ISCAR Vancouver 2015

In vivo and in vitro assessments for SCAR – What's new? What's next?

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No conflict of interest

No off-label use of medical devices, products or pharmaceuticals

rug patch tests

At least one month after the CADR (> 6 months after DRESS)

mercialized products for drug patch testing at 10% in pet.

(Chemotechnique laboratory,Velinge, Sweden)

n G, potassium salt
lin trihydrate
illin sodium salt hydrate
m sodium salt
in monohydrate
line hydrochloride
ycin base
cin base
mycin
ycin
xazole
cine
xacine hydrochloride
azepine
in
n hydrochloride
il
alicylic acid
ac sodium salt
fene
m
nophen
ir
nol
zine hydrochloride
lorotiazide
ycin phosphate
ne
ine
en

Commercialized forms provided by the patients themselves of drugs diluted to **30% in petrolatum**

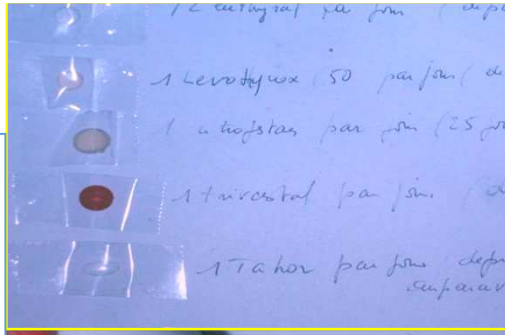
(ESCD criteria, Barbaud A et al. CD 2001)

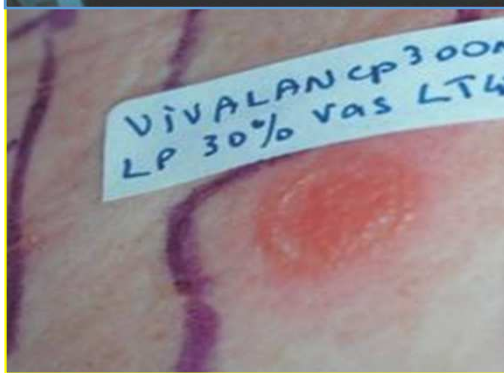
Or 20%

(ENDA criteria, Brockow K et al. Allergy 2002)

Patch tests are read on Day 2 and Day 4 or 5

According to ICDRG criteria





is • Original Article

COD
Contact Dermatitis

ant concentrations and amounts of active ingredient in drug
ts

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What is the concentration of active ingredient in preparations for drug patch tests **(dilution at 30%)**

STUDY

- **5558 Drug Patch tests** done for CADR
- 2007 – end of 2012

Non irritant concentrations

- With most of **the 89** drugs tested
- Many positive (+) results with Telaprevir/Amiodarone /Trimebutine/Pantoprazole BUT drugs with moderate or high imputability in all the patients tested
- →10 controls= all negative

Amount of active ingredient

- 0.05% digoxine – 30% paracetamol lyophilisate (powder)
- 0.05% to 27.08% when tablets were used
- Mean concentration: 9.8%
- 25% of DPT: Concentration < 2%
- 25% of DPT: Concentration > 16%

Brajon D et al. Contact Dermatitis, 2014, 71: 170–17

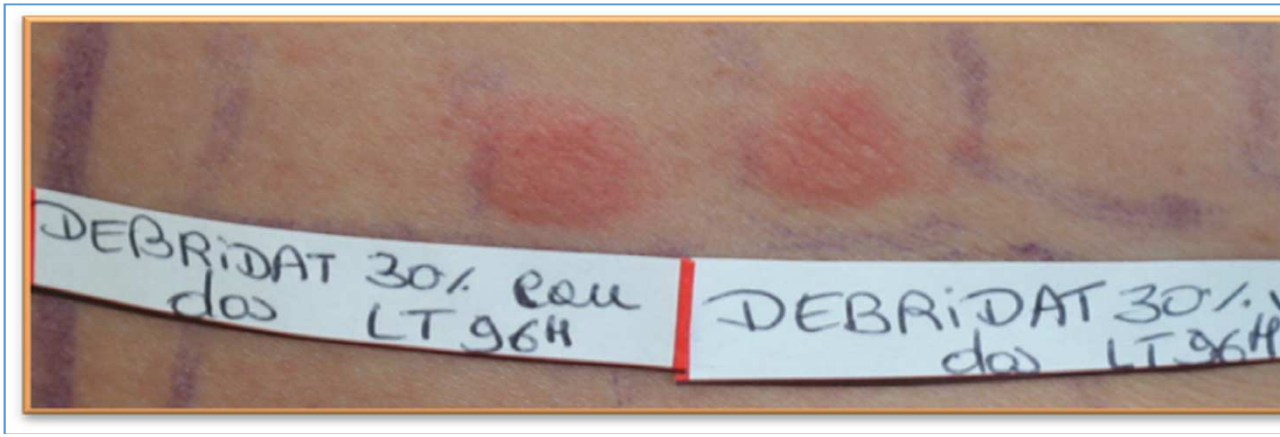
Drug patch tests with the commercially available drugs: give the concentration of active ingredient

In multicenter studies on DPTs, it would be convenient

- to either use the same generic forms of drugs
- or consider only AI concentrations to enable standardization of methods among center

It is better to use

- Lyophilisates (powder)
- Commercialized material for drug patch tests (but many drugs are missing!)



