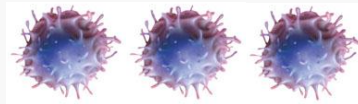


# **Lymphocyte transformation test – a practical approach - short**

*Werner J. Pichler, MD*

*werner.pichler@adr-ac.ch*



**ADR-AC GmbH**

*Adverse Drug Reactions –*

*Analysis and Consulting*

*Holligenstr 91, CH 3008 Bern,*

*Switzerland*

# When skin and in vitro tests?

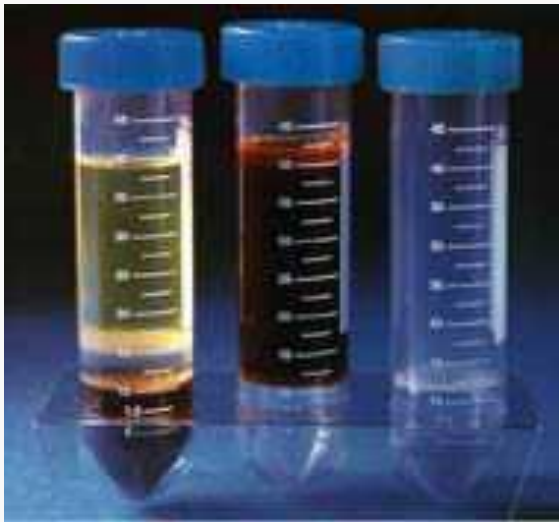
- **Not in acute stage:**
  - **IgE:** mast cells degranulated: effect of acute treatments
  - **T-cells:** T cells still activated, effect of treatments
- **Better in remission**
  - **IgE:** >1 week and within 6 (-12) months
  - **T cells:** > 4 weeks after event, best within 6 - 12 months; it is possible later !
- **Persistence:** variable: 1yrs to >20yrs

# LTT-procedure I



Anti-coagulated blood  
(heparin, EDTA, citrate)

Send at room temperature,  
max. 24 hrs, processing <36hrs  
= lab needs to be informed

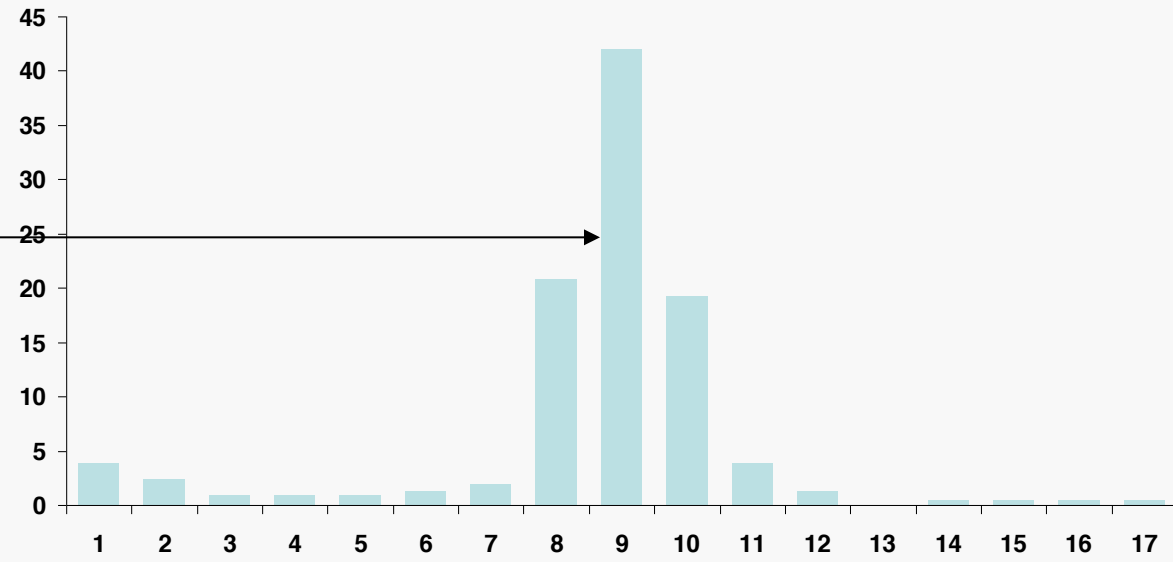


Cell separation - ca.  $30 \times 10^6$   
lymphocytes from 30ml blood

Culture with drug: **pure drug substance**  
(Sigma, companies,... = powder solved in NaCl,  
PBS, DMSO, NaOH... , injection fluid)

