GENETIC SUSCEPTIBILITY TO CARBAMAZEPINE-INDUCED CUTANEOUS ADVERSE DRUG REACTIONS

Thesis

submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai

for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

THE FACULTY OF MEDICINE AND MEDICAL SPECIALTIES

by

Dr. M.R.Sivakumar MD, DM

Department of Experimental Medicine,
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69, Anna Salai, Guindy, Chennai 600 032.

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CERTIFICATE

This is to certify that the thesis entitled "**GENETIC SUSCEPTIBILITY TO CARBAMAZEPINE-INDUCED CUTANEOUS ADVERSE DRUG REACTIONS**" is an original research work done by me, and it was not used previously either partly or fully for the award of any Degree, Diploma, Associate ship, fellowship or other similar title.

Place: Chennai

Date: 15.06.2012

Dr. M.R. Sivakumar

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CERTIFICATE

This is to certify that the thesis entitled " **GENETIC SUSCEPTIBILITY TO CARBAMAZEPINE-INDUCED CUTANEOUS ADVERSE DRUG REACTIONS**" submitted by Dr. M. R. SIVAKUMAR for the Award of the degree of Doctor of Philosophy in Medicine is a bonafide record of research done by him during the period of study under our supervision and guidance, and that it has not formed the basis for the award of any Degree, Diploma, Associate ship, Fellowship or other similar title. We also certify that this thesis is his original independent work. We recommend that this thesis should be placed before the examiners for their consideration for the award of the Ph.D Degree.

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This Thesis is dedicated to my Parents M.R.Rajappa and Late Mrs. Saraswathy Rajappa, and all my Teachers especially Late Prof. K. Jagannathan.

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M.R. Sivakumar

CONTENTS

PREFACE

Idiosyncratic Drug Reaction

An idiosyncratic drug reaction may be defined as 'any adverse effect that cannot be explained on the basis of the known mechanisms of action of the drug and occurs mostly unpredictably in susceptible individuals only, irrespective of dosage'. Idiosyncratic reactions are relatively common in patients treated with antiepileptic drugs (AEDs), and can be life-threatening. Over the years, evidence has accumulated that many of these reactions depend on genetic factors.

Cutaneous hypersensitivity reactions

Immune-mediated cutaneous hypersensitivity reactions are the most common idiosyncratic reactions to AEDs, and affect 5-15% of patients started on treatment with carbamazepine (CBZ), phenytoin (PHT), phenobarbital and lamotrigine (LTG). These reactions usually consist of mild erythematous or maculopapular rashes. However, the same AEDs are also associated with a risk of potentially life-threatening Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-related rash with eosinophilia and systemic symptoms (DRESS), with a frequency that ranges between 1 and 10 per 10 000 new users of these drugs.

Observations in identical twins and in families suggest a genetic predisposition to these reactions, although the underlying mechanisms are not fully understood.

Because the studies investigating the association between *HLA-B*1502* and CBZ-induced SJS/TEN used a case-control design, they provide data on sensitivity and specificity (in this case, 100 and 97%, respectively) but not on positive and negative predictive values, which are dependent upon the frequency of the reaction in the population being tested. Assuming a SJS frequency of 0.25% among CBZ users in high-risk ethnic populations, it may be estimated that presence of the *HLA-B*1502* allele has a 7.7% positive predictive value for CBZ-induced SJS/TEN, and its absence a 100% negative predictive value.

Although much attention has been focused on *HLA-B*1502*, other HLA genotypes are being investigated as potential predictors of AED-induced cutaneous reactions. In a study in Chinese, maculopapular reactions to CBZ were found to be associated with single nucleotide polymorphisms (SNPs) in the HLA-E region and a nearby allele, *HLA-A*3101*, whereas DRESS was associated with SNPs in the motilin gene located terminal to the HLA class II genes. A study in Caucasians suggested that the *HLA-B*0702* allele is potentially protective against severe CBZ-induced hypersensitivity. Further investigations are required to clarify the clinical significance of these findings, and the potential mechanisms involved.

Pharmacogenomic research is impacting clinical practice by improving our understanding of genetic profiles as risk factors for idiosyncratic reactions. Most findings come from association studies, and the quality of evidence is sometimes inconclusive. The greatest progress has been in the definition of HLA-related genes

as predictors of AED-induced SJS and TEN. Results demonstrate the complexity of genetic modulation of drug responses, including drug-related and ethnic specificity, and their practical application in making possible the identification of patients at risk of developing severe reactions to CBZ and, possibly, other AEDs.

Recent studies have investigated the role of genetic factors in the development of antiepileptic drug-induced cutaneous reactions, carbamazepine and valproate-induced liver toxicity, vigabatrin-induced visual field defects, and antiepileptic drug-induced teratogenicity.

INTRODUCTION

Adverse drug reactions (ADRs) are a major clinical problem. According to a widely cited meta-analysis, ADRs was ranked between the fourth and sixth most common cause of death (Lazarou[1] et al., 1998), and cause 6-7% of hospitalization (JAMA, 2006). Potentially serious cutaneous ADRs account for about 2-3% of all hospital admissions (Bigby[2] et al., 1986). Although drug eruptions may be mild to moderate, such as maculopapular rash, erythema multiforme (EM), urticaria, and fixed drug eruption, more severe cutaneous ADRs such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN; Lyell's syndrome) are life-threatening and frequently result in death.

SJS is characterized by high fever, malaise, and a rapidly developing blistering exanthema of macules and target-like lesions accompanied by mucosal involvement. TEN has similar presentations with an even more extensive skin detachment and a higher mortality rate (30 to 40%). Although the incidence of SJS/TEN is rare with an annual estimated incidence of 3-5 per million people, these conditions can kill or severely disable previously otherwise healthy people (Roujeau[3] and Stern, 1994). The severity of the condition has prompted pharmaceutical companies to withdraw a few newly released drugs.

Almost all SJS/TEN cases are caused by drugs, most commonly sulfonamides, anticonvulsants, allopurinol, nonsteroidal anti-inflammatory drugs (NSAIDs), and antimalarials (Roujeauet[4]et al., 1995). In Taiwan, anticonvulsants

(carbamazepine, phenytoin and phenobarbital), and allopurinol are the most common drugs causing SJS/TEN.

Sushma M et al from, Bangalore, India, reported on the cutaneous adverse drug reactions in a retrospective hospital-based study. Of a total of 3541 patients, 404 (11.4%) were diagnosed as cutaneous ADRs, of which 52% were males and 48% females. A majority of the patients were in the age group of 21-40 years. The most common type of ADR was maculopapular rash (42.7%), followed by Stevens-Johnson syndrome (SJS) (19.5%) and fixed drug eruption (11.4%). The drug class implicated was antibiotics (45%), followed by antiepileptics (19%) and NSAIDs (19%). The incidence of life threatening cutaneous ADRs like SJS and TEN were found to be higher compared to studies published abroad.

KompellaVB[5]et al reported on the ophthalmic complications and management of Stevens-Johnson syndrome at a tertiary eye care center in South India. They retrospectively reviewed the medical records of patients with Stevens-Johnson syndrome seen between1987-1998. A total of 95 patients were diagnosed with SJS during the 11-year period. The total number of patients seen in the outpatient department during this period was 226,760. Thus, the prevalence of SJS in their center was 0.0004%.

Recent developments of **pharmacogenomics** have implied that the susceptibility to ADRs is associated with genetic variants. A successful example of application of pharmacogenomic study to prevent drug-induced side effect is genotyping thiopurine methyltransferase (TPMT) before prescribing azathioprine, a

drug for rheumatologic or cancer diseases (Yates et al., 1997). An individual's genomic polymorphism(s) of TPMT can cause enzyme deficiency and slow metabolizing rate, resulting in leukocytopenia.

Although susceptibility to SJS/TEN on certain drugs is thought to be genetically determined (Gennis M A, 1991; Edwards[6] SG, 1999), the responsible genetic factors have yet to be identified and currently there is no method clinically useful that can be used to predict who will develop SJS/TEN or to which drugs.

Incidence of adverse cutaneous drug reactions and prevalence of *HLA B*1502* in normal population and carbamazepine-induced SJS and TEN

The prevalence of *HLA-B*1502* in major populations

Continent and ethnicity	Incidence of allele (%)	No
North America:		
Asian	5	396
African	<1	251
Hispanic	0	240
European	0	287
Native American	0	235
Asia:		
Singapore	12	86
Han Chinese	10	572
Malay	8	101
Thai 6 99		
India (Khandesh Pawra)	6	50
Filipino	5	94
India (North Hindi)	2	91
India (Mumbai Marathas)	1	72
Korean	<1	200

REVIEW OF LITERATURE

Background

Stevens-Johnson syndrome (SJS) is an immune-complex–mediated hypersensitivity complex that typically involves the skin and the mucous membranes. While minor presentations may occur, significant involvement of oral, nasal, eye, vaginal, urethral, gastrointestinal (GI), and lower respiratory tract mucous membranes may develop in the course of the illness. GI and respiratory involvement may progress to necrosis. Stevens-Johnson syndrome is a serious systemic disorder with the potential for severe morbidity and even death.

The syndrome was first described in 1922, when the American paediatricians Albert Mason Stevens and Frank Chambliss Johnson[7] reported the cases of 2 boys aged 7 and 8 years with "an extraordinary, generalized eruption with continued fever, inflamed buccal mucosa, and severe purulent conjunctivitis." Both cases had been misdiagnosed by primary care physicians as haemorrhagic measles. The condition was characterized by severe purulent conjunctivitis, severe stomatitis with extensive mucosal necrosis, and purpuric macules. It became known as SJS and was recognized as a severe mucocutaneous disease with a prolonged course and potentially lethal outcome that is in most cases drug-induced.

Erythema multiforme (EM), originally described by von Hebra in 1866, was part of the differential diagnosis in both cases but was excluded because of the "character of skin lesions, the lack of subjective symptoms, the prolonged high fever, and the terminal heavy crusting." Despite the presence of leucopoenia in both cases,

Stevens and Johnson in their initial report suspected an infectious disease of unknown aetiology as the cause.

In 1950, Thomas divided EM into 2 categories: erythema multiforme minor (von Hebra) and erythema multiforme major (EMM). Since 1983, erythema multiforme major and Stevens-Johnson syndrome had been considered synonymous.

In the 1990s, however, Bastuji and Roujeau each proposed that erythema multiforme major and Stevens-Johnson syndrome are 2 distinct disorders[8]. They suggested that the denomination of erythema multiforme should be restricted to patients with typical targets or raised oedematous papules, with or without mucosal involvement. This clinical picture is in accordance with the original description by von Hebra.

Bastuji and Roujeau further proposed that the denomination of Stevens-Johnson syndrome should be used for a syndrome characterized by mucous membrane erosions and widespread small blisters that arise on erythematous or purpuric maculae that are different from classic targets.

According to this clinical classification, erythema multiforme major and Stevens-Johnson syndrome could be 2 distinct disorders with similar mucosal erosions, but different patterns of cutaneous lesions. This hypothesis is supported further by a strong correlation between clinical classification and the probable cause.

Several investigators propose that Stevens-Johnson syndrome and toxic epidermal necrolysis (TEN) represent the same disease at different levels of severity.

A unifying classification of "acute disseminated epidermal necrosis" or "exanthematic necrolysis" has been suggested.

A simple classification of the disease is as follows[9].

1. Stevens-Johnson syndrome - A "minor form of TEN," with less than 10% body surface area (BSA) detachment
2. Toxic epidermal necrolysis - Detachment of more than 30% BSA
3. Overlapping Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) - Detachment of 10-30% BSA

In 1956, Alan Lyell described four patients with an eruption resembling scalding of the skin which he called toxic epidermal necrolysis or TEN[10]. It was only as more patients with TEN were reported in the years following Lyell's original publication, that it became clear that TEN was drug induced, and that certain drugs such as sulfonamides, pyrazolones, barbiturates, and antiepileptics were the most frequent triggers of TEN. Increasingly to date, SJS and TEN are considered to be two ends of a spectrum of severe epidermolytic adverse cutaneous drug reactions, differing only by their extent of skin detachment.

Epidemiology

SJS and TEN are rare diseases in absolute numbers with an incidence of 1.89 cases of TEN per million inhabitants per year reported for Western Germany and Berlin in 1996[11]. La Grenade et al report similar results, with 1.9 cases of TEN per million inhabitants per year based on all cases reported to the FDA AERS database in the USA[12]. Lower incidence rates were reported by Chan et al in Singapore[13]. Certain infectious diseases may have an impact on the incidence of TEN, and this is clearly the case for HIV where the annual incidence is approximately 1000-fold higher than in the general population, with approximately 1 case per thousand per year in the HIV-positive population[14]. In a study of HIV positive patients of the greater Paris area in the late eighties and early nineties, 15 cases of SJS/TEN were reported in patients with AIDS compared to 0.04 expected cases[15]. In another study only ten out of 50 cases of SJS/TEN in HIV patients could be clearly attributed to the use of medications, whereas in the other cases a cause could not be determined due to lack of data of drug intake or details[16].

Regional differences in drug prescription, the genetic background of patients (HLA, metabolizing enzymes), the coexistence of cancer, or concomitant radiotherapy[17, 18], can have an impact on the incidence of SJS and TEN.

Other infections have occasionally been reported as the sole cause. Mycoplasma *pneumoniae* infections are widely documented to cause SJS and TEN without initial exposure to drugs [19, 20].

Wetter et al[21] examined the clinical, etiologic, and histologic features of Stevens-Johnson syndrome and identified possible correlates of clinical disease severity related to etiologic and histopathologic findings.

Herpes simplex virus was recognized in several cases of SJS, especially in children[22]. Single case reports describe Lupus erythematosus[23] or reactivation of Herpes simplex under treatment with azithromycin as potential causes of SJS[24]. The occurrence of TEN in a patient with severe aplastic anaemia after allogeneic hematopoietic stem cell transplantation has also been reported[25]. However, in about 25-50% of cases no obvious identifiable cause can be found.

Strom et al reviewed Medicaid billing data from 1980-1984 in Michigan, Minnesota, and Florida to determine the incidence of Stevens-Johnson syndrome; the incidence rates were 7.1, 2.6, and 6.8 cases per million populations per year, respectively[26]. Cases tend to have a propensity for the early spring and winter.

For overlapping SJS and TEN, oxicam NSAIDs (piroxicam, meloxicam, tenoxicam) and sulfonamides are most commonly implicated in the United States and other western nations[27].

SJS occurs with a worldwide distribution similar in aetiology and occurrence to that in the United States. However, a study from Germany reported only 1.1 cases per 1 million person-years.

In contrast to the drugs most often implicated in western nations, allopurinol is the most common offending agent in Southeast Asian nations, including Malaysia, Singapore, Taiwan, and Hong Kong.

Stevens-Johnson syndrome has been described worldwide in all races, although it may be more common in whites. Interestingly, disease is not limited to humans; cases have been reported in dogs, cats, and monkeys. The proportion of females has been estimated to be 33-62%. The largest series reports 39.9% of females in a group of 315 patients with Stevens-Johnson syndrome. In a large cohort, the mean age of patients with Stevens-Johnson syndrome was 25 years. In a smaller series, the mean age of patients with Stevens-Johnson syndrome was reported as 47 years. However, cases have been reported in children as young as 3 months.

Clinical Features

History

Typically, Stevens-Johnson syndrome (SJS) begins with a nonspecific upper respiratory tract infection. This usually is part of a 1- to 14-day prodrome during which fever, sore throat, cough productive of a thick purulent sputum, chills, headache, and malaise may be present. Vomiting and diarrhoea are occasionally noted as part of the prodrome.

Mucocutaneous lesions develop abruptly. Clusters of outbreaks last from 2-4 weeks. The lesions are typically nonpruritic.

A history of fever or localized worsening should suggest a superimposed infection; however, fever has been reported to occur in up to 85% of cases.

Involvement of oral and/or mucous membranes may be severe enough that patients may not be able to eat or drink. Patients with genitourinary involvement may complain of dysuria or an inability to void.

A history of a previous outbreak of Stevens-Johnson syndrome or of erythema multiforme may be elicited. Recurrences may occur if the responsible agent is not eliminated or if the patient is re-exposed.

Patients may complain of a burning rash that begins symmetrically on the face and the upper part of the torso. This may be accompanied by ocular symptoms.

In addition to the skin, lesions in Stevens-Johnson syndrome may involve the following parts of the body:

- Oral mucosa
- Oesophagus
- Pharynx
- Larynx
- Anus
- Trachea
- Vagina
- Urethra

Ocular symptoms include the following:

- Red eye

- Tearing
- Dry eye
- Pain
- Blepharospasm
- Itching
- Grittiness
- Heavy eyelid
- Foreign body sensation
- Decreased vision
- Burn sensation
- Photophobia
- Diplopia

Delineation of a drug exposure time line is essential, especially in the 1-3 weeks preceding the cutaneous eruption.

Physical Examination

The rash can begin as macules that develop into papules, vesicles, bullae, urticarial plaques, or confluent erythema. The center of these lesions may be vesicular, purpuric, or necrotic.

The typical lesion has the appearance of a target; this is considered pathognomonic. However, in contrast to the typical lesions of erythema multiforme, these lesions have only two zones of colour. The core may be vesicular, purpuric, or

necrotic; that zone is surrounded by macular erythema. Some have called these ‘targetoid’ lesions.

Lesions may become bullous and later rupture, leaving denuded skin. The skin becomes susceptible to secondary infection. Extensive sloughing can occur.

Urticarial lesions typically are not pruritic. Infection may be responsible for the scarring associated with morbidity.

Although lesions may occur anywhere, the palms, soles, dorsum of the hands, and extensor surfaces are most commonly affected. Desquamation on the foot is common.

The rash may be confined to any one area of the body, most often the trunk. Mucosal involvement may include erythema, oedema, sloughing, blistering, ulceration, and necrosis.

Although some have suggested the possibility of Stevens-Johnson syndrome without skin lesions, most believe that mucosal lesions alone are not enough to establish the diagnosis. Cases without skin lesions have been termed "atypical" or "incomplete[28]." These authors suggested that the combination of urethritis, conjunctivitis, and stomatitis established the diagnosis of Stevens-Johnson syndrome in a patient with *Mycoplasma pneumonia*– induced signs and symptoms.

The following signs may be noted on examination:

- Fever
- Orthostasis
- Tachycardia

- Hypotension
- Altered level of consciousness
- Epitasis
- Conjunctivitis
- Corneal ulcerations
- Erosive vulvovaginitis or balanitis
- Seizures
- Coma

The following signs may be noted on external examination:

- Conjunctival hyperemia
- Entropion
- Skin lesions
- Nasal lesions
- Mouth lesions
- Discharge (i.e. catarrhal, mucous, membranous)

The following ocular signs may be noted on slit lamp examination

Eyelids: Trichiasis, distichiasis, meibomian gland dysfunction, blepharitis

Conjunctiva: Papillae, follicles, keratinization, subepithelial fibrosis, conjunctival shrinkage, foreshortening of fornices, symblepharon and ankyloblepharon

Cornea: Superficial punctate keratitis, epithelial defect, stromal ulcer, neovascularization, keratinization, limbitis, conjunctivalization, stromal opacity and perforation.

The clinical features may be described in 3 phases.

1. **Acute Phase**

Initial symptoms of toxic epidermal necrolysis (TEN) and Stevens Johnson Syndrome (SJS) can be nonspecific and include symptoms such as fever, stinging eyes and discomfort upon swallowing.

Typically, these symptoms precede cutaneous manifestations by a few days. Early sites of cutaneous involvement are the presternal region of the trunk and the face, but also the palms and soles. Involvement (erythema and erosions) of the buccal, genital and/or ocular mucosa occurs in more than 90% of patients, and in some cases the respiratory and gastrointestinal tracts are also affected. Ocular involvement at the onset of disease is frequent, and can range from acute conjunctivitis, eyelid oedema, erythema, crusts, and ocular discharge, to conjunctival membrane or pseduomembrane formation or corneal erosion, and, in severe cases, to cicatrizing lesions, symblepharon, fornix foreshortening, and corneal ulceration. The severity of acute ocular manifestation is not however predictive of late complications[29].

The morphology of early skin lesions includes erythematous and livid macules, which may or may not be slightly infiltrated, and have a tendency to rapid

coalescence. The above mentioned skin signs associated with mucosal involvement are clear danger signs and warrant the initiation of rapid diagnostic confirmation with immediate cryosections of a skin biopsy. Histological examination including direct immunofluorescence analysis of the skin biopsy is also important in order to rule out differential diagnoses such as autoimmune blistering diseases, bullous fixed drug eruption, acute generalized exanthematous pustulosis, and staphylococcal scalded skin syndrome.

2. **In a second phase**, large areas of epidermal detachment develop. In the absence of epidermal detachment, more detailed skin examination should be performed by exerting tangential mechanical pressure on several erythematous zones (Nikolsky sign). The Nikolsky sign is positive if mechanical pressure induces epidermal detachment, but is not specific for TEN or SJS, as it can also be positive in, for example, autoimmune bullous skin diseases.

   The extent of skin involvement is a major prognostic factor. It should be emphasized that only necrotic skin, which is already detached (e.g. blisters, erosions) or detachable skin (Nikolsky positive) should be included in the evaluation of the extent of skin involvement.

3. **Late phase and sequelae**

Sequelae are common features of late phase TEN. According to the study of Magina et al[30] following symptoms are found: hyper- and hypopigmentation of the skin (62.5%), nail dystrophies (37.5%), and ocular complications. According to a

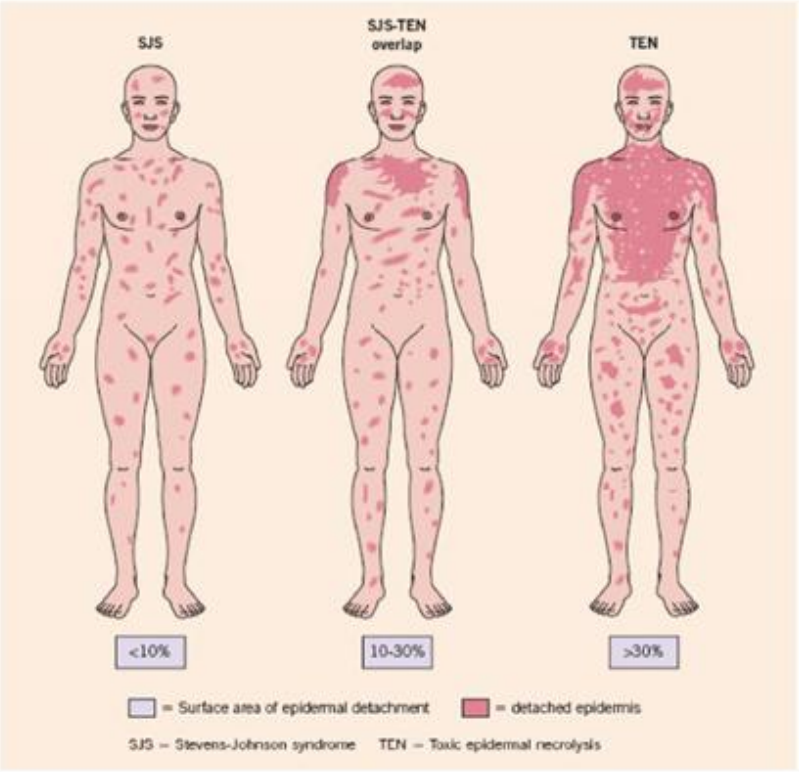
study of Yip et al. 50% of patients with TEN develop late ocular complications including, by order of decreasing frequency, severe dry eyes (46% of cases), trichiasis (16%), symblepharon (14%), distichiasis (14%), visual loss (5%), entropion (5%), ankyloblepharon (2%), lagophthalmos (2%), and corneal ulceration (2%). Hypertrophic scars are only seen in very few patients[31].

Clinical features that distinguish SJA, SJS-TEN overlap, and TEN

Clinical entity	**SJS**	**SJS-TEN overlap**	**TEN**
Primary lesions	Dusky red lesions Flat atypical targets	Dusky red lesions Flat atypical targets	Poorly delineated erythematous plaques Epidermal detachment Dusky red lesions Flat atypical targets
Distribution	Isolated lesions Confluence (+) on face and trunk	Isolated lesions Confluence (++) on face and trunk	Isolated lesions (rare) Confluence (+++) on face, trunk, and elsewhere
Mucosal Involvement	Yes	Yes	Yes
Systemic symptoms	Usually	Always	Always
Detachment (% Body surface area)	< 10	10-30	> 30

Bastuji-Garin et al. proposed classifying patients into three groups according to the degree of skin detachment.

Fig.1. Pictorial representation of SJS, SJS-TEN overlap and TEN showing the surface of epidermal detachment



Long-term complications of mucosal involvement occur in 73% of patients who present mucosal involvement in the acute phase, and the mucosal sequelae involve mainly the oral and oesophageal mucosa, and to a lesser extent lung and genital mucosa[32].

In a small post SJS/TEN study seven out of nine patients had either xerostomia or keratoconjunctivitis or both, resembling Sjögren-like syndrome[33]. Additionally, another group reported a patient with Sjögren-like pluriglandular exocrine insufficiency including exocrine pancreatic impairment[34].

Gastrointestinal Involvement

Both oral and pharyngeal cavities are typically involved in Stevens-Johnson syndrome. Oral lesions, which commonly begin as vesicles, rupture, forming a gray-white membrane. The lips are often painful and crusted with blood. Many patients suffer severe dysphagia, preventing them from ingesting adequate amounts of fluids and nutrition. Oral mucous membranes are usually severely eroded for 10 to 14 days; however, prolonged ulceration of the gastrointestinal tract months after recovery has been reported. Although typically the oropharyngeal cavity is involved, erosion and sloughing may extend the entire length of the gastrointestinal tract, resulting in malnutrition, pain, and bleeding. The most severe gastrointestinal complication is bleeding. Mortality is rarely associated with this complication however, transfusion may be required.