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ctive ingredient INN name (registered trade name)	Dilution	T–	% of AI in DPT
steroids			
ncinonide	0.1% pet.	364	ND
tamethasone (Célestène®) 2-mg tablet	30% aq./pet./alc.	18	0.22
tamethasone dipropionate (Diprostene®) 7 mg/ml injectable form	0.1% alc.	13	ND
rtivazol (Altim) 3.75 mg/1.5 ml injectable form	0.1% alc.	13	ND
xamethasone 21-phosphate disodium salt	1% pet.	450	1.00
xamethasone (Dectancyl®) 0.5-mg tablet	0.1% alc.	12	ND
drocortisone	1% pet.	397	1.00
ethylprednisolone (Solumedrol®) 62.5 mg/ml injectable form	0.1% alc.	11	0.10
ethylprednisolone (Solumedrol®) 62.5 mg/ml injectable form	30% aq./pet./alc.	30	ND
ometasone furoate (Nasonex®) 50 µg	Pure	356	ND
ednisone (Cortancyl®) 20-mg tablet	0.1% alc.	13	0.001
ednisone (Cortancyl®) 20-mg* tablet	30% aq./pet./alc.	7	3.51
ednisolone	1% pet.	355	1.00
ednisolone (Solupred®) 20-mg tablet	30% aq./pet./alc.	22	3.51
pump inhibitors			
omeprazole (Inexium®) 40-mg tablet	30% aq./pet./alc.	36	2.92
nsoprazole (Ogast®) 30-mg capsule	30% aq./pet./alc.	11	ND
neprazole (Mopral®) 10-mg capsule	30% aq./pet./alc.	48	2.59
ntoprazole (Eupantol®) 40-mg tablet	30% aq./pet./alc.	37	6.09
beprazole (Pariet®) 20-mg tablet	30% aq./pet./alc.	15	ND
etic drugs			
rmperidone (Motilium®) 10-mg* tablet	30% aq./pet./alc.	8	3.00
etoclopramide (Primperan®) 10-mg tablet	30% aq./pet./alc.	12	2.42
dansetron (Zophren®) 8-mg tablet	30% aq./pet./alc.	10	9.23
sics			
etaminophen (Dafalgan®) 500-mg capsule	30% aq./pet./alc.	72	30.00
etaminophen codeine (Codoliprane®) 400-mg/20-mg tablet	30% aq./pet./alc.	18	24.69/1.23
rphine sulfate (Skenan LP®) 200-mg capsule	30% aq./pet./alc.	10	ND
rphine sulfate (Actiskenan®) 30-mg capsule	30% aq./pet./alc.	10	ND
rphine chlorhydrate 10 mg/ml injectable form	30% aq./alc.	16	ND
fopam (Acupan®) 20-mg injectable form	30% aq./pet./alc.	13	ND
madol (Topalgic®) LP 100-mg tablet	30% aq./pet./alc.	40	9.32
erferon			
g-interferon alpha-2a (Pegasys®) 180 µg	Pure	17	ND
g-interferon alpha-2a (Pegasys®) 180 µg	30% aq./alc.	17	ND
g-interferon alpha-2b (Viraferon®) 100 µg	Pure	16	ND
g-interferon alpha-2b (Viraferon®) 100 µg	30% aq./alc.	16	ND
combinant peg-interferon alpha-2b (Introna®) 10 m IU	Pure	10	ND
combinant peg-interferon alpha-2b (Introna®) 10 m IU	30% aq./alc.	10	ND
combinant peg-interferon alpha-2a (Roferon®) 12 m IU	Pure	11	ND
combinant peg-interferon alpha-2a (Roferon®) 12 m IU	30% aq./alc.	11	ND
l agents (HCV)			
bavirine (Rebetol®) 200-mg capsule	30% aq./pet./alc.	15	20.69
aprevir (Incivo®) 375-mg tablet	30% aq./pet./alc.	11	11.11
tamines			
tirizine (Cetirizine Arrow®) 10-mg tablet	30% aq./pet./alc.	16	ND
sloratadine (Aerius®) 5-mg tablet	30% aq./alc./pet.	18	1.42
xchlorpheniramine (Polaramine®) 2-mg tablet	30% aq./pet./alc.	13	0.85
droxyzine (Atarax®) 100-mg tablet	30% aq./pet./alc.	31	11.54
vocetirizine (Xyzal®) 5-mg tablet	30% aq./pet./alc.	14	1.47
iazepines			
brazolam (Alprazolam EG®) 0.25-mg tablet	30% aq./pet./alc.	13	0.06
omazepam (Bromazepam Arrow®) 6-mg tablet	30% aq./pet./alc.	11	0.62
zepam (Diazepam Ratiopharm®) 10-mg tablet	30% aq./pet./alc.	10	1.79
trazepam (Myolastan®) 50-mg tablet	30% aq./pet./alc.	10	7.69
rhythmic drugs			
niodarone (Cordarone®) 200-mg tablet	30% aq./pet./alc.	13	17.05
goxin 0.25-mg* tablet	30% aq./pet./alc.	2	0.05

