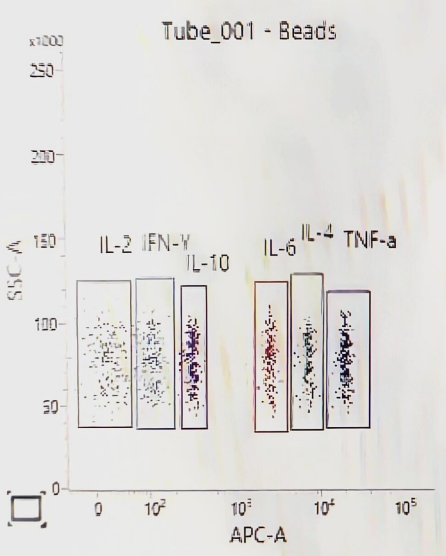
CD4+ T cells can be divided into multiple subsets, with different subsets secreting distinct cytokines. These secreted cytokines further participate in immune responses by regulating the differentiation and proliferation of immune cells through positive or negative feedback mechanisms.

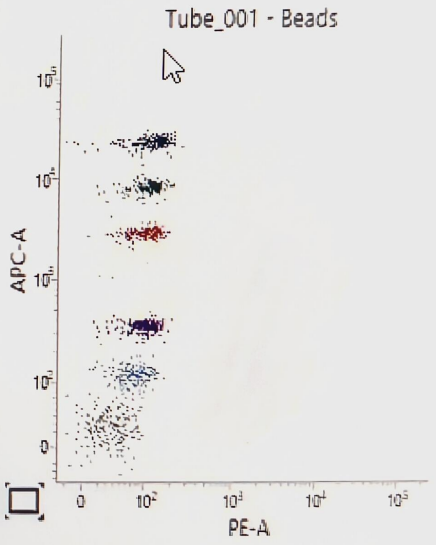
As signaling molecules, cytokines mediate and regulate immune responses and inflammatory reactions, stimulating hematopoietic cell production and tissue repair. Under normal conditions, their expression and secretion are strictly controlled by the body. However, under pathological conditions, cytokine expression becomes abnormal. Therefore, studying cytokine expression at the single-cell level through flow cytometry holds significant value for the diagnosis and treatment of certain diseases.



Each microsphere is coated with a specific capture antibody to bind the target cytokine, forming a specific interaction. Fluorescein-labeled antibodies are then added to create a sandwich-like immune complex of microsphere-cytokine-fluorescein-labeled antibody.

序	项目名称	代号	结果	参考值	单位
1	白介素-2	IL-2	0.307	0-7.394	pg/ml
2	白介素-4	IL-4	4.097	0-7.541	pg/ml
3	白介素-6	IL-6	6.19	0-9.1	pg/ml
4	白介素-10	IL-10	2.662	≤8.051	pg/ml
5	干扰素-γ	IFN-γ	1.136	0-11.25	pg/ml
6	肿瘤坏死因子-α	TNF-α	1.603	0-14.19	pg/ml





This report is the result of our hospital's cytokine test.We can detect the IL-2, 4, 6, 8, 10, IFN-γ，TNF-α.

(4) Detection of platelet function

CD61+ CD63+ anti-platelet membrane glycoprotein (platelets from aged activated state)

