Negative Response of Lymphocyte Transformation Test (LTT) in a Patient Diagnosed as Stevens-Johnson Syndrome: A Case Report

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ABSTRACT
Background: Evidences for the key role of T-lymphocytes in the pathophysiology of Stevens-Johnson syndrome (SJS) may be evaluated by drug patch test (DPT) and lymphocyte transformation test (LTT). **Purpose:** This LTT technology may reveal the role and function of T-lymphocytes for both diagnostic and research purposes. **Case:** A 33 year-old woman was admited in Dermatology and Venereology Ward at Dr. Soetomo General Hospital with skin and mucous membrane lesions after taking oral medication. Clinical and laboratory examination were performed, establishing the diagnosis of SJS caused by suspect amoxycillin and paracetamol. **Case management:** The suspected drug was discontinued immediately. Patient was given appropriate supportive treatment, systemic antibiotic, and intravenous dexamethasone with initial adjusted dose of 0.1-0.2 mg/kg/day daily according to clinical improvement. The DPT and LTT were performed 6 months after the lesions healed completely. Both DPT and LTT revealed negative results. LTT is based on the principle that T-cells proliferate in the presence of a specific-antigen, with sensitivity and specificity of 60-70% and 85%, respectively. The LTT revealed negative response, stimulation index (SI<2). Patients with SJS often show weak positive or even negative LTT response. **Conclusions:** Negative result of DPT in SJS does not exclude suspected drug. LTT is more objective and specific than DPT, however the clinical severity is not associated with high SI values.

Key words: Stevens-Johnson syndrome (SJS), drug patch test (DPT), lymphocyte transformation test (LTT).

ABSTRAK
Latar belakang: Peran limfosit-T dalam patofisiologi sindrom Stevens-Johnson (SSJ) dapat dievaluasi dengan uji tempel obat (UTO) dan uji transformasi limfosit (UTL). **Tujuan:** Teknologi UTL dapat menunjukkan peran dan fungsi limfosit-T untuk tujuan diagnostik dan penelitian. **Kasus:** Seorang wanita berusia 33 tahun dirawat di Instalasi Rawat Inap (IRNA) Kesehatan Kulit dan Kelamin Rumah Sakit Umum Daerah (RSUD) Dr. Soetomo Surabaya dengan lesi pada kulit dan membran mukosa setelah minum obat. Pemeriksaan klinis dan laboratorium dilakukan, dan ditegakkan diagnosis SSJ dengan obat penyebab yang dicurigai yaitu amoksisilin dan parasetamol. **Penatalaksanaan:** Obat yang dicurigai segera dihentikan, kemudian diberikan terapi suportif, antibiotik sistemik, dan deksametason intravena dengan dosis awal 0,1- 0,2mg/kg/hari dengan penyesuaian dosis harian sesuai kemajuan klinis. Pemeriksaan UTO dan UTL yang dilakukan bulan ke-6 setelah lesi sembuh menunjukkan hasil negatif. Prinsip dasar UTL adalah sel T berproliferasi dengan paparan antigen spesifik, memiliki sensitivitas 60-70% dan spesifisitas 85%. Kasus ini menunjukkan respons UTL negatif, indeks stimulasi (IS<2). Pasien SJS sering memberi respons UTL positif lemah atau negatif. **Simpulan:** Hasil negatif UTO pada SSJ tidak menyingkirkan obat yang dicurigai. Walaupun UTL lebih objektif dan spesifik dibandingkan UTO, keparahan klinis tidak berhubungan dengan tingginya nilai IS.

Kata kunci: sindrom Stevens-Johnsons (SSJ), uji tempel obat (UTO), uji transformasi limfosit (UTL).

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INTRODUCTION

One of the most severe forms of cutaneous adverse drug reaction (CADR) is Stevens-Johnson syndrome (SJS), which was first described in 1922 by two phsycians (Stevens and Johnson). SJS is defined as a rare mucocutaneous disease that can be acute, life threatening, and characterized by extensive necrosis and detachment of the epidermis.[1,2]

Based on retrospective study at Dr. Soetomo General Hospital during period of 2009-2011, SJS is the most frequent diagnosis from the entire drug eruption cases on the hospitalization patients in Dermatology and Venereology Ward of Dr. Soetomo General Hospital, with the total number of patients were 43 patients (23.4%) from 2828 hospitalized patients.