Table 1. Concentrations of active ingredient (AI) in drug patch tests (DPTs) and their non-irritating concentrations

Drug/active ingredient INN name (registered trade name)	Dilution	T–	% of AI
NSAIDs			
Celecoxib (Celebrex®) 100-mg tablet	30% pet.	10	11.
Ibuprofen (Advil®) 400-mg tablet	30% aq./pet./alc.	18	16.
Ketoprofen (Ketum® gel)	1% pet.	44	1.0
Niflumic acid (Niflugel®)	Pure	135	NI
Antibiotics			
Fluoroquinolones			
Ciprofloxacin (Ciflox®) 500-mg tablet	30% aq./pet./alc.	10	18.
Levofloxacin (Tavanic®) 500-mg tablet	30% aq./pet./alc.	74	24.
Moxifloxacin (Izilox®) 400-mg tablet	30% aq./pet.	46	NI
Ofloxacin (Oflocet®) 200-mg tablet	30% pet./aq./alc.	64	15.
Pefloxacin (Peflacine®) 400-mg tablet	30% aq./pet./alc.	66	15.
Pristinamycin			
Pristinamycin (Pyostacine®) 500-mg tablet	30% aq./pet.	44	20.
Macrolides			
Azithromycin (Azadose®) 600-mg tablet	30% pet.	79	16.
Josamycin (Josacine®) 1000-mg tablet	30% pet.	89	15.
Roxithromycin (Rulid®) 100-mg tablet	30% pet.	75	20.
Spiramycin injectable form	Pure	71	NI
Telithromycin (Ketek®) 400-mg tablet	30% pet.	61	19.
β-Lactams			
Amoxicillin (Clamoxyl®) 500-mg* tablet	30% pet.	8	25.
Amoxicillin–clavulanic acid 1000-mg tablet	30% pet.	157	15.
Amoxicillin–clavulanic acid 1000-mg tablet	30% aq./alc.	14	15.
Cefamandole (Kefandol®) 750-mg injectable form	30% pet.	176	30.
Cefamandole (Kefandol®) 750-mg injectable form	Reconstituted	217	NI
Cefixim (Cefixim Arrow®) 200-mg tablet	30% pet.	395	12.
Cefotaxime (Claforan®) 1-g injectable form	Reconstituted	216	NI
Cefotaxime (Claforan®) 1-g injectable form	30% pet./aq.	218	30.
Cefotiam (Taketiam®) 200-mg tablet	30% pet.	368	NI
Cefpodoxim (Cefpodoxim Arrow®) 100-mg tablet	30% pet.	395	12.
Ceftriaxone (Rocephine®) 1-g injectable form	30% pet.	179	30.
Cloxacillin (Orbenine®) 500-mg* capsule	30% pet.	7	27.
Oxacillin (Bristopen®) 1-g capsule	30% aq.	237	30.
Oxacillin (Bristopen®) 1-g capsule	30% pet.	169	30.
Penicillin V tablet	30% pet.	369	NI
Glycopeptides			
Teicoplanin (Targocid®) 400-mg injectable form	30% aq./pet./alc.	35	30.
Vancomycin (Vancocin®) 1000-mg injectable form	30% aq./pet./alc.	26	29.
Cyclins			
Clindamycin (Dalacin®) 300-mg capsule	30% aq./pet./alc.	15	14.
Doxycycline (Doxycycline Arrow®) 100-mg tablet	30% aq./pet./alc.	12	11.
Other antibiotics			
Cotrimoxazole (Bactrim forte®) 800-mg tablet	30% aq./pet./alc.	27	24.
Rifampicin (Rifadine®) 300-mg capsule	30% aq./pet./alc.	30	24.
Imidazole derivatives			
Fluconazole (Fluconazole Actavis®) 100-mg* capsule	30% aq./pet./alc.	9	17.
Metronidazole (Flagyl®) 500-mg tablet	30% aq./pet./alc.	23	21.
Heparins and heparinoids			
Calcium heparinate (Calciparine®)	25 000 UI/ml	39	NI
Dalteriparin sodium (Fragmine®)	12 500 UI/ml	37	NI
Danaparoid (Orgaran®)	1250 UI/ml	35	NI
Enoxaparine (Lovenox®)	10 000 UI/ml	41	NI
Heparin	5000 UI/ml	35	NI
Nadroparin calcium (Fraxiparine®)	9500 UI/ml	40	NI
Reviparin sodium (Clivarine®)	5726 UI/ml	29	NI
Tinzaparine sodium (Innohep®)	20 000 UI/ml	40	NI

Drug prick tests with delayed readings (at 24 h)