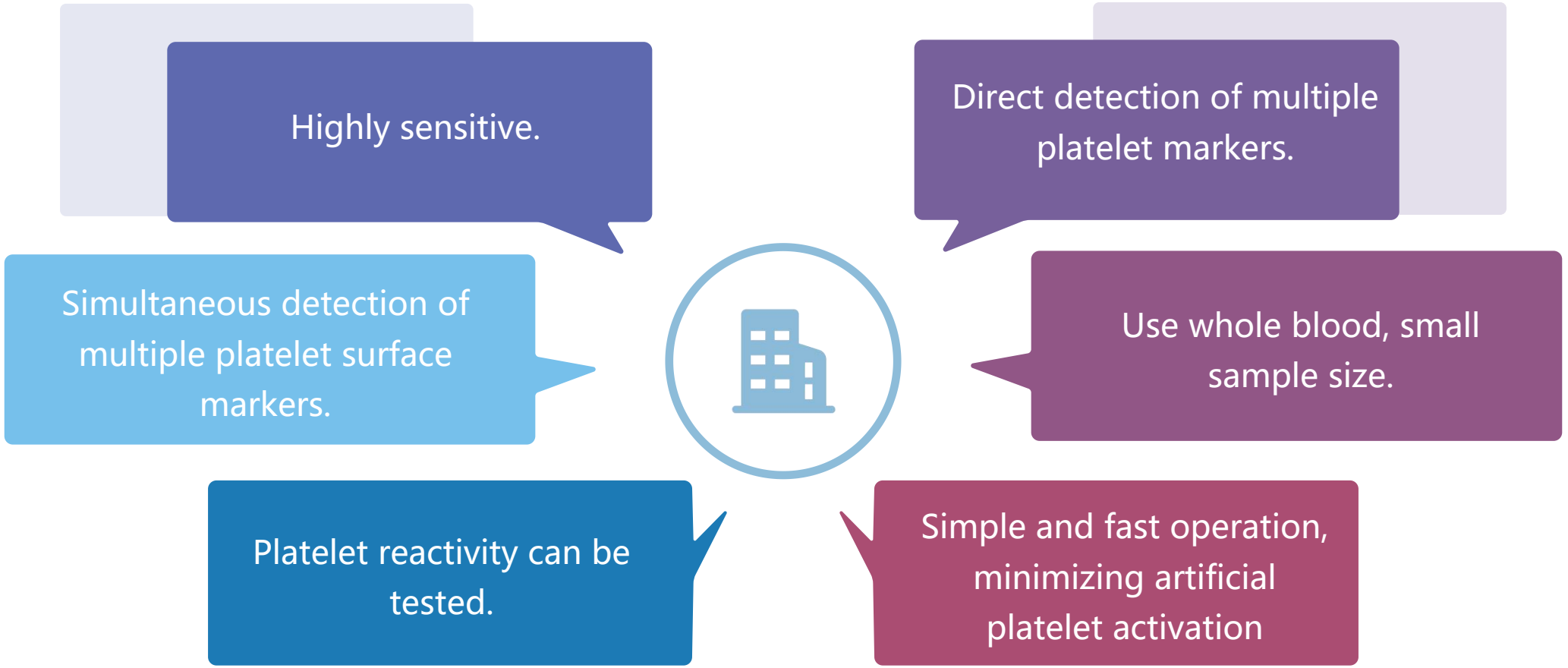
CD61+ anti-platelet membrane glycoprotein (total platelets)

CD61+ CD62+ platelet α granule protein (newly activated platelets)

Platelet-related tests include the following items:

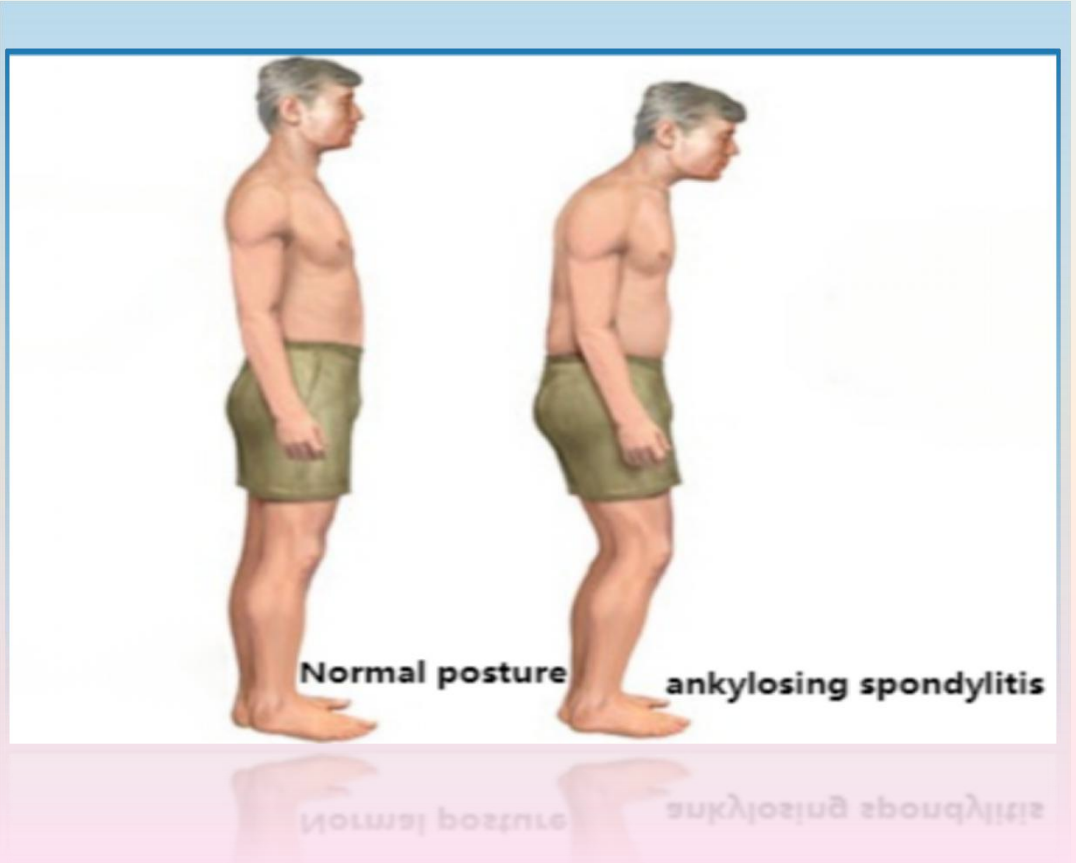
By analyzing platelet count, structure (GP), activation (P-selectin), and platelet-associated antibodies, flow cytometry plays a vital role in the clinical diagnosis and risk assessment of thrombotic/hemostatic disorders, hereditary platelet membrane glycoprotein deficiencies, acquired platelet dysfunction, immune thrombocytopenia, inflammatory peripheral vascular diseases, and atherosclerosis. It demonstrates unparalleled advantages over traditional diagnostic methods.