Respiratory Manifestations

Respiratory complications are frequently associated with Stevens-Johnson syndrome and are often the cause of death. Pneumonitis, bronchial pneumonia, and bronchiolitis have been reported to occur in approximately 30 percent of cases with severe Stevens-Johnson syndrome. More than half of these individuals subsequently develop respiratory failure, requiring intubation and ventilatory support to prevent increased morbidity and mortality. Respiratory failure is associated with mucus retention and sloughing of the tracheobronchial mucosa, which contributes to the prolonged need for support when such failure occurs. Many patients experience a

late respiratory deterioration. This deterioration may be caused by immune complexes that are formed in response to an inciting antigen. Such immune complexes have been isolated from cutaneous lesions during early stages. Immune complexes have been known to produce alveolar disease, but the antigen has not been identified.

Infections

Patients presenting with Stevens-Johnson syndrome will have complicating infections in 60 to 75 percent of cases. Impaired host defence probably contributes to the high incidence of infection. Infections are the single greatest cause of mortality in these patients. Common infections are gram-negative pneumonias and septicaemias. Organisms that have been isolated include Pseudomonas aeruginosa, Klebsiella, Staphylcoccus aureus, and Candida species. Leucopoenia generally occurs within 72 hours after the skin lesions have appeared. Aetiology of this leucopoenia is uncertain. Evidence suggests that humoral toxins may be released into the circulation, suppressing the bone marrow. Another cause could be the silver sulfadiazine. As noted, skin sloughing oftentimes resembles that of a burn patient and is commonly treated with silver sulfadiazine. This drug has been associated with leucopoenia in burn patients.

Etiology and pathogenesis

Various etiologic factors (e.g. infection, drugs, malignancies) have been implicated as causes of Stevens-Johnson syndrome however, as many as half of cases are idiopathic. The four etiologic categories are as follows:

- Infectious
- Drug-induced
- Malignancy-related
- Idiopathic

Stevens-Johnson syndrome is idiopathic in 25-50% of cases. Drugs and malignancies are most often implicated as the aetiology in adults and elderly persons. Paediatric cases are related more often to infections.

Infectious causes

Viral diseases that have been reported to cause Stevens-Johnson syndrome include the following:

- Herpes simplex virus (possibly; remains a debated issue)
- AIDS
- Coxsackie viral infections
- Influenza
- Hepatitis
- Mumps
- In children, Epstein-Barr virus and Enteroviruses have been identified.

More than half of the patients with Stevens-Johnson syndrome report a recent upper respiratory tract infection.

Bacterial aetiologies include the following:

- Group A beta-haemolytic streptococci

- Diphtheria
- Brucellosis
- Lymphogranuloma venereum
- Mycobacteria
- *Mycoplasma pneumoniae*[35]
- Rickettsial infections
- Tularemia
- Typhoid

Fungal causes include coccidioidomycosis, dermatophytosis, and histoplasmosis.

Protozoal causes reported include Malaria and Trichomoniasis.

Drug-induced

Drug exposure and a resulting hypersensitivity reaction is the cause of the very large majority of cases of SJS/TEN.

Risk estimates of Drugs in Common Use

Drug	**Drug case** (N = 245)	**Controls** (N=1147)	**Crude** RR	**Multivariate** RR(**95% CI)**
Contraceptive pills	11 (19)	53 (20)	1.0	0.4(0.1–1.5)
Benzodiazepines	28 (11)	84 (7)	1.6	0.3(0.1–0.7)
Phenothiazines	11 (5)	16 (1)	3.3	1.6(0.5–5.4)
Sulfonylureas	4 (2)	13 (1)	1.4	0.7(0.1–3.2)

Thiazide diuretics	17 (7)	44 (4)	1.9	1.4(0.5–2.8)
Hydrochlorothiazide	12 (5)	33 (3)	1.7	1.2(0.3–4.6)
Other diuretic agents	19 (8)	55 (5)		
Amiloride	5 (2)	15 (1)	1.6	1.1(0.2–5.2)
Furosemide	4 (2)	16 (1)	1.2	0.3(0.05–1.6)
Fibrate antihyperlipids	10 (4)	21 (2)	2.3	1.0(0.4–2.8)
Angiotensin-converting–Enzyme inhibitors	14 (6)	35 (3)	1.9	1.2(0.5–2.8)
Captopril	6 (2)	23 (2)	1.2	0.9(0.2–3.1)
Calcium-channel blockers	16 (7)	38 (3)	2.0	1.5(0.7–3.5)
Nifedipine	8 (3)	14 (1)	2.7	1.4(0.4–4.6)
Beta-blockers	8 (3)	23 (2)	1.7	1.4(0.4–4.2)
Other antihypertensive and vasodilating agents	20 (8)	48 (4)	2.0	1.0(0.3–3.4)
Isosorbide	8 (3)	19 (2)		
Digitalis Glycosides	9 (4)	16 (1)	2.7	0.8(0.2–2.7)
H1 antihistamines	23 (9)	36 (3)	3.2	1.7(0.8–3.7)
H2 antihistamines	12 (5)	20 (2)	2.9	1.5(0.5–4.2)
Biguanides	4 (2)	15 (1)	1.2	0.8(0.2–3.0)
Levothyroxine	6 (2)	17 (2)	1.7	0.6(0.1–2.5)
Corticosteroids	35 (14)	16 (1)	12	4.4(1.9–10)

*Drugs with a prevalence of use among the controls of at least 1 percent. CI denotes confidence interval.

Estimates of Relative Risk according to the duration of Therapy

CASE DRUG	DURATION (MO)	PATIENTS (N=245)	CONTROLS (N = 1147)		CRUDE RELATIVERISK (95%CI)
Sulfonamides	≤ 2	29	1	156	(66 – 367)
	> 2	2	0	∞	(1.5 – ∞)

		1			
Aminopenicillins	≤ 2	15	12	6.2	(3.1 – 12)
	> 2	0	0		
Quinolones	≤ 2	11	2	18	(7 – 46)
	> 2	0	2	0	(0 – 26)
		1			
Cephalosporins	≤ 2	14	3	23	(9.7 – 55)
	> 2	0	0		
Phenobarbital	≤ 2	18	2	45	(19 – 108)
	> 2	7	6	5.8	(2.2 – 82)
		3	1		
Carbamazepine	≤ 2	13	0	∞	(19 – ∞)
	> 2	0	6	0	(0 – 32)
Phenytoin	≤ 2	8	0	∞	(11 – ∞)
	> 2	0	3	0	(0 – 12)
Valproic acid	≤ 2	4	0	∞	(4.3 – ∞)
	> 2	6	4	7.3	(2.5 – 22)
Oxicam NSAIDs	≤ 2	15	1	72	(25 – 209)
	> 2	0	2	0	(2.5 – 22)
Propionic acid	≤ 2	10	9	5.4	(2.4 – 12)
NSAIDs	> 2	2	4	2.4	(0.4 – 13)
Allopurinol	≤ 2	11	1	52	(16 – 167)
	> 2	1	9	0.5	(0.1 – 4)
		1	1		
Chlormezanone	≤ 2	13	1	62	(21 – 188)
	> 2	0	0		
Corticosteroids	≤ 2	20	2	54	(23 – 124)
	> 2	15	14	5.8**	(3– 11)

*CI denotes confidence interval.

This large case–control study determined with substantial precision the risks of toxic epidermal necrolysis and Stevens–Johnson syndrome associated with the use of the most commonly prescribed drugs. It confirms that the use of antibacterial sulfonamides, oxicam NSAIDs, chlormezanone, anticonvulsant agents, and

allopurinol is associated with substantial relative increases in the risk of toxic epidermal necrolysis and Stevens–Johnson syndrome. Significant associations were also observed for many antibiotics and, unexpectedly, for corticosteroids. With all drugs associated with the conditions, the excess risks were low.

The highest rate, for sulfonamides, was 4.5 cases per million users per week. For many drugs, the risk of Stevens–Johnson syndrome and toxic epidermal necrolysis was highest in the first weeks of use. This confirms the clinical impression and has implications for understanding the mechanisms of these disorders and for therapy. Sulfonamides have often been implicated as a cause of Stevens–Johnson syndrome and toxic epidermal necrolysis. In this study, trimethoprim–sulfamethoxazole was the sulfonamide most frequently used by case patients. Despite their structural relations to antibacterial sulfonamides, thiazide diuretics and sulfonylureas were not associated with increased risks.

Many antibiotics have previously been implicated in at least a few case reports. Because fever may begin a few days before the skin manifestations, the reaction might be related to infection rather than to the drugs. The authors found significant associations for most classes of antibiotics, including Cephalosporins, quinolones, aminopenicillins, tetracycline, and imidazole antifungal agents. An association for all anti-infective drugs could suggest some confounding by indication. The associations remained significant, with lower point estimates, when a term for recent infection was included in the multivariate model. This result and the reports of cases related to prophylactic administration of long-acting sulphonamides suggest that antibiotics and not infection cause the reaction.

Among NSAIDs, butazone derivatives (phenylbutazone and oxyphenbutazone) have long been implicated. Because these drugs are now seldom used, no information about them was available for the current study. Oxicam derivatives were also suspected. Isoxicam was withdrawn from the market in France after having been associated with 13 cases of toxic epidermal necrolysis. The two currently marketed Oxicams, piroxicam and tenoxicam, were significantly associated, and risks were significantly higher for them than for diclofenac and propionic acid derivatives. The risks were linked to recently initiated therapy. When the analysis was restricted to treatment of two months or less, the risk increased with Oxicams but not with propionic acid derivatives. The prevalence of the use of other NSAIDs was too low to permit an analysis of individual drugs.

Severe adverse cutaneous reactions, including Stevens–Johnson syndrome and toxic epidermal necrolysis, have long been associated with the use of aromatic anticonvulsant drugs (phenobarbital, phenytoin, and carbamazepine). The current study demonstrated that valproic acid, often viewed as safer with respect to cutaneous reactions, had a significant risk that was similar to that of aromatic anticonvulsants. For all anticonvulsants, the risk was greatest in the first two months of treatment, although some increased risk persisted among long-term users of phenobarbital and valproic acid.

Allopurinol, which is most often administered for long periods, is frequently cited as a cause of Stevens–Johnson syndrome and toxic epidermal necrolysis. The

risk is not constant over time. The relative risk with any use underestimates the risk during the first two months of therapy and overestimates the risk with long-term therapy.

Chlormezanone is a minor tranquilizer not related to the benzodiazepines that has muscle-relaxing properties and sedative effects. Frequently prescribed in Europe together with NSAIDs and analgesics, chlormezanone has been suspected of inducing severe cutaneous reactions. The results of their study indicate a high relative risk.

Since Stevens–Johnson syndrome and toxic epidermal necrolysis are probably mediated immunologically and corticosteroids prevent other types of drug reactions, the significant increase in risk associated with exposure to corticosteroids was surprising. Topical and systemic corticosteroids, however, can induce contact dermatitis and other skin reactions. Toxic epidermal necrolysis can occur in spite of high doses of systemic corticosteroids. No explanation is apparent for the high risk observed with recently initiated corticosteroid therapy. This association does not appear to be due to underlying diseases for which the drugs were sometimes used (e.g. collagen vascular diseases and brain tumours) or to the use of other drugs associated with Stevens–Johnson syndrome and toxic epidermal necrolysis. The

relative risk remained significantly elevated when subjects with these factors were excluded.

Acetaminophen was not a significant risk factor in France. In contrast, the multivariate risk was 9.3 in other countries. The rates of use in the various countries were similar among case patients but quite different among controls: 13 percent in France as compared with 2.5 percent in the other countries (range, 1 to4 percent). The annual sales of acetaminophen in France are 10 and 20 times those in Germany and Italy, respectively. In other studies in Italy and France, estimated acetaminophen use in controls was similar to that in ours. These findings support their observation about variation in use among controls. Acetaminophen is used mainly as an antipyretic in Italy, Germany, and Portugal and as an analgesic in France. Confining the analysis to subjects with fever, however, did not explain the difference in risk between countries. One hypothesis is that the prevalence of use in France is so high that repeated exposures could lead to either the selection of patients who do not react or the induction of tolerance. It has recently been suggested that people's past experience with NSAIDs decreases the overall risk of NSAID-associated bleeding in the upper gastrointestinal tract, perhaps because susceptible people select themselves out of the population at risk.

In absolute case numbers, allopurinol is the most common cause of SJS/TEN in Europe and Israel[36], and mostly in patients receiving daily doses of at least 200 mg.

In a case control study of patients hospitalized for SJS/TEN in selected hospitals in France, Germany, Italy and Portugal between 1989 and 1993,

Roujeau et al. reported that the following drugs are at increased risk of inducing SJS/TEN when used over a short period of time:

- Trimethoprim-sulfamethoxazole and other sulfonamide-antibiotics
- Aminopenicillins
- Cephalosporins
- Quinolones
- Chlormezanone
- Modafinil
- Allopurinol[37]
- Mirtazapine
- TNF-alpha antagonists (e.g. infliximab, etanercept, adalimumab)[38]
- Cocaine

The following anticonvulsants have been implicated:

- Phenytoin
- Carbamazepine
- oxcarbazepine
- Valproic acid
- Lamotrigine
- Barbiturates

Among drugs usually taken for longer periods of time (carbamazepine, phenytoin, phenobarbital, valproic acid, non-steroidal anti-inflammatory drugs of the oxicam-type, allopurinol and corticosteroids), the highest risk of induction of

SJS/TEN occurs during the first 2 months of treatment with a sharp drop of incidence thereafter. However, although these drugs have a high relative risk compared to other drugs, the actual risk remained low with 5 cases or less per million users per week.

Studies in different populations indicate that the risk of developing SJS/TEN is highest when the drug has been recently initiated, and subsequently declines within 8 weeks or more of administration[39]. Interestingly, the long term use of gluco-corticosteroids for a variety of diseases does not change the incidence of the occurrence of SJS/TEN for the incriminated drugs, but it appears that gluco-corticoids lengthen the interval between the beginning of the intake of the drug and onset of SJS/TEN[40]. A recent survey of TEN in children identified similar drugs to those in adults, as well as an increased susceptibility to acetaminophen (paracetamol) [41].

Photo-induced TEN or SJS are only reported in very rare cases. Case reports exist for hydroxchloroquine[42], naproxene[43] and clobazam[44].

Antibiotics are the most common cause of Stevens-Johnson syndrome, followed by analgesics, cough and cold medication, NSAIDs, psycho epileptics, and anti-gout drugs.

Of antibiotics, penicillins and sulfa drugs are prominent. Ciprofloxacin[45], antiretroviral drugs like nevirapine, abacavir, other non-nucleoside reverse transcriptase inhibitors[46] and Indinavir have all been implicated.

An often addressed issue is the induction of TEN or SJS after vaccination. The vaccine adverse event reporting system concludes that despite the plausibility of a relationship between vaccination and SJS/TEN, the very small number of reports compared to the large amount of vaccinations and the benefits of vaccinations outweighs the potential risk of SJS/TEN[47].

Genetic factors

Genetic factors associated with drug hypersensitivity are a complex issue that has been studied in different populations and a variety of ethnic backgrounds. A unique and strong association between HLA, drug hypersensitivity and ethnic background was discovered by Chung et al. who showed a strong association in Han Chinese between the *HLA-B*1502*, SJS and carbamazepine[48]. This high association with an odds ratio of 2504 led to further studies in a similar ethnical group of Hong Kong Han Chinese with severe adverse reactions to antiepileptic drugs[49]. Another study confirmed the susceptibility of individuals with *HLA-B*1502* to carbamazepine in a Thai population[50]. A smaller Indian based study however showed only a weak correlation between *HLA-B*1502* and carbamazepine induced severe drug allergy.

A genetic correlation could not however be shown in Japanese or Europeans[51-53]. Indeed, in a large European study (RegiSCAR), HLA-B genotyping was performed in patients with severe cutaneous adverse reactions caused by the two previously mentioned drugs (carbamazepine, allopurinol) and another three high risk drugs (sulfamethoxazole, lamotrigine, NSAID's of oxicam-type). This RegiSCAR study revealed that *HLA-B*1502* is neither a marker for carbamazepine,

sulfamethoxazole, lamotrigine, or NSAID's of oxicam-type induced SJS/TEN nor a sufficient explanation for the cause of the disease in Europeans[54]. This leads to the conclusion that this genetic constellation (*HLA-B*1502*) is not a population independent marker for SJS/TEN in carbamazepine exposed individuals. Severe cutaneous reactions in *HLA-B*1502* subjects were not only associated with the drug carbamazepine, but also, to a lesser extent (lower odds ratio), with phenytoin and lamotrigine.

Carbamazepine[55] causes various forms of hypersensitivity reactions, ranging from maculopapular exanthema to severe blistering reactions. The *HLA-B*1502* allele has been shown to be strongly correlated with carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS-TEN) in the Han Chinese and other Asian populations but not in European populations.

McCormack M et al[55] performed a genome wide association study of samples obtained from 22 subjects with carbamazepine-induced hypersensitivity syndrome, 43 subjects with carbamazepine induced maculopapular exanthema, and 3987 control subjects, all of European descent. This study is discussed in detail later.

A second strong association between HLA genotype and SJS/TEN has been reported for allopurinol. 100% of Han Chinese patients with a severe adverse drug reaction to allopurinol were HLA-B*5801 positive[56]. Subsequently, a strong association between SJS/TEN and HLA-B*5801 was found in Japanese patients,

Thai patients, and also, to a lesser extent (55% of cases), in patients of European origin.

There is strong evidence for a genetic predisposition to severe cutaneous adverse drug reactions such as Stevens-Johnson syndrome. Carriage of the following human leukocyte antigens has been associated with increased risk:

- *HLA-B*1502*
- HLA-B*44
- HLA-A29
- HLA-B12
- HLA-DR7
- HLA-A2
- HLA-B*5801
- HLA-A*0206
- HLA-DQB1*0601

Certain of these HLA alleles are associated with an increased probability of developing Stevens-Johnson syndrome upon exposure to specific drugs. The US Food and Drug Administration (FDA) and Health Canada advise screening for *HLA-B*1502* in patients of south-eastern Asian ethnicity before starting treatment with carbamazepine. (The risk is much lower in other ethnic populations, making screening impractical in them). Pre-treatment screening is not readily available.

Whites with HLA-B*44 appear to be more susceptible to develop Stevens-Johnson syndrome. HLA-A29, HLA-B12, and HLA-DR7 are frequently associated

with sulfonamide-induced Stevens-Johnson syndrome, while HLA-A2 and HLA-B12 are often encountered in Stevens-Johnson syndrome induced by nonsteroidal anti-inflammatory drugs (NSAIDs).

HLA-A*0206 and HLA-DQB1*0601 allele have been shown to be was strongly associated with Stevens-Johnson syndrome with ocular disease[57]. Nevertheless, whether the presence of those genes constitutes a predisposition to Stevens-Johnson syndrome or whether those genes are in linkage disequilibrium with more relevant adjacent genes is unknown[58].

Kisalay Ghosh, Gautam Banerjee, Asok Kumar Ghosal, and Jayoti Nandi[59] in their article on 'Cutaneous Drug Hypersensitivity: Immunological and Genetic Perspective' stated that drug hypersensitivity is an unpredictable, immunologically mediated adverse reaction, clustered in a genetically predisposed individual. The role of "hapten concept" in immune sensitization has recently been contested by the "pharmacological interaction" hypothesis. After completion of the "human genome project" and with the availability of high-resolution genotyping, genetic susceptibility to hypersensitivity for certain drugs has been proved beyond doubt though the trend is ethnicity and phenotype dependent. Application of this newly acquired knowledge may reduce or abolish the morbidity and mortality associated with cutaneous drug hypersensitivity.

The morbidity and mortality associated with adverse reactions to different drugs remain a major threat to present-day therapeutics. Many drug reactions involve the skin either in an isolated manner or as a component of systemic involvement. Some of these cutaneous adverse drug reactions (CADR) like

maculopapular rash (MPR) or urticaria are mild, while some others like drug hypersensitivity syndrome (DHS) and Stevens–Johnson syndrome (SJS) are associated with poorer outcomes. Adverse drug reactions (ADRs) can sometimes be explained by the pharmacological property of the drug and are predictable. This is known as “type A” ADR[54]. However, neither certain drug reactions can be explained by the pharmacological nature of the drug nor their occurrence can be predicted. This is categorized as “type B” ADR[60]. The majority of these are caused by immunological response of the host against the parent drug or its active metabolite. The term “drug hypersensitivity reaction” (DHR) is restricted to these immune-mediated “type B” ADRs. Many of these DHRs show familial and ethnic clustering that can only be explained by genetic susceptibility. Drug hypersensitivity is an immunologically mediated response restricted to a few genetically predisposed individuals. If the genetic basis can be used to identify the “at-risk population” or the immunogenic component of the drug can be neutralized, many CADR may be predicted and prevented. This constitutes the basis of “personalized medicine”.

Basic Immunology of Drug Hypersensitivity Reaction

Like all hypersensitivity reactions, DHR can be classified according to Gell and Coomb's classification[61] into four different groups, and the fourth group has recently been classified further into four subgroups (IVa–IVd) [62,63]

Most CADR are caused by type IV or delayed type hypersensitivity reaction (DTHR). The offending drug or its metabolite either diffuse through stratum corneum or enter via systemic circulation and provoke sensitization (*vide infra*). This leads to

stimulation of Langerhans cells (LC) with liberation of inflammatory signals culminating in maturation of LC and their migration to regional lymph node. Consequently, antigen presentation to T-cells takes place through major histocompatibility complex (MHC), which causes expansion of clonal T-cells. Presence of co-stimulatory signals potentiates this expansion. Recently, these T-cells have been found to have heterogeneous function (cytokine production, inflammatory cellular response and subsequent nature of CADR)

Hapten theory

Though sensitization and activation of immune system is thought to occur in all drug hypersensitivities, there is much controversy about the process of sensitization. In 1935, Landsteiner introduced the “hapten concept” to explain the mechanism of sensitization[64]. This concept has so far been used to explain the initiation of sensitization in DHR. A hapten, by definition, is a protein with low molecular weight (<1000 Da) which binds covalently *in vivo* to some protein structure, be it soluble or cell bound. [Prohaptens are proteins which generate hapten after metabolism *in vivo*]. This hapten-carrier complex is then taken up and processed by antigen presenting cells (APC) and presented to T-cells through MHC. As a result, T-cell activation takes place and primary immunological response is generated.

Pharmacological-interaction theory

In recent years, this “haptenation” theory has been challenged by a novel concept called pharmacological-interaction (P-I) theory[62, 65]. According to this theory, the drug can bind directly to the T-cell receptor (TCR) via a non-covalent bond.

Neither is there a need for the drug to bind to any carrier protein nor the drug needs to be metabolically broken down into “haptens”. It has been postulated that for this concept to be significant, the T-cell should express a TCR that can bind the drug and induce stimulatory signal. The T-cells should also have a lower threshold for stimulation so that they are stimulated by minor signal generated by “drug binding to TCR”. Lastly, the TCR should interact with MHC on APC to enhance the immune response[66].

Hapten theory or pharmacological-interaction concept?

The proponents of the hapten theory have cited the example of sulfamethoxazole (SMX)-nitroso which is a metabolic derivative of SMX and acts as a hapten to initiate SMX hypersensitivity. This SMX-nitroso is deactivated *in vivo* by non-protein thiol (glutathione) or ascorbate and it has been shown that people with lower level of thiol and ascorbate (like those suffering from HIV/AIDS) generate more SMX-nitroso and develop SMX hypersensitivity more frequently than those in the normal population[67]. However, using the same SMX model, it was shown that addition of glutathione did not reduce, but rather increased the immune stimulation[68], though SMX-nitroso generation stopped and this could only be explained by the “P-I concept”.

The nickel model: Futile unifying effort or fact?

These two contradictory views could be unified using the nickel model. In this, the nickel ion is shown to act both as a “hapten” as well as a metal ion directly binding to TCR simultaneously[69]. But the concept that priming of T-cells with reactive

metabolites (hapten theory) may result in secondary reactivity toward the parent drug (P-I concept) has recently been refuted[70].

Danger hypothesis: Redefining hapten theory

There was one chink in the armour of "hapten theory" - if certain drug metabolites can act as a hapten, why does not a drug always lead to drug hypersensitivity? The quest for the answer has generated the "danger hypothesis"[71]. According to this hypothesis, the primary signal initiated by the hapten–carrier complex must be strengthened by co-stimulatory signals (danger signals) lest the primary signal should wean off and "tolerance" develops instead of hypersensitivity.

Further substantiation of danger hypothesis

The actual genre of danger signals is presently indefinite, yet the probable candidates are high mobility group box 1 protein (HMGB1), heat shock proteins (HSPs), and S100 proteins[72]. These molecules act through toll-like receptors. Concurrent presence of physical, chemical or viral stress is known to induce cell death and release these danger signals[73], explaining the increased occurrence of drug hypersensitivity in these conditions. Recent studies have shown that polymorphism of the genes involved in synthesis of these "danger signals" (HSP[74, 75] tumour necrosis factor or TNF[76,77]), their "receptors" (toll-like receptor 3[78]) or the "signalling pathway" (proapoptotic Fas-L[79] and IL-4 receptor/IL-13 signalling pathway[80]. can influence the occurrence and severity of drug hypersensitivity.

Genetic Basis of Drug Hypersensitivity Reaction

The idiosyncratic behaviour and familial clustering of the drug hypersensitivities have intrigued researchers to find out a genetic basis. In the earlier days, studies of genetic background in drug hypersensitivity meant twin studies (monozygotic and dizygotic) where both twins suffered from the same disease and got the same treatment. In practical sense, it meant an extremely large and economically unfeasible study. The elementary role of MHC in drug hypersensitivity allured initial researchers to concentrate on human leukocyte antigen (HLA), but the outcome of their studies was confusing. The reason may be small number of cases, inadequate case definition or insensitive serological typing[81].