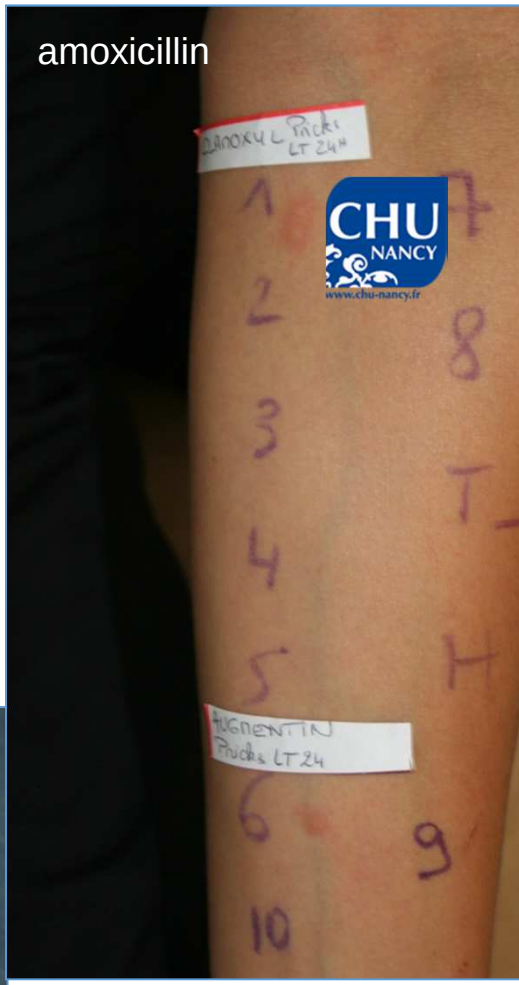
Could be discussed for AGEP and DRESS, when patch tests are negative

Done on the forearm.

Read at 20 minutes, results compared to those obtained with saline .9% and histamine (10 mg/mL).

But also <u>read one day later.</u>

Delayed positive reactions in drug prick tests: erythematous and infiltrated reaction



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Can we perform IDT in severe CADR (follow the new guidelines fo performing intradermal tests (IDT) by ENDA group ?
Highly debatable

ever in SJS/TEN

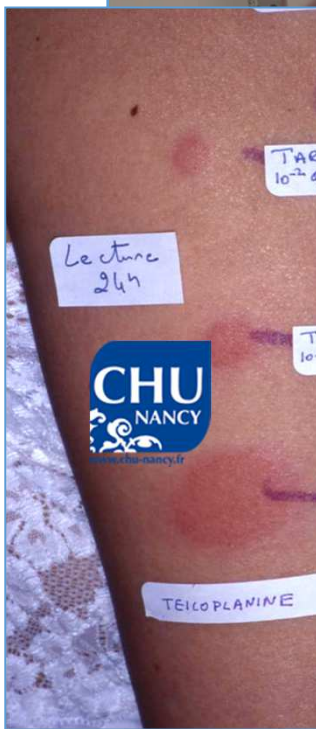
AGEP or DRESS, to be discussed when and only when a CADR occurs

- In a patient with a multiple regimen of drugs (in resuscitation unit)
- For absolutely necessary drugs,
  - without any possible substitution with another chemical class,
  - That have a low responsibility, according to chronological criteria.
  - Mainly in order to find a substitute drug

- In DRESS, when there is no virus reactivation, controlled by negative PCR.

- Injecting only 0.02 ml (new guidelines by ENDA group)

- Read after 24 hours or later: considered positive when there is an infiltrated and erythematous reaction.



Drug Patch tests in severe CADR: French group (« FISARD »).

Barbaud A et al. Toxidermies group FISARD of the French Society of Dermatology. A multicentre study to determine the value and safety of drug patch tests for the three main classes of severe cutaneous adverse drug reactions. Br J Derma 2013 168: 555–562

Multicentre study with drug patch tests with commercialized form provided by the patients themselves a 30% in pet. or pure drug at 10% in pet. *(ESCD guidelines)*

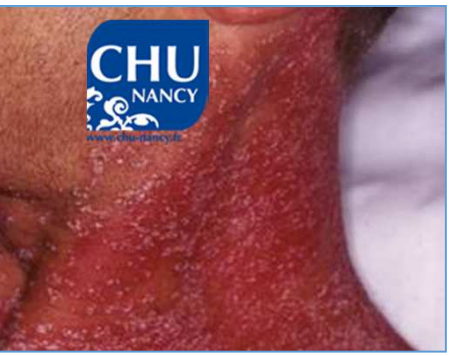
In the 12 months following the disappearance of the CADR

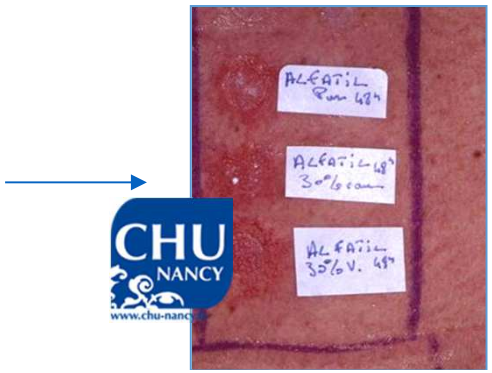
With all drugs recently introduced

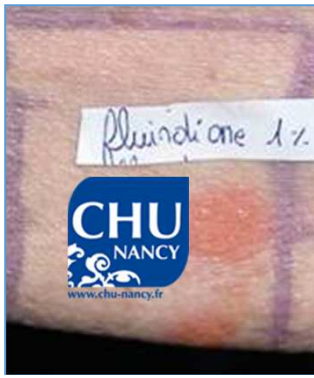
134 included patients (48 males, 86 females, mean age: 51.7 years) with severe CADR: 72 DRESS, 45 AGEP, 17 SJS/TEN.