Evidences for the key role of T-lymphocytes in the pathophysiology of CADR may be evaluated by the positive result of drug patch test (DPT) reactions and the lymphocyte transformation test (LTT).[3,4] This LTT technology is very promising for future development of studies upon the role and function of T-lymphocytes for both the diagnostic purposes and studies to improve our understanding of the pathophysiology of drug allergic reactions.[3]

We reported a case of SJS caused by amoxycillin and paracetamol, in a 33-year-old female patient. The diagnosis was established by history taking, physical, laboratory examination, and also invivo and invitro examination to identify the causative drugs. The false negative results of DPT in this case had been confirmed by LTT. Based on the LTT result, the drugs inducing SJS in this patient were amoxycillin and paracetamol. This report discusses the clinical manifestation, diagnosis, management, and also invivo and invitro examination to etablish the causation of this CADR.

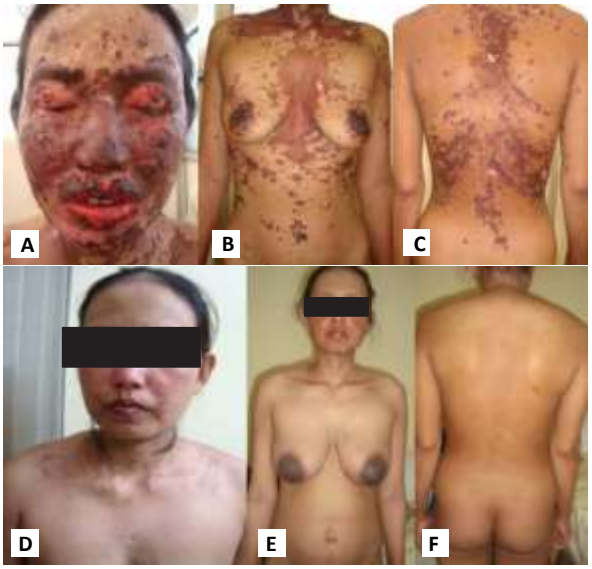
CASE REPORT

A 33-year-old Maduranese woman was admittted to the Dermatology and Venereology Ward of Dr. Soetomo General Hospital on March 29th until April 18th 2012 with chief complaint erythematous lesions on almost of her skin since 6 days before. She had developed symptoms of fever, cold, cough, and malaise 10 days before admission, and was treated by general practitioner (GP). The skin lesion developed on the fourth day after taking oral medicines (amoxycillin and paracetamol), and this was the first episode of such a reaction. Lesions initially appeared on the face, lips, chest, upperback, and gradually spread to several area of the body. Patient also complained of pain in swallowing and foreign body sensation in both eyes. She found difficulty to eat and drink since 4 days before admission. According to the patient's history, one month ago she suffered from flu-like symptoms and took the same oral medicines precribed by the same GP. There was no history of taking traditional medicine, applying any topical medication, or getting drug injection. There were also no history of diabetes mellitus and malignancy.

Based on physical examination in the fist day of admission, patient looked weak and ill. Blood pressure was 100/70 mmHg, heart rate was 90 beats per minute and regular, respiratory rate was 22 times per minute, and body temperature was 38.4^{0}C. There were no signs of anemia, icteric, cyanosis, and dyspnea, or respiratory distress. There was no abnormality on the heart, lungs, abdominal, and extremities. There was no enlargement of lymphnodes. From dermatological examination on the regio facialis, coli, thoracalis, and extremities, there were irregularly shaped erythematous macules, which progressively coalesce with epidermal detachment (Nikolsky's sign) less than 10% of body surface area (BSA). There was conjunctival congestion or hyperemia, discharges on both of her eyes, erosions, hemorrhagic crust of the lips, and also oral mucous erosions. There was no lesion on the genital area. Based on laboratory examination, the blood examination was within normal limit. Urine examination revealed hematuria +2 (2-5 per field of view), proteinuria +1, and slight alteration of serum electrolyte level. The level of blood ureum nitrogen (BUN) and random blood glucose were 23 mg/dl (<27 mg/dl) and 119 mg/dl (<250 mg/dl), respectively. Serum bicarbonate was not examined. The score of toxic epidermal necrosis (SCORTEN) scale of this patient was 1.