A shift of idea: Multigenic concept in drug hypersensitivity reaction

Drug hypersensitivity was previously believed to originate from a single gene defect. But now we know that an intricate interaction between many genes as well as environmental factors culminates in drug hypersensitivity. The different genes can be classified function-wise into four groups: a) bio-activation genes, b) bio-inactivation genes, c) genes controlling immune-responsiveness, and d) genes controlling tissue injury and repair[74].

Metabolic gene: A suspect in earlier studies

Many investigators concentrated on polymorphisms of genes responsible for the synthesis of drug metabolizing enzymes. It was postulated that carbamazepine 10-11 epoxide, a major metabolite of carbamazepine (CBZ) both *in vitro* and *in vivo*, leads to immune sensitization, and defective detoxification of this metabolite causes CBZ induced DHR. *In vitro* lymphocyte cytotoxic assay also pointed to a

detoxification defect underlying the propensity of CBZ hypersensitivity. But no specific polymorphism in microsomal epoxide hydrolase gene (responsible for CBZ 10-11 epoxide detoxification) could be detected in two separate studies[82,83] Earlier studies demonstrated that slow acetylator phenotype arising from acetylator gene polymorphism is associated with SMX hypersensitivity both in normal population[84] and in HIV infected patients[85], but recently this claim has been refuted.[86,87] Similarly, in abacavir hypersensitivity, polymorphism of metabolically important genes has been found to be insignificant[76].

Re-exploration of major histocompatibility complex and high-resolution genotyping:

After failure of the "metabolic gene hypothesis", most investigators again turned their focus on MHC. In contrast to earlier studies using serology for HLA-typing, nowadays, high-resolution genotyping is being used for this purpose. Knowledge of linkage disequilibrium pattern[88, 89] has further helped researchers to reach definitive conclusions, particularly in relation to an anti-retroviral drug, abacavir; antiepileptic drugs (AEDs) like carbamazepine (CBZ), oxcarbamazepine (OXC), phenytoin (PHT), lamotrigine (LTG); and the anti-gout drug, allopurinol.

Abacavir and Pharmacogenomics

Abacavir is a nucleoside reverse transcriptase inhibitor with good activity against HIV-1 virus. About 5% of patients develop hypersensitivity reaction to abacavir. Majority of the reactions appear in the initial 6 weeks of drug introduction and present as skin rash, fever, gastrointestinal and respiratory manifestations[90]. They start subsiding within 72 hours of drug withdrawal and recur with more severity

within hours of drug reintroduction[91].Familial clustering and lower risk in Afro-Americans[92] suggested genetic susceptibility in abacavir hypersensitivity. High-resolution genetic assessment has found a strong association of abacavir hypersensitivity with HLA-B*5701, HLA-DR7 and HLA-DQ3 haplotypes (odds ratio>100)[93]. Further studies using recombinant haplotype mapping has placed the responsible locus in the ancestral haplotype 57.1. Haplotypic polymorphism in the TNF promoter region has also been associated with abacavir hypersensitivity[76, 94] and this may control severity by influencing TNF synthesis. It was shown recently[75] that concurrent presence of HLA-B*5701 and haplotypic Hsp 70-Hom variant was more specifically associated with abacavir hypersensitivity (odds ratio 3893, *P*<0.00001). HLA-B*5701 association to abacavir hypersensitivity was later confirmed in White males[95].

Does ethnicity really matter in abacavir hypersensitivity?

The strong association of HLA-B*5701 allele to abacavir hypersensitivity could not be demonstrated in Afro-Americans and this discrepancy was attributed to linkage disequilibrium[95]. A different genetic basis in non-White population might be another possibility. But this ethnic bias has lately been questioned. Saag et al. have found that in immunologically (by patch test) confirmed (but not in clinically suspected) abacavir hypersensitivity, HLA-B*5701 allele is a 100% sensitive marker in Afro-Americans[96]. Besides, screening for HLA-B*5701 has been shown to reduce the incidence of abacavir hypersensitivity among ethnically mixed population[97].

Bridging the gap: Clinical application of pharmacogenomics in abacavir hypersensitivity

Depending on a West Australian HIV cohort, Mallal et al. proposed routine HLA-B*5701 genotyping before prescribing abacavir to Whites[93]. Further studies among predominantly White population confirmed that screening is effective in reducing abacavir hypersensitivity[98, 99] and is cost-effective[100]. Genetic screening has recently been demonstrated to be cost-effective even in multi-ethnic French population[97].

Quest for newer genotyping method: Cheap, easy and quick

The complex and time-consuming HLA typing method using sequence-based primer intrigued scientists to invent an alternative path. Recently, an HIV cohort in Switzerland demonstrated a strong association between HCP5 single-nucleotide polymorphism (SNP) and abacavir hypersensitivity. This SNP, known as rs2395029, is in linkage disequilibrium with HLA-B*5701 in European ancestry and was found in all 98 HLA-B*5701 positive individuals, but absent in 999 of 1005 HLA-B*5701 negative persons. This HCP5 genotyping may be useful in place of sequence-based HLA typing for screening abacavir hypersensitivity[101].A prospective Spanish study[102] found this method easier, cheaper and quicker compared to the conventional sequence based method.

Carbamazepine Hypersensitivity: Second Milestone in Pharmacogenomics

CBZ, SJS/TEN and the Han Chinese

The strongest genetic predisposition for drug hypersensitivity has been documented with CBZ. It is an AED associated with different clinical manifestations of hypersensitivity reaction including maculopapular rash (MPR), drug

hypersensitivity syndrome (DHS) and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The last adverse effect is noted with excess frequency among people from Southeast Asian lineage, particularly Han Chinese, than their European or Japanese counterparts. In a path-finding study among Han Chinese in Taiwan, *HLA-B*1502* allele was found among 100% patients who developed SJS/TEN after taking CBZ. The same allele was present only in 3% of CBZ-tolerant patients and the odds ratio was greater than 2504.49 and corrected *P*-value (P_c) was 3.13×10^{-21}.[103]

Ethnicity matters in carbamazepine hypersensitivity

Early studies suggested that severe hypersensitivity to CBZ may be associated with a genetic polymorphism of the promoter region of the proinflammatory cytokine tumour necrosis factor-α, and with the heat shock protein-70 gene cluster, which may be involved in reactions to stress.

Fig.2. Illustration of the frequency distribution of the *HLA-B*1502* allele in native ethnic groups in different regions of the world based on studies that excluded individuals from populations that migrated in the past 1000 years

The overall average frequency is 1.39%, but frequency is substantially lower among European Caucasians (about 0.001%) and higher in Asia populations originating from the regions highlighted in grey. Because considerable migration has occurred over the centuries, the frequency distribution shown in this illustration does not necessarily reflect the frequency of the allele in populations currently living in the highlighted areas. In particular, high-frequency pockets exist within communities of Asian ancestry living outside Asia. White areas (n.a., no data available) may include regions where only populations that migrated in the past 1000 years were studied. (Based on a meta-analysis of 171 native population samples).

The allelic predisposition to development of SJS/TEN after CBZ intake could not be demonstrated in patients of Japanese[104] or European origin[105]. But multiple studies in non-Chinese Southeast Asian population could replicate the initial result.[106-108] (Among these, the Indian study[108] was methodologically weak as it did not consider the CBZ-tolerant patients). The exact cause of this ethnic difference is not yet clear. Some have attributed this to low prevalence of *HLA-B*1502* allele in non-Southeast Asian population[104]. Another explanation comes from the multigenic concept of drug hypersensitivity which claims that other genes may be responsible in non-Southeast Asian population. A recent study demonstrated that HLA-B*5901 may be one of the alleles responsible for CBZ-induced SJS in Japanese[109]. But the most acceptable concept till date is “linkage disequilibrium” which claims that *HLA-B*1502* is in strong linkage disequilibrium in Southeast Asian population but not in Japanese or European.

Genetic predisposition is phenotype specific

It should be remembered that the association of *HLA-B*1502* allele is only valid for CBZ-induced SJS/TEN, but not for other CBZ hypersensitivities like MPR[106, 110]or DHS[110]. They may be caused by different allelic association (MPR has been associated with SNP in the HLA-E region and HLA-A*3101 and DHS with SNP in the motilin gene) [110] or by different immune mechanism.

Black-box warning for carbamazepine use

USFDA has issued a black-box warning stating that persons of genetically high risk ancestry should be screened for *HLA-B*1502* allele before initiating treatment with CBZ[111]. Similar warning has been issued by Health Canada[112].

Newer method of detecting the allele

In USA and Canada, many commercial laboratories now perform high-resolution genetic testing with sequence specific primer (SSP) using patient's blood or buccal swab[113]. These methods are costly and time-consuming (take around 1 week). Recently, a new (loop-mediated isothermal amplification or LAMP) method for detecting *HLA-B*1502* genotype has been shown to be accurate (100% concordant with SSP), yet inexpensive, rapid and simple[114]. They may be useful in future in the clinical practice.

Does *HLA-B*1502* allele confer a drug effect or a class effect?

There was another unanswered question: “Is this genetic susceptibility restricted to the drug (CBZ) or the class (drugs with similar aromatic ring)?” In clinical practice, cross-reactivity is known to exist between CBZ and three structurally similar drugs, phenytoin (PHT), lamotrigine (LTG) and oxcarbazepine (OXC). A large Thai

cohort, in 2008, showed PHT-induced SJS to be associated with *HLA-B*1502* allele[106]. In a recently published study, Hung et al. showed that OXC, PHT and LTG share the common risk allele *HLA-B*1502* while inducing SJS. They recommended that CBZ, OXC and PHT should be avoided in the *HLA-B*1502* carrier and LTG should be used with caution[115].

Does other gene contribute in CBZ hypersensitivity?

Like abacavir, HSP gene polymorphism was also found to be associated with severe CBZ hypersensitivity, but it was not clear whether the association was causal or related to linkage disequilibrium with another gene in MHC.

Allopurinol Hypersensitivity and Genetic Predisposition

Again the Han Chinese

The anti-gout drug, allopurinol, also has shown strong genetic predisposition for developing hypersensitivity reaction. It is the commonest cause of SJS/TEN in Europe[116] and one of the most frequent causes of DHS in the world[117]. In a case–control association study among Han Chinese, Hung et al[118] found that the HLA-B*5801 allele was present in all 51 (100%) patients with allopurinol-induced severe cutaneous adverse reaction (30 DHS and 21 SJS/TEN) but only in 20 of 135 (15%) allopurinol-tolerant patients [odds ratio 580.3 (95% confidence interval, 34.4–9780.9), corrected P value = 4.7×10^{-24}].

Ethnic bias: Probably present

In Japanese population, a strong association of HLA-B*5801 allele to allopurinol-induced SJS/TEN[104, 119] and DHS[119] was also evident. The association was moderate [odds ratio = 80 (confidence interval = 34–157), $P<10^{-6}$] in the people of European ancestry[120]. This weaker association is probably due to lower frequency of the allele in the European population. A study among Thai population which has a high frequency of HLA-B*5801 allele has shown a strong association [odds ratio of 348.3 (95% confidence interval = 19.2–6336.9, $P = 1.6 \times 10$)].[121] Further study with large cohort is needed for a definitive conclusion.

Does phenotype matter?

The study among Han Chinese[118] showed, unlike CBZ hypersensitivity, the genetic predisposition, here, is related to both DHS and SJS/TEN. This was confirmed by one Japanese study[119]. But the other studies[120, 121] did not look into allopurinol-induced DHS.

Is prior genotyping mandatory for allopurinol use?

Tassaneeyakulet al[121] demonstrated strong association between genotyping for HLA-B*5801 allele and allopurinol-induced SJS/TEN (sensitivity 100%, specificity 87%, positive predictive value 1.52 and negative predictive value 100%) in Thai population. This proves the allele to be a valid genetic marker among these people. But Fernando and Broadfoot recommended screening of all patients irrespective of ethnicity before allopurinol therapy, without much statistical strength. Till date, there is no recommendation for prior genetic screening. Moreover, genotyping for HLA-B*5801 allele is complex and available only in research laboratories. These have impeded the implementation of routine genetic testing before starting allopurinol.

Other Drug: Newer Allele

Apart from the above, weaker association of rare alleles to SJS/TEN has been documented in European study[120]. These include HLA-B*38 for SMX or LTG and HLA-B*73 for oxicam. Recently, HLA-B*5901 allele has been claimed to be strongly associated with methazolamide-induced SJS/TEN in Korean patients[122]. It is too early to comment on the significance of these studies.

Glutathione-*S*-transferase genotype and antiepileptic drug-induced liver toxicity

A survey conducted in 192 CBZ-treated Japanese patients assessed the relationship between the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the genotype for glutathione-*S*-transferase (*GST*) and microsomal epoxide hydrolase (*mEH*), two enzymes involved in the detoxification of reactive metabolites. The levels of ALT and AST were higher in patients who were *GSTM1*-negative (null genotype) than in those who were *GSTM1*-positive, whereas there was no relationship with *mEH* genotypes. The authors' suggestion that the *GSTM1* null genotype may be a risk factor for CBZ-induced liver toxicity should be interpreted with caution, because the mechanisms underlying a mild elevation of liver enzyme may differ from those involved in severe liver toxicity. In another Japanese study conducted with similar methodology, the *GSTM1* null genotype and the combined *GSTM1* null genotype/*GSTT1* null genotype were suggested to represent a risk factor for an increase in serum γ-glutamyl transferase among patients with epilepsy treated with valproic acid (VPA) polytherapy. Again, these genotypes may not necessarily be a marker of VPA-induced liver toxicity.

More genes predisposing to systemic idiosyncratic reactions to VPA

Several inborn errors of metabolism are known to represent a risk factor for severe idiosyncratic reactions to VPA, including liver toxicity. Many studies have focused on the interaction between VPA and mitochondrial function, and the role of mitochondrial disorders, such as Alpers' syndrome, as conditions predisposing to severe VPA toxicity. Recent studies in this area reinforced, in particular, evidence that certain mutations in the gene coding for the catalytic subunit of mitochondrial DNA (mtDNA) polymerase γ (POLG) can lead to a range of clinical phenotypes which predispose to development of fatal liver failure after exposure to VPA, although a single case report suggests that there may be mutations in the *POLG1* gene associated with reversibility of the hepatotoxicity. Other single case reports have also suggested an association between mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like acidosis (MELAS) and VPA-induced seizure aggravation, between the C88393T (pro→Ser/MTATP8) mtDNA heteroplasmic mutation and VPA-induced cognitive regression associated with brain pseudo atrophy, and between a t(8;16) chromosomal translocation and reversible VPA-associated acute myeloid leukemia. Overall, these findings reinforce the need to consider the possibility of a genetic defect whenever systemic idiosyncratic-like disorders occur in patients given VPA.

Vigabatrin-induced visual field constriction

Vigabatrin causes irreversible visual field constriction in over a third of treated individuals. The underlying mechanisms have not been fully clarified, but one hypothesis is that susceptibility to the retinal toxicity may be related to variation in genes coding for the presumed transporters of Vigabatrin into the retina, its target enzyme GABA transaminase, and the $GABA_C$ receptor. In a study that tested this hypothesis, polymorphisms of six candidate genes (*SLC6A1, SLC6A13, SCL6A11, ABAT, GABRR1* and *GABRR2*) were assessed in 73 patients exposed to Vigabatrin for at least 1 year. The degree of visual field constriction correlated with three SNPs (*GABRR1/2* tSNP6, *GAT1/3* tSNP7 and *GAT2* tSNP6) and with one haplotype at *GAT2*. However, these findings could not be confirmed in a second independent cohort of 58 patients, suggesting that the initial results were false-positives, or expression of weak effect. This study highlights the pitfalls of drawing conclusions from association studies, particularly when confirmatory investigations in independent samples are not conducted.

In the future, newer genetic association of drug hypersensitivity will be explored, focusing on their pathogenesis. However, to translate these research findings into clinical application, several points require stringent persuasion:

1. Different immunological hypotheses of drug hypersensitivity need to be verified, unified and rejected (if needed) by practical experiments.
2. The phenotypic diagnostic criteria of individual drug hypersensitivity involving the skin (MPR, SJS/TEN and DHS) must be clearly

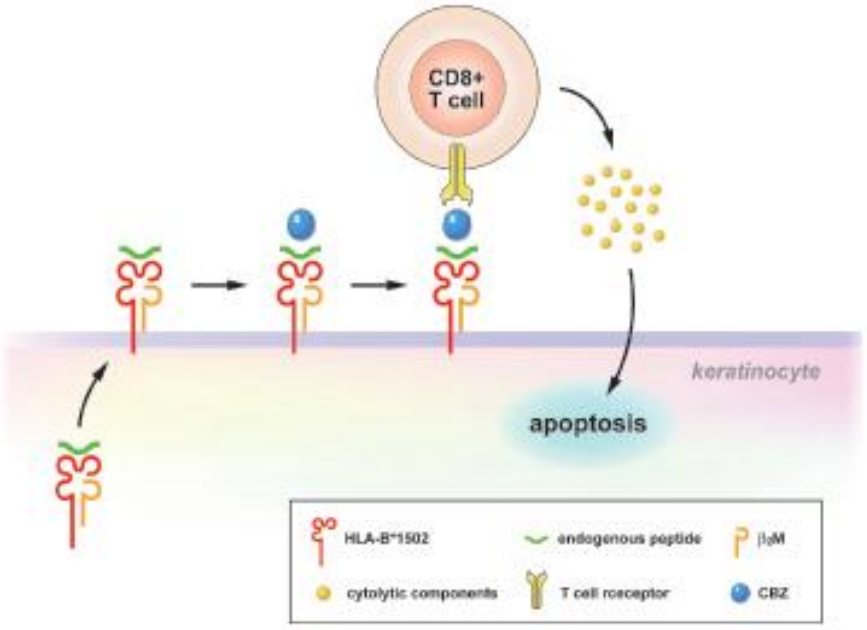
delineated and the genetic association of each of them separately explored.

3. To add statistical strength to these studies, a large number of cases should be included. Considering the rarity of certain drug hypersensitivities, this may necessitate multicenter and even multinational study.
4. Genetic susceptibility to individual drugs should be re-evaluated in the light of ethnic backgrounds.
5. Availability and cost-effectiveness of the screening test. Designing easier, quicker and cheaper tests is essential in this regard.
6. Finally, experimental bias toward immune genes should be avoided. Genetic susceptibility may be conferred by “metabolic” genes and novel “nonimmune” genes[123] as well.

Pathogenesis and Pathology of skin lesions in SJS/TEN

The pathogenesis of SJS/TEN is not fully understood but is believed to be immune-mediated, as re-challenging an individual with the same drug can result in rapid recurrence of SJS/TEN[124,125]

Fig.3. A model of keratinocyte apoptosis induced by the interaction of CBZ-*HLA-B*1502*-TCR in SJS/TEN



The *HLA-B*1502* protein first forms a complex with β2-microglobulin (β2M) and an endogenous peptide in the endoplasmic reticulum (ER). Next the peptide-loaded *HLA-B*1502* is transported to the cell surface. When CBZ is encountered, the peptide-loaded *HLA-B*1502* directly interacts with CBZ and subsequently activates CBZ-specific CD8+ T cells. Massive amounts of cytolytic components, such as

granulysin, perforin, and granzyme B, are unregulated and released, leading to the apoptosis of keratinocytes.

Riichiro Abe, Tadamichi Shimizu, Akihiko Shibaki, Hideki Nakamura *et al*[126] in their paper on 'Toxic Epidermal Necrolysis and Stevens-Johnson Syndrome are Induced by Soluble Fas Ligand' explained about the pathogeneses of toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS), both severe blistering diseases usually associated with drug intake, are not fully elucidated. Histologically, both TEN and SJS are characterized by extensive keratinocyte apoptosis. Previous studies have shown that keratinocyte apoptosis in TEN and SJS was induced by a suicidal interaction between Fas and Fas ligand (FasL), which are both expressed by keratinocytes.

However, the preliminary examinations of Abe et al demonstrated that FasL is hardly detected on keratinocytes. They hypothesized that soluble FasL (sFasL) is secreted by peripheral blood mononuclear cells (PBMCs) and this interacts with the Fas expressed on keratinocytes in TEN and SJS. To justify this hypothesis, They investigated whether sFasL secreted by PBMCs could induce the keratinocyte apoptosis in TEN and SJS. Enzyme-linked immunosorbent assay analysis demonstrated that there was no significant sFasL increase in any samples of healthy controls (<40 pg/ml, *n* _ 14) and patients with an ordinary erythema multiforme-type drug eruption (41.5 _ 3.1 pg/ml, *n* _ 14), whereas high concentrations are detected in all samples of TEN and SJS patients (TEN: 131.5_57.4 pg/ml, *n*_8; SJS: 119.1_41.0 pg/ml, *n* _ 14) ($P < 0.0001$). *In vitro* analysis using cultured keratinocytes revealed that the sera of TEN and SJS patients induced abundant keratinocyte apoptosis

compared to erythema multiforme-type drug eruption sera. Furthermore, on stimulation with the causal drug, PBMCs obtained from TEN and SJS patients secreted high levels of sFasL. Taken together, these results indicate that sFasL secreted by PBMCs, not keratinocytes, plays a crucial role in the apoptosis and pathogenesis of TEN and SJS, and that the serum sFasL level maybe a good indicator for the early diagnosis of TEN and SJS.

TEN and SJS are severe blistering diseases usually associated with drug intake in which apoptotic keratinocyte cell death results in the separation of large areas of skin at the dermo-epidermal junction, producing the appearance of scalded skin. The pathophysiology of these diseases is not well known, although immune mechanisms and altered metabolism of drugs have been postulated. TEN and SJS are usually a pauci-inflammatory process since TEN and SJS lesions contain relatively few inflammatory cells. Abe et al initial hypothesis was that apoptosis is not mediated by lesional infiltrating cells, but by circulating soluble factors.

Their study demonstrates that PBMCs from TEN and SJS patients secrete sFasL on stimulation with the causal drug. In addition, patient's sera induced apoptosis in cultured keratinocytes, indicating that sFasL produced by PMBCs may contribute to the pathogenesis of TEN and SJS. The precise mechanisms responsible for enhanced keratinocyte-specific apoptosis in TEN and SJS remain unclear. It remains controversial that keratinocyte apoptosis is mediated by soluble factors such as tumour necrosis factor-alpha, nitric oxide, or peripheral blood cells, particularly cytotoxic T cells. For example, Nassif and colleagues reported that T lymphocytes present within the lesions of TEN patients might exhibit, without any

restimulation, a drug-specific cytotoxicity against autologous cells. On the other hand, proinflammatory cytokines including tumour necrosis factor-alpha, which mediates apoptosis, are likely to be involved in TEN. Although several therapeutic approaches for TEN targeting tumour necrosis factor- alpha were assessed, almost all trials failed to decrease mortality. Viard and colleagues[127] reported that Fas-FasL interaction is directly involved in the epidermal necrolysis of TEN. They showed that keratinocytes of TEN patients express lytically active FasL. However, the exposure of Fas-sensitive target cells to cryostat sections of skin that express FasL does not result in target cell apoptosis. The reason why keratinocyte FasL is non-functional within the keratinocytes remains unclear, but may be because of its cellular localization. Another possibility may be because of other control mechanisms known to regulate the lytic potential of FasL, such as metalloproteinase-mediated surface cleavage and inactivation. Unexpectedly, their data showed a lack of correlation between sFasL levels and the degree of skin detachment in both TEN and SJS. They expected that the degree of detachment would be parallel to FasL levels. TEN and SJS are very rare diseases, for example, the incidence of TEN is 0.4 to 1.2 cases per million. The authors therefore suggest that the patient background may be a strong contributing factor, for example HLA typing, indicate heterogeneous disease severity. In addition, they suggest the trigger levels may be different between individuals, so severity and sFasL levels do not show correlation.

TEN is associated with a mortality rate of >30%, most frequently as a result of sulfonamide, anticonvulsant, or nonsteroidal anti-inflammatory drug use. Particularly, before the bullae stage is reached, impending TEN and SJS is difficult to distinguish

from other drug eruptions. As these disorders progress so rapidly, a gold standard marker for diagnosis is urgently required. Their study revealed that high levels of serum sFasL were detected in TEN and SJS patients, especially in the early stages, suggesting the possibility of using this as a diagnostic marker of TEN and SJS.

There is no specific treatment for TEN at present. Some retrospective studies have claimed a benefit in the use of corticosteroids, whereas other reports showed no benefit or even increased morbidity and mortality. Other trials that included cyclosporine and monoclonal chimeric IgG anti-tumour necrosis factor-alpha antibodies, have been used in isolated cases and in short uncontrolled series, allowing no conclusions on their efficacy.

The extent of epidermal detachment is the main prognostic factor in TEN patients, therapies with a potential to stop this process of epithelial apoptosis might be highly useful during the initial phase of the disease. As demonstrated in the study, sFasL secreted by PBMCs may mediate keratinocyte apoptosis, which might significantly contribute to the pathogenesis of TEN and SJS. Therefore, a reduction in serum sFasL might be a new therapeutic approach in the treatment of TEN and SJS.

The knowledge of sFasL is pivotal in understanding the pathogenesis in TEN and SJS, and opens a future for more focused diagnostic and therapeutic applications.

Till date, it was not recognised that innate immunity plays a critical role in the pathophysiology of SJS/TEN, by way of bridging between the acute response to invading non self molecules and chronic local immune inflammation. Ueta M et al[134]

previously reported that while human corneal epithelium harbours messages for most TLRs, TLR3 is most highly expressed. In conjunctival, as in corneal, epithelium, TLR3 is the TLR with the highest expression level at the messenger RNA level. The cell-surface TLR3 of human corneal epithelial cells responds to virus dsRNA-mimic polyI:C to generate proinflammatory cytokines and interferon b, and that the innate immune responses in human corneal epithelial cells differ from those in immune-competent cells[128].