**NOT** tablets or sirups or suspensions...

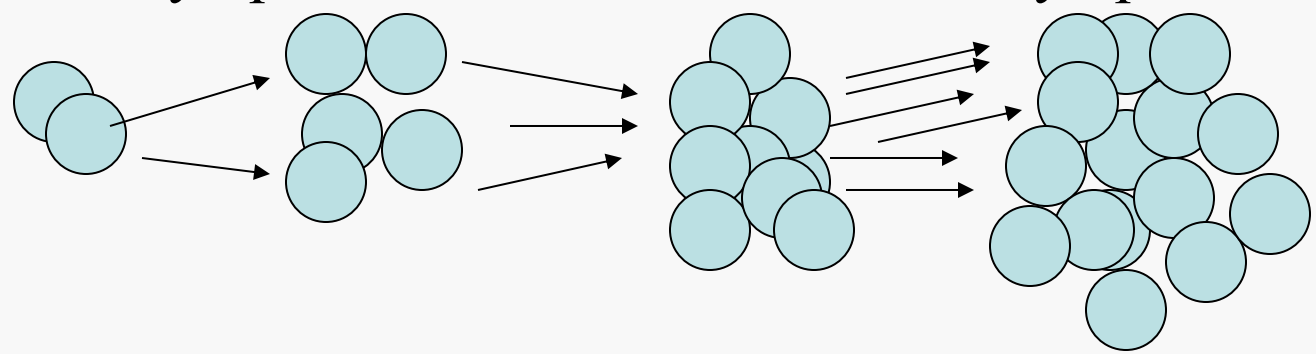
# Delayed reaction



1 2 3 4 5 6 7 8 9 10 days

No symptoms

symptoms



Few precursor cells ... Expansion....

Symptoms arise if a certain amount of specific T-cells exerts effector function (in tissue): homing

# LTT procedure II

$2 \times 10^5$  lymphocytes / ml in  
AB serum or autologous plasma

Quadruplicate cultures

3-5 drug concentrations  
often 0.1 -1 -10 -100mg/ml

**ca. 150 pure substances:**  
**1. Each drug concentration  
tested for toxicity in vitro**

**2. Lack of spontaneously  
inducing proliferation**



# Stimulation Index (SI)

- No (moderate, spontaneous) proliferation in control cultures (without antigen): background control
- Enhanced proliferation in positive control (TT)
- SI: 
$$\frac{\text{cpm with drug}}{\text{cpm without drug}}$$
- SI: **2**  $\frac{8000 \text{ cpm}}{4000 \text{ cpm}}$   $\Delta = 4000 \text{ cpm}$  or  $\frac{1000 \text{ cpm}}{500 \text{ cpm}}$ ;  $\Delta = 500 \text{ cpm}$

## LTT, example

Medikament/Substanz	Konz. <b>microg</b>	Autol. Plasma <b>SI</b>	AB-Serum <b>SI</b>
Phenytoin RS	1	<b>16.2</b>	<b>18.7</b>
	10	<b>55.1</b>	<b>81.7</b>
	50	<b>93.3</b>	<b>72.9</b>
	100	<b>63.5</b>	<b>63.8</b>
Lamotrigin RS	0.1	1.4	1.5
	1	1.6	1.9
	10	<b>2.6</b>	<b>4.4</b>
	100	<b>2.9</b>	<b>19.6</b>
Pantoprazol RS	0.1	1.2	1.1
	1	<b>2.0</b>	1.0
	10	1.1	<b>2.4</b>
	50	0.2	0.3

Positive LTT for Phenytoin and Lamotrigine (DRESS)

dose dependence, reaction in both, AB serum and autolog. plasma

But Pantoprazole: ??? INTERPRETATION NEEDED

# LTT – technical aspects

Fresh heparinized (citrated, EDTA) blood,  
**Moon EXPRESS (< 24-36hrs)**

LTT in AB-Serum and autologous Plasma  
(may differ)

Dosis-urve (1, 10, 100 $\mu$ g/ml)

Interpretation: the better, the more information  
calling, e-mail....