Advantages of flow detection of platelet function



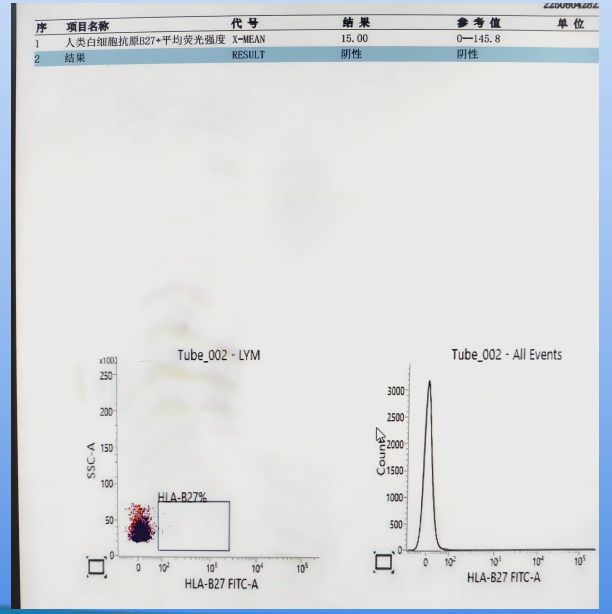
(5) Detection of Human leukocyte antigen B27

Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease affecting the spine and adjacent tissues, classified as a subtype of spondyloarthropathies. It primarily involves the sacroiliac joints, spinal joints, paravertebral soft tissues, and limb joints.



- Human leukocyte antigen B27 (HLA-B27) is strongly associated with various spinal joint diseases, particularly ankylosing spondylitis (AS).
- The HLA-B27 positivity rate in AS patients ranges from 80% to 95%, while the general population shows a rate of 5% to 10%.
- Among patients with other spinal joint diseases, those who are HLA-B27-positive are more likely to develop or progress to ankylosing spondylitis (AS) and exhibit systemic manifestations.

The report on the right is the HLA-B27 test item from our hospital.

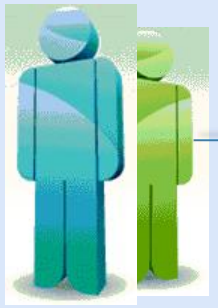


序	项目名称	代 号	结 果	参 考 值	单 位
1	人类白细胞抗原B27+平均荧光强度	X-MEAN	15.00	0—145.8	
2	结果	RESULT	阴性	阴性	