In the current study, the association between Japanese patients with SJS/TEN and TLR3 gene polymorphisms were clarified. This raises the possibility that abnormalities of TLR3 on the ocular surface might contribute to ocular surface inflammation such as SJS/TEN. In addition, the association between the onset of SJS/TEN and viral infections raises the possibility of an association between SJS/TEN and a disordered innate immune response. The association between TLR polymorphisms and human diseases has been suggested. Polymorphisms in the TLR3 gene could be associated with type 1 diabetes in black people from South Africa[129]. In the children of European farmers, there was a strong association between TLR2 polymorphisms and allergic diseases[130]. Toroket al[131] reported an association between a functional polymorphism in TLR4 and ulcerative colitis. The specific link between exposure to environmental triggers and the induction of a highly restricted autoimmune process remains to be detected, and the innate immune system could constitute a link between the environment and the adaptive immune system.

The histopathology of SJS/TEN lesions show that keratinocyte apoptosis followed by necrosis is the pathogenic basis of the widespread epidermal detachment observed in SJS/TEN.

The clinical, histopathological and immunological findings in SJS/TEN support the currently prevalent concept, that SJS and TEN are specific drug hypersensitivity reactions in which cytotoxic T lymphocytes (CTL) play a role in the initiation phase. In the early phase of disease, blister fluid contains mainly cytotoxic CD8+T lymphocytes[132,133], suggesting that a major histocompatibility (MHC) class-I restricted drug presentation leads to clonal expansion of CD8+ CTLs, and the subsequent immune reaction that causes SJS/TEN. These CD8+ T cells express common cutaneous leukocyte antigen (CLA) and are negative for CD45RA and CD28. Nassif et al. were able to demonstrate that blister T cells from patients exert drug specific cytototoxic activity against both autologous B-lymphocyte cell lines and keratinocytes[134], and furthermore demonstrated that this cell-mediated cytotoxicity was mediated by granzyme B. The discrepancy between the paucity of the infiltration of immune cells (including CTLs) in the skin of patients with SJS/TEN and the overwhelming keratinocyte apoptosis has however lead to the search for cytotoxic proteins and/or cytokines that may "amplify" the extent of keratinocyte apoptosis that CTLs alone could induce upon cell-cell contact. The strongest evidence suggests a key contribution of the cytotoxic molecules FasL and granulysin as molecules responsible for the disseminated keratinocyte apoptosis in SJS/TEN[135, 136].

The role of the membrane form of the death ligand FasL and its cognate death receptor Fas in the signalling that triggers keratinocyte apoptosis is supported by

research performed using an ex-vivo experimental set up with TEN lesional skin biopsy cryostat section overlays with Fas-expressing lymphoid target cells. However, the functional relevance of up-regulated keratinocyte membrane FasL, and thus its ability to induce keratinocyte cell death, has been questioned by some as the above ex-vivo demonstration of the lytic ability of keratinocyte FasL in TEN was limited in its effect on lymphoid target cells and not demonstrated with keratinocytes as target cells. It is well known that primary keratinocytes are sensitive to the cytolytic effect of FasL in vitro, and this sensitivity can be further enhanced by interferon gamma, a cytokine known to be present in the skin during TEN[137-139]. However, it is still not fully understood what causes the up-regulation of FasL/Fas on keratinocytes, and how the immune system, including T cells found in blister fluid at the onset of disease may regulate this.

The role of soluble FasL (sFasL) in SJS/TEN remains controversial. It appears clear now that increased levels of sFasL can be found in the serum of patients with SJS/TEN, and levels of sFasL are consistently elevated when analysis is performed preceding skin detachment[140]. Soluble FasL as opposed to membrane-bound FasL is, however, very poorly cytolytic, and it is therefore unlikely to be a cause of keratinocyte apoptosis in TEN[141]. Nevertheless, one study showed that sera of SJS/TEN were able to induce abundant keratinocyte apoptosis and furthermore that peripheral blood mononuclear cells of patients stimulated by the causative drug excreted high levels of sFasL[142], but it should be noted that sera can contain small membrane vesicles with membrane bound FasL that can account for the observed activity.

Gene expression analysis of blister fluid cells, and analysis of blister fluid from patients with SJS/TEN has also recently identified secretory granulysin (a cationic cytolytic protein secreted by CTL's, NK cells and NKT cells) as a key molecule responsible for the induction of keratinocyte death in TEN. Blister fluid cells express high levels of granulysin mRNA, the protein is found in increased concentrations in blister fluid, and most importantly, recombinant Granulysin mimics features of SJS/TEN when injected intradermally in mice. The finding that elevated serum granulysin levels apparently discriminate between serious and non-blistering adverse drug reactions, serum granulysin levels being normal in the latter, lends further support an important role of granulysin in SJS/TEN[143].

Antigen presentation and production of tumour necrosis factor (TNF)–alpha by the local tissue dendrocytes results in the recruitment and augmentation of T-lymphocyte proliferation and enhances the cytotoxicity of the other immune effector cells[144]. A "killer effector molecule" has been identified that may play a role in the activation of cytotoxic lymphocytes[145]. The activated CD8+ lymphocytes, in turn, can induce epidermal cell apoptosis via several mechanisms, which include the release of granzyme B and perforin.

Apoptosis of keratinocytes can also take place as a result of ligation of their surface death receptors with the appropriate molecules. Those can trigger the activation of the caspase system, leading to DNA disorganization and cell death[146]. Apoptosis of keratinocytes can be mediated via direct interaction between the cell-death receptor Fas and its ligand. Both can be present on the surfaces of the

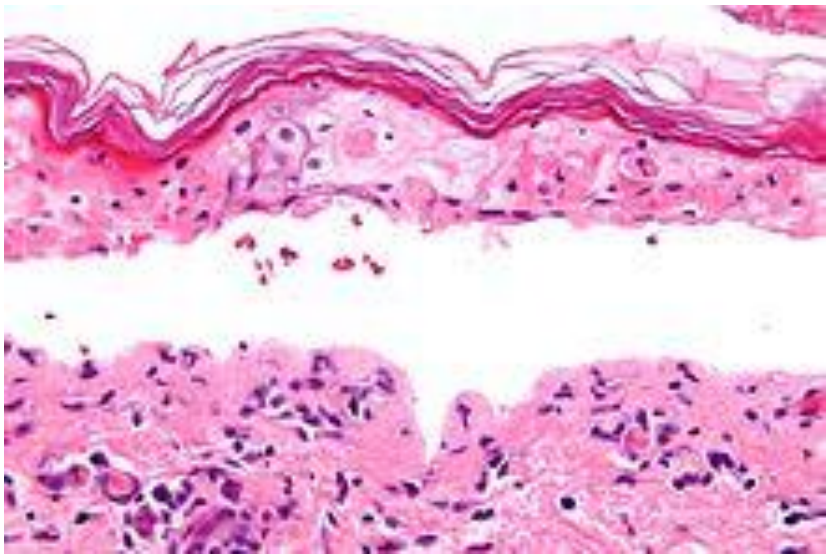
keratinocytes. Alternatively, activated T-cells can release soluble Fas ligand and interferon-gamma, which induces Fas expression by keratinocytes.

The death of keratinocytes causes separation of the epidermis from the dermis. Once apoptosis ensues, the dying cells provoke recruitment of more chemokines. This can perpetuate the inflammatory process, which leads to extensive epidermal necrolysis[147].

Slow acetylators are people whose liver cannot completely detoxify reactive drug metabolites. For example, patients with sulfonamide-induced toxic epidermal necrolysis have been shown to have a slow acetylator genotype that results in increased production of sulfonamide hydroxylamine via the P-450 pathway. These drug metabolites may have direct toxic effects or may act as haptens that interact with host tissues, rendering them antigenic[148, 149].

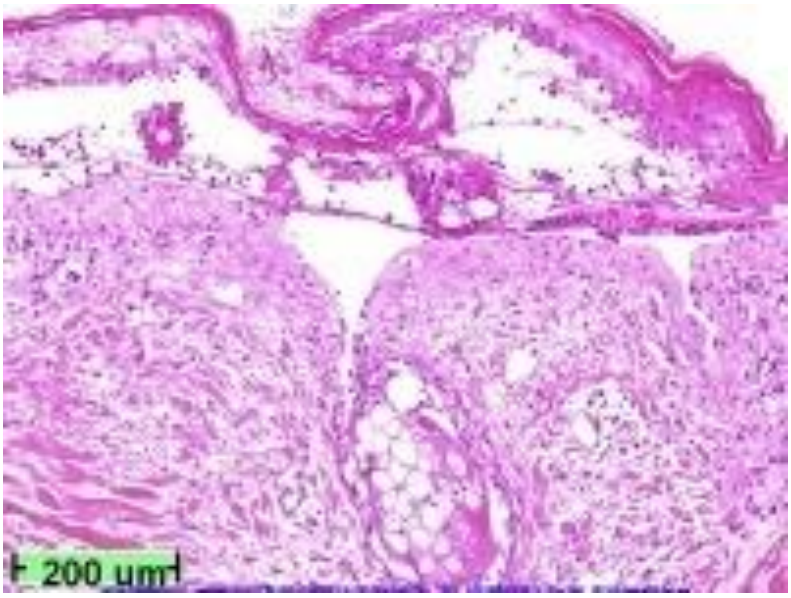
Histologic Findings

Fig.4. Micrograph showing full thickness epidermal necrosis with a basket weave-like stratum corneum and separation of the dermis and epidermis (Skin biopsy H&E stain).



Minimal dermal inflammatory cell infiltrate and full-thickness necrosis of epidermis are typical histopathologic findings in patients with Stevens-Johnson syndrome. The epidermal-dermal junction shows changes, ranging from vacuolar alteration to subepidermal blisters. The dermal infiltrate is superficial and mostly perivascular. Keratinocytes undergo apoptosis.

Fig.5. Low-power view showing full-thickness epidermal necrosis



In the dermis, $CD4^+$ T lymphocytes predominate, whereas in the epidermis, the T cells are predominantly $CD8^+$. The dermoepidermal junction and epidermis is infiltrated mostly by $CD8^+$ T lymphocytes. Complement 3 component and immunoglobulin G (IgG) deposits at the dermoepidermal junction and around small dermal vessels were interpreted as the result of a nonspecific exudative phenomenon. The activated state is underlined by human leukocyte antigen (HLA)-DR expression on keratinocytes, similar to other skin inflammatory disorders. $CD8^+$ T cells that recognize major histocompatibility complex I (MHC-I) modified by an antigen may produce skin lesions of Stevens-Johnson syndrome, or they may be produced by T cells that recognize an antigen that is restricted by MHC-I.

Histological work up of immediate cryosections or conventional formalin-fixed sections of the skin revealing wide spread necrotic epidermis involving all layers confirms the diagnosis. In order to rule out autoimmune blistering diseases, direct immune fluorescence staining should be additionally performed and no immunoglobulin and/or complement deposition in the epidermis and/or the epidermal-dermal zone should be detected.

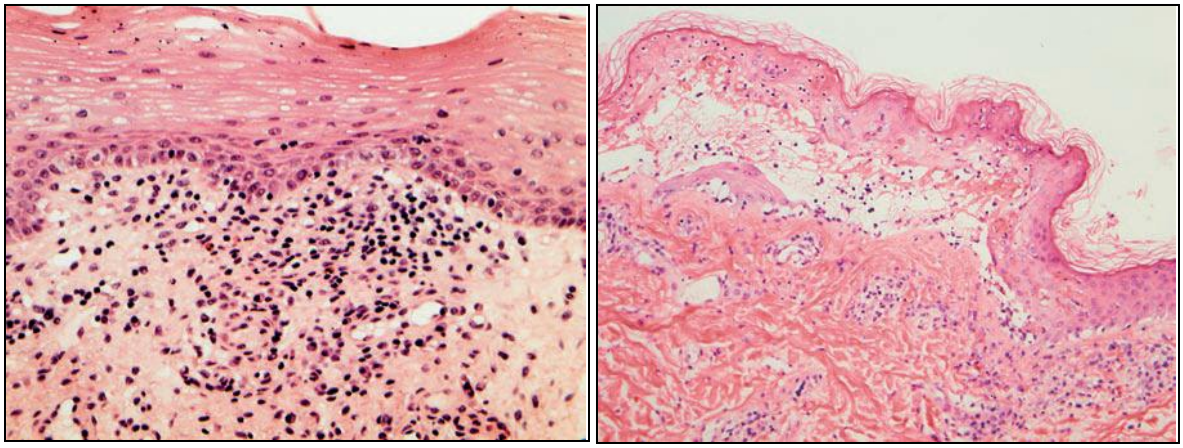


Fig.6. Histopathology of Stevens-Johnson syndrome: Left, Focal basal cell vacuolar change with dense superficial dermal lymphocytic inflammation and occasional eosinophils in patient with Stevens-Johnson syndrome secondary to lamotrigine therapy (hematoxylin-eosin, original magnification ×40). Right, Full-thickness necrosis, basal vacuolar change, and subepidermal bullae in patient with Stevens-Johnson syndrome secondary to *Mycoplasma pneumoniae* infection (hematoxylin-eosin, original magnification ×20

Complications

Of patients with Stevens-Johnson syndrome, 27-50% progress to severe ocular disease. Ocular complications of Stevens-Johnson syndrome include the following:

- Chronic cicatrizing conjunctivitis
- Corneal epithelial defects
- Corneal stromal ulcers
- Corneal perforation
- Endophthalmitis

Other complications may include the following:

- Gastroenterological - Oesophageal strictures
- Genitourinary - Renal tubular necrosis, renal failure, penile scarring, vaginal stenosis
- Pulmonary - Tracheobronchial shedding with resultant respiratory failure
- Cutaneous - Scarring and cosmetic deformity, recurrences of infection through slow-healing ulcerations

Lesions may continue to erupt in crops for as long as 2-3 weeks. Mucosal pseduomembrane formation may lead to mucosal scarring and loss of function of the involved organ system. Oesophageal strictures may occur when extensive involvement of the oesophagus exists. Mucosal shedding in the tracheobronchial tree may lead to respiratory failure.

Blindness may develop secondary to severe keratitis or panophthalmitis in 3-10% of patients. Vaginal stenosis and penile scarring have been reported. Renal complications are rare.

Cutaneous lesions may resolve with a patchwork of hyper pigmentation and hypopigmentation. Fingernails and toenails may regrow abnormally. Lesions of the genitourinary system may lead to phimosis or vaginal synechiae.

Diagnostic Considerations

The gravity of the diagnosis must be recognized. Because patients with Stevens-Johnson syndrome (SJS) who present early in the development of the

disease may not yet be critically ill, the clinician may misdiagnose the condition and discharge the patient. SJS should be considered in all patients with target lesions and mucous membrane involvement.

Diagnosis and diagnostic methods

The diagnosis relies on the one hand on clinical symptoms and on the other hand on histological features.

Typical clinical signs initially include areas of erythematous and livid macules on the skin, on which a positive Nikolsky sign can be induced by mechanical pressure on the skin, followed within minutes to hours by the onset of epidermal detachment characterized by the development of blisters. It should be noted, however, that the Nikolsky sign is not specific for SJS/TEN. Mucosal, including ocular, involvement develops shortly before or simultaneously with skin signs in almost all cases. To distinguish SJS, SJS-TEN and TEN the surface area of the detachment is the main discriminating factor.

Differential Diagnosis

Major differential diagnosis of SJS/TEN are:

- Autoimmune blistering diseases, including linear IgA dermatosis and paraneoplastic pemphigus
- Pemphigus vulgaris and bullous pemphigoid
- Acute generalized exanthematous pustulosis (AGEP)
- Disseminated fixed bullous drug eruption

- Staphylococcal scalded skin syndrome (SSSS). SSSS was one of the most important differential diagnoses in the past, but the incidence is currently very low with 0.09 and 0.13 cases per one million inhabitants per year.
- Exfoliative Dermatitis
- Atopic Keratoconjunctivitis
- Ocular Burns
- Sjögren Syndrome
- Thermal Burns in Emergency Medicine
- Toxic Shock Syndrome
- Trachoma
- Chemical Burns in Emergency Medicine
- Irradiation
- Trauma
- Progressive systemic sclerosis (scleroderma)
- Erythroderma ichthyosiform congenita
- Porphyria cutaneatarda
- Epidermolysis bullosa acquisita
- Linear immunoglobulin A bullous disease
- Bullous systemic lupus erythematous
- Corynebacterium diphtheriae conjunctivitis
- Sebaceous cell carcinoma
- Adenoviral conjunctivitis
- Intraepithelial epithelioma

Approach Considerations

Serum levels of the following are typically elevated in patients with Stevens-Johnson syndrome:

- Tumour necrosis factor (TNF)-alpha
- Soluble interleukin 2-receptor
- Interleukin 6
- C-reactive protein

However, none of these serologic tests is used routinely in diagnosing and managing Stevens-Johnson syndrome.

A complete blood count (CBC) may reveal a normal white blood cell (WBC) count or a nonspecific leucocytosis. A severely elevated WBC count indicates the possibility of a superimposed bacterial infection. Electrolytes and other chemistries may be needed to help manage related problems.

Skin and blood cultures have been advocated because the incidence of serious bacterial bloodstream infections and sepsis contribute to morbidity and mortality[150]. In addition, cultures of urine and wounds are indicated when an infection is clinically suspected. Determine renal function and evaluate urine for blood.

Skin biopsy specimens demonstrate that the bullae are sub epidermal. However, skin biopsy is not an emergency department (ED) procedure. Epidermal cell necrosis may be noted. Perivascular areas are infiltrated with lymphocytes.

Bronchoscopy, esophagogastroduodenoscopy (EGD), and colonoscopy may be indicated. Chest radiography may indicate the existence of a pneumonitis when clinically suspected. Otherwise, routine plain films are not indicated.

Conjunctival biopsies from patients with active ocular disease show subepithelial plasma cells and lymphocyte infiltration. Lymphocytes also are present around vessel walls. The predominant infiltrating lymphocyte is the helper T cell. Immunohistology of the conjunctiva reveals numerous HLA-DR–positive cells in the substantia propria, vessel walls, and epithelium. In the epithelium, HLA-DR is presented by Langerhans cells, macrophages, and activated T cells. Immunoreactant deposition in vessel walls, comprised of immunoglobulin and complement components, is another prominent feature.

On transmission electron microscopy, the conjunctivae of patients with episodic conjunctival inflammation revealed squamous epithelial metaplasia, vascular basement membrane zone disruption, reduplication, and thickening.

In vivo confocal microscopy may be a useful tool for therapeutic indications and follow-up of ocular problems associated with Stevens-Johnson syndrome[150].

Pre-hospital and Emergency Department Care

Paramedics should recognize the presence of severe fluid loss and should treat patients with Stevens-Johnson syndrome as they would patients with thermal burns.

Most patients present early and prior to obvious signs of hemodynamic compromise.

The single most important role for the ED physician is to detect Stevens-Johnson syndrome/toxic epidermal necrolysis early and initiate the appropriate ED and inpatient management.

Withdrawal of the suspected offending agent is critically important. Timing of withdrawal has been linked to outcome. Underlying diseases and secondary infections must be identified and treated.

Patients should be treated with special attention to airway and hemodynamic stability, fluid status, wound/burn care, and pain control. Care in the ED must be directed to fluid replacement and electrolyte correction.

Treatment is primarily supportive and symptomatic. Some have advocated corticosteroids, cyclophosphamide, plasmapheresis, haemodialysis, and immunoglobulin.

Oral lesions are managed with mouthwashes. Topical anaesthetics are useful in reducing pain and allowing the patient to take in fluids.

Skin lesions are treated as burns. Areas of denuded skin must be covered with compresses of saline or Burow solution.

Tetanus prophylaxis should be addressed.

Allergological testing

A detailed drug history is very important when striving to identify the culprit drug in SJS/TEN. In some cases several drugs are possible candidates and allergological testing can be of help in identifying the most likely candidate.

In principle, the severity of SJS and TEN does not allow re-challenge and intradermal testing with the culprit drugs due to the feared risk of re-inducing a second episode of SJS/TEN, although two case reports describe intradermal testing without triggering of a second episode of TEN[151]

Induction of SJS/TEN has, however, been documented following local eye treatment[152]

Patch-testing is an investigational option, but not a routine diagnostic option at the moment. Data from Wolkenstein et al. has shown that low sensitivity is a problem with patch testing in SJS/TEN, as only two of 22 tested patients had a relevant positive patch test[153].

Currently the focus of allergological testing lies more on *ex vivo/in vitro* tests.

The lymphocyte transformation test (LTT), that measures the proliferation of T cells to a drug *in vitro* has shown a sensitivity of 60-70% for patients allergic to beta-lactam antibiotics[154]. Unfortunately the sensitivity of the LTT is still very low in SJS/TEN even if performed within one week after onset of the disease[155].

Another recently reported approach looks for up-regulation of CD69 on T-lymphocytes two days after lymphocyte stimulation in vitro as a sign of drug hypersensitivity[156]. Novel *in vitro* methods to help identify culprit drug in SJS/TEN are still needed[157].

Prognosis

SJS and TEN are severe and life-threatening. The average reported mortality rate of SJS is 1-5%, and of TEN is 25-35%; it can be even higher in elderly patients and those with a large surface area of epidermal detachment. Mortality is determined primarily by the extent of skin sloughing. When body surface area (BSA) sloughing is less than 10%, the mortality rate is approximately 1-5%. However, when more than 30% BSA sloughing is present, the mortality rate is between 25% and 35%, and may be as high as 50%.[158]

Bacteraemia and sepsis appear to play a major role in increased mortality.

In order to standardize the evaluation of risk and prognosis in patients with SJS/TEN, different scoring systems have been proposed. The SCORTEN is now the most widely used scoring system and evaluates the following parameters: age, malignancy, tachycardia, initial body surface area of epidermal detachment, serum urea, serum glucose, and bicarbonate.

Yun et al. reported recently that lactate dehydrogenase (LDH) may be an additional useful parameter in the evaluation of disease severity[159].

More than 50% of patients surviving TEN suffer from long-term sequelae of the disease. These include symblepharon, conjunctival synechiae, entropion, ingrowth of eyelashes, cutaneous scarring, irregular pigmentation, eruptive nevi, and persistent erosions of the mucous membranes, phimosis, vaginal synechiae, nail dystrophy, and diffuse hair loss.

The SCORTEN score (a severity-of-illness score for toxic epidermal necrolysis) calculates the risk for death in both SJS and TEN on the basis of the following variables:

- Age >40 years
- Malignancy
- Heart rate >120
- Initial percentage of epidermal detachment >10%
- Blood urea nitrogen (BUN) level >10 mmol/L
- Serum glucose level >14 mmol/L
- Bicarbonate level < 20 mmol/L

Each variable is assigned a value of 1 point. Mortality rates are as follows:

- 0-1 points, ≥3.2%
- 2 points, ≥12.1%
- 3 points, ≥35.3%
- 4 points, ≥58.3%
- 5 or more points, ≥90%

Other negative prognostic factors include persistent neutropenia (defined as neutropenia lasting more than 5 days), hypoalbuminemia (usually < 2 g/dL), and persistent azotemia.

Although some patients rapidly progress to lose very large areas of the epidermis in a matter of days, the process suddenly ceases in others and reepithelialisation begins a few days later. Predicting the course of disease in a given patient at the initial presentation is not possible. Reepithelialisation is usually

complete within 3 weeks, but pressure and mucosal areas may remain eroded and crusted for 2 weeks or longer.

Survivors of Stevens-Johnson syndrome may experience numerous long-term sequelae; the most disabling are those of the eye. Cicatrization of conjunctival erosions may lead to the following:

- Inverted eyelashes
- Photophobia
- A burning sensation in the eyes
- Watery eyes
- A sicca like syndrome
- Corneal and conjunctival neovascularization

As many as 40% of survivors of toxic epidermal necrolysis have residual potentially disabling lesions that may cause blindness.

Anticonvulsants and SJS/TEN

Common drug causing SJS/TEN in different countries

	Europe	India	Singapore	Malaysia	Taiwan
Case no.	n-245	n-57	n-23	n-14	n-230
Carbamazepine	5%	19%	27.7%	35.7%	26%
Phenytoin	3%	19%	4.35%	14.3%	4.3%
Phenobarbital	12%	5.3%	0%	0%	3.48%
Sulphonamides	13%	15.8%	0%	14.3%	2.2%
Aminopenicillins	6%	NA	4.3%	21.4%	4%
Allopurinol	5%	NA	13%	7.1%	6.9%
*NSAIDS	29%	3.5%	17.4%	0%	7%
Method of Data collection	a case/control study; data from 245SJS/ TEN during a 4-year period (Feb.1989~Jan.1993) through surveillance networks in France, Germany, Italy, and Portugal)	A prospective study; Data from 57 consecutive SJS/ TEN during a 6-year period of Nehru Hospital, India.	A retrospective study; Data from 23 SJS/TEN during a 6-year period (1989-1994) of Singapore General Hospital, Singapore.	A retrospective study; Data from 14 SJS/TEN during a 8-year period (1987-1994) of Hospital University Science Malaysia, Malaysia.	A retrospective study; Data from 230 SJS/TEN during a 5-year period (1997-2002) Chang Gung Memorial Hospital Health System, Taiwan.

*NSAIDs: non –steroidal anti-inflammatory drugs.

N is total number of SJS/TEN cases accumulated.

NA: data not available.

Man CB, Kwan P, Baum L, Yu E et al [160], in their article entitled 'Association between *HLA-B*1502* allele and antiepileptic drug-induced cutaneous reactions in Han Chinese' state that a previous study conducted in Taiwan found a 100% association between *HLA-B*1502* allele and carbamazepine-induced Stevens-Johnson syndrome (SJS) in Han Chinese subjects, with an extremely high odds ratio compared with carbamazepine-tolerant subjects (odds ratio = 2,504). The authors examined this association in 24 Hong Kong Han Chinese subjects who had cutaneous adverse reactions induced by different antiepileptic drugs (AEDs). They were matched with 48 AED-tolerant controls. *HLA-B*1502* was associated with severe cutaneous reactions (SCR) induced by AEDs, which included carbamazepine, phenytoin, and lamotrigine ($p = 0.001$, odds ratio = 17.6), but was not associated with maculopapular exanthema (MPE) ($p = 0.32$). Identification of genetic polymorphisms predisposing to development of AED-induced SCR offers the possibility of avoiding these high-risk drugs in genetically susceptible individuals.