76/134 (57.5%) had at least one positive drug patch tests

- 58% (26/45) for AGEP,
- 64% (46/72) among cases with DRESS,
- and 23.5% (4/17) for SJS/TEN.







Drug patch tests are of value in Acute Generalized Exanthematous Pustulosis (AGEP)

- Positive patch tests in **7/14 AGEP (50%)**

Wolkenstein P. Contact Dermatitis 1996

- **26/45 AGEP (58%)** in the French multicenter study *:*
  - 8 for betalactams,
  - 8 for pristinamycin,
  - 3 for corticosteroids,
  - 2 for RCM, 2 for dextropropoxyphene in combination with paracetamol (acetaminophen),
  - and 1 each for fluindione, non-fractionated heparin, pseudoephedrine (at 1% in petrolatum), tetrazepam, clindamycin, and varenicline. .

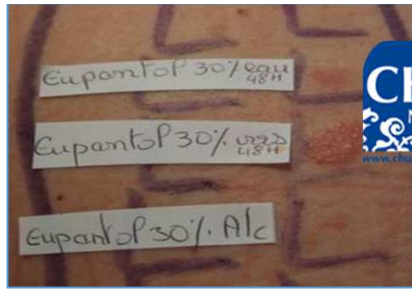
1 relapse during patch tests with pristinamycin

Barbaud A et al. Br J Dermatol 2013

- **From literature, positive patch tests in AGEP**
- allylisopropylacetylurea,
- betalactams,
- bleomycin,
- carbimazole,
- celecoxib,
- ciprofloxacine,
- clindamycin,
- corticosteroids,
- diltiazem,
- metamizole,
- methoxalene,
- metronidazole,
- morphine,
- nimesulide,
- pseudoephedrine,
- ranitidine
- Tetrazepam

A.Barbaud Curr Allergy Asthma Rep, 2014,14: 442

Positive drug patch tests are frequent in DRESS



In a Portuguese study

- Among 56 patients with DRESS, mainly due to anti-convulsant drugs (33 cases, mainly carbamazepine) or to allopurinol (19 cases),
- **18 PT+/56 (32%)**, of the patients had positive drug patch tests
- 13 + /18 with carbamazepine
- 0/ 19 with allopurinol

French multicenter study

- Selected DRESS with with a score ≥4 according to the criteria of DRESS of Kardaun criteria
- In testing all drugs introduced within the 2 months prior to the onset of DRESS
- **46/72 (64%)** of the cases with DRESS (26 M, 46 F, mean age: 51.22 years) had positive patch tests
- Mean delay between tests and DRESS: 6.1 months
- No positive results with allopurinol (8 cases) or salazopyrine (5 cases)

- *Santiago F et al. Contact Dermatitis 2010, 62: 47-53*
- *Barbaud et al. groupe "Toxidermie de la SFD" Br J Dermatol* 2013 168: 555–562

46 +/72 patch tests in DRESS (64%): Drugs with positive patch tests

Barbaud A et al. Br J Dermatol 2013

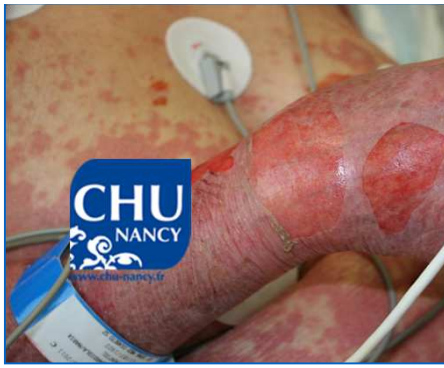
- Multiple drug reactivity is not rare: 13 cases (18%) versus 7/1925 (0.3%) in non severe CADR *(Studer et al. Ann Dermatol Venereol 2012)*

- In the French multicentre study, positive patch tests were observed with:
  - carbamazepine (11positive PTs/13 suspected cases),
  - betalactams (14 cases),
  - proton pump inhibitors (PPIs): 5 cases, vancomycin (4 cases), pristinamycin (3 cases), other miscellanous drugs.