Based on the history taking, physical, and laboratory examination, the diagnosis of SJS was established. The two suspected drugs (amoxycillin and paracetamol) were stopped immediately to prevent worsening of condition. Then the patient was given appropriate supportive treatment with intravenous fluid drips, corticosteroid injection (dexamethasone), systemic antibiotic, and other topical treatments. Initial dose of dexamethasone 0.1-0.2 mg/kg/day was given intravenously with tapering dose as the clinical condition improved. Topical treatments for the skin lesions were normal saline wet dressing, topical fusidic sodium 2% cream for the skin erosions, and kenalog in orabase (triamcinolone) for mucous membrane lesions. The patient was also advised not to manipulate the lesions.

Within 6 days, the inflammation subsided and complete re-epithelization was reached in 20 days. The



Picture 1. Progression of the patient. A,B,C. Before treatment D, E, F. After treatment.

patient was discharged after 20 days of hospitalization with advice to follow up and a cautionary advice regarding not to take amoxycillin and paracetamol in the future.

To identifY the suspected causal agent, invivo test using DPT was perfomed 6 month after all the lesions completely healed. The DPT pannel used on patient consist of 10 drugs, such as amoxycillin trichidrate, cefotaxim sodium, erythromycin, cotrimoxazole, carbamazepine, acetyl salycilic acid, piroxicam, acetaminophen (paracetamol), ciprofloxacin, and sodium diclofenac. The DPT series were applied on the back, and read in 48, 72, 96, and 168 hours (day-7) afterward, as suggested in International Contact Dermatitis Research Group (ICDRG) guideline. However, the DPT in this patient revealed negative result.

Because of the negative result of the DPT, we considered performing lymphocyte transformation test (LTT). The result of LIT showed stimulation index (SI)<2 for each drug (SI for amoxycillin and paracetamol were 1.508 and 1.178, respectively) which revealed negative LIT response of this patient.

Picture 2. DPT results (day-2 until day-7).

DISCUSSION

SJS and toxic epidermal necrolysis (TEN) are the most severe type of hypersensitivity reactions affecting the skin that can be life threatening, characterized by extensive epidermal detachment and mucous membrane erosion.[1,2,4,5] In this case, the patient suffered from skin lesions on the fourth day after taking oral medicine (amoxycillin and paracetamol) from general practitioner because of fever, cold, cough, and malaise. She gradually developed 6 days history of skin lesions, and this was the first episode of such a reaction. There was history of taking the same medicine 1 month before because of the same complaint. For the first time the lesions appeared on the face, lips, chest, upperback, and then gradually spread to several area of the body. The lesions were especially appeared on face and upper part of the body, minimally involving distal portions of arms and legs accompanied with pain in swallowing, foreign body sensation in both eyes, but no complaint of pain when mixturition.

The clinical manifestations were similar to the literature, for which the eruption is initially symetrically distributed on the face, upper trunk, and proximal part of the limbs, including involvement of the mucous membrane (eyes and oral mucous). The distal portions of the extremities are relatively spared. The rash can mpidly extend to the rest of the body within a few days and even a few hours. The initial skin lesions are characterized by erythematous, dusky red, purpuric macules, irregularly shaped, which progressively coalesce. Nikolsk:y's sign was positive in erythematous zones and was less than 10%. The extent of the epidermal detachment lesions are classified into one of three groups which is:SJS, less than 10% ofBSA;TEN, more than 30% ofBSA, and SJSITEN overlap, between 10-30% of BSA. It is also possible to have visceral involvement (pulmonary, digestive, renal complication).'