Cross reactivities among the aromatic AED (Carbamazepine, phenytoin, phenobarbital) in inducing cutaneous adverse reaction is well recognized. The *HLA-B1502* is associated with AED-Induced SJS/TEN which were not limited to Carbamazepine alone, but also included phenytoin, phenobarbital and lamotrigine.

P Brent Ferrell Jr and Howard L McLeod[161] in their paper on 'Carbamazepine, *HLA-B*1502* and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations' states that recently, the USA FDA has made a labelling change to the drug information contained in carbamazepine. Owing to recent data implicating the HLA allele *B*1502* as a marker for carbamazepine-induced Stevens-

Johnson syndrome and toxic epidermal necrolysis in Han Chinese, the FDA recommends genotyping all Asians for the allele. This allele is seen in high frequency in many Asian populations other than Han Chinese, but there are few data on whether the allele is a marker for this severe outcome in anyone other than Han Chinese. In fact, the association has not been found in Caucasian patients. They review the data that prompted this recommendation, list data for other ethnic groups, both Asian and non-Asian, and briefly discuss the implication of this recommendation for clinical practice.

FDA ALERT [12/12/2007]**:** Dangerous or even fatal skin reactions (Stevens Johnson syndrome and toxic epidermal necrolysis), that can be caused by carbamazepine therapy, are significantly more common in patients with a particular human leukocyte antigen (HLA) allele, *HLA-B*1502*. This allele occurs almost exclusively in patients with ancestry across broad areas of Asia, including South Asian Indians. Genetic tests for *HLA-B*1502* are already available. Patients with ancestry from areas in which *HLA-B*1502* is present should be screened for the *HLA-B*1502* allele before starting treatment with carbamazepine. If they test positive, carbamazepine should not be started unless the expected benefit clearly outweighs the increased risk of serious skin reactions. Patients who have been taking carbamazepine for more than a few months without developing skin reactions are at low risk of these events ever developing from carbamazepine. This is true for patients of any ethnicity or genotype, including patients positive for *HLA-B*1502*. This new safety information will be reflected in updated product labelling.

Carbamazepine, an anticonvulsant and a mood-stabilizing drug, is the main cause of the Stevens–Johnson syndrome (SJS) and its related disease, toxic epidermal necrolysis (TEN), in Southeast Asian countries. Carbamazepine-induced SJS–TEN is strongly associated with the *HLA-B*1502* allele.

Cutaneous adverse drug reactions are among some of the most frequent adverse events associated with a number of our commonly used aromatic ring–containing antiepileptic drugs (AEDs), including carbamazepine, phenytoin, oxcarbazepine and lamotrigine.

In its mildest form, maculopapular exanthema may occur in perhaps up to 10% of patients receiving carbamazepine. In some patients, however, more severe dermatologic hypersensitivity reactions such as drug reaction with eosinophilia and systemic symptoms [DRESS]) may occur and has been associated with a mortality rate of about 10%. This hypersensitivity syndrome is associated with rash, fever, and organ dysfunction such as nephritis or hepatitis, and most commonly presents within the first 2 months of therapy, with flu-like symptoms such as fever and malaise. For patients receiving carbamazepine or phenytoin, the incidence of DRESS is estimated to be 1in 5,000.

Among the most severe of these reactions are Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN).Treatment with commonly used AEDs such as carbamazepine, phenytoin, phenobarbital, and lamotrigine is considered to increase the risk of SJS/TEN. For carbamazepine, the risk of developing SJS/TEN in individuals of European decent is about1 to 6 cases of

10,000 patients exposed. SJS and TEN are considered to be variants of the same process, with the mortality rate for patients developing TEN approaching 30%. Clearly, it would be advantageous to be able to identify patients at possible risk for developing any sort of hypersensitivity reaction to these commonly used medications.

Until relatively recently, dermatologic reactions were considered to be idiosyncratic events, and thus, largely unpredictable. This notion changed however following the demonstration of a relationship between these potentially life-threatening reactions and the human leukocyte antigen (HLA)-*B*1502* in various Asian populations.

Avoidance of carbamazepine in patients carrying the *HLA-B*1502* allele has recently been shown to substantially reduce the incidence of SJS/TEN in Asian patients. The discovery of the involvement of human leukocyte antigen– dependent presentation of a drug for T-cell activation has led to the recognition that a direct, noncovalent binding between a drug and T-cell receptor with an HLA molecule is responsible, leading to T-cell activation and clonal expansion of CD8+ cytotoxic T cells in the skin.

Clinically, recognition of the association of this allelic variant represent an important step forward in their ability to predict (and presumably prevent) serious hypersensitivity reactions to carbamazepine. Important questions, however, remained unanswered. For example, would genotype screening for *HLA-B*1502* be of value in those patients of other ethnic Asian or European decent? Could these

genetic variants be relevant for treatment with other aromatic ring–containing AEDs, such as phenytoin, oxcarbazepine, or lamotrigine?

With respect to generalizability to the broader population, while the *HLA-B*1502* allele is quite prevalent in individuals from Southern China (~15%), as well as those from several other Southeast Asian countries (~2–8%), this allele is quite uncommon in Japanese and European Caucasians (<1%). Although the incidence of SJS/TEN is lower in European Caucasians than certain Asian populations, it does clearly still occur.

Chen et al recently reported that prospective screening for the *HLA-B*1502* allele and subsequent avoidance of carbamazepine prescription in genetically susceptible Han Chinese patients did in fact reduce the occurrence of SJS/TEN, as compared with historical controls. Interestingly, the absence of the *HLA-B*1502* allele did not appear to reduce the incidence of more mild rash and itching in these patients.

Finally, it is reasonable to speculate that given the strong association between either *HLA-B*1502* in certain Asian populations or *HLA-B*3101* in Northern Europeans and severe carbamazepine hypersensitivity, that we might expect to see similar risk in those patients receiving alternative AEDs such as oxcarbazepine, phenytoin, or lamotrigine. Unfortunately, the supportive evidence is still lacking.

With respect to Han Chinese carriers of *HLA-B*1502*, the data is conflicting. Hung and co-workers suggest an increased risk for oxcarbazepine and phenytoin in these patients. For lamotrigine, however, the presence of this allele (or any other)

does not appear to be associated with increased risk of either maculopapular exanthema or SJS/TEN.

The U.S. FDA now recommends that patients of Asian ancestry be screened for *HLA-B*1502* prior to starting treatment with carbamazepine. While helpful, until the study by McCormack et al., clinicians were left with a great deal of uncertainty as to how to apply this information to other patient groups.

Will screening for HLA-A*3101 prove to be as beneficial inpatients of European (and perhaps Japanese) ancestry as it appears that *HLA-B*1502* identification is in Han Chinese? Only additional prospective studies will be able to answer this. For now, however, it seems only reasonable that screening for HLA-A*3101 be strongly considered prior to initiation of carbamazepine in those patients. One can now realistically envision a time when genomic profiling will lead to safer pharmacological management in patients with epilepsy.

Wen-Hung Chung, Shuen-Iu Hung, Jui-Yung Yang, Shih-Chi Su, *et al*[162] in their paper 'Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis' described that Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening adverse drug reactions characterized by massive epidermal necrosis, in which the specific danger signals involved remain unclear. Here they show that blister cells from skin lesions of SJS-TEN primarily consist of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, and both blister fluids and cells were cytotoxic. Gene expression profiling identified granulysin as the most highly expressed cytotoxic

molecule, confirmed by quantitative PCR and immunohistochemistry. Granulysin concentrations in the blister fluids were two to four orders of magnitude higher than perforin, granzyme B or soluble Fas ligand concentrations, and depleting granulysin reduced the cytotoxicity. Granulysin in the blister fluids was a 15-kDa secretory form, and injection of it into mouse skin resulted in features mimicking SJS-TEN. Their findings demonstrate that secretory granulysin is a key molecule responsible for the disseminated keratinocyte death in SJS-TEN and highlight a mechanism for CTL- or NK cell—mediated cytotoxicity that does not require direct cellular contact.

Chung WH, Hung SI and Chen YT[163] in their paper on 'Genetic predisposition of life threatening antiepileptic-induced skin reactions' reiterate the fact that recent advances in pharmacogenetic studies have uncovered increasingly more genes that predispose individuals to adverse drug reactions. Aromatic antiepileptic drugs (AEDs) are a frequent cause of severe cutaneous adverse reactions (SCAR). A strong genetic association between *HLA-B*1502* and carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) has been shown in Han Chinese patients. This article reviews and updates genetic information associated with CBZ and other AEDs causing SCAR in different ethnic populations. Independent studies from different countries confirmed that patients carrying the *HLA-B*1502* are at high risk of SJS/TEN when exposed to CBZ. The US FDA and similar regulatory agencies in Canada and Taiwan have updated the CBZ drug label to include the genetic information. Available data also suggest that *HLA-B*1502* is a risk allele for SJS/TEN caused by other aromatic AEDs with a similar structure to

CBZ. Screening for *HLA-B*1502* allele before starting treatment with CBZ is justified in patients from high-risk populations as recommended by regulatory agencies. Similar chemicals should also be avoided in individuals who test positive for *HLA-B*1502*.

Yang G, Deng YJ, Qin H, Zhu BF, *et al*[164] described the HLA-B*15 subtypes distribution in Han population in Beijing, China, as compared with those of other populations. To identify *HLA-B*15* subtypes distribution in Han population in Beijing, People's Republic of China, 826 unrelated healthy individuals were typed using the polymerase chain reaction-sequence-based typing method. Within the 246 *HLA-B*15* positive individuals, 29 *HLA-B*15* alleles were identified, the most predominant of which is B*1501 (40.07%), followed by *B*1502* (12.87%), B*1511 (12.87%), B*1518 (9.19%) and B*1532 (3.31%). The distribution of *HLA-B*15* subtype frequencies was compared between the Beijing Han, eight other Chinese ethnic minorities and six Chinese populations covering the mainland of China, Taiwan, Hong Kong and Singapore. A neighbour-joining phylogenetic tree was constructed and revealed that the Beijing Han population clustered into the northern populations group and had a closer relationship with northern Han and Hui than with southern Han or other ethnic minorities. These results thus provide useful information that can be used in anthropology, selection for bone marrow transplantation as well as in disease-association study, such as in carbamazepine (CBZ)-induced Stevens-Johnson syndrome and toxic epidermal necrolysis.

Aihara M[165]. in his paper on 'Pharmacogenetics of cutaneous adverse drug reactions', commented that drug-induced hypersensitivity reactions are of major medical concern because they are associated with high morbidity and high mortality. In addition, individual patients' reactions are impossible to predict in each patient. In the field of severe cutaneous adverse drug reactions (cutaneous ADR) such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome (DHIS) or drug rash with eosinophilia and systemic symptoms (DRESS), major advances have recently been gained through studies of an association between HLA alleles and drug hypersensitivity induced by specific drugs. The results of these pharmacogenomic studies allow prediction of the risk of adverse reactions in patients treated with certain drugs, including carbamazepine and other aromatic antiepileptic drugs, allopurinol and abacavir. However, different ethnic populations show variations in the genetic associations. A strong association between carbamazepine-induced SJS/TEN and *HLA-B*1502* has been found in Southeast Asian patients but not in Caucasian and Japanese patients. Moderate associations between aromatic amine anticonvulsants and other HLA alleles have been proposed in Japanese patients. In contrast, HLA-B*5801 was found to be associated with allopurinol-induced cutaneous ADR, including SJS/TEN and DIHS/DRESS, in Caucasian and Asian patients, including the Japanese. These differences may, at least in part, be due to the differences in allele frequency in different ethnic populations. This article reviews the progress in pharmacogenomics, associated mainly with carbamazepine and allopurinol in different ethnic populations. Pharmacogenetic screening based on associations between adverse reactions and

specific HLA alleles helps to avoid serious conditions associated with drug hypersensitivity.

Munir Pirmohamed[166] in his article 'Pharmacogenetics of Idiosyncratic Adverse Drug Reactions' in the chapter on Adverse Drug Reactions, Handbook of Experimental pharmacology, noted that Idiosyncratic adverse drug reactions are unpredictable and thought to have an underlying genetic etiology. With the completion of the human genome and Hap Map projects, together with the rapid advances in genotyping technologies, they have unprecedented capabilities in identifying genetic predisposing factors for these relatively rare, but serious, reactions. The main roadblock to this is the lack of sufficient numbers of well-characterized samples from patients with such reactions. This is now beginning to be solved through the formation of international consortia, including developing novel ways of identifying and recruiting patients affected by these reactions, both prospectively and retrospectively. This has been led by the research on abacavir hypersensitivity – its association with HLA-B*5701 forms the gold standard of how they need to identify associations and implement them in clinical practice. Strong genetic predisposing factors have also been identified for hypersensitivity reactions such as are associated with carbamazepine, allopurinol, flucloxacillin, and statin-induced myopathy. However, for most other idiosyncratic adverse drug reactions, the genetic effect sizes have been low to moderate, although this may partly be due to the fact that only small numbers have been investigated and limited genotyping strategies have been utilized. It may also indicate that genetic predisposition will be

dependent on multiple genes, with complex interactions with environmental factors. Irrespective of the strength of the genetic associations identified with individual idiosyncratic adverse drug reactions, it is important to undertake functional investigations to provide insights into the mechanism(s) of how the drug interacts with the gene variant to lead to a phenotype, which can take a multitude of clinical forms with variable severity. Such investigations will be essential in preventing the burden caused by idiosyncratic reactions, both in healthcare and in industry.

Pirmohamed M, Friedmann PS, Molokhia M, Loke YK, *et al*[167] in their paper on 'Phenotype Standardization for Immune-Mediated Drug-Induced Skin Injury' stated that advances in genetic research and molecular biology techniques have made it possible to begin to characterize the underlying genetic factors that predispose patients to serious forms of drug-induced skin injury (DISI). To facilitate research in this area, they have set out standardized phenotypic definitions for (i) Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), (ii) acute generalized exanthematous pustulosis (AGEP), and (iii) hypersensitivity syndrome (HSS; also known as drug reaction with eosinophilia and systemic symptoms (DRESS) and drug-induced hypersensitivity syndrome (DIHS)). A DISI Expert Working Group comprising participants with varied expertise reviewed and debated current terminology and diagnostic criteria for DISI and agreed on the minimum phenotypic criteria for selected forms of DISI (SJS/TEN, AGEP, and HSS). In addition, an algorithm has been developed to aid appropriate clinical categorization of patients with DISI. These standardized criteria will be important in facilitating

adequate and accurate patient recruitment in order to advance research in pharmacogenomic, immunological, mechanistic, and epidemiological studies.

Kate Traynor[168] in his paper 'FDA, Researchers focus on genetics of drug hypersensitivity' states that the anticonvulsant drug carbamazepine is associated with serious skin hypersensitivity reactions, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis, in patients who have the *HLA-B*1502* allele. Labelling for the drug carries warnings against using it in patients with this genetic marker. FDA in November announced that it is investigating data on whether *HLA-B*1502* is associated with SJS and other skin reactions in people treated with another anticonvulsant, phenytoin.

Diego Franciotta, Patrick Kwan and Emilio Perucca[169] in their article on 'Genetic basis for idiosyncratic reactions to antiepileptic drugs' state that in recent years, there has been an explosion of genetic research in epilepsy, including a search for genetic markers of adverse reactions to antiepileptic drugs. The article focussed on recent findings concerning genetic factors affecting susceptibility to idiosyncratic reactions to antiepileptic drugs. Recent studies have investigated the role of genetic factors in the development of antiepileptic drug-induced cutaneous reactions, carbamazepine and valproate-induced liver toxicity, vigabatrin-induced visual field defects, and antiepileptic drug-induced teratogenicity. The greatest progress has been an improved definition of the role of human leukocyte antigen-related genes as predictors of the risk of serious antiepileptic drug-induced

cutaneous reactions. This has led to the recommendation that patients of Asian ancestry be tested for the *HLA-B*1502* allele, in order to identify those at high risk of developing Stevens–Johnson syndrome and toxic epidermal necrolysis after administration of carbamazepine and, possibly, phenytoin and other antiepileptic drugs. Future research will probably lead to discovery of additional genetic predictors of susceptibility to adverse reactions to antiepileptic drugs. Identification of genetic markers should, in turn, allow unravelling of the molecular mechanisms underlying these reactions. Ultimately, these advances should lead not only to improved personalization of therapy but also to development of safer drugs.

Wolfgang Loscher, Ulrich Klotz, Fritz Zimpric and Dieter Schmidt[170] in their paper on ‘ The clinical impact of pharmacogenetics on the treatment of epilepsy’ state that drug treatment of epilepsy is characterized by unpredictability of efficacy, adverse drug reactions, and optimal doses in individual patients, which, at least in part, is a consequence of genetic variation. Since genetic variability in drug metabolism was reported to affect the treatment with phenytoin more than 25 years ago, the ultimate goal of pharmacogenetics is to use the genetic makeup of an individual to predict drug response and efficacy, as well as potential adverse drug events. However, determining the practical relevance of pharmacogenetic variants remains difficult, in part because of problems with study design and replication. This article reviews the published work with particular emphasis on pharmacogenetic alterations that may affect efficacy, tolerability, and safety of antiepileptic drugs (AEDs), including variation in genes encoding drug target (SCN1A), drug transport

(ABCB1), drug metabolizing (CYP2C9, CYP2C19), and human leukocyte antigen (HLA) proteins. Although the current studies associating particular genes and their variants with seizure control or adverse events have inherent weaknesses and have not provided unifying conclusions, several results, for example that Asian patients with a particular HLA allele, *HLA-B*1502*, are at a higher risk for Stevens-Johnson syndrome when using carbamazepine, are helpful to increase their knowledge how genetic variation affects the treatment of epilepsy. Although genetic testing raises ethical and social issues, a better understanding of the genetic influences on epilepsy outcome is the key to developing the much needed new therapeutic strategies for individuals with epilepsy.

Jose de Leon[171] in his paper on 'The future (or lack of future) of personalized prescription in psychiatry' explained the rapid technological advances in genetics have created conceptual chaos regarding the genetics of drug response. Terms for differing concepts are used interchangeably: pharmacogenetics with pharmacogenomics, personalized medicine with personalized prescription. Biomarker has many definitions. The author prefers the concept of personalized prescription and uses it with implications beyond pharmacogenetics by considering all scientific information valid for prescribing medication. Genetics may not be crucial for all drugs. In this comprehensive view, clinicians must consider genetic, environmental and personal variables when prescribing medication and incorporate some basic pharmacological principles: (1) safety and efficacy, (2) pharmacokinetics and pharmacodynamics, (3) therapeutic window and prescriber's role, and (4)

idiosyncratic and dose-related adverse drug reactions. Personalized prescription in the clinical environment can be expressed in two main ways: as personalized selection of the drug and as personalized dosing. The future or lack of future, of personalized drug selection and of personalized dosing in psychiatry is reviewed. Currently, the author thinks that, in psychiatry, pharmacogenetic tests have some potential in two areas: (1) excluding some drugs for some unusual patients (*HLA-B*1502* genotyping in Asians for carbamazepine), and (2) using pharmacokinetic genes for personalizing dosing in narrow therapeutic window drugs. In the short term, there is dubious potential for other pharmacogenetic tests and no potential for pharmacogenetic testing to ascertain the best drug for each patient. Personalized dosing has immediate application if one understands it as the use of their current scientific knowledge of genetic, environmental and personal variables to determine dosing; its sole requirement is well-trained psychiatrists.

Christine Lonjou, Nicolas Borot, Peggy Sekula, Neil Ledger, *et al*[172] in their article on 'A European study of HLA-B in Stevens–Johnson syndrome and toxic epidermal necrolysis' related to five high-risk drugs described Stevens–Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are rare but life-threatening cutaneous adverse reactions to drugs, especially to allopurinol, carbamazepine, lamotrigine, phenobarbital, phenytoin, sulfamethoxazole, oxicam and nevirapine. Recently, a strong association between carbamazepine and allopurinol induced SJS or TEN has been described with respectively, HLA-B*. Their objective is to further investigate the relationship between SJS/TEN and HLA-B in a

large number of patients in a European population. HLA-B genotyping was performed on 150 patients included in a European study (RegiSCAR) of SJS and TEN. They focused on patients related to 'high-risk' drugs including: 31 cases related to allopurinol, 28 to sulfamethoxazole, 19 to lamotrigine and 14 to oxicam. Results Sixty-one percent of 31 allopurinol-induced SJS/TEN patients carried the HLA-B*5801 allele and the figure was 55% for 27 patients of European ancestry [odds ratio = 80 (34–187)], (P<10–6) as previously observed in Han Chinese. For other drugs, two rare alleles showed a weaker association with SJS/TEN in a limited number of patients: B*38 for sulfamethoxazole or lamotrigine-related patients, and B*73 for oxicam. At variance with prior results in Asia, this study shows that even when HLA-B alleles behave as strong risk factors, as for allopurinol, they are neither sufficient nor necessary to explain the disease. Further investigations are necessary to delineate the exact role of the HLA region in SJS/TEN, and to look for other associations in other regions of the genome.

Perry W. Payne, Jr[173] in his paper 'Currents in Contemporary Ethics' states that the term "personalized medicine" increasingly has come to mean the use of genetic testing in prescribing pharmaceutical products. The scientific basis of this approach to medicine is that, because of genetic variations, humans differ in their response to treatments. This observation is the cornerstone of pharmacogenetics and pharmacogenomics. Ethical problems sometimes arise when this principle is applied on a group basis. For example, if humans could be divided into two genetic groups, a group with genotype A and a group with genotype B, members of each

group might respond differently to a particular drug. The group with genotype A might have an adverse reaction to a drug. The group with genotype B might have a therapeutic response to the drug. Personalized medicine focuses on ensuring that individuals likely to respond positively to a drug receive it, and individuals likely to respond negatively are not given the drug. Although often defined as a way of individually tailoring treatments, personalized medicine is better characterized as a way of tailoring treatments to groups of people with some shared genetic trait or traits. Hence, personalized medicine may be viewed as “subgroup medicine.”

Nahoko Kaniwa, Yoshiro Saito, Michiko Aihara, Kayoko Matsunaga, *et al*[174] in their paper on ‘HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis’ stated that Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening severe adverse drug reactions with mucosal and cutaneous disorders, and very often accompanied by high fever and systemic complications. Some investigators have proposed that SJS and TEN are variations of the same disease expressed with different severity, although this is controversial. Although SJS and TEN incidence is very low (0.4-6 per million per year),more than 100 different causative drugs have been reported. The diseases are probably T-cell-mediated delayed allergic reactions, and typically begin within 1-3 weeks after exposure to a drug. Recently, an extremely strong association (odd ration: ~2504) between human leukocyte antigen *(HLA)-B*1502* and carbamazepine-induced SJS/TEN in Han Chinese in Taiwan was reported. Another Taiwanese study showed that HLA-B*5801

was detected in all Han Chinese patients with SJS/TEN or drug-induced hypersensitivity (DIHS) induced by allopurinol. The involvement of *HLA-B*1502* was also confirmed in SJS/TEN caused by other aromatic epileptic agents such as phenytoin in Han Chinese or Thai population. However, such a strong association between *HLA-B*1502* and carbamazepine-induced SJS/TEN was not detected in Caucasian patients. These reports suggested that HLA involvement in severe cutaneous adverse reactions may be drug-specific as well as ethnic group-specific. Thus, they started a retrospective case-control study to explore genetic biomarkers related to SJS and TEN in Japanese patients living in Japan.

John W. Miller[175] in his paper 'Of Race, Ethnicity, and Rash: The Genetics of Antiepileptic Drug-Induced Skin Reactions' states that serious allergic cutaneous reactions, especially Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), are among the most feared complications of antiepileptic drug (AED) therapy. SJS and TEN are characterized by a blistering exanthema with mucosal involvement and skin detachment. TEN is defined by more extensive skin involvement than SJS (>30%) and has a higher mortality rate, 25% or more. The risk of these rare conditions colours many epilepsy treatment decisions, influencing the choice of an AED and the speed at which it is initiated. For this reason, the emerging evidence that genetic factors strongly predict occurrence of SJS and TEN will most certainly lead to changes in clinical practice.

R. Yuliwulandari, K. Kashiwase, H. Nakajima, J. Uddin, T., *et al*[176] in their paper on 'Polymorphisms of HLA genes in Western Javanese (Indonesia): close affinities to Southeast Asian populations' described about the identification of human leukocyte antigen (HLA) antigens that are known as the highest polymorphic genes has become a valuable tool for tissue transplantation, platelet transfusion, disease susceptibility or resistance, and forensic and anthropological studies. In the present study, the allele and haplotype frequencies of HLA-A, HLA-B, and HLA-DRB1 were studied in 237 unrelated healthy Western Javanese (Indonesia) by the high-resolution polymerase chain reaction-Luminex method. A total of 18 A, 40 B, and 20 DRB1 alleles were identified. The most frequent HLA-A, -B, and -DRB1 alleles were HLA-A*2407 (21.6%), *HLA-B*1502* (11.6%) and HLA-B*1513 (11.2%), and DRB1*1202 (37.8%), respectively. The most frequent two-locus haplotypes were HLA-A*2407-B*3505 (7%) and HLA-B*1513-DRB1*1202 (9.2%), and three-locus haplotypes were HLA-A*3401-B*1521-DRB1*150201 (4.6%), HLA-A*2407-B*3505-DRB1*1202 (4.3%), and HLA-A*330301-B*440302-DRB1*070101 (4.2%). HLA allele and haplotype frequencies in addition to phylogenetic tree and principal component analyses based on the four-digit sequence-level allele frequencies for HLA-A, HLA-B, and HLA-DRB1 showed that Western Javanese (Indonesia) was closest to Southeast Asian populations.