(6) Immunophenotyping of Leukemia



homo sapiens group



Male



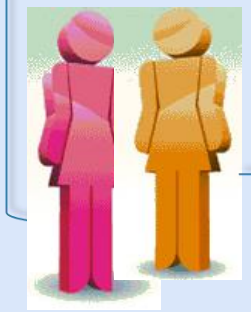
Boy



Adult male



Elderly male



Female



Girl

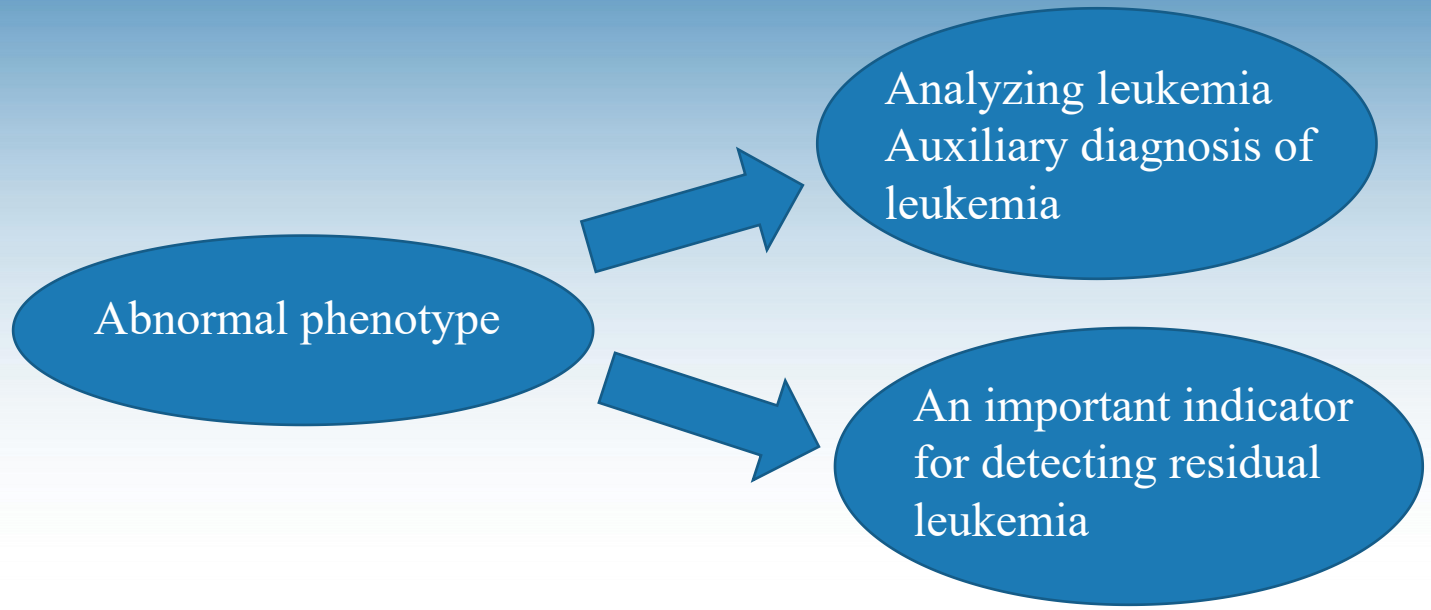


Adult female



Elderly women

The immunological classification of leukemia is similar to the classification of population into men and women, men into boys, middle-aged men and elderly men, and women into girls, middle-aged women and elderly women.



The expression of abnormal antigen of leukemia can be used to diagnose leukemia and detect the important index of residual leukemia.

The expression of leukemia cell antigens is highly complex, often requiring simultaneous detection with dozens or even scores of antibodies. Multicolor fluorescence-labeled flow cytometry significantly improves the efficiency and accuracy of detection.