In DRESS,

- Test more than 6 months after the disappearance of the DRESS
- Do patch tests and if negative, prick tests with delayed readings
- With all drugs taken the 2 month before and the week following the onset of the DRE
- Reactivation has not been reported, relapse of the rash is rare

Drug patch tests in SJS/ TENs



Intradermal tests are contra indicated

2 PT+/22 SSJ or TENs **(9%)**
Wolkenstein P. Contact Dermatitis 1996 , 35 : 234

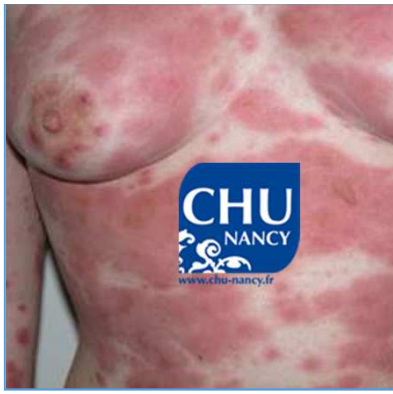
39 SSJ/TENs: **25.6%** of positive patch tests
Duong T et al. Journees Dermatologiques de Paris 2009

SJS/TENs: **23.53%** (4 PT+/17) *("Fisard" group Br J Dermatol 2013)*

Positive PTs when cotrimoxazole was tested on cutaneous sites previously affected by necrolysis
Klein CE. Contact Dermatitis 1995

We did not observe any differences in testing skin areas that were or were not previously involved.

SSJ/TENs, the value of patch tests could depend on the drug considered and also on HLA alleles



arbamazepine (5 negative results / 5 cases) *Barbaud A et al. Br J Dermatol 2013*

0 PT+/16 patients with SJS/TENs due to carbamazepine

n YT et al. J Eur Acad Dermatol Venereol. 2013;27:356-64.

- In carbamazepine-induced SJS/TEN or DRESS, patch tests were found to be positive in 62% of SJS/TEN HLA-B*1502+ patients

literature, positive patch tests have been found positive in SSJ/TEN with:

- Antibiotics: betalactam antibiotics, cotrimoxazole, glycopeptides,
- and ramipril, lamotrigine, tetrazepam, PPI, pseudoephedrin, Nigelle essential oil.

	Patch tests	Prick tests	IDT
generalized nematous pustulosis	useful *58% (26/45)	unknown value	Unknown value
SS	useful *64% (46/72) 6 months after DRESS	unknown value, delayed readings can be of low value	Unknown: could be dangerous. Only done with drugs with a low imputability
ENs	Can be done but with a low value.*23.5% (4/17)	No value	Not allowed because th could be dangerous

Barbaud A,Collet Evelyne; Milpied Brigitte et al. Br J Dermatol 2013 168: 555–562. Groupe FISARD

Negative drug skin tests are not sufficient to eliminate the responsibility of a drug in inducing a CADR
In non severe ADR, they have to be followed by provocation tests

Poor relevance of a lymphocyte proliferation assay in Stevens–Johnson syndrome or toxic epidermal necrolysis

ıcubation of peripheral blood mononuclear cells (PBMC) with the incriminated drug and the proliferative response

In 23 patients with SJS or TEN who reacted to lamotrigine, positive LTT were observed [1]

- in 3/6 cases of mild eruptions,
- 1/9 SJS/TEN-cases tested during the acute phase
- 3/14 SJS/TEN-cases tested after recovery.

From literature, the sensitivity of LTT response is low: 11–21%

Among 18 SJS/TENs, the sensitivity of LTT was 27% (CI: 8–55%)

- Reactive cells are rarely detected in these reactions.
- Drug-specific CD8+ T cells are refractory to further stimulation in vitro,
- Similar to 'exhausted' CD8+ T cells in HIV or chronic infections
- Related to enhanced Treg cell function or PD-1 expression?[2]
- or not related to T-reg [1].

- *1. Tang YH et al.Clin Exp Allergy. 2012;42:248-54. 2. Porebski G et al. Clin Exp Allergy. 2013 ;43:1027-37.*

ELIspot and DRESS and Abacavir hypersensitivity or DRESS/DIHS

- The value of ELIspot interferon-gamma has been reported in maculopapular exanthem due betalactam antibiotics
- **Abacavir hypersensitivity and Elispot**
- ELIspot interferon-gamma was done from PBMC of Abacavir treated patients with

	spot forming cells per million PBMC	
Confirmed HSR: 5 cases	82.3±23.0	
Suspected HSR: 12 cases	10.5±4.5	$p < .005$
42 controls without HSR	0.5±1.0	

Esser S et al. Inflamm Allergy Drug Targets. 2012;11:227-34.

Enzyme-linked immunospot (ELISPOT) assay and DRESS

2 days after the initiation of steroids, Day 5 of DRESS, IFN-γ–releasing cells in the peripheral blood were measured by ELISPOT assay

Significant numbers of IFN-γ–secreting cells were demonstrated (1,048 spots forming cells/106 PBMCs) upon incubation with 100 µg/mL **sulfasalazine**,

but not with other drugs administered concurrently (ceftriaxone), previously (amoxicillin), or never (ceftazidime)

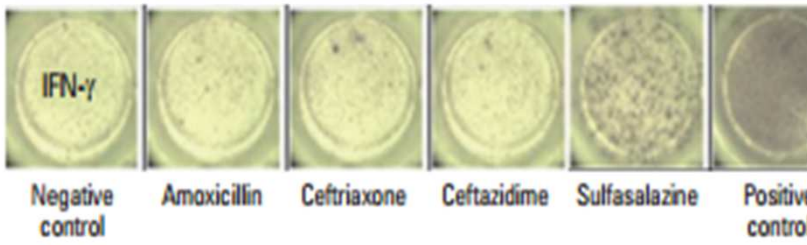


Fig. 2. Sulfasalazine-specific interferon-gamma responses as demonstrated enzyme-linked immunospot assay.

Potential role of IFN-γ ELISPOT assays in identifying the culprit dru in patients with a history of multipl drug use.

tharacharukul, J Klaewsongkram. Allergy Asthma Immunol Res. 2013;5:415-417.

Ex vivo IFN-gamma ELISPOT assay and sFasL ELISA remain positive a long time after the remission of SJS/TEN

Methods

- Ex vivo IFN-g ELISpot assay and by sFasL ELISA
- From 8 patients analyzed in clinical remission of SJS/TENs

Results

- In all 8 patients with SJS and TEN, IFN-gamma ELIspot was positive (2565- 4400 SFU per million T cells).
- A substantial cultured IFN-g ELISpot response was observed as long as 3 years later.

M et al. PLoS One. 2012;7:e45516.

Table 1. Patient characteristics.

Patient	Age/ gender	Causal drugs	Irrelevant drug	Disease	LTT	Intervals*
1	32 years/f	CFZ (50 ug/ml)	NMS (7 ug/ml)	TEN	+	3 years
2	37 years/f	NMS (7 ug/ml)	CFZ (50 ug/ml)	TEN	+	2 years
3	57 years/f	AMX (40 ug/ml)	NMS (7 ug/ml)	SJS	+	1 year
4	80 years/m	PNC (40 ug/ml)	NMS (7 ug/ml)	TEN	+	1 year
5	64 years/m	NMS (7 ug/ml)	CFZ (50 ug/ml)	SJS	+	1 year
5	3 years/m	APAP (50 ug/ml)	CFZ (50 ug/ml)	TEN	+	1 year
6	77 years/m	AP (50 ug/ml)	CFZ (50 ug/ml)	TEN	+	3 month
7	26 years/m	AMX (40 ug/ml)	NMS (7 ug/ml)	SJS	+	2 months
8	15 years/f	HCQ (40 ug/ml)	CFZ (50 ug/ml)	TEN	+	1 month
9	48 years/f	CBZ (25 μg/mL)	AMX (40 ug/ml)	MPE	+	2 years
10	46 years/m	APAP (50 ug/ml)	CFZ (50 ug/ml)	MPE	+	1.5 years
11	21 years/f	AMX (40 ug/ml)	NMS (7 ug/ml)	MPE	+	1 year
12	45 years/m	PNC (40 ug/ml)	NMS (7 ug/ml)	MPE	+	1 year
13	34 years/f	CBZ (25 μg/mL)	AMX (40 ug/ml)	MPE	+	3 months
14	14 years/f	CFZ (50 ug/ml)	NMS (7 ug/ml)	MPE	+	1 month

Drug concentrations used for stimulation in *in vitro* experiments. f, female; m, male; CFZ, cephazolin; NMS, nimesulide; AMX, amoxicillin; PNC, penicillin; APAP, acetaminophen; HCQ, hydroxychloroquine; CBZ, carbamazepine; AP, allopurinol; TEN, toxic epidermal necrolysis; SJS, Stevens–Johnson syndrome; MPE, maculopapular exanthema; LTT, lymphocyte transformation test. *Interval between acute allergy and present analysis.

doi:10.1371/journal.pone.0045516.t001

Combine 3 different cellular assays may help to overcome or bypass the assumed refractoriness of PBMC of SJS/TEN patients

Methods

15 patients with well defined SJS/TEN (ALDEN score ≥ 6) vs 18 controls

11 assay end-points based on drug-induced cell proliferation, cytokine production and measurement of cell mediated, drug-induced cytotoxicity by measuring granzyme B secretion (ELISpot) or granulysin production (flow cytometry).

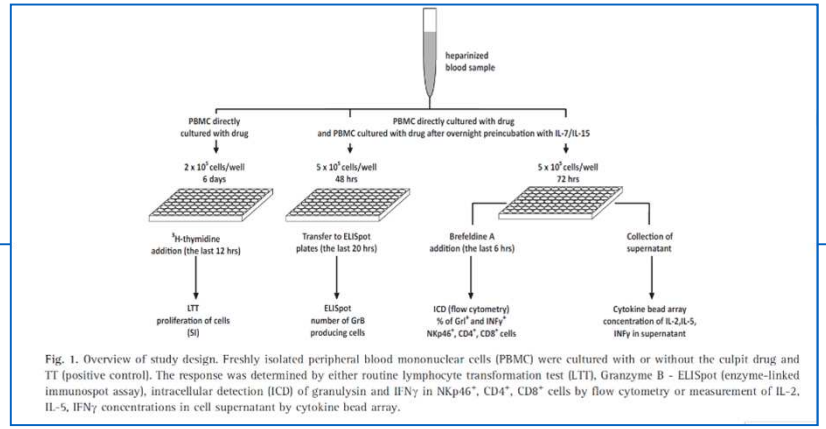


Fig. 1. Overview of study design. Freshly isolated peripheral blood mononuclear cells (PBMC) were cultured with or without the culpit drug and TT (positive control). The response was determined by either routine lymphocyte transformation test (LTT), Granzyme B - ELISpot (enzyme-linked immunospot assay), intracellular detection (ICD) of granulysin and IFNγ in NKp46⁺, CD4⁺, CD8⁺ cells by flow cytometry or measurement of IL-2, IL-5, IFNγ concentrations in cell supernatant by cytokine bead array.

- no single assay achieved a sufficient sensitivity (max. 53%), BUT combining 3 tests enabled to identify the causative drug in 80% (12/15) of patients
- with high specificity (95%).

Table 2. Results of tests in the patients group. The sensitivity and specificity were calculated at the cut-offs (bolded) determined from the ROC curves. Results are expressed as delta (Δ) values (the difference between the response in the presence of tested drugs minus negative control - culture medium).

Consecutive patient	Culprit drug	LTT‡ (SI)	% Granulysin⁺ cells NKp46⁺	CD8⁺	CD4⁺	GrB – ELISpot (sfc/well)	Cytokines (pg/mL) IFNγ	IL-5	IL-2	Time interval (months)
1.	SPD	5.2*	1.8	−5.5	2.4†	0.5	0	0	0	6
2.	CBZ	2.9†	34.8†	2.3	0.3	29.5*	20†	9*	83†	3
3.	ALP	1.7	−5.7	0.3	4†	21.5*	28.2†	0	−0.2	60
4.	CBZ	0.8	2.4	−0.4	0	37*	0.3	0.3	−1.5	12
5.	CBZ	0.7	−0.4	−2.2	−1.3	−2.5	−0.2	−0.2	−0.2	96
6.	SPD	1.4	1.7	0.4	0	24*	nd	0	nd	6
7.	SDX	1.2	5.9	−0.1	0.3	2	2.6*	3.4*	−4.7	24
8.	LTG	1.5	3.1	−0.6	0.2	0.7	0	−0.2	−0.7	3
9.	MA	2.02*	3.5	2.3	1.1*	0	−0.2	−0.2	3.4	3
10.	SDX	0.9	−0.30	−0.2	−0.3	1.5	nd	nd	nd	4
11.	CBZ	1.9	14.1*	0.2	0.65*	−6.5	18.7*	1	2.9	2
12.	CBZ	1.1	19.6*	−0.2	1*	1	15.7*	13.2†	6.2†	1
13.	CBZ	1.8	30.0†	0.8	0.7*	5	22.9†	11.8*	14.5†	5
14.	LTG	1.1	26.2†	2.7	1.8†	−9	nd	13.9†	41.2†	12
15.	LTG	2.3†	26.0†	8.7*	4.4†	59.5*	nd	22.3†	7†	1
Sensitivity % (CI)		27 (8–55)	40 (16–68)	7 (2–32)	53 (27–79)	33 (12–62)	55 (23–83)	43 (18–71)	38 (14–68)	
					73 (45–92)					
					80 (52–96)					
Specificity % (CI)		100 (90–100)	96 (85–100)	100 (90–100)	100 (90–100)	98 (85–100)	95 (80–99)	100 (90–100)	98 (85–100)	
					98 (85–100)					
					95 (80–99)					

The grey cells represent positive results (obtained in *one or †two concentrations of the drug). ‡The value of the cut-off (SI = 2) chosen arbitrary, based on the previous evidence [10]. Time intervals between acute symptoms and testing are shown.
SPD, sulfapyridine; CBZ, carbamazepine; ALP, allopurinol; SDX, sulfadoxine; MA, mefenamic acid; LTG, lamotrigine; nd, not done; CI, confidence interval.

Porebski G et al. Clin Exp Allergy. 2013 ;43:1027-37.

Combine 3 different cellular assays may help to overcome or bypass the assumed refractoriness of PBMC of SJS/TEN patients

To determine causality in SJS/TEN patients.

- the first-choice could be
- Granulysin expression in CD4+
- Granzyme B-ELISpot
- and IFN gamma secretion in cell supernatant

- Or
- Granulysin in NKp46+ cells
- Granzyme B-ELISpot
- and IFN gamma secretion in ce supernatant

Porebski G et al. Clin Exp Allergy. 2013 ;43:1027

3 Key messages

- Standardization of methods for drug skin test are absolutely necessary to compare our results and more over to compared ex vivo and in vivo tests
- Drug patch tests are useful and well tolerated in SCARs
- Elispot IFN gamma could be useful in determining the responsible drug in DRESS while, in SSJ/TEN delayed immune responses can be long-lasting but requiring different in vitro methods to be proven