Shuen-Iu Hung, Wen-Hung Chung and Yuan-TsongChen[177] in their paper on 'Genetics of Severe Drug Hypersensitivity Reactions in Han Chinese' demonstrated drug hypersensitivity, an immune-related idiosyncratic adverse reaction, was

historically referred to as being unpredictable. However, recent studies in Han Chinese have revealed that several types of severe drug hypersensitivity reactions have a strong genetic predisposition and might be predicted, particularly with the use of the genes coding for major histocompatibility complex (MHC), a key molecule for immune response. The genetic predisposition is drug- and phenotype-specific, HLA-B * 1502 is associated with carbamazepine-induced Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), and HLA-B * 5801 with allopurinol-SJS/TEN/hypersensitivity syndrome. These genetic associations are not limited to the Han Chinese, and their significance in other ethnic populations depends on the allele frequency of the populations. The genetic studies in Han Chinese support the concept that MHC-restricted presentation of drug is involved in the pathogenesis of immune-mediated severe drug hypersensitivity reactions. These genetic markers have the potential to be used for further development of tests to identify individuals at risk for these drug-related life-threatening conditions, as well as for an increased understanding of the pathogenesis of these clinical syndromes.

Chih-Wen Ou Yang,Shuen-Iu Hung, Chiun-Gung Juo, Ya-Ping Lin, *et al*[178] in their article on '*HLA-B*1502*–bound peptides: Implications for the pathogenesis of carbamazepine-induced Stevens-Johnson syndrome' demonstrated Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) can involve MHC-restricted presentation of a drug or its metabolites for T-cell activation. *HLA-B*1502* tightly associated with carbamazepine (CBZ) induced these conditions in a Han Chinese population. They sought to identify *HLA-B*1502*–bound peptides that might

be involved in CBZ-induced SJS/TEN. Methods: Soluble *HLA-B*1502* was used to identify bound peptides in the presence and absence of CBZ by using liquid chromatography–tandem mass spectrometry. Peptide-binding assays were performed to detect the specific interaction between the HLA molecule and the identified peptides. Mass spectra were compared to detect CBZ-modified peptides. They identified more than 145 peptides bound to HLAB* 1502. In 13 of 15 peptides examined, they functionally confirmed their specificity with binding assays. Preferable uses of these peptides at the anchoring residues P2 and P9 were similar to those observed in other HLA-B alleles in the Han Chinese population. However, the preferable use of serine residues at the nonanchoring position (P) 5, P6, P7, and P8 appeared to be unique for the B*1502 peptides. No specific CBZ-modified peptides were detected when they compared the mass spectra of peptides detected in the presence or absence of the drug. They concluded noncovalent interaction between a drug and an HLA complex might contribute to cytotoxic T cell–mediated cell death in patients with SJS/TEN. Clinical implications: An understanding of pharmacologic interaction of drugs with an HLA complex might lead to safer drugs that avoid SJS/TEN.

Michael W. Mann and Gerard Pons[179] in their paper on 'Various Pharmacogenetic Aspects of Antiepileptic Drug Therapy' evaluated pharmacogenetics concerns the influence of an individual's genetic background on the pharmacokinetics and pharmacodynamics of xenobiotics. Much of the pharmacogenetic data in the field of epilepsy deals with the pharmacokinetics of

antiepileptic drugs (AEDs). In particular, two polymorphisms of cytochrome P450 2C9 are known to slow down the metabolism of phenytoin to a degree that increases the risk of the neurotoxic adverse effects of 144 Mann & Pons this drug among carriers of these polymorphisms. A significant number of patients with epilepsy do not respond to AEDs and such pharmacoresistance is a major, largely unsolved, problem that is likely to be multifactorial in nature. In this regard, genetic factors may influence transmembrane drug transporter proteins, thereby modifying the intracerebral penetration of AEDs. Monogenic idiopathic epilepsies are rare and frequently associated with ion channel mutations; however, to date, a consistent relationship between changes in channel properties and clinical phenotype has not been established nor has any association between genotype and response to specific treatment options. Polymorphisms of drug targets may represent another genetic facet in epilepsy: a recent study demonstrated for the first time a polymorphism of a drug target (the α-subunit of a voltage-gated sodium channel) associated in clinical practice with differing response to two classic AEDs. Adverse drug reactions and teratogenicity of AEDs remain a major concern. Whole-genome single nucleotide polymorphism profiling might in the future help to determine genetic predisposing factors for adverse drug reactions. Recently, in Han Chinese treated with carbamazepine and presenting with Stevens-Johnson syndrome, a strong association was found with *HLA-B*1502*. If genetically targeted drug development drugs for relatively rare diseases could become economically viable for the pharmaceutical industry. The synergy of lower trial costs and efficacy-based prescribing may reduce the cost of medical treatment for a particular disease. This

hypothetical advantage of the practical use of pharmacogenetics is, however, counterbalanced by several possible dangers, including illicit data mining and the development of a human 'genetic underclass' with the risk of exclusion from, for example employment or health insurance, because of an 'unfavourable' genetic profile.

Chung WH, Hung SI, Hong HS, Hsih *et al*[180] in their paper 'Medical genetics: a marker for Stevens-Johnson syndrome' explained that Stevens-Johnson syndrome and the related disease toxic epidermal necrolysis are life-threatening reactions of the skin to particular types of medication. They showed that there is a strong association in Han Chinese between a genetic marker, the human leukocyte antigen *HLA-B*1502*, and Stevens-Johnson syndrome induced by carbamazepine. It should be possible to exploit this association in a highly reliable test to predict severe adverse reaction, as well as for investigation of the pathogenesis of Stevens-Johnson syndrome.

Hiroyuki Gatanaga, Haruhito Honda and Shinichi Oka[181] in their article on 'Pharmacogenetic information derived from analysis of HLA alleles' reported a large amount of pharmacogenetic information has, in particular, accumulated on the association between human leukocyte antigen (HLA) alleles and hypersensitivity to certain drugs. Prospective HLA typing has dramatically reduced the risk of abacavir hypersensitivity because of its strong association with HLA-B*5701. Significant predisposition to nevirapine hypersensitivity has been reported in Caucasian

Australians harbouring HLA-DRB1*0101 with high CD4+ T-cell counts, and Sardinians and Japanese harbouring HLA-Cw8. A strong association between carbamazepine hypersensitivity and *HLA-B*1502* has been reported in Han Chinese. Most Han Chinese individuals with allopurinol-induced severe cutaneous adverse reactions are positive for HLA-B*5801. HLA typing can stratify risk of hypersensitivity to certain drugs and allow personalized treatment, although the patients should be monitored closely even if they are negative for HLA alleles associated with hypersensitivity.

J.L. Guéant, R.M. Guéant-Rodriguez, I. AimoneGastin, J.A. Cornejo-García *et al*[182] in their paper on 'Pharmacogenetic Determinants of Immediate and Delayed Reactions of Drug Hypersensitivity' discussed about drug allergy and referred to a hypersensitivity reaction for which either an IgE or T-cell-mediated mechanism is demonstrated. The recognition of the drug by B and T cells is influenced by variants of HLA genes. The genetic factors involved in IgE-mediated mechanisms have been studied mainly in β-lactam reactions, and they appear to be related to human leukocyte antigen presentation (HLA A2 and DRw52), TNFA –308G>A, class switching to IgE by B cells (variants of IL-13 and of IL-4RA), and expression of IgE receptors on target cells (variant of the FcεRIβ gene). Delayed Tcell-mediated reactions are also associated with HLA alleles. Studies have reported an association of *HLA-B*1502* and HLA-B*5801 in patients with the Stevens-Johnson syndrome or toxic epidermal necrolysis provoked by carbamazepine, as well as of HLA-B*5701 with abacavir hypersensitivity. HLA-B*5701 seems to be a

strong predictor in whites, but not in Hispanics or Africans. Carbamazepine hypersensitivity is also influenced by gene variants of cytochrome P450 enzymes on the generation of reactive metabolites, while CYP2C9*2 and CYP2C9*3 polymorphisms influence the bioactivation of sulfamethoxazole in prohapten. Pharmacogenetic studies on aspirin hypersensitivity have identified distinct types of predictors, such as HLA genotypes, a polymorphism in the promoter of the FcεRIβ gene, and variants in genes of enzymes from the arachidonic acid pathway. In the future, identification of genetic predictors will benefit from genomewide association studies that also take ethnic differences into account. Ideally, predictors will help to prevent adverse reactions, as suggested by a recent study on the effectiveness of prospective HLA-B*5701 screening to prevent hypersensitivity reactions to abacavir in HIV patients.

Y.-T. Lin, Y.-C.Chang, R.C.-Y.Hui, C.-H. Yang, *et al*[183] in their article on A patch testing and cross-sensitivity study of carbamazepine-induced severe cutaneous adverse drug reactions demonstrate the usefulness of the drug patch testing for Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS／TEN) is still controversial. Recent studies have shown that *HLA-B*1502* is strongly associated with CBZ-SJS／TEN in Chinese and Southeast Asian populations. They have evaluated the usefulness of patch tests for patients with carbamazepine (CBZ)-induced SJS, TEN and drug reaction with eosinophilia and systemic symptoms (DRESS) and the cross-reactivity in patch tests among the aromatic antiepileptic drugs. They measure the frequency of positive patch test reactions and cross-

sensitivity to structure-related aromatic anti-epileptic drugs (AEDs) for patients after SJS／TEN or DRESS episodes caused by CBZ. CBZ and other structure-related AEDs used for patch testing were prepared in 10% and 30% petrolatum. Secondary measures included the association of *HLA-B*1502* genotype and frequency of possible side effects from the patch tests. They found positive patch test reactions to 30% CBZ in the CBZ-SJS／TEN were 62.5% (10／16), and 70% (7／10) in the CBZ-DRESS. None of the 10 healthy controls displayed a positive reaction to tested agents. Cross-sensitivity to other aromatic AEDs was observed in both CBZ-SJS／TEN and the CBZ-DRESS. Only the *HLA-B*1502* genotype was present and strongly associated with the CBZ-SJS／TEN, but not with the CBZ-DRESS. Drug patch testing is a safe and useful method for the identification of CBZ as the culprit drug of SJS／TEN as well as DRESS. Testing of chemically or pharmacologically related AEDs may provide information on cross-reactivity for these patients.

Wen Yi Ding, Chew Kek Lee, and Siew Eng Choon[184] in their paper Cutaneous adverse drug reactions seen in a tertiary hospital in Johor, Malaysia stated that adverse drug reactions are most commonly cutaneous in nature. Patterns of cutaneous adverse drug reactions (ADRs) and their causative drugs vary among the different populations previously studied. Their aim is to determine the clinical pattern of drug eruptions and the common drugs implicated, particularly in severe cutaneous ADRs in their population. This study was done by analysing the database established for all adverse cutaneous drug reactions seen from January 2001 until December 2008. A total of 281 cutaneous ADRs were seen in 280 patients. The

most common reaction pattern was maculopapular eruption (111 cases, 39.5%) followed by Stevens-Johnson Syndrome (SJS: 79 cases, 28.1%), drug reaction with eosinophilia and systemic symptoms (DRESS: 19 cases, 6.8%), toxic epidermal necrolysis (TEN: 16 cases, 5.7 %), urticaria/angioedema (15 cases, 5.3%) and fixed drug eruptions (15 cases, 5.3%). Antibiotics (38.8%) and anticonvulsants (23.8%) accounted for 62.6% of the 281 cutaneous ADRs seen. Allopurinol was implicated in 39 (13.9%), carbamazepine in 29 (10.3%), phenytoin in 27 (9.6%) and cotrimoxazole in 26 (9.3%) cases. Carbamazepine, allopurinol and cotrimoxazole were the three main causative drugs of SJS/TEN accounting for 24.0%, 18.8% and 12.5% respectively of the 96 cases seen whereas DRESS was mainly caused by allopurinol (10 cases, 52.6%) and phenytoin (3 cases, 15.8%). The reaction patterns and drugs causing cutaneous ADRs in their population are similar to those seen in other countries although they have a much higher proportion of severe cutaneous ADRs probably due to referral bias, different prescribing habit and a higher prevalence of *HLA-B*1502* and HLA-B*5801 which are genetic markers for carbamazepine induced SJS/TEN and allopurinol-induced SJS/TEN/DRESS respectively. The most common reaction pattern seen in their study population was maculopapular eruptions. Antibiotics, anticonvulsants and NSAIDs were the most frequently implicated drug groups. Carbamazepine and allopurinol were the two main causative drugs of severe ADRs in their population.

Chun-Yu Wei, Wen-Hung Chung, Hsiao-Wen Huang, Yuan-Tsong Chen *et al*[185] in their article on 'Direct interaction between HLA-B and carbamazepine

activates T cells in patients with Stevens-Johnson syndrome' emphasized increasing studies have revealed that HLA alleles are the major genetic determinants of drug hypersensitivity; however, the underlying molecular mechanism remains unclear. They adopted the *HLA-B*1502* genetic predisposition to carbamazepine (CBZ)–induced Stevens-Johnson syndrome(SJS)/toxic epidermal necrolysis (TEN) as a model to study the pathologic role of HLA in delayed-type drug hypersensitivity. They in vitro expanded CBZ-specific cytotoxic T lymphocytes (CTLs) from patients with CBZ-induced SJS/TEN and analysed the interaction between HLA-B and CBZ analogues based on CTL response, surface plasmon resonance, peptide binding assay, site-directed mutagenesis, and computer modelling. The endogenous peptide–loaded *HLA-B*1502* molecule presented CBZ to CTLs without the involvement of intracellular drug metabolism or antigen processing. The *HLA-B*1502*/ peptide/b2-microglobulin protein complex showed binding affinity toward chemicals sharing 5-carboxamide on the tricyclic ring, as with CBZ. However, modifications of the ring structure of CBZ altered *HLA-B*1502* binding and CTL response. In addition to *HLA-B*1502*, other HLA-B75 family members could also present CBZ to activate CTLs, whereas members of the HLA-B62 and HLA-B72 families could not. Three residues (Asn63, Ile95, and Leu156) in the peptide binding groove of *HLA-B*1502* were involved in CBZ presentation and CTL activation. In particular, Asn63 shared by members of the B75 family was the key residue. Computer simulations revealed a preferred molecular conformation of the 5-carboxamide group of CBZ and the side chain of Arg62 on the B pocket of *HLA-B*1502*. This demonstrates a direct interaction of HLA with drugs, provides a detailed

molecular mechanism of HLA-associated drug hypersensitivity, and has clinical correlations for CBZ-related drug–induced SJS/TEN

Werner J. Pichler, Dean J. Naisbitt, and B. Kevin Park[186] in the paper on Immune pathomechanism of drug hypersensitivity reactions stated that drug hypersensitivity research has progressed enormously in recent years, and a greater understanding of mechanisms has contributed to improved drug safety. Progress has been made in genetics, enabling personalized medicine for certain drugs, and in understanding drug interactions with the immune system. In a recent meeting in Rome, the clinical, chemical, pharmacologic, immunologic, and genetic aspects of drug hypersensitivity were discussed, and certain aspects are briefly summarized that small chemicals, including drugs, can induce immune reactions by binding as a hapten to a carrier protein. Park (Liverpool, England) demonstrated that drug haptens bind to protein in patients in a highly restricted manner and that irreversibly modified carrier proteins are able to stimulate CD41 and CD81 T cells from hypersensitive patients. Drug haptens might also stimulate cells of the innate immune system, in particular dendritic cells, and thus give rise to a complex and complete immune reaction. Many drugs do not have hapten-like characteristics but might gain them on metabolism (so-called prohaptens). The group of Naisbitt found that the stimulation of dendritic cells and T cells can occur as a consequence of the transformation of a prohapten to a hapten in antigen-presenting cells and as such explain the immune-stimulatory capacity of prohaptens. The striking association between HLA-B alleles and the development of certain drug reactions was discussed

in detail. Mallal (Perth, Australia) elegantly described a highly restricted HLA-B*5701–specific T-cell response in abacavir hypersensitive patients and healthy volunteers expressing HLAB*5701 but not closely related alleles. Expression of *HLA-B*1502* is a marker known to be necessary but not sufficient to predict carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in Han Chinese. The group of Chen and Hong (Taiwan) described the possible ‘‘missing link’’ because they showed that the presence of certain T-cell receptor (TCR) clonotypes was necessary to elicit T-cell responses to carbamazepine. The role of TCRs in drug binding was also emphasized by Pichler (Bern, Switzerland). Following up on their ‘‘pharmacological interactions of drugs with immune receptors’’ concept (p-i concept), namely that drugs can bind directly to TCRs, MHC molecules, or both and thereby stimulate T cells, they looked for drug-binding sites for the drug sulfamethoxazole in drug-specific TCRs: modelling revealed up to 7 binding sites on the CDR3 and CDR2 regions of TCR Va and Vb. Among many other presentations, the important role of regulatory T cells in drug hypersensitivity was addressed.

Nahoko Kaniwa[187] in his paper on ‘Exploratory study on biomarkers associated with severe cutaneous adverse reactions’ described that most of adverse drug reactions (ADRs) occur as an extension of pharmacological effects. They occur dependently on their blood concentrations and can be potentially reduced by controlling their dose. On the other hand, ADRs categorized as Type B usually occur irrelevantly to their pharmacological effects at different organs from their target, and are often life-threatening and unpredictable. The incidences of Type B ADRs are

very low. Severe cutaneous adverse reactions including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are delayed allergic reactions in which T-cells are involved and categorized as Type B ADRs. Recent progress of pharmacogenomic studies has revealed that particular types of human leukocyte antigen (HLA) class I antigens have strong associations with severe cutaneous adverse reactions and that the associations are specific to causative drugs, phenotypes of adverse reactions and ethnic groups. They established a research group in 2006 with professionals of pharmacogenomics, dermatologists, ophthalmologists and psychiatrists to explore genetic biomarkers associated with Japanese SJS/TEN patients. To date, they have collected more than 100 Japanese SJS/TEN patients through participating institutes and a case-collecting system covering all over Japan constructed by us. No carriers of *HLA-B*1502* which was reported to have extremely strong association with carbamazepine-induced SJS/TEN in Han Chinese and south Asians, although a moderate association between allopurinol-induced SJS/TEN and HLA-B*5801 detected in Han Chinese was observed.

AIMS AND OBJECTIVES OF THE STUDY

To study the prevalence of *HLA-B*1502* among patients with seizures who on exposure to treatment with Carbamazepine developed severe cutaneous adverse drug reaction (Stevens Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN).

To test patients with seizures for the presence of *HLA-B*1502* allele prior to starting treatment with Carbamazepine.

Preliminary data (retrospective data) on the Incidence of Carbamazepine induced SJS in the local population is estimated to be about 1- 6 per 1000 new users in Asian countries.

Prevalence of the Allele in the local population in Chennai is unknown. No such study has been done earlier and published from South India. This will be the first study to look at the prevalence of *HLA-B*1502* in the local population and the incidence of *HLA-B*1502* induced SJS/TEN due to initiation of carbamazepine (CBZ) therapy for patients with seizures.

The correlation with *HLA-B*1502* is most significant for carbamazepine-induced SJS/TEN, wherein all the patients tested have the *HLA-B*1502* allele. Hence these two serious cutaneous reactions alone are considered in the present study.

Milder cutaneous reactions induced by carbamazepine, such as maculopapular rash, erythema multiforme (EM), urticaria, and fixed drug eruption,

are particularly associated with another allele, HLA-B*4601. In the present study, only SJS/TEN was considered. Testing for HLA-B*4601 will be done at a future date for mild cutaneous reactions listed above.

MATERIALS / PATIENTS AND METHODS

The present research provides a method of assessing the risk of a patient developing a cutaneous adverse drug reaction in response to carbamazepine. The research pertains to performing HLA typing for the presence of an HLA-B allele, *HLA-B*1502*, using a biological sample (blood) from the patient, using Sequence Specific Primers (SSP).

The allele can be detected by using an oligonucleotide that specifically hybridizes with the nucleic acid coding for the allele. DNA extracted from the peripheral blood of the patient is employed in the determination.

Sequence-specific oligonucleotide probes (SSOP) are readily available as commercial kits from Qiagen, supplied by Biotron Healthcare India Pvt. Ltd, 301,Coral Classic, 20th Road, Chembur, Mumbai-71.

Sample Population:

Patients were referred from the Madras Institute of Neurology and Government General Hospital, Chennai.

Patients started on CBZ were divided into 3 groups:

a.) Patients who developed mild cutaneous adverse reactions

b.) Patients who developed severe cutaneous adverse drug reaction (Stevens Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)

c.) Patients who tolerated CBZ well and did not develop any cutaneous adverse reactions.

The Patients on CBZ who developed severe cutaneous adverse drug reactions SJS and TEN and those who did not develop any cutaneous adverse reactions, were tested for the presence of *HLA-B*1502*. Patients who do not have *HLA-B*1502* are less likely to develop SJS and TEN on exposure to CBZ.

Sample size Calculation

Formula: $n = \frac{Z^2 P (1-P)}{d^2}$

Where:

n = Sample size

Z = is Confidence = 95 % (1.96)

P is expected prevalence which is 60/10000 = 0.6 per 100

d is precision which is = 0.05

$\frac{1.96 \times 1.96 \times 0.6 \times 0.4}{0.05 \times 0.05} = 369$

Anticipating non response or missing data to be about (10 - 20%)

Sample size will work out to (406 - 443)

Sample size: 400 – 450 would be the appropriate sample size.

Prospective interventional cohort study: *HLA-B*1502* testing were done in patients with seizures before starting CBZ and the drug was avoided in those testing positive for *HLA-B*1502.*

Subjects on CBZ who had earlier developed cutaneous adverse drug effects, if found to have the *HLA-B*1502* allele, were switched over to some other antiepileptic drug like Clonazepam or Levetiracetam which are not known to cause SJS/TEN.

Statistical analysis was performed using standard SPSS software and analysed using the following methods:

a. Prevalence of SJS and *HLA-B*1502* in epileptic patients exposed to CBZ
b. RR Ratio
c. p value
d. Sensitivity and Specificity of the HLA B* 1502 test
e. Positive and Negative Predictive values.

The cost of performing *HLA-B*1502*from peripheral blood per patient was about Rupees 1000/- .

Inclusion Criteria

1. Patients on CBZ who have had Cutaneous ADR, esp. Stevens Johnson Syndrome/Toxic Epidermal Necrolysis.
2. Patients on CBZ who had no cutaneous ADR to Carbamazepine.
3. Epileptic patients who are likely to be started on carbamazepine.

Site selection

1. Madras Institute of Neurology and Govt. General Hospital, Chennai – 600 003.
2. Patients referred to the Department.

Duration of study: 4 years

Specimen: Whole blood

Volume: 7 mL whole blood

Container: Lavender-top (EDTA) tube

Storage Instructions: Maintain whole blood at room temperature or refrigerate.

Causes for Rejection: Haemolysis, clotted specimen and insufficient volume of DNA.

Limitations: This test has been recommended for patients of Asian descent who are being considered for Carbamazepine therapy. The utility of this test for patients of

other ethnic backgrounds has not been established. Even with appropriate precautions, an occasional specimen may not be satisfactory for testing. In such cases, an additional specimen should be collected for retesting.

Methodology: Polymerase chain reaction (PCR) using sequence-specific oligonucleotide probes (SSOP).

The required equipments for performing HLA typing by molecular methods (Polymerase Chain Reaction) are available at The Department of experimental medicine, The Tamilnadu Dr. MGR Medical University.

I was trained at the Karolinska Institute, Stockholm, Sweden, for performing HLA typing using PCR based kits and SSP techniques, and hence have the required expertise to perform these tests by myself.

Family H/o ADR for drugs, especially Carbamazepine was recorded in the patient case record form.

After an informed consent, 7 ml of blood in ethylene diamine tetra acetic acid (EDTA) was collected and DNA separation were done using Qiagen kits and 30 ng/µl of purified DNA sample were used for HLA-B genotyping.

*HLA–B*1502* were tested by conventional Polymerase Chain Reaction amplification and gel electrophoresis, using Olerup *HLA–B*1502* kits by SSOP (Sequence Specific Oligopeptide) method.

DNA Extraction

DNA separation was done using Qiagen kits.

DNA separation were done using Qiagen kits and simple QIAamp spin and vacuum procedures, which are ideal for simultaneous processing of multiple samples and yield pure DNA ready for direct amplification in just 20 minutes. The DNA purified using QIAamp kits is up to 50 kb in size, with fragments of approximately 20-30 kb predominating. DNA of this length denatures completely during thermal cycling and can be amplified with high efficiency.

Checking for DNA purity

The G-Quant Photometer was used for rapid and reliable quantification of DNA. It is also ideal for determining the sample purity using the 260/280 nm purity method.

260 (DNA) absorbance should be higher than the 280 (protein), or else we will have too much interference during PCR.

A purity of 1.8 to 2 is ideal.

The concentration of each DNA sample was finally adjusted to 30 ng/µl, which was used for HLA-B genotyping.

PCR amplification was done as per Protocol for *HLA–B*1502 typing.*

PCR AMPLIFICATION

For one HLA-B*15 typing, add at room temperature in a 0.5 ml tube:

53 x 2 µl= 106 µl DNA (30 ng/µl)

53 x 3 µl= 159 µl PCR Master Mix complete with Taq - mix well before taking your aliquot

53 x 5 µl= 265 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 48 wells of an HLA-S*15 typing. Well No.1 of the 48 well PCR plate is marked with '1', Close the 48 well PCR plate with the provided lids.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be <1°C.

PCR cycling parameters:

1. 1 cycle	94°C	2 min	denaturation
2. 10 cycles	94°C	10 sec.	denaturation
	65°C	60 sec.	annealing and extension
3. 20 cycles	94°C	10 sec.	denaturation
	61°C	50 sec.	annealing
	72°C	30 sec.	extension

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% *(w/v)* agarose gel in 0.5 x TBE buffer. Dissolve the agarose by

boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml),5µlper 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. Note: Ethidium bromide is a powerful carcinogen.

