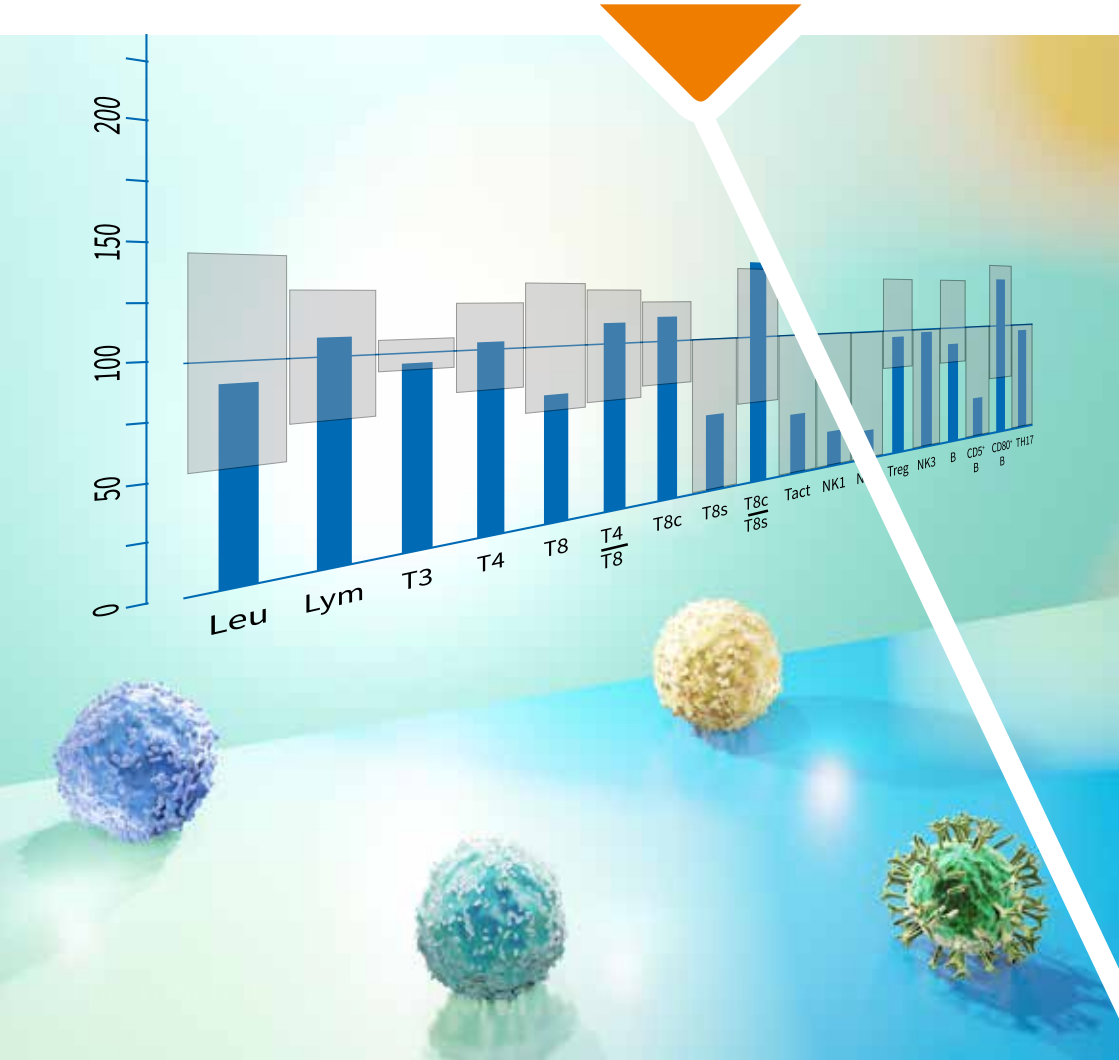
Micro-immunotherapy
International Medical Experience

Interpretation of Lymphocyte Typing



Please note: All statements made in this brochure are for guidance only. Please do not make a diagnosis solely on the basis of this brochure. Always analyse every case individually and assess the immune status in the context of the patient's clinical presentation. Ideally, the results of other laboratory tests (in particular serologies and serum protein profile) should be taken into account in order to permit a more precise interpretation. It is also important to always consider not only the absolute values but also the relation of the parameters to each other.

Further information can be found in the book on diagnostic methods in integrated medicine, which may be ordered in French, German or Spanish via www.microimmuno.fr, www.megemit.org or www.aemi.es, respectively.

The International Micro-immunotherapy Associations assume no liability for any of the decisions you may make based on the information provided in this brochure.

The statements made in this brochure are based on the knowledge and experience of doctors and therapists of the International Micro-immunotherapy Associations and have been validated by Dr Petra Blum (Germany).

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1. Introduction to lymphocyte typing

What is lymphocyte typing?