Management ofSJSITEN consists of symptomatic treatments, specific treatments in acute stage, and also sequele treatments. The mainstay of treatment in SJSffEN is symptomatic and supportive care (fluids and electrolyte replacement), early nutritional support, control of infection, topical skin care, and eye care.The specific treatment given to the patient was corticosteroids, and sequeletreatment.•

Standard treatment in treating SJS cases in hospitalized patient at Dermatology and Venereology Ward of Dr. Soetomo General Hospital over period of 2009-2011 depends on both the general condition ofthe patient and skin lesions.[6] Most of patient had been treated with systemic corticosteroid (the dosage must be adjusted daily according to the clinical improvement), antihistamine, and antibiotic. This patient was treated with systemic antibiotic because of using high dose steroid in the initial treatment.

Education about prevention to the patients and family is very important. The most important issues are to evaluate drug causality. Invivo and invitro test can be useful in the exploration of drug allergy.[1]

In recent years, invivo and invitro tests have been developed to detect T-cells in type IV hypersensitivity reaction.[5,7-9] Evidence for the key role ofT-Lymphocytes in the pathophysiology of allergic drug reactions were proven by performing DPT and LIT.[3,...,10]

DPT is performed similar to patch test, in analogy for contact sensitizers, where the drug diluted in solution or petrolatum is applied to the skin on the upper back using standard materials such as Finn chambers on Scanpor•tape for 24-48 hours.'·" Positive reactions rely on the development of a localized inflammatory response based on activation of drug-specific T cells acting as cytotoxic effector cells and recruitment of inflammatory cells.'

Any reaction is scored according to the International Contact Dermatitis Research Group (ICDRG) as follows: +? = doubtful reaction (mild redness only); + = weak, positive reaction (red and slightly thickened skin); ++ = strong positive reaction (red swollen skin with individual small waters blisters), +++ = extreme positive reaction (intense redness and swelling with coalescent large blisters or spreading reaction) and IR =Irritant reaction."·"

Conventionally, it is important to perform early readinginOPT, 15-30minutesafterremovingocclusion strips to eliminate false positive reaction (transient erythema)." Generally, readings should be performed on day 2, 3, 4, and 7. Two days occlusion ensures that adequate allergen penetration has occi!ITed to provoke reaction on that site. In case of negative result on day 2, additional readings on subsequent days (day 3,4,7) are recommended. Reading at day 7 is recommended, especially if the result is negatif at 96 hours."'·"

DPT is usually recommended to be performed within 6 weeks to 6 months after complete healing of the CADR lesions.""·"DPT may be negative if delayed for more than 6 months."Inthis case, OPT was performed 6 months after all the skin lesions were completely healed. Perhaps, the delayed timing of performing DPT caused the negative result in this patient

It is not known for how long the skin sensitivity persist and whether drug reactivity last longer. Based on literature, many patients who were tested after 10 years still give positive reactions. It is important to realize that negative OPT does not exclude the possibility of the drug in causing CADR' The drug may not reach the immunocompetent cells in ammount sufficient to elicit a visible response."The factors affecting false negative of DPT were incorrect patch test methods, too low **concentration, inappropriate vehicle, skin barrier** function, topical corticosteroid use on the site of DPT, and patients undergoing systemic corticosteroid or immnnomodulator treatment. Genetic factors of drug metabolism, drug molecular weight, and solubility may have a role in this insensitivity."""

OPT is not an invasive procedure, and should be used as the first line of investigation or defming relevant drug in severe type ofCADR including SJS, because of its safety profile. The OPT has lowest posibility to re-induce severe drug hypersensitivity reaction."·" The sensitivity of DPT can vary depending on the vehicle used and the dmg tested, ranging from 10.8 to 50% in CADR patient, based on previous stodies."·"·" The greatest sensitivity is obtained with antibiotics, mostly those in J3-lactarn family and especially amoxycillin."

We considered performing LTT in this case, because the OPT yielded negative result. Newer findings proved that LTT is a well established invitro assay to detect drug specific T-cells proliferation or activation. This invitro investigation can help to confirm the causation in individual cases. II has been used for more than three decades."·" LTT is based on the principle that T-cells can divide and proliferate in the presence of a specific antigen. It has been the most widely used test to detect T-cell sensitization to drug invitro.'·'-'"·"Interestingly, positive LTT can be observed after I0 or more years after reaction without further exposure to the drug." This technology is very promising for future developments upon the role and function of T-lymphocytes for both diagnostic and research purposes to improve our understanding of the patophysiology of dmg allergic reactions.'