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row. Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 *V/cm.*

DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.

Record the presence and absence of specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.

Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the *lot-specific Interpretation and Specificity Tables.*

PCR MASTER MIX

The PCR Master Mix with *Taq* contains:

Taq polymerase	0.4 unit per 10 µl SSP reaction
Nucleotides	final concentration of each dNTPis 200 µM
PCR buffer	final concentrations: 50 mM KCI, 1.5 mM $MgCl_2$, 10 mMTris-HCI pH 8.3, 0.001 % *w/v* gelatin final concentration of glycerol is 5% Final concentration of cresol red is 100 µg/ml

Patient recruitment and sample collection

This is a prospective cohort study.

After an informed consent, 7 ml of peripheral blood was collected in ethylene diamine tetra acetic acid (EDTA) coated sterile vials.

Fig.7. Gel Electrophoresis showing negative control, positive molecular marker and positive samples

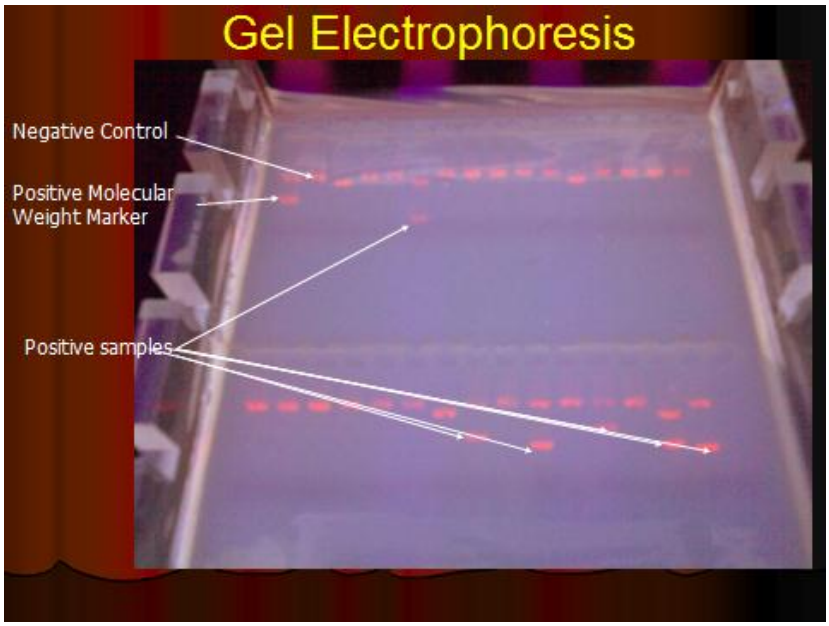


Fig.8. Gel Electrophoresis showing negative control, negative molecular marker and negative samples

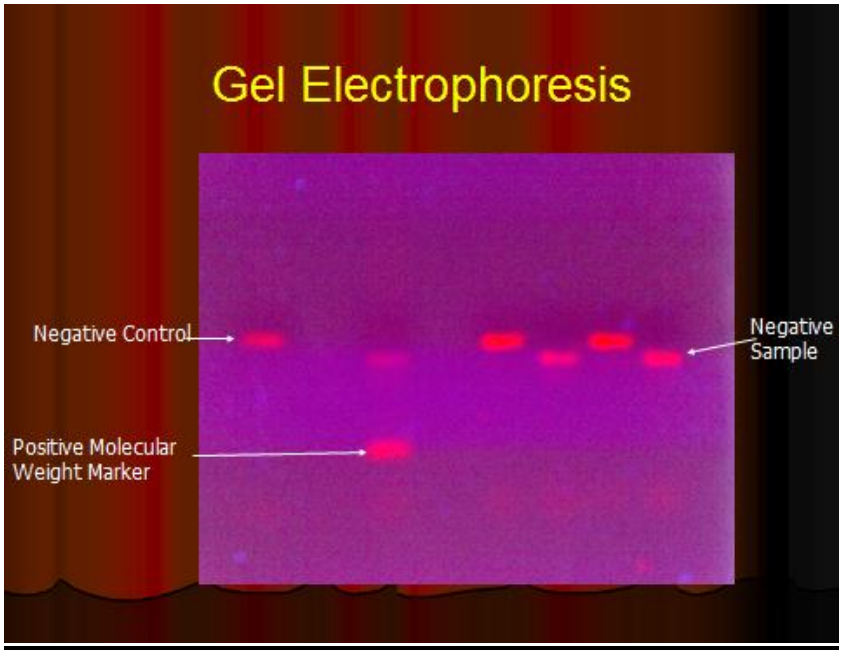
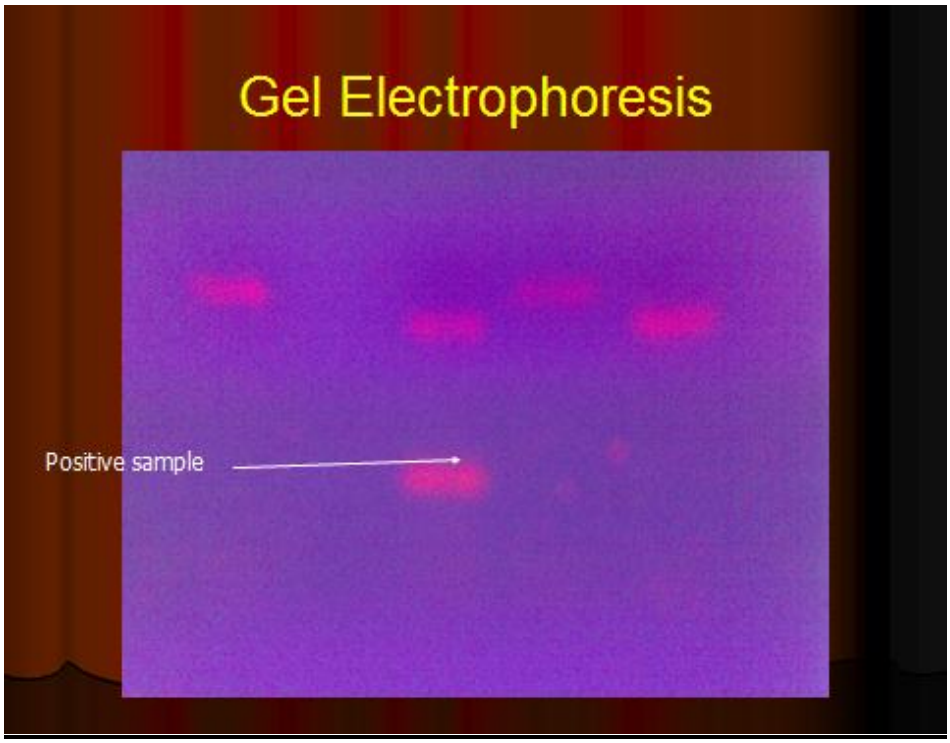


Fig.9. Gel Electrophoresis showing positive sample



RESULTS AND ANALYSIS

From July 2008 till March 2012, we enrolled 352 patients with seizures and 100 healthy subjects

Total number of Seizure patients tested for *HLA-B*1502*	: 352
Men: Women ratio	: 165: 187
Mean age	: 31.77 years (9–72)
Total number of patients on CBZ tested for *HLA-B*1502*	: 330
Number of above patients who tested positive for *HLA-B*1502*	: 66
Patients on carbamazepine who developed SJS/TEN	: 54
Patients on carbamazepine who tested negative for *HLA-B*1502* developed SJS/TEN	: 2
Positive Predictive Value for development of SJS/TEN on exposure to CBZ	: 81.82 %
Negative Predictive Value	: 99.30%
Sensitivity of *HLA-B*1502* Test	: 96.43%
Specificity of *HLA-B*1502* Test	: 95.95%
Presence of *HLA-B*1502* in the epileptic group	: 82 (18.20%)

The Mean time from exposure to CBZ and development of SJS : 14 days (5-28)

Patients on Combined therapy with CBZ and Sodium Valproate who developed SJS and had *HLA-B*1502* : 2

Total number of healthy controls tested for *HLA-B*1502* : 100

Mean age of healthy controls : 32.05 years (9–58)

Men: Women ratio (healthy controls tested for *HLA-B*1502*) : 50: 50

Presence of *HLA-B*1502* in 100 healthy controls : 7 (7%)

Association of HLAB1502 with SJS amongst patients with Seizures

	B*1502 Negative	B*1502 Positive	P<0.001
No SJS	284	12	296
SJS	2	54	56
	286	66	352

Odds Ratio and 95% confidence interval = 614.25 (133.66 - 2823)

Sensitivity = 54 /(54+2) = 96.43%

Specificity = 284/(284+12)=95.95%

Positive Predictive Value= 54/(54+12)= 81.82%

Negative Predictive Value = 284/(284+2)=99.30%

Association of HLAB1502 with SJS amongst patients on Carbamazepine

	B*1502 Negative	B*1502 Positive	P<0.001
No SJS	262	12	274
SJS	2	54	56
	264	66	330

Odds Ratio and 95% confidence interval = 562.5 (122.34 - 2586.23)

Sensitivity= 54/(54+2)=96.43%

Specificity=262/(262+12)=95.62%

Positive Predictive Value=54/(54+12)=81.82%

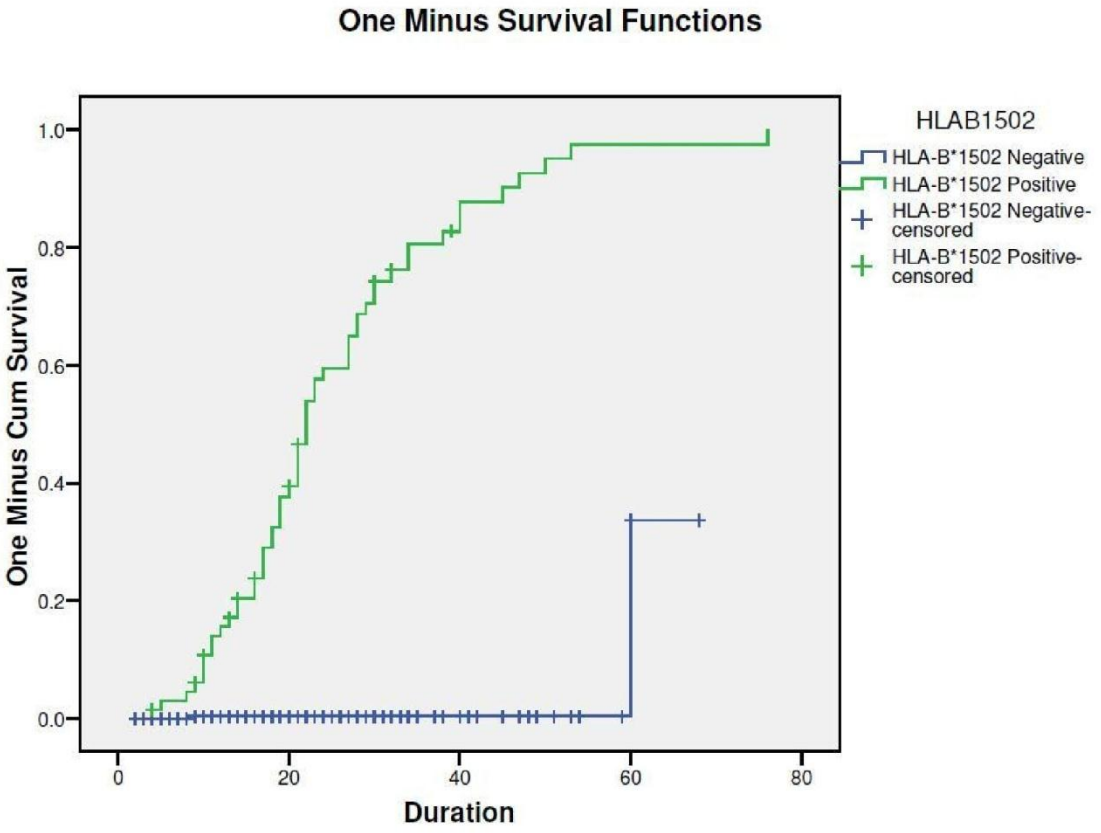
Negative Predictive Value=262/(262+2)=99.24%

Prevalence of HLAB1502 amongst seizure patients and controls

	B*1502 Negative	B*1502 Positive	P=0.003
GTCS	286	66 (19.4%)	352
Control	93	7(7%)	100
	379	73	452

*HLA-B*1502* is increased in subjects with seizures. Odds Ratio 3.19 (1.41 to 7.2)

Probability of Developing Steven Johnson syndrome among those with *HLA-B*1502* and taking Carbamazepine

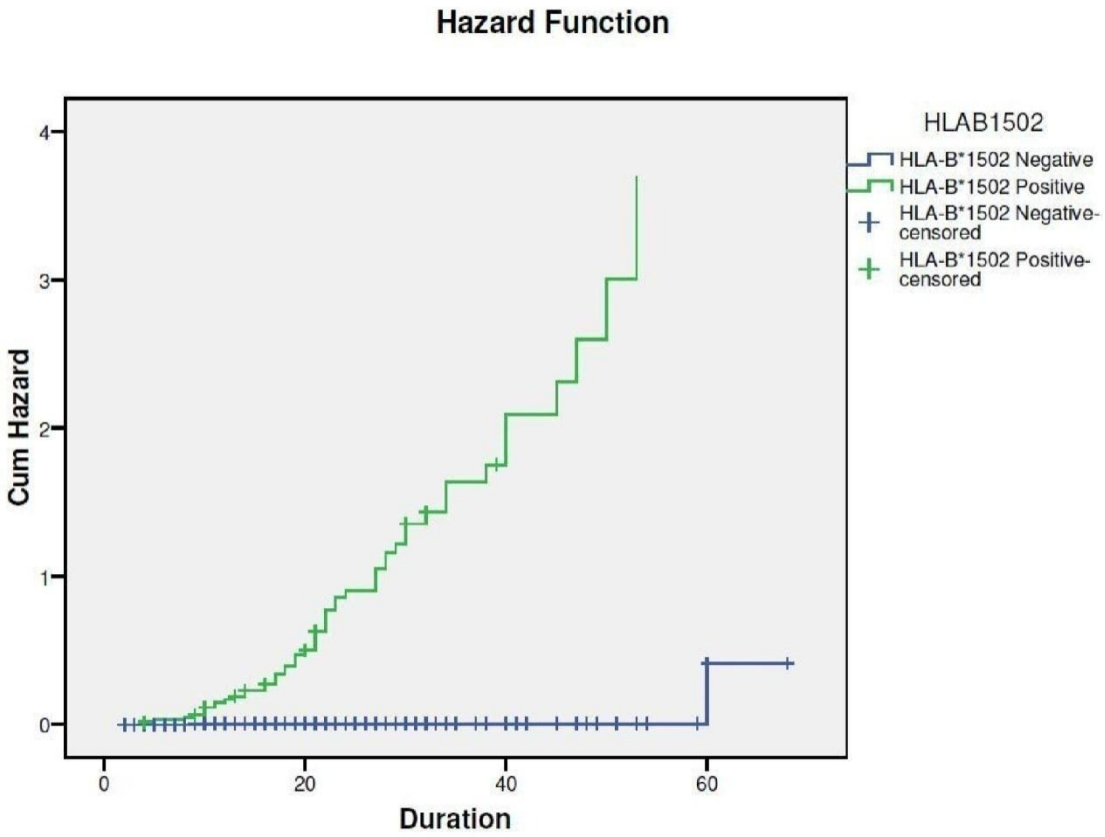


Proportion Surviving:

This proportion is computed as 1 minus the proportion failing

This proportion is computed as the ratio of the number of cases failing in the respective interval, divided by the number of cases at risk in the interval.

Hazard function for Steven Johnson Syndrome among patients with *HLA-B*1502* and taking carbamazepine; Log rank test P<0.001



Hazard Function

The term hazard function/ rate was first used by Barlow, in 1963. It is computed as the number of failures per time units in the respective interval, divided by the average number of surviving cases at the mid-point of the interval.

Minor Adverse Events

Mild and transient rash and itching developed in 18 (4%) combination of rash, itching, and localized blisters and limited oral ulcers in 12 (2.6%).

Of the 18 subjects with rash or itching, none were *HLA-B*1502*–positive.

Of the 12 patients with limited oral ulcers, none were *HLA-B*1502*–positive.

Clinical characteristics of patients who developed SJS/TEN

Case No.	Age	Sex	Type	AEDs	*HLA-B*1502*	CADR	Onset of rash(Days)
1	22	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	18
2	57	M	GTCS	CBZ	Detected	SJS-oral, eyes	21
3	55	M	GTCS	CBZ	Detected	SJS	9
4	24	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	18
5	27	M	GTCS	CBZ	Detected	SJS-oral, eyes	21
6	43	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	14
7	47	M	GTCS	CBZ	Detected	SJS-oral, eyes	11
8	28	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	17
9	27	F	GTCS	CBZ	Detected	SJS-oral, eyes	5
10	56	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	10
11	18	M	GTCS	CBZ	Detected	SJS-oral, eyes	12
12	50	F	GTCS	CBZ	Detected	SJS-oral, eyes	18
13	37	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	19
14	21	F	GTCS	CBZ	Detected	SJS-oral, eyes	10
15	29	F	GTCS	CBZ	Detected	SJS-oral, eyes	15
16	19	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	11
17	17	M	GTCS	CBZ	Detected	SJS-oral, eyes	15
18	32	M	GTCS	CBZ	Detected	SJS-oral, eyes	17
19	19	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	19
20	72	F	GTCS	CBZ	Detected	SJS-oral, eyes	28
21	38	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	10
22	18	F	GTCS	CBZ	Detected	SJS-oral, eyes	10
23	36	F	GTCS	CBZ	Detected	SJS-oral, eyes	12
24	37	F	GTCS	CBZ	Detected	SJS-oral, eyes	14
25	53	F	GTCS	CBZ	Detected	SJS-oral, eyes	11
26	27	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	12
27	44	F	GTCS	CBZ	Detected	SJS-oral, eyes	11
28	41	M	GTCS	CBZ	Detected	SJS-oral, eyes	18

29	11	F	GTCS	CBZ	Detected	TEN-oral, eyes, genital*	20
30	9	F	GTCS	CBZ	Detected	SJS-oral, eyes	12
31	37	F	GTCS	CBZ	Detected	SJS-oral, eyes	9
32	25	M	GTCS	CBZ+ Sod Val	Detected	SJS-oral, eyes, genital	15
33	34	F	GTCS	CBZ	Detected	SJS-oral, eyes	12
34	28	M	GTCS	CBZ+ Sod Val	Detected	SJS-oral, eyes	10
35	9	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	14
36	21	M	GTCS	CBZ	Detected	TEN-oral, eyes, genital	10
37	32	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	12
38	23	M	GTCS	CBZ	Detected	SJS-oral, eyes	10
39	33	F	GTCS	CBZ	Detected	SJS-oral, eyes	12
40	35	M	GTCS	CBZ	Detected	TEN-oral, eyes, genital*	19
41	48	F	GTCS	CBZ	Detected	SJS-oral, eyes	12
42	33	F	GTCS	CBZ	Detected	SJS-oral, eyes, genital	14
43	19	F	GTCS	CBZ	Detected	SJS-oral, eyes	11
44	15	F	GTCS	CBZ	Detected	SJS-oral, eyes	17
45	60	M	GTCS	CBZ	Detected	SJS-oral, eyes	14
46	20	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	21
47	21	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	16
48	33	M	GTCS	CBZ	Detected	SJS-oral, eyes	9
49	29	F	GTCS	CBZ	Detected	SJS-oral, eyes	11
50	25	M	GTCS	CBZ	Detected	SJS-oral, eyes, genital	10
51	29	M	GTCS	CBZ	Detected	SJS-oral, eyes	12
52	32	M	GTCS	CBZ	Detected	SJS-oral, eyes	21
53	37	F	GTCS	CBZ	Detected	SJS-oral, eyes	11
54	72	M	GTCS	CBZ	Detected	SJS-oral, eyes	18
55	48	F	GTCS	CBZ	Not Detected	SJS-oral, eyes	12
56	27	M	GTCS	CBZ	Not Detected	SJS-oral, eyes	16

*Denotes expired patients.

ANALYSIS OF RESULTS

The Men: Women ratio among 352 seizure patients tested for *HLA-B*1502* allele was 1: 1.13. There was a slight increase in females.

The sex ratio was almost equal (29 Men and 27 Women) in the 56 patients who developed SJS/TEN.

The Mean age of 352 Epileptic patients was : 31.77 years.

The Mean age of patients who developed SJS/TEN was : 32.83 years.

The age distribution of 56 patients who developed SJS/TEN

0-10	2
11-20	9
21-30	17
31-40	14
41-50	7
51-60	5
61-70	0
71-80	2

The Maximal incidence of SJS/TEN was seen in the age groups between 11-40 years

54 out of 56 patients (96.40 %) who developed SJS were positive for *HLA-B*1502* allele and in two patients *HLA-B*1502* allele was negative (3.60 %).

The mean interval between CBZ administration and the development of skin rash was 14 days (Range: 5-28 days).

All the patients developed SJS within 30 days from exposure to CBZ.

Patients who did not develop any skin rash for more than 30 days after starting CBZ, tolerated CBZ without developing SJS/TEN for the entire duration of treatment up to three years.

In our study CBZ was stopped immediately when patients developed rashes after exposure to CBZ. Even *HLA-B*1502*–negative subjects who developed skin rashes or oral mucosal lesions were not re-exposed to CBZ.

In our study, the alternate anti-epileptic drugs used in *HLA-B*1502* carriers were: Levetiracetam, Gabapentin, Pregabalin, Zonisamide, Lacosamide and Clonazepam.

In our study *HLA-B*1502* screening was routinely performed prior to the administration of CBZ.

In our study, the presence of the *HLA-B*1502* allele was strongly correlated to the development of SJS/TEN. The Positive Predictive Value for development of SJS/TEN on exposure to CBZ was 81.82 % with a very high Negative Predictive Value of 99.30%. The Sensitivity of *HLA-B*1502* Test was 96.43% with a Specificity of 95.95%

In our study, two patients on combined therapy with CBZ and Sodium Valproate tested positive for *HLA-B*1502* allele and both developed SJS.

In our study, *HLA-B*1502* allele was present in the epileptic group in 82 out of 352 patients studied (18.20%).

In our study, we tested 100 healthy controls (50 men and 50 women) with a mean age of 32.05 years (range: 9–58) for the presence of *HLA-B*1502* allele. *HLA-B*1502* was detected in 7 subjects (7%).

In our study, *HLA-B*1502* allele is increased in subjects with seizures when compared to Controls (18.20% Vs 7% in Controls) with a Odds Ratio of 3.19 (1.41 to 7.2).