Leukemia can be classified into the following types：

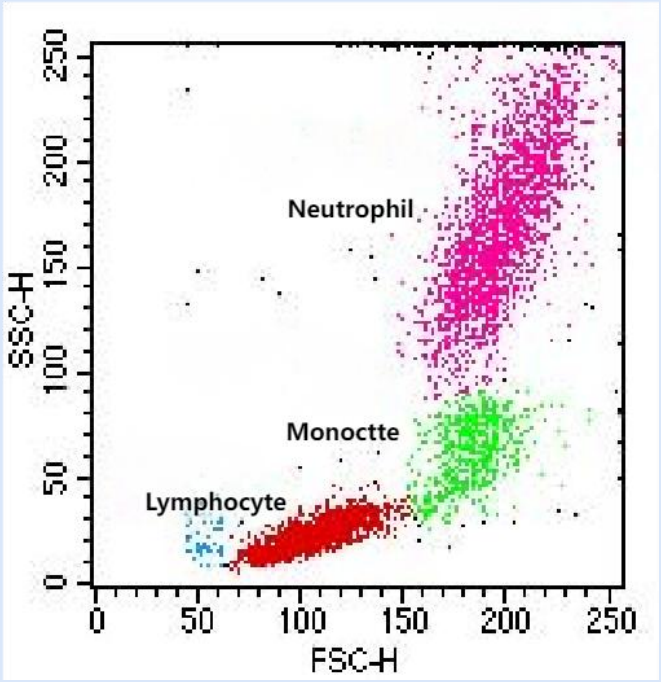
1. AML （M0-M7）

2. ALL （B-ALL、T/NK-ALL）

3 Mixed phenotype acute leukemia (B/Myel, T/Myel)

4.Myeloid Proliferative Disorders (CML, CEL, Mastocytosis, CMML, MDS, etc.)





5 Lymphoproliferative diseases

（1）Mature B-cell tumors：CLL/SLL，SMZL、B-PLL、HCL、LPL、PCN、FL、DLBCL

（2）Mature T/NK cell tumors：T-PLL、T-LGL、CLPD-NK、ATLL、MF、SS、ALCL

6 MM

7 AML-MRD、ALL-MRD、CLL-MRD

（7） MRD

MRD–Minimal Residual Disease

Refers to the state of a small number of residual leukemia cells in the body after complete remission induced by leukemia chemotherapy or bone marrow transplantation, which may be the root cause of leukemia recurrence.

Clinicians want to know::

- **Does the patient respond to chemotherapy?**
- **Are the tumor cells in the patient's body completely removed?**
- **Are there still a small number of tumor cells left?**

The significance of monitoring MRD

- **Prognosis (Acute lymphoblastic leukemia in children)**
- **Should treatment begin, continue, or stop**
- **Layered Treatment**

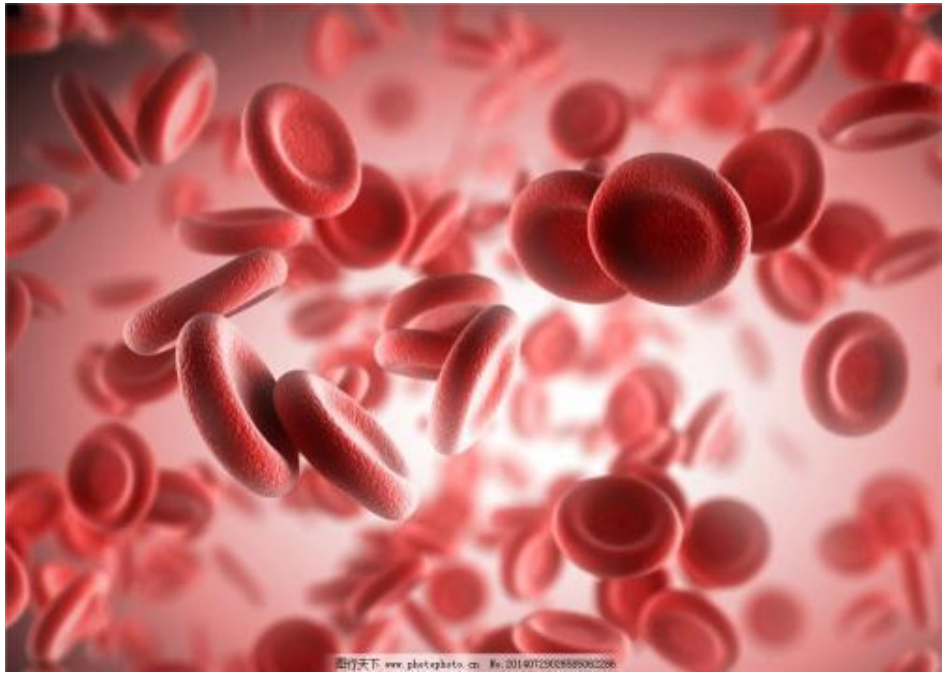


（8） PNH

Paroxysmal nocturnal hemoglobinuria

- Fundamentals of flow cytometry: CD55 and CD59 are widely expressed in various cells, inhibiting the formation of the attack complex. Patients with PNH exhibit a deficiency in both CD55 and CD59.