In several stodics pcrfomcd and other reports published to date, the LTT had a general sensitivity of 60-70%, 85% specificity, and was often superior to skin testing for nonimmediate type reactions. A lower sensitivity rate of 33% was reported by Barna and colleagues. A prominent retrospestive analysis that included 923 patients with various adverse dmg reactions indicated that the sensitivity of the LTT depends on the dmg tested.">'

A retrospective evaluation of the sensitivity and spesificity of the LTT with a hlgh amount of LTT reactions tc J3-lactarns antibiotics revealed a sensitivity of78% and specificity of85% to 93%, confirming that LTT is the best diagnostic tool in patient allergic to P-lactams."""" The LTT sensitivity is higher than other skin test (62%)." Pichler and Tilch recommended that LTT should be considered a useful invitro diagnostic tool to identify subjets allergic to penicillins, especially patients with nonimmediate reactions where the LTT has a better diagnostic value than skin test."Whereas the sensitivity of the OPT was lower than LTT, and

specificity ofDPT is similar to LTT.' Overall, with DPT and LTT together allowed the identification of the eliciting drug in 76% of the patient." Furthermore, this test is not available everywhere and is still considered a **researchtool.**[5,19]

Pichler and Tilch had observed positive reactions of LTT 10-20 years after the original treatment of - lactmns, which had originally had caused a delayed reaction. Some patients appear to lose reactivity in 3-4 years. At present, one cannot predict whether the drug reactivity will persist or not, and whether those which have lost its reactivity will tolerate the drug again. Therefore it is recommended to perform the test within 2-3 years after the reaction.'

Inthis case, LTT was performed 6 months after the acute event. The possibility of LTT negative response, because it was performed in recovery stage. Based on the studies, LTT results in most cases of SJS or TEN show only weak positive or even negative response, especially in recovery stage. Many authors recommend performing LTT dnring the acute stage, within 1 week after the disease onset (or less than 3 to 6 months after the acute event) to get the highest sensitivity.""-"

Based onliterature, it is considered to apply cut-off value for a positive response if SI greater than 2 and greater 3 for fl-lactams (SI between 2 and 3 is considered to be weakly positive). The relevance of such a low proliferation is hard to judge without additional clinical information or other tests. Weak response of LTT could already indicate sensitization. Drug exposed but not allergic individuals do not mount a proliferative reaction to a drug."·"The result ofLTT in this case showed SI<2 for each drug (SI for amoxycillin was 1.508 and SI for paracetamol was 1.178), revealed negative respons of LTT."

High Sl values in the LTT are not necessarily associated with the severity of clinical symptoms of CADR. Patients with severe forms of drug hypersensitivity, such as SJS or TEN often show only a weak positive or even negative response ofLTT."-"The LTT reflects only the reactivation and proliferation of memory cells that are present in the peripheral blood of allergic patients, and the high precursor frequency of these cells is not necessarily associated with more severe clinical symptoms.Infact, the clinical severity of a CADR seems to be more closely related to the effector function ofthc reactive T-cclls. Thus, in some forms of drug hypersensitivity, a cytok:ine-based invitro assay could be more useful than proliferation-based assays like the LTT."

In the case of severe CADR including SJS, the most important thing is to establish the suspected causal drug. DPT is should be used as first line of investigation relevant drug, however negative result of DPT like in this case docs not exclude the suspected drug. The LTT had better diagnostic value than skin test. Both DPT and LTT should be performed within 6 weeks to 6 months after complete healing of skin lesions. The positive cut-offvalue ofLTT is SI greater than 2 and greater 3 for - lactmns. Weak response of LTT could already indicate sensitization. The relevance of such a low proliferation is hard to judge without additional clinical information or other tests. High SI value in LTT is not associated with the severity of clinical symptoms. Patients with severe drug hypersensitivity reaction often show weak positive **or even negative LTT response, as in this case.** Cytotoxicity or a cytokine-based invitro assay is considered to be more useful than proliferation-based assays like the LTT.

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