Fig.10. SJS presenting as Palmar rash

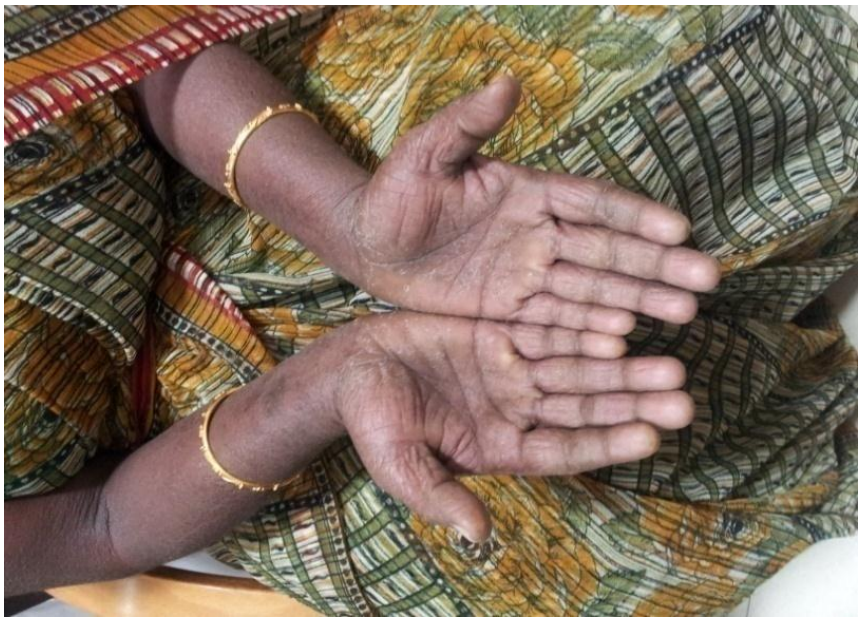


Fig.11. SJS presenting as erythematous rashes in legs

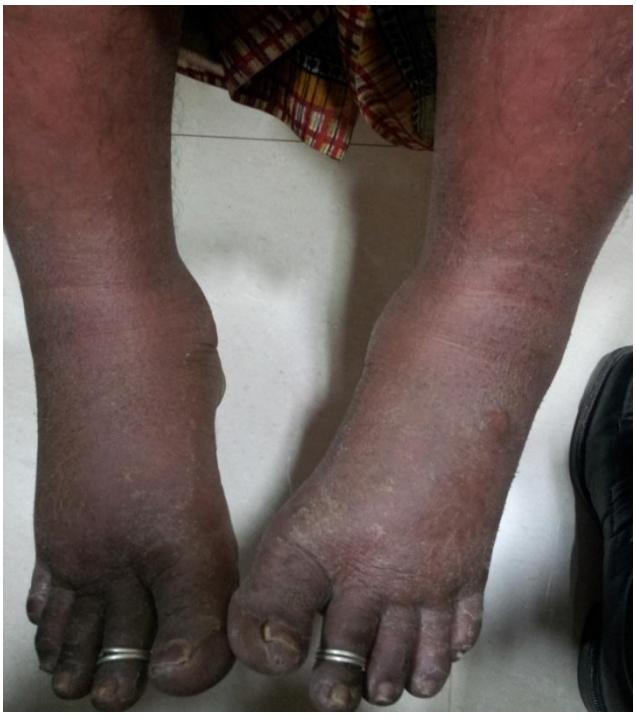


Fig.12. SJS presenting as erythematous rashes in face

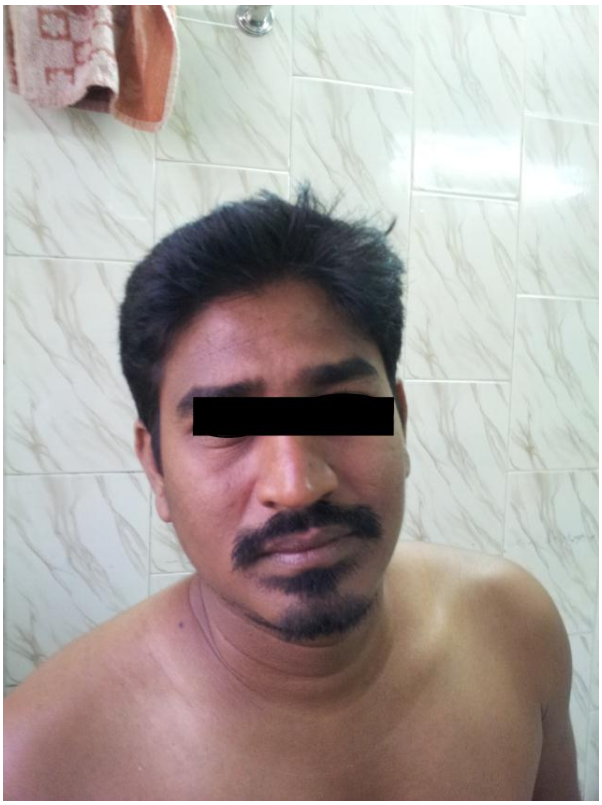


Fig.13. SJS presenting as erythematous rashes in chest and trunk

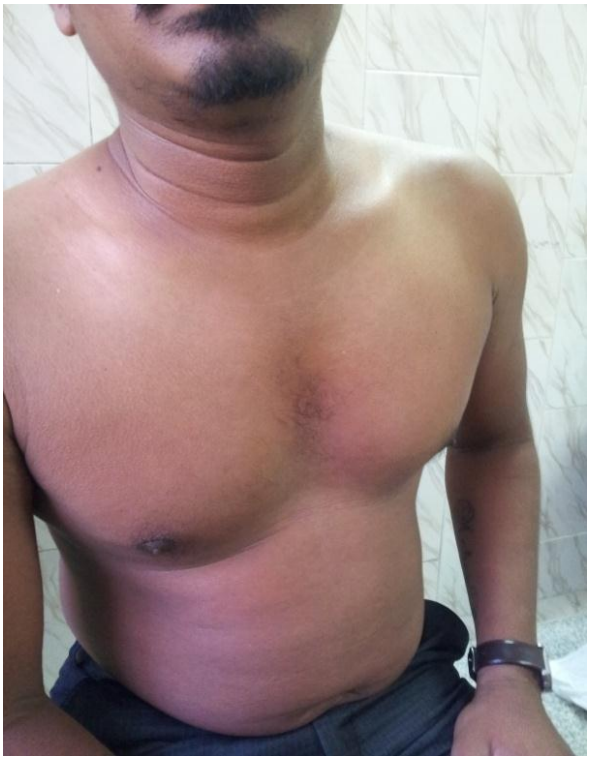


Fig.14. SJS presenting as erythematous rashes in the back

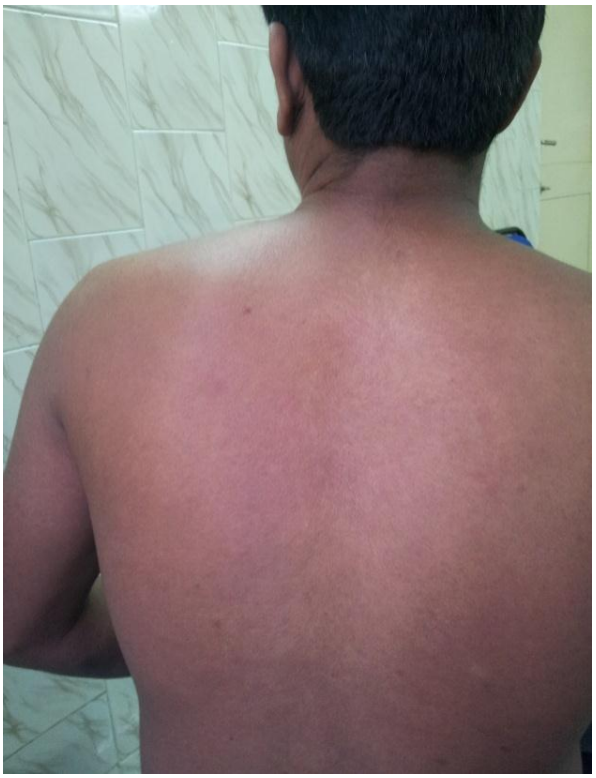


Fig.15. SJS presenting as erythematous rashes in palm

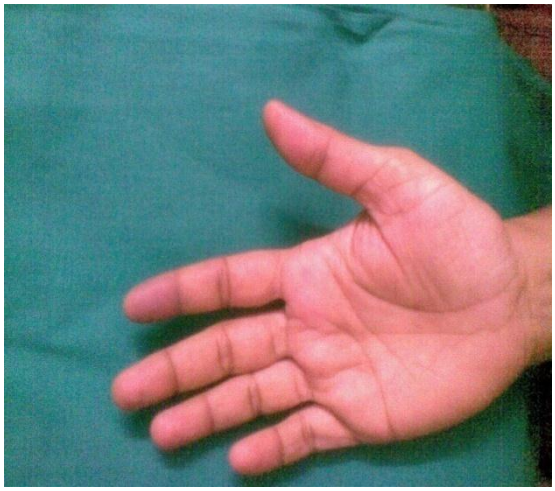


Fig.16. SJS presenting as erythematous rashes in palms

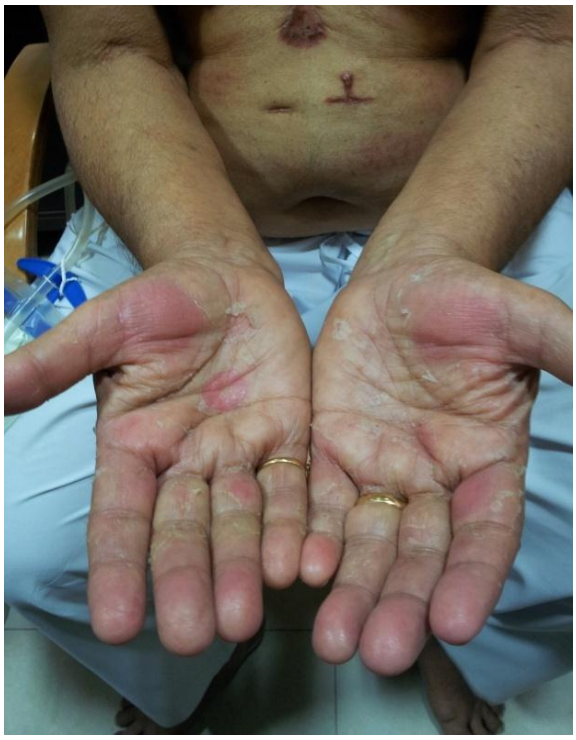


Fig.17. SJS presenting as erythematous rashes in the back

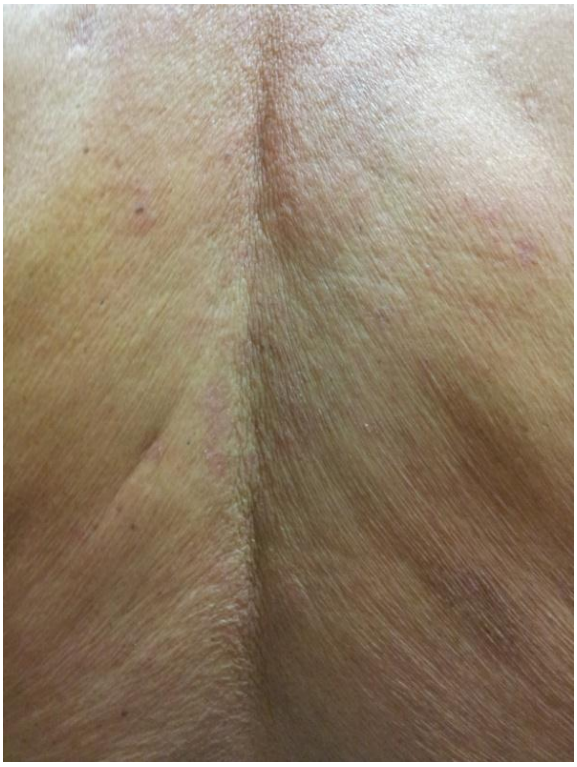


Fig.18. SJS presenting with rashes in the face, neck, arms and back

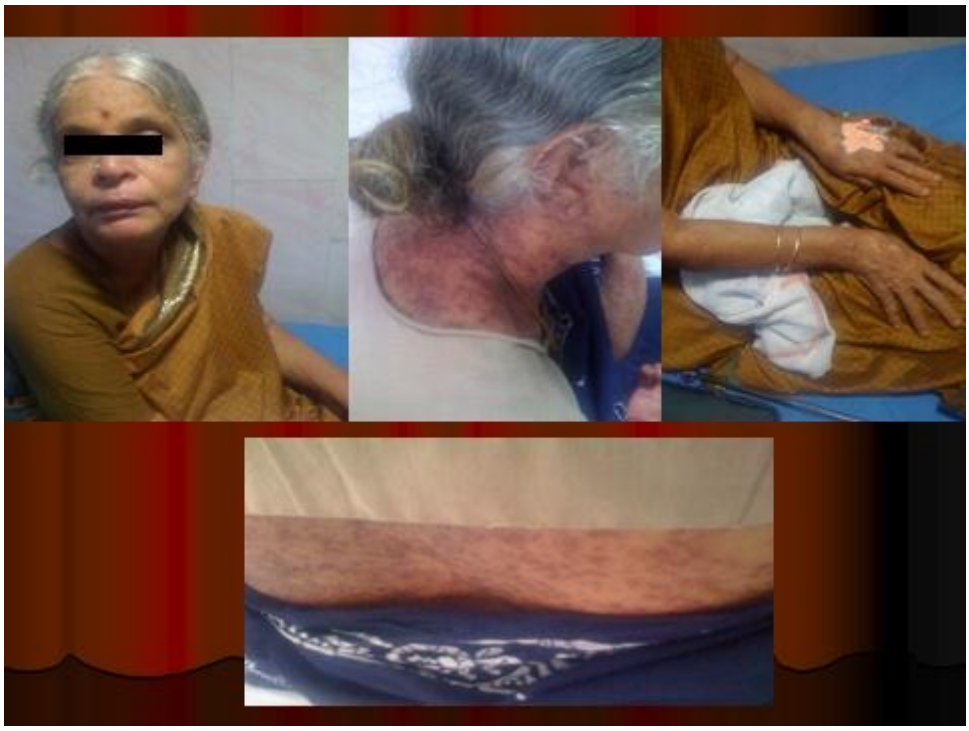


Fig.19. SJS presenting as erythematous rashes in legs

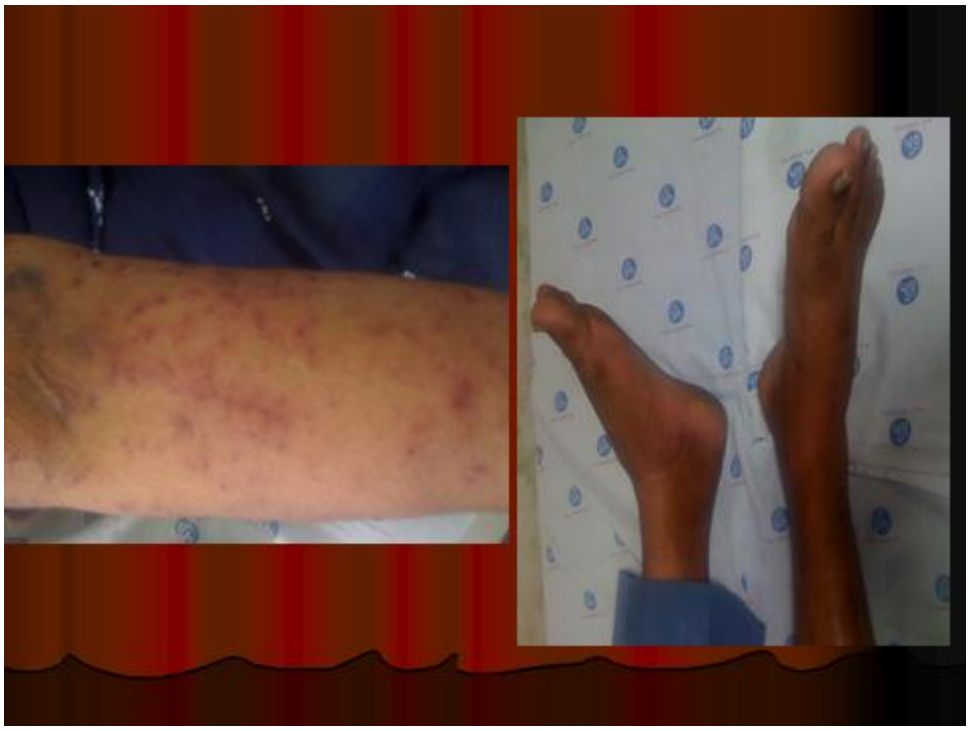
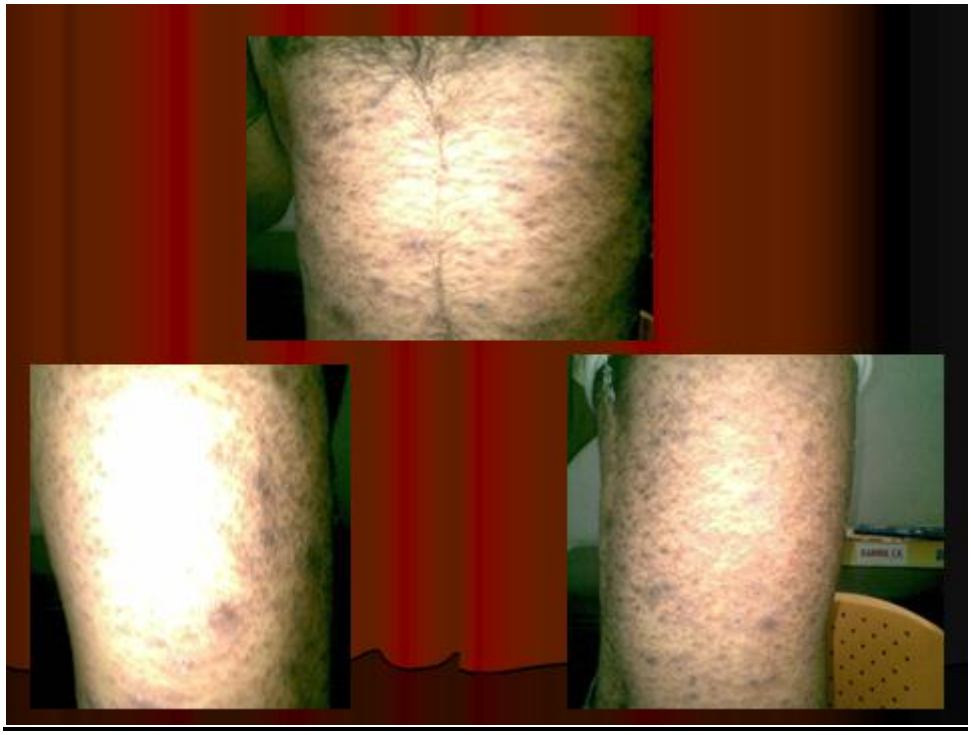


Fig.20. Healed SJS lesions in chest and legs



DISCUSSION

Wetter[188] in a retrospective review of patients seen at Mayo Clinic between January 1, 2000, and December 31, 2007, of 27 patients (mean age, 28.1 years), 22 (81%) had involvement of 2 or more mucous membranes, and 19 (70%) had ocular involvement. Medications, most commonly antibiotics and anticonvulsants, were causative in 20 patients. *Mycoplasma pneumonia* infection caused 6 of the 27 cases. Corticosteroids were the most common systemic therapy. No patients with mycoplasma-induced Stevens-Johnson syndrome had internal organ involvement or required treatment in the intensive care unit, in contrast to 4 patients each in the drug-induced group. Three patients had chronic ocular sequelae, and 1 died of complications. Biopsy specimens from 13 patients (48%) showed epidermal necrosis (8patients), basal vacuolar change (10 patients), and sub epidermal bullae (10 patients). Biopsy specimens from 11 patients displayed moderate or dense dermal infiltrate. Histologic features in drug induced cases included individual necrotic keratinocytes, dense dermal infiltrate, red blood cell extravasations, pigment incontinence, parakeratosis, and substantial eosinophils or neutrophils. Their clinical and etiologic findings corroborate those in previous reports. *M pneumoniae*–induced Stevens-Johnson syndrome manifested less severely than its drug-induced counterpart. The limited number of biopsies precludes unequivocal demonstration of histopathological differences between drug-induced and *M pneumoniae*–induced Stevens-Johnson syndrome.

They mention that much confusion exists regarding the nosology of SJS, which poses particular challenges for researchers conducting studies of SJS and reviewing previously reported cases. The distinction between SJS and EM major is controversial for some researchers, and they suggest instead that the 2 entities are

synonymous; others contend that more extensive mucous membrane involvement in SJS differentiates it from EM major. To most accurately review the 27 cases of SJS in the current study and compare them with those reported in the medical literature, the authors used an accepted consensus definition of SJS that relies on the pattern and distribution of skin lesions, rather than on the extent and severity of mucous membrane involvement.

Although the degree of mucous membrane involvement is not an absolute criterion for SJS according to the consensus definition, mucosal erosions were reported to be present in more than 90% of patients by this classification scheme; another study of 33 patients with SJS demonstrated mucous membrane involvement in all the patients, with 23 having 2 or more affected membranes. Their findings are in keeping with these data because all 27 of their study patients had mucous membrane involvement, and 22 (81%) had involvement of 2 or more mucous membranes.

In the present study oral and eye involvement was present in 35 out of 56 patients who developed SJS/TEN (62.5%). Additional involvement of the genitalia was present in 20 patients (35.71%).

The mean age of their patients was 28.1 years, with nearly half younger than 18 years. This finding is concordant with the emphasis in the medical literature on SJS as a disease that occurs predominantly in younger patients.

In our present study, the mean age of the patients who developed SJS/TEN was 32.83 years. The maximal incidence of SJS was present in the age groups 21-50 years. There were no gender difference in the occurrence of CBZ induced SJS. 29 Men and 27 women developed SJS.

54 out of 56 patients (96.40 %) who developed SJS were positive for *HLA-B*1502* allele and in two patients *HLA-B*1502* allele was negative (3.60 %).

The patients who developed CBZ were exposed to doses of 200 to 800 mg prior to the onset of skin rashes or mucosal lesions of SJS. There is no study which has looked into the initial dose of carbamazepine and the time to onset of SJS lesions. Since carbamazepine induced SJS is an idiosyncratic hypersensitivity drug reaction the initial dose may not be causally related to the development of SJS.

In Mayo patients with drug-induced SJS, the mean interval between drug administration and onset of cutaneous findings was 15.3 days. Previous reports in the medical literature describe similar intervals, with the greatest risk of development of SJS occurring in the first 2 months of drug treatment and an interval of 4 to 28 days being the most suggestive of drug causality in SJS.

The mean interval between CBZ administration and the development of skin rash was 14 days. All patients developed SJS within 28 days from exposure to CBZ. Patients who did not develop any skin rash 30 days after starting CBZ, tolerated CBZ without developing SJS/TEN for the entire duration of treatment upto three years.

A recent multinational case-control study in Europe called EuroSCAR (European Study of Severe Cutaneous Adverse Reactions) showed a high risk of SJS with the following medications: trimethoprim-sulfamethoxazole and other anti-infective sulfonamides, lamotrigine, carbamazepine, phenytoin, phenobarbital, allopurinol, nevirapine, and oxicam nonsteroidal anti-inflammatory drugs. Among recently marketed drugs, nevirapine and lamotrigine were strongly associated with SJS, with sertraline showing a lower but still significant risk as well.

M pneumoniae is also a well-known cause of SJS. It usually affects children and young adults and has been reported as the most common infectious agent associated with SJS. Nearly all patients manifest oral involvement, and approximately two-thirds of patients have ocular involvement. Previous studies suggest that *Mycoplasma* induced SJS is associated with less frequent and less severe complications than those resulting from other causes. In their study, 6 cases were due to *M pneumoniae* as confirmed by positive serologic findings; previous studies have documented 10% to 29% of SJS cases as secondary to *M pneumoniae* infection. In this series, the mean age of patients with mycoplasma-induced SJS (19 years) was younger than that of patients with drug-induced SJS (31.40 years). No patient with mycoplasma-induced SJS manifested internal organ involvement or required treatment in the intensive care unit, and the mean hospital stay was shorter than that observed in patients with drug-induced SJS, which augments previous assertions that mycoplasma-induced SJS may manifest less severely than its drug induced counterpart.

Serological tests for Mycoplasma *pneumonia* were not performed in the present study.

In the study of Pei Cheng, screening patients for the *HLA-B*1502* allele before the initiation of carbamazepine treatment and withholding carbamazepine from *HLA-B*1502*–positive patients can reduce the incidence of carbamazepine-induced SJS–TEN among Han Chinese. In estimating the historical incidence of this condition, they defined new carbamazepine recipients as those who had not received carbamazepine during the previous year and who were prescribed carbamazepine for at least 14 days in the year of interest, because carbamazepine-induced SJS–

TEN is a delayed hypersensitivity reaction that usually takes at least 14 days to develop. However, even if they had included all new carbamazepine recipients, regardless of the duration of treatment, as historical controls, the difference in the incidence of SJS–TEN would still be significant (P = 0.01,P = 0.02, and P = 0.02 for 2002, 2003, and 2004,respectively). Since they estimated the historical incidence of carbamazepine-induced SJS–TEN on the basis of data obtained from the NHIRD, the reliability of these data is critical for the validity of their estimation.

The NHIRD was established in Taiwan when the government launched the National Health Insurance system in 1995. This mandatory single-payer health insurance system, which is administered by the Taiwanese government, provides health care for almost all people in Taiwan, with enrolment of 99.5% of the population in 2008. Of the health care facilities in Taiwan, 92.5% have been contracted by the National Health Insurance System. The NHIRD data were therefore likely to be comprehensive. To estimate both the percentage of subjects with SJS–TEN among those with ICD-9-CM diagnostic code 695.1 (indicating erythema multiforme) and the percentage of cases of carbamazepine induced SJS–TEN in Taiwan, they based their review on the medical records of 700 cases with diagnostic code 695.1 during a 5-year period at the Chang-Gung Memorial Hospital, the largest hospital system in Taiwan, with several regional centers. This hospital provides health care for about 12% of the Taiwanese population, and its patients are thought to be representative of the general population.

It is possible that some of the drug-related adverse reactions they observed were early SJS lesions or that early withdrawal of carbamazepine may have prevented a more severe SJS–TEN or TEN-like reaction. However, we think that this

is unlikely, since once patients are sensitized by carbamazepine and have early blisters or ulcers, SJS–TEN progresses, even after the withdrawal of the drug.

SJS–TEN did not develop in any of the subjects who completed the 2-month follow-up. Adverse cutaneous reactions, including blisters, oral lesions, and rash, that occurred in the subjects were mild, localized, and transient. In some subjects, blisters developed only after the rash subsided (i.e. blisters were sporadic and tended not to occur at the same time as rash). Furthermore, many *HLA-B*1502*–negative subjects resumed taking carbamazepine without the occurrence of skin lesions. More important, SJS–TEN did not develop in these subjects, a finding that is consistent with the concept that the incidence of carbamazepine-induced SJS–TEN in *HLA-B*1502*–negative persons is very low.

This large series suggested the value of *HLA-B*1502* screening to prevent carbamazepine-induced SJS–TEN. However, as for any new pharmacogenomic test, it is important to document the use and safety of the alternative medications. Of the 367 *HLA-B*1502* carriers, 215 (58.6%) were given an alternative medication, such as gabapentin, valproic acid, oxcarbazepine, clonazepam, or lamotrigine; the remainder continued to take their pre-study medication. Among the 215 *HLA-B*1502* carriers who took alternative drugs, the only symptom seen during the 2-month follow-up was mild, transient rash in 5 subjects (2.3%).

In our study CBZ was stopped immediately when patients developed rashes after exposure to CBZ. Even *HLA-B*1502*–negative subjects who developed skin rashes or oral mucosal lesions were not re-exposed to CBZ.

In our study, the alternate antiepileptic drugs used in *HLA-B*1502* carriers were: Levetiracetam, Gabapentin, Pregabalin, Zonisamide, Lacosamide and Clonazepam.

A strong association between *HLA-B*1502* and carbamazepine-induced SJS–TEN has been found in Asian populations other than the Han Chinese, including Malay, Thai, and South Asian Indians. In Malaysia, Thailand, and India, studies have shown that carbamazepine was the major cause of drug-induced SJS–TEN. Since the contribution of *HLA-B*1502* to carbamazepine-induced SJS–TEN has been proved to be causal, the authors speculated that in these countries, in which *HLA-B*1502* is relatively prevalent, *HLA-B*1502* screening could provide a benefit.

In our study *HLA-B*1502* screening was routinely performed prior to the administration of CBZ.

*HLA-B*1502* as a marker for carbamazepine-induced SJS is well established in the Han Chinese by different studies. The allele frequency of *HLA-B*1