It is a practical and precise laboratory test that uses the CD (*cluster of differentiation*) molecules on the surface of immune cells to determine various subgroups of lymphocytes (especially of T and B cells) in the blood. This diagnostic tool thus provides a picture of the patient's immune system at a certain point in time, which must be interpreted in the context of the patient's clinical presentation.

Patient values are shown in a histogram in percentages and compared with a normal reference range of the performing laboratory. This graphical representation in percentiles can be used to evaluate, at a glance, the adaptive potential (adaptability) of the immune system in relation to the clinical picture.

The lymphocyte typing test is also often referred to as micro-immune status or just immune status.

In which cases is it useful to perform lymphocyte typing?

Lymphocyte typing has proven to be extremely useful in the following cases:

- Unexplained, persistent or increasing fatigue
- Multiple or recurrent infections by bacteria, viruses, parasites or fungi
- Severe allergies
- Autoimmune diseases
- Neoplastic, haematological and malignant diseases
- More or less permanent states of stress
- Long-term medication affecting the immune system

What is the purpose of lymphocyte typing?

Lymphocyte typing is very useful in daily clinical practice for detecting immune disorders that are involved in numerous diseases. It can be used:

- To determine the patient's immune status in relation to the clinical condition
- To aid interpretation of other test results (e.g. serology, protein profile), and as a starting point to determine further diagnostic steps
- To determine treatment orientation (especially when micro-immunotherapy is used)
- To monitor therapy (keeping an interval of 9-12 months between each individual lymphocyte typing test)
- As a preventive measure

What practical considerations need to be taken into account when performing lymphocyte typing?

- The blood samples must be taken in EDTA tubes, and they can only be taken Mondays to Wednesdays before midday (very latest on Thursday morning) as they need to be accepted by the laboratory within 24 hours. Conditions of couriers / collection services need to be considered.
- It should generally not be carried out during the acute phase of an infection or illness.
- After extreme stress (e.g. marathon, long haul flight), a recovery period of 4-5 days should be left before taking the blood samples.
- In general, lymphocyte typing should not be done in children and adolescents under the age of 15 (as there is not enough valid comparative data available for this age group).
- In oncological patients, lymphocyte typing should generally not be performed during chemotherapy and up to 6 months after.
- In case of patients undergoing an immunosuppressive therapy or a corticotherapy, it is important to assess the extent to which a lymphocyte typing is necessary.

2. Steps to interpretation

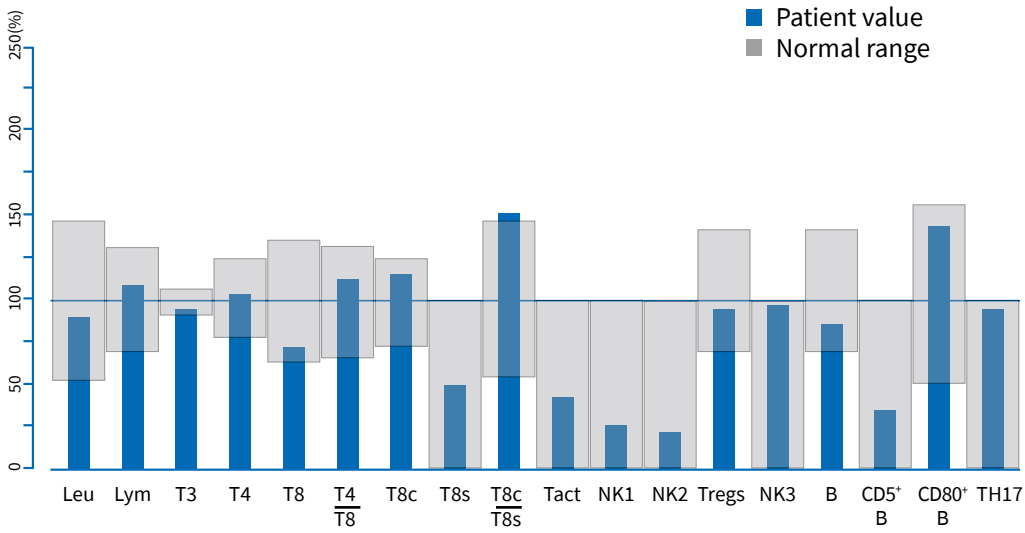
2.1.
Assessment of the overall condition of the immune system: adaptation / non-adaptation

2.2.
Assessment of the immunocompetence in case of microbial burden

2.3.
Assessment of deviations of immunological parameters and possible clinical implications

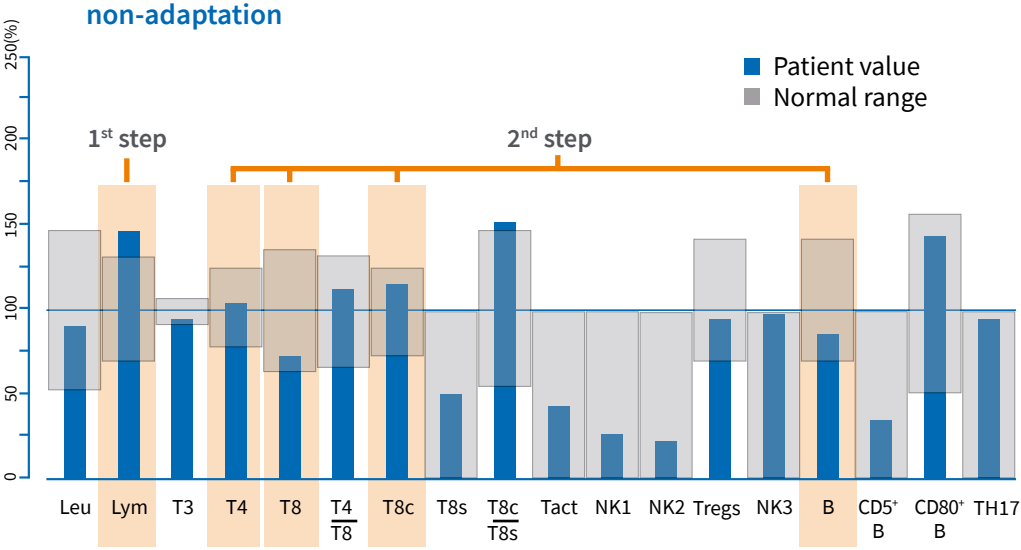
Picture of an (almost) ideal lymphocyte typing

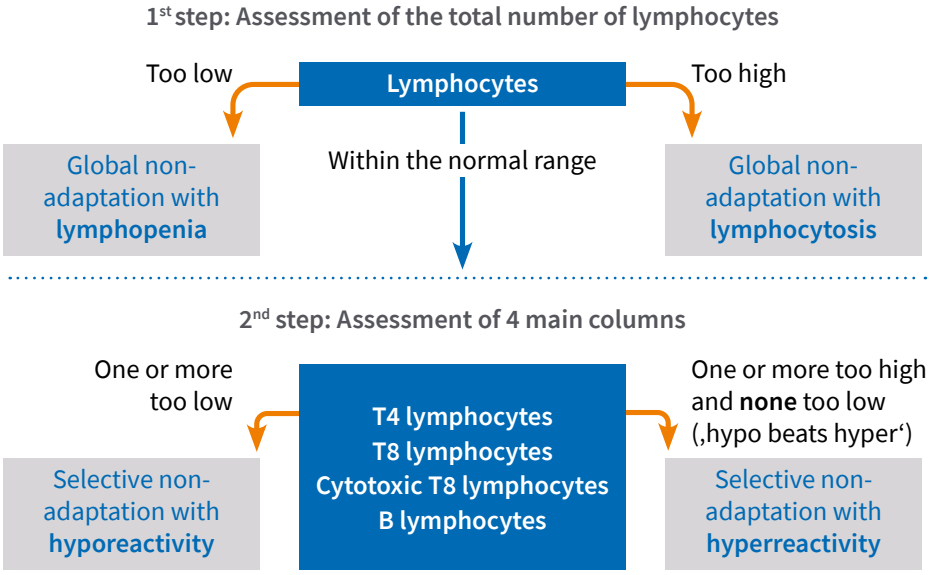
Note: The visual representation of a lymphocyte typing may vary depending on the different laboratories.



- Leu: Leukocytes
- Lym: Lymphocytes
- T3: T lymphocytes
- T4: T4 lymphocytes
- T8: T8 lymphocytes
- T8c: Cytotoxic T8 lymphocytes
- T8s: Senescent T8 lymphocytes
- Tact: Activated T lymphocytes
- NK1: CD57+ Natural killer cells
- NK2: NK-like T cells
- Tregs: Regulatory T lymphocytes
- NK3: Natural killer cells
- B: B lymphocytes
- CD5+ B: CD5+ B lymphocytes
- CD80+ B: CD80+ B lymphocytes
- TH17: TH17 lymphocytes

2.1. Assessment of the overall condition of the immune system: adaptation / non-adaptation





2.2. Assessment of the immunocompetence in case of microbial burden

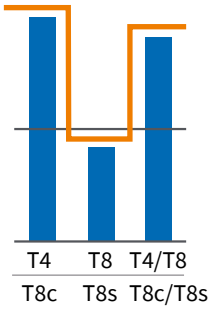
Characteristic pictures may appear in two areas of the overall diagram:

Extracellular range
Columns T4, T8 and T4/T8 ratio
↓
Represents reactivity of the immune system to e.g. bacteria, fungi or parasites

Intracellular range
Columns T8c, T8s and T8c/T8s ratio
↓
Represents reactivity of the immune system to e.g. viruses or intracellular bacteria

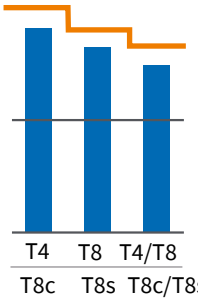
Cathedral

T4 and T4/T8 > T8 or
T8c and T8c/T8s > T8s
Picture indicates a high ability of the immune system to react to microbial burden.
Good treatability



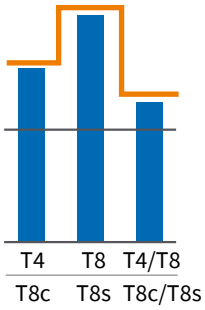
Stairs

T8 increases or
T8s increses
Picture indicates a limited ability of the immune system to react to microbial burden.
Limited treatability (possibly longer treatment period)



Podium

T8 > T4 and T4/T8 or
T8s > T8c and T8c/T8s
Picture indicates that the immune system is "blocked".
Difficult treatability (most likely prolonged treatment period)



Practical advice: In case of the picture of a "stair" or "podium", the doctor or therapist should look for further immune stressors. In the extracellular range, bacterial infections (e.g. streptococci) should be ruled out diagnostically. In the intracellular range, these presentations are often related to inflammation (induced, among others, by gut dysbiosis or focal infections of the teeth), an increase in cortisol or even depression. In those cases, an anti-inflammatory/anti-stress therapy should be considered first.

Choice of formulas after determination of the immune status and identification of a pathogen by serology *(based on the clinical experience of the doctors and therapists of the International Micro-immunotherapy Associations (IFMi, MeGeMIT and AEMI)*

Infectious agent	Non-adaptation with lymphopenia/hyporeactivity ▶ The immune system must be **supported**.	Non-adaptation with lymphocytosis/hyperreactivity ▶ The immune system must be **modulated** • 1-2 months, then switch to the corresponding **support** formula)
Epstein-Barr virus	**EBV*:** 1 capsule/day	**XFS:** 1 capsule/day
Cytomegalovirus	**CMV*:** 1 capsule/day	**XFS:** 1 capsule/day
Toxoplasma gondii	**TOXO*:** 1 capsule/day	**XFS:** 1 capsule/day
Hepatitis B, C and D virus	**HC*:** 1 capsule/day	**HCX:** 1 capsule/day
Other non-specific cases	**EID*:** 1 capsule/day	**EAI:** 1 capsule/day
		• 1-2 months
Herpes simplex virus 1 and 2	**HERP:** 1 capsule/day	**+ EAI:** 1 capsule/day
Chlamydia trachomatis	**CHLA:** 1 capsule/day	**+ EAI:** 1 capsule/day
Hepatitis A virus	**HA:** 1 capsule/day	**+ EAI:** 1 capsule/day
Human papillomavirus	**PAPI:** 1 capsule/day	**+ EAI:** 1 capsule/day
Varicella zoster virus	**ZONA:** 1 to 4 capsules/day	**+ EAI:** 1 capsule/day

* In acute cases, the dosage may be increased until symptoms disappear (to be assessed according to medical criteria).

Practical advice: Experience has shown that, in the majority of cases, the immune system is in a state of non-adaptation with hyporeactivity; hyperreactivity occurs less frequently.

2.3. Assessment of deviations of immunological parameters and possible clinical implications

CD: Surface markers (CD) 📖: Brief description ↑: Increased levels ↓: Decreased levels

Leukocytes (Leu)

CD	Varies according to subgroup
📖	Group of immune cells. Divided into monocytes, granulocytes, mast cells, dendritic cells and lymphocytes
↑	Leukocytosis: Immune response in the context of infections/inflammation; possibly also related to leukaemia
↓	Leukopenia: Often induced by therapy/medication; possibly also related to bone marrow diseases or some infections (e. g. HIV)

Lymphocytes (Lym)

CD	Varies according to subgroup
📖	Subgroup of leukocytes. Divided into T lymphocytes, B lymphocytes and NK cells
↑	Lymphocytosis: Immune response in the context of infections/inflammation; possibly also related to stress
↓	Lymphopenia: Often induced by therapy/medication or associated with immunosenescence; possibly also in the late stage of an infection or in case of chronic infections

T lymphocytes (T3)

CD	CD3+
📖	Immune cells that mature in the thymus and are responsible for the cellular adaptive immunity
↑	Immune response in the context of infections/inflammation; possibly also related to autoimmunity
↓	Often induced by therapy/medication or associated with immunosenescence; possibly also in the late stage of an infection

CD4+ T lymphocytes (T4)

CD3+ CD4+

‘Coordinators’ of the cellular adaptive immunity, recognising extracellular antigens presented predominantly on MHC II molecules and inducing the activation and proliferation of other immune cells

Immune response in the context of infections; possibly also related to autoimmunity

Often induced by therapy/medication or associated with immunosenescence; possibly also in the late stage of an infection or in case of persistent viral infections (EBV, HBV, CMV)

CD8+ T lymphocytes (T8)

CD3+ CD8+

Immune cells of the cellular adaptive immunity, recognising intracellular antigens presented predominantly on MHC I molecules and, amongst others, inducing lysis of infected or abnormal (cancer) cells

Immune response in the context of infections (in particular of viral origin) or associated with acute allergies; possibly also related to stress

Often induced by therapy/medication or associated with immunosenescence; possibly also in the late stage of an infection or in case of chronic infections or reactivations

T4/T8

Ratio of T4 to T8 cells. Together with the previous two columns, allows prediction of the therapeutic outcome

Often in the context of autoimmunity

Often induced by therapy/medication or related to infections

Practical advice: During an acute flare of an autoimmune disease, increased levels of T4 lymphocytes, of the T4/T8 ratio and possibly of activated T cells can be observed. These results are usually accompanied by increased levels of inflammatory proteins (C-reactive protein, haptoglobin, alpha-1-acid glycoprotein) and IgG or even IgA in the serum protein profile.

Cytotoxic CD8+ T lymphocytes (T8c)

CD3+ CD8+ CD57-

Cytotoxic T8 lymphocytes with high proliferative capacity, essential for antimicrobial and antitumour defence

Immune response in the context of infections (in particular intracellular viruses and bacteria); possibly also related to oncological processes

Often induced by therapy/medication or associated with immunosenescence

Senescent CD8+ T lymphocytes (T8s)

CD3+ CD8+ CD57+

Highly differentiated T8 lymphocytes with cytotoxic potential but reduced proliferative capacity

Decreased efficiency in the defence against intracellular pathogens, e.g. due to stress or age

T8c/T8s

Ratio of T8c to T8s cells. Together with the previous two columns, allows prediction of the therapeutic outcome

Increased cytotoxic potential of the immune system

Reduced cytotoxic potential of the immune system

Activated T lymphocytes (Tact)

CD3+ HLA-DR+

T lymphocytes that have already recognised their antigen but are not yet fully differentiated

Immune response in the context of infections/inflammation; often also compensatory increase in case of a “blocking” in the extra- or intracellular range

CD57+ Natural killer cells (NK1)

CD3- CD8- CD57+

Highly differentiated NK cells with high antigen experience and cytotoxic potential but with reduced proliferative capacity

Immune response in the context of acute infections (in particular of viral origin); often also compensatory increase in case of decreased levels of T8c

NK-like T cells (NK2)

CD3+ CD16+ CD56+

T lymphocytes that express both T cell receptors and NK molecules and are involved in the immune response to infected or abnormal (cancer) cells

Immune response in the context of acute infections (in particular of viral origin); often also compensatory increase in case of decreased levels of T8c

Practical advice: In case of infections with some herpesviruses, such as the varicella-zoster virus, antibody levels may remain elevated for several years without virus reactivation. In order to better interpret the results of the serology, a lymphocyte typing may be helpful. Increased levels of T8 lymphocytes, cytotoxic T8 cells and activated T lymphocytes together with a low T4/T8 ratio usually indicate virus reactivation. These deviations have to be interpreted in relation with the clinical presentation of the patient.

Regulatory T lymphocytes (Tregs)

CD4+ CD25+ CD127low

T4 lymphocytes which downregulate the function of effector cells and thus can limit inflammatory processes, ensure tolerance, avoid autoimmunity and maintain homeostasis

Increased immune tolerance (e.g. in case of undetected focal infections over a longer period of time) with associated risk of chronic infections and tumours

Reduced immune tolerance with associated risk of allergic or autoimmune diseases

Natural killer cells (NK3)

CD3- CD16+ CD56+

Immune cells of the cellular innate immunity, which have a high cytotoxic potential and play an important role in the defence against infected or abnormal (cancer) cells

Immune response in the context of acute infections (in particular of viral origin); often also related to stress; if accompanied by decreased levels of IgM and increased IgA, alpha-1-acid glycoprotein, haptoglobin and C-reactive protein, indicative of malignancy; if accompanied by increased levels of Tregs, indicative of chronic infection

Practical advice: An increase in regulatory T cells may be indicative of a focal infection. This often involves the teeth, the sinuses or chronic processes such as fibroids or disorders of the gallbladder or appendix.

B lymphocytes (B, B Lymph)

CD19+

Immune cells that mature in the bone marrow and are responsible for the humoral adaptive immunity (antibody production)

Often related to toxicity, allergies, EBV or gut dysbiosis

Weakened humoral defence with associated risk of infection

CD5+ B lymphocytes (CD5+ B, CD5+ B Lymph)

CD19+ CD5+

Subgroup of B lymphocytes that is potentially autoreactive

Tendency towards allergic or autoimmune processes

CD80+ B lymphocytes (CD80+ B, CD80+ B Lymph)

CD19+ CD80+

Activated B lymphocytes that play an important role in antigen presentation

High antigen load, e.g. in chronic infections, allergies or autoimmune processes

Weakened humoral defence with associated risk of infection

TH17 lymphocytes (TH17)*

CD4+ CD154+ and IL-17 secretion assay

T4 lymphocytes which play an important role in the activation of neutrophils and the maintenance of intestinal mucosal integrity

High inflammatory activity, e.g. in chronic inflammatory bowel disease; if accompanied by low levels of Tregs, indicative of autoimmunity

**This cell type is not always part of lymphocyte typing. In some laboratories, it may be requested separately.*

3. Summary and examples

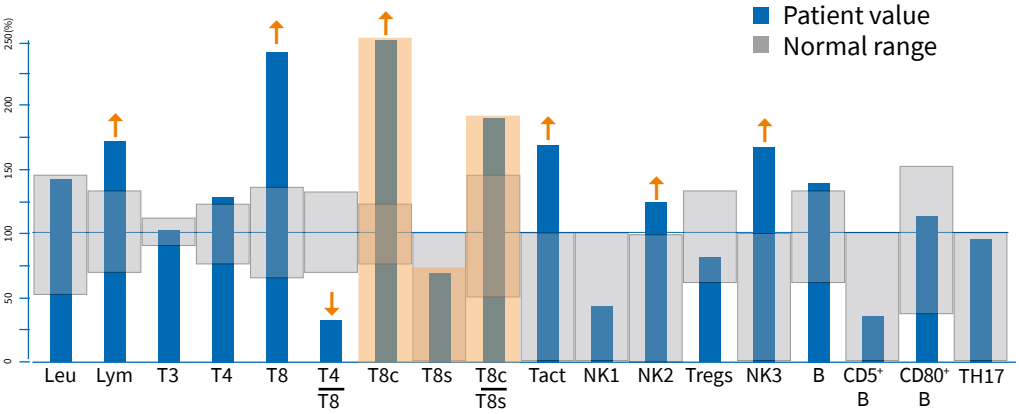
Parameters that are particularly helpful in the evaluation of the immune status in the context of various clinical presentations

Note: It is only tendencies that are listed in this table; each patient has a specific immune response. An interpretation cannot be made without knowledge of the clinical condition of the patient. It is also important to take into account the results of other laboratory tests (in particular serologies and serum protein profile).

Acute viral infection / reactivation	Chronic, persistent viral infection	Chronic inflammatory / autoimmune processes	Allergy
T8 ↑	T4 (EBV, HBV, CMV) ↓	T4 ↑	T8 ↑
T4/T8 ↓	T8s ↑	T4/T8 ↑	Tregs ↓
T8c ↑	NK3 (associated with increased Tregs) ↑	Tact ↑	B Lymph ↑
T8c/T8s ↑	Tregs ↑	Tregs ↓	CD5+ B Lymph ↑
Tact (often as compensation for a „blocking“ in the extra- or intracellular range) ↑		CD5+ B Lymph ↑	CD80+ B Lymph ↑
NK1, NK2, NK3 (often as compensation for low T8c) ↑		CD80+ B Lymph ↑	
		TH17 (in particular in the gut) ↑	

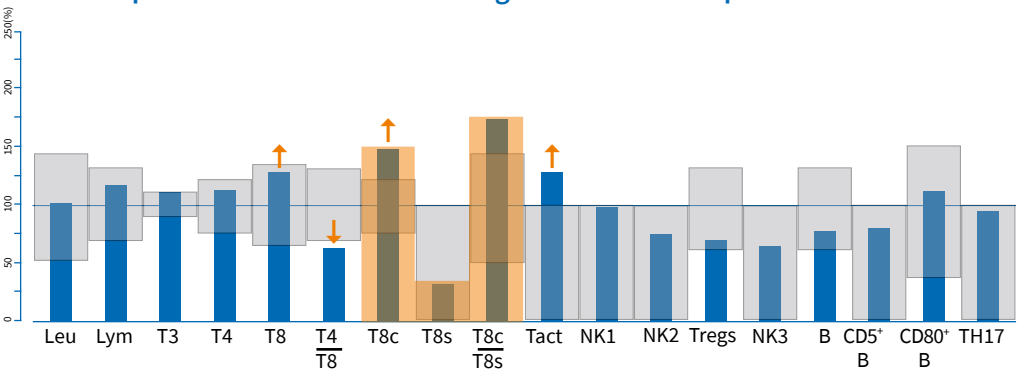
↑ Increased levels ↓ Decreased levels Particularly favourable constellation Unfavourable constellation

Example 1: Acute primary infection



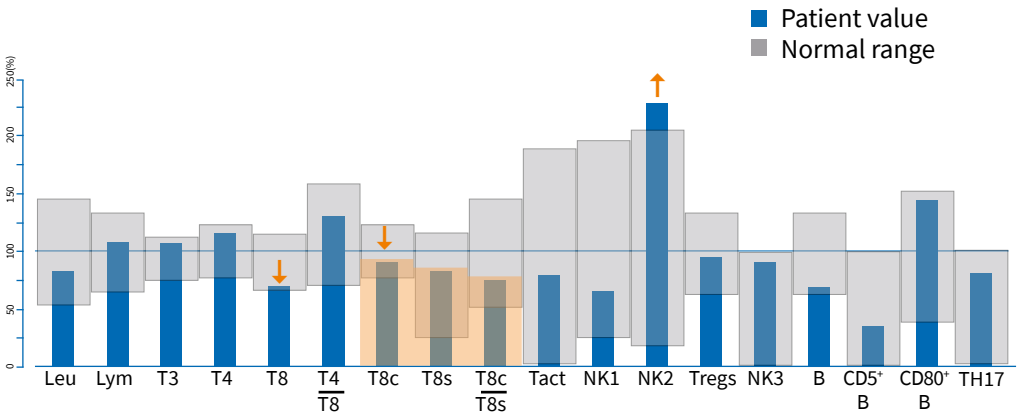
In an acute primary infection, a temporary, pronounced increase of various immune cells and of the total number of lymphocytes (lymphocytosis) can be observed. In particular, the T8 and T8c cells, as well as the NK2 and NK3, are recruited in high numbers. Viral infections are often also associated with a reduction of the T4/T8 ratio. In this example, T8c, T8s and the T8c/T8s ratio form the picture of a "cathedral", indicating good treatability.

Example 2: Virus reactivation with good immunocompetence



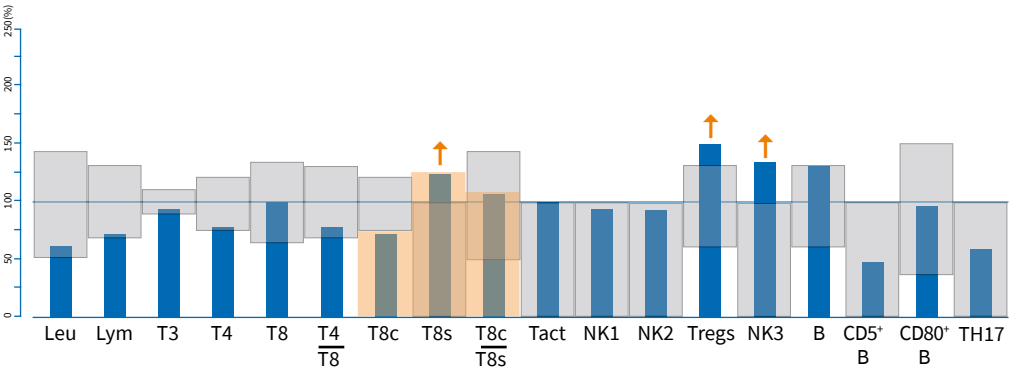
During virus reactivation, normal levels of lymphocytes, as well as NK cells, are usually observed. T8 cells, Tact and especially T8c are often in the upper normal range or elevated (though not as pronounced as in the acute stage). The picture of a "cathedral" formed by the T8c, T8s and T8c/T8s ratio indicates good immunocompetence. Additionally, a reduction of the T4/T8 ratio may be observed.

Example 3: Virus reactivation with limited immunocompetence



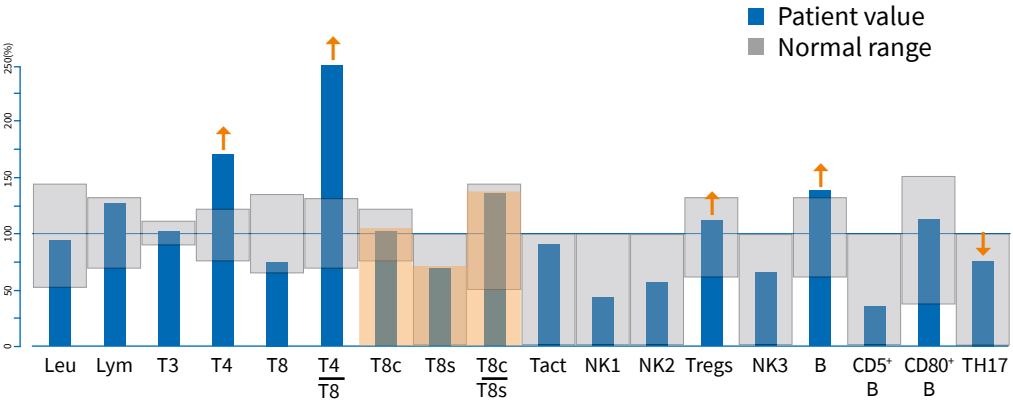
The T8 and T8c cells, which are important for virus control, are in the lower normal range. This aspect, together with the picture of "stairs" in the T8c, T8s and T8c/T8s ratio, indicates a reduced efficiency in the defence against viruses. The NK2 are increased in compensation for the reduced number of T8 cells.

Example 4: Chronic, persistent viral infection



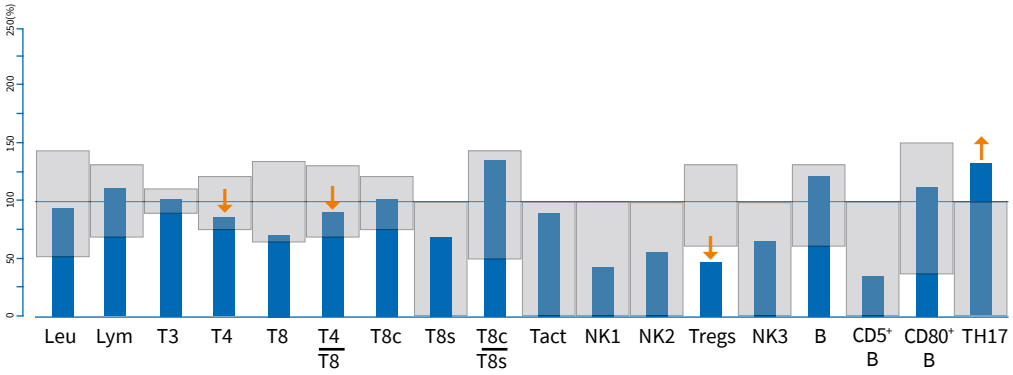
An increase of T8s as a result of repeated antigen exposure is observed. The picture of a "podium" in the T8c, T8s and T8c/T8s ratio indicates that the antiviral defence is hindered. The NK3, however, increase in a compensatory manner. The increase in Tregs indicates an increased immune tolerance, which impairs the ability of the effector cells to control the virus.

Example 5: Autoimmunity with good prognosis



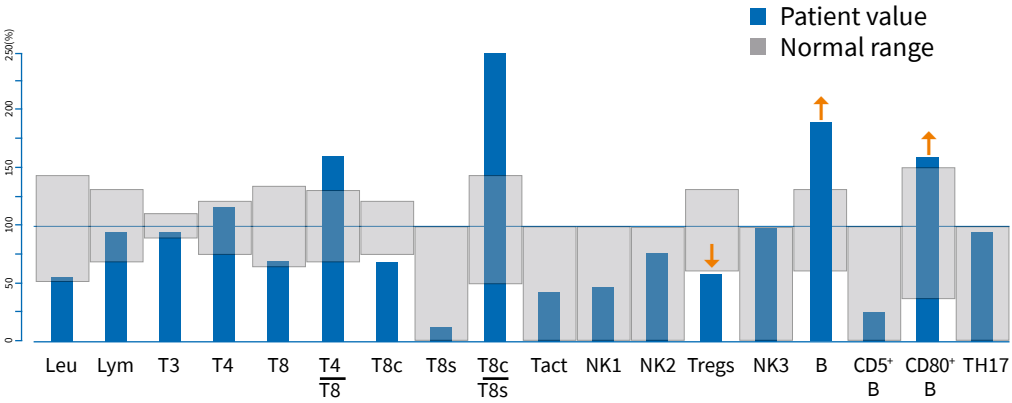
Patients with autoimmune diseases usually show increased levels of T4 cells and an elevated T4/T8 ratio. B lymphocytes may also be increased. In this example, the overall condition is favourable: the Tregs, which generally have an immunosuppressive effect, are in the upper normal range. The picture of a "cathedral" formed by the T8c, T8s and the T8c/T8s ratio indicates good treatability. Other immune cells that are associated with autoimmunity (including TH17 cells) are in the normal range.

Example 6: Autoimmunity with less favourable prognosis



In contrast to the previous example, the decreased levels of Tregs and increased TH17 cells indicate a poorer prognosis. In such cases, it is quite possible that T4 cells are decreased or in the lower limit of the normal range, whereas the T4/T8 ratio is within the normal range.

Example 7: Allergy



Allergy often presents with increased B lymphocytes and CD80+ B lymphocytes. The slightly decreased number of Tregs indicates a reduced immune tolerance and thus a higher risk of exaggerated defensive response.

Practical advice: The examples shown in this section are simplified representations of the immune status in the context of different clinical presentations. They are intended to be a useful tool when initiating into the interpretation of lymphocyte typing and to help you identify the possible clinical implications of deviations of different immunological parameters. However, please bear in mind that real cases in daily clinical practice and the constellations in the corresponding lymphocyte typings may differ from the ones presented in this leaflet for each clinical condition. If the patient presents with a global immune imbalance, the values of the different parameters in the lymphocyte typing might deviate from the ones expected from a properly responding immune system. Hence, lymphocyte typing should always be interpreted taking into account the patient's clinical picture.

4. Conclusion

Nowadays, it is well known that many diseases are associated with underlying immune disorders. Therefore, it is important to include the immune system in all diagnostic and therapeutic strategies.

Lymphocyte typing makes it possible to determine the cellular immune status of a patient at a given time in the context of the clinical presentation. Whilst the test cannot be used to establish a definite diagnosis, it provides – together with other diagnostic tools such as serum protein profile or serologies – valuable support for a better diagnostic and therapeutic orientation (especially when micro-immunotherapy is used).

Contact us / visit our website:

mi@micro-immunotherapy.com

www.micro-immunotherapy.com