# ***When is a LTT positive ?***

**Easy:** SI >4 (mostly relevant...)

clear dose dependence

**Difficult:**

SI >2-4: in more than one concentration,  
in both AB-serum and autol. plasma

Consider: quadruplicate values (CV)

positive control (TT)

background proliferation

experience with drug in LTT

**It requires careful interpretation! The more data are available, the better the interpretation**

# SI reflects the intensity of T cell proliferation, but

- The SI does not correlate to severity of symptoms
- SI may be marginal in fatal SJS/TEN, but very high in «harmless» MPE
- Type of drug, way of stimulation (p-i vs hapten) may also influence T cell proliferation

# ***Sensitivity & Specificity of the lymphocyte transformation test (LTT)***

Author	n	sensitivity	specificity	disease
Nyfeler & Pichler, 1997	100	74	85*	All
Luque E et al., 2001	50	62	93	Penicillins (imm. & non-immediate)
Hari Y et al, 2001	21	67	98	MPE & bullous E.
Naisbitt D et al, 2003	36	94	100	DRESS/ DiHS

The sensitivity of LTT is dependent of the type of reaction !!

# Specificity of LTT

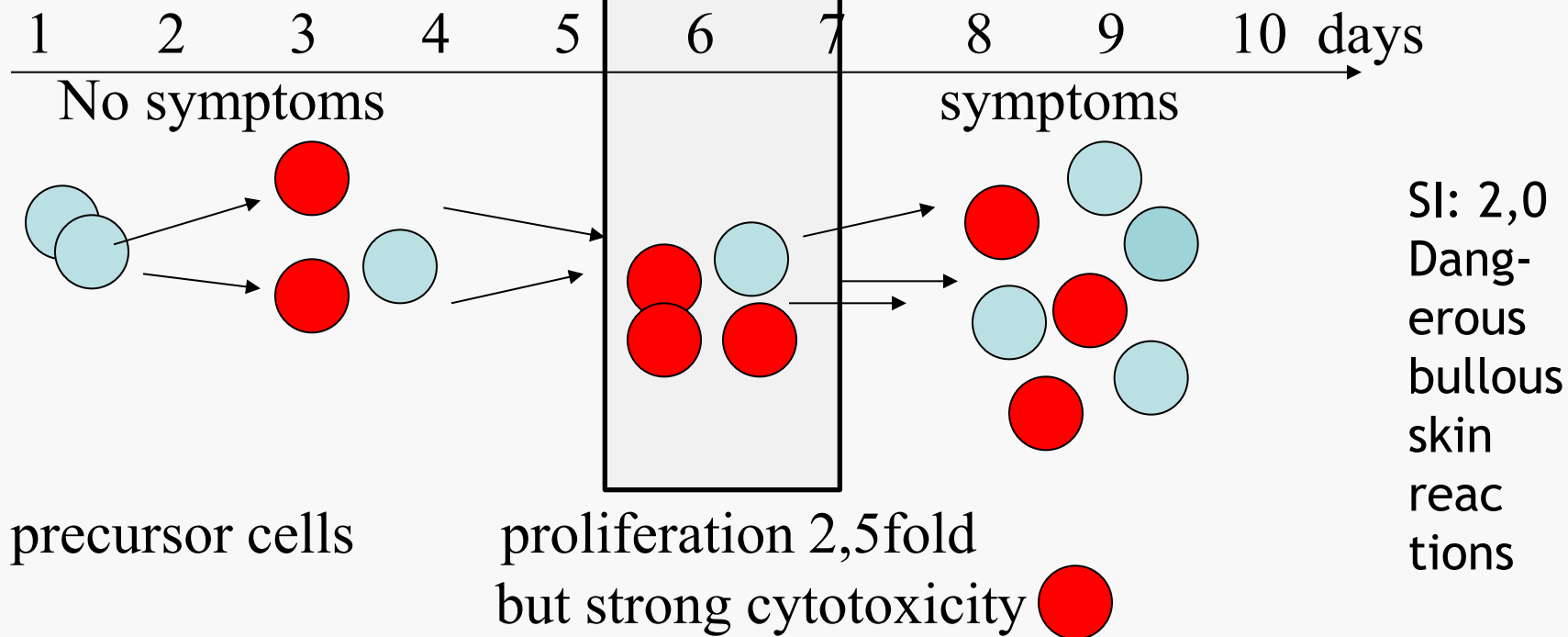
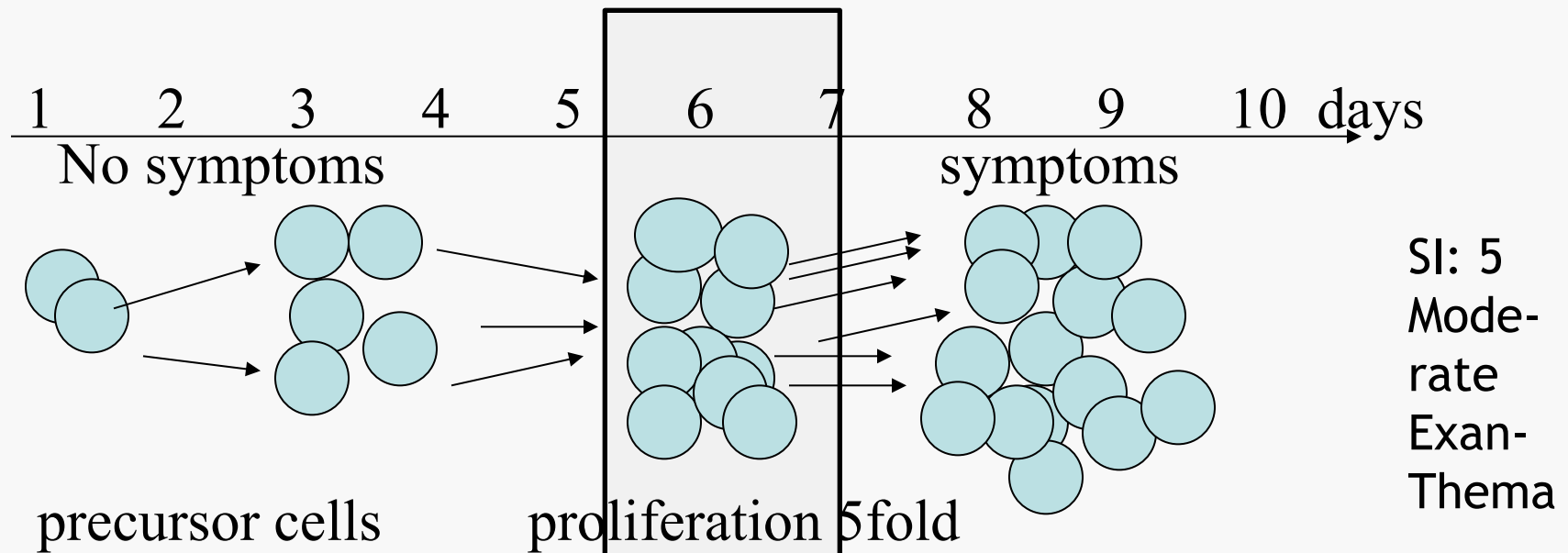
- **Excellent 85, 93, 95, 98 & 100%**  
**= ca. 95%**
- Unspecific expansion of cells in vitro to drugs is rare and limited to a few drugs only (paracetamol, vancomycin, .... )
- penicillin G causes a moderate increase in some donors (cut off SI 2,5 – 3) cut off SI=3

**If you have a positive LTT, it is meaningful !!**

The **specificity** of LTT is acceptable  
the **sensitivity** is 60-70% in  
studies\*

\*) ca. 10-20% of LTT analysed in routine lab are positive  
max. 50% if drug allergy considered highly likely....

A negative result does not rule an drug hypersensitivity



# ***In vitro* drug causality assessment in Stevens-Johnson syndrome – alternatives for lymphocyte transformation test.**

*G Porebski et al, CEA 2013*

- 15 patients with SJS/TEN (ALDEN score  $\geq 6$ )
- 18 drug-exposed controls

	+	sensitivity	CI
• <b>LTT</b>	<b>4/15</b>	<b>27%,</b>	<b>8-55%</b>
• granzyme B-ELISpot	5/15	33%,	12-62%
• granulysin (NKp46 <sup>+</sup> )	6/15	40%,	16-68%
• granulysin (CD3 <sup>+</sup> CD4 <sup>+</sup> )	8/15	53%,	27-79%
• IL-2 + IL-5	6/14	43%	18-71%
• IFN $\gamma$	6/11	55%,	23-83%
• <b>IFN<math>\gamma</math>, GranzB, GrlyCD4</b>	<b>12/15</b>	<b>80%,</b>	<b>52-96%</b>

Specificities of tested assays were in the range of 95 (CI:80-99%)-100% (CI:90-100%).

# Punktescore zur Bewertung der LTT Resultate, ohne Berücksichtigung von Zusatzinformationen

Bewertung SI:

< 2: keine Sensibilisierung nachgewiesen

2-3: Sensibilisierung möglich

>2,5-4: schwache Sensibilisierung

> 4: Sensibilisierung sehr wahrscheinlich (falls mehr als ein Wert)

	Punkte
Schwankungen der Quadruplikate: > 50%	-1 /betroffenes Quadruplikat
„Ausreisser“: nur ein Wert hoch (meist Aggregate?)	-1
Werte mit SI > 2.5 nur im autologen Serum oder AB Serum	-1
SI Wert >2.5 (>3 bei Penizillin)	+1/Wert
SI Wert > 4	+2/Wert
Dosisabhängigkeit über mindestens 2 Dosen	4



# Common mistakes

- Drug mixtures: cough sirup, mixtures....
- too many drugs
- wrong ideas: an in vitro proliferation assay is not suitable to explain pruritus to a drug headache, diarrhoe,.....
- Macular exanthema, rash, delayed urticaria, minimal exanthema...: not enough T cells in circulation
- Tablets: doubtful results ...

# Value of LTT with different diseases

## ***often positive:***

- *DHS/DRESS (35/36= 97%)*
- *generalised maculopapular exanthema*
- *bullous exanthema*
- *AGEP*
- *anaphylaxis (IgE-mediated, generalised, severe symptoms)+*

## ***occasionally positive:***

- *hepatitis\**
- *pancreatitis°*
- *nephritis (dependent on type of drug)*
- *interstitial lung disease°*
- *urticaria, angioedema+*
- *SJS/TEN*

°) *seldom examined*

\*) *strongly dependent on drug*

+) *positive in IgE-penicillins*



# Value of LTT with different diseases

***seldom or never positive (<10% or less):***

- *Vasculitis<sup>°</sup>*
- *macular exanthema (without cell infiltration)*
- *Guillain-Barré<sup>°</sup>*
- *blood diseases as ITP<sup>°</sup>, haemolytic anaemia<sup>°</sup>*
- *fixed drug exanthema*

*°) seldom examined*



# The main cause for a negative LTT in spite of very likely sensitization is

- Disease became clinically manifest due to cofactors
- It was **no** strong T cell reaction
  - No more detectable in peripheral blood cells
  - (But may remain detectable in skin (homing))
- It was more a cytotoxic reaction and thus LTT / proliferation was not the correct choice (see example SJS or abacavir)

# Suitable drugs for LTT

- **antibiotics:** b-lactams, quinolones, macrolids, sulfonamides, tetracycline, vancomycin+...
- **antiepileptics:** phenytoin, carbamazepin, lamotrigine, gabapentin...
- **ACE-inhibitors:** enalapril, ...
- **antituberculous drugs:** isoniazid, rifampicin
- **diuretics:** hydrochlorothiazide, furosemide, indapamid, ...
- **NSAID** (Cox 1 and Cox 2 inhibitors): diclofenac, celecoxib, mefenaminic acid,
- **pyrazolones:** propyphenazone
- **HMG-CoA-reductase inhibitors:** acrivastatin
- **morphin-derivatives:** pethidin, codein, ...
- **radio-contrast media+:** iohexol, iopamiro, ....
- **muscle relaxants:** suxamethoniumchlorid, ...
- **vitamins:** cyancobalamin (Vit. B12), folic acid, ...
- **contact allergens:** p-phenylendiamine
- **Metals:** NiCl<sub>2</sub>, CrSO<sub>4</sub>
- **varia:** .....

*always as PURE SUBSTANCES*



# lymphocyte transformation test

+

in vitro, not dangerous  
positive result  
meaningful as good  
specificity  
sensitivity +/-  
cross-reactivity  
research tool

-

laborious, time consuming  
complicated logistic,  
need of fresh cells  
sensitivity +/-  
  
*(limited availability,  
radioactivity, expensive)*