- Advantages of flow cytometry: higher specificity than acid hemolysis and sucrose hemolysis tests, and faster than other CD55/CD59 detection methods



Clinical applications of flow cytometry

Differential diagnosis and progression of infectious diseases:

(1) Viral infection: Decreased CD4+ T cells and inverted CD4/CD8 ratio

(2) Intracellular bacterial and parasitic infections: NK cells and monocyte proliferation

(3) Bacterial infection: no changes above

Disease progression assessment:

Viral infections: The severity of the condition is strongly associated with reduced CD4+ T cell counts and an inverted CD4/CD8 ratio, with HIV showing particularly pronounced effects.

Clinical applications of flow cytometry

Prognosis of tumor diseases and evaluation of surgical and chemotherapy effects:

(1) Early-stage tumor: CD4+ T cells decrease, while NK cells show a significant proportional increase.

(2) Advanced tumor: Both CD4+ T and NK lymphocytes are reduced

(3) Disease progression: The ratio of suppressor T cells (CD8+CD28-T cells) and regulatory T cells (CD4+CD25+ T cells) shows a positive correlation with disease progression.

Clinical applications of flow cytometry

- **Monitoring of post-transplant rejection:**

（1）Elevated CD3+/CD25+ T cells by 5-10% above normal indicates impending or existing rejection;

（2）Decreased CD4/CD8 ratio suggests concurrent severe infection, with <0.2% requiring discontinuation of immunosuppressants;

（3）Increased CD3+/HLA-DR+ cells by 5-10% without CD25+ elevation suggests cytomegalovirus infection;

（4）CD4+/CD45RA+ and Th17 cell counts positively correlate with acute rejection severity;

（5）NK cells (CD3-CD16/56+) are key effector cells in acute immune rejection;

（6）Th1 (CD4+INF+) mediates both acute and chronic immune rejection.

Clinical applications of flow cytometry

- **Assessment of rheumatic and immune disorders: diagnostic tools and prognosis evaluation.**

（1）Laboratory diagnosis of ankylosing spondylitis (AS): Three key clinical indicators include:

1) Low back pain; 2) X-ray evidence of sacroiliac joint fusion; 3) Positive HLA-B27 markers.

（2）Systemic lupus erythematosus (SLE) is characterized by elevated CD4+/CD8+ T cell counts, with CD8+ reduction levels reflecting disease activity and organ involvement.

（3）Immunodeficiency disorders such as gamma globulin deficiency, thymic dysplasia, and severe combined immunodeficiency (SCID) result in decreased immune cell populations.

Clinical applications of flow cytometry

Diagnosis and prognosis of hematologic diseases:

（1）Immunotyping of leukemia and lymphoma;

（2）Evaluation of treatment efficacy for PNH, anemia, and ITP.

（3）Aplastic anemia: Patients with immune phenotype abnormalities (e.g., CD4/CD8 inversion, CD8+ or activated T cells, or abnormal proliferation of TCRγδ) typically present with more severe clinical manifestations, yet demonstrate relatively better efficacy with immunosuppressive therapy.

（4）Thrombocytopenic disorders: When platelet count is <20,000/μL, the results from hematology analyzers show significant errors, with flow cytometry being the most accurate counting method;

（5）Therapeutic efficacy evaluation of anemia: Reticulocyte/platelet count;

（6）ITP: Detection of platelet-associated antibodies.

- **Differential diagnosis of bronchitis and asthma:** CD23, the receptor for IgE, reflects the severity of type 1 hypersensitivity reactions.
- **Allergy test:** Basophil activation test can reflect the condition of allergic reaction
- **Pregnancy loss warning:** Elevated Th1/Th2 cell ratios (CD3+/CD25+ and CD3+/HLA-DR+) may indicate pregnancy loss. After excluding infections and other factors, this reflects maternal immune rejection of the fetus. Rh-negative pregnant women showing maternal blood fetal red blood cell cross-reactivity may have Rh hemolytic disease.
- **Immune-related diseases: Glomerulonephritis:** In patients developing acute vascular occlusion or severe restenosis within 24 hours post-PTCA, elevated activated platelet CD62p and CD63 levels can predict the risk of acute ischemic reinfarction following PTCA.
- **Cardiovascular disease:** In patients developing acute vascular occlusion or severe restenosis within 24 hours post-PTCA, elevated activated platelet CD62p and CD63 levels can predict the risk of acute ischemic reinfarction.

The important question in this lesson：

If you were to work in the laboratory department in the future, what flow cytometry-related testing projects would you develop?

Thank you for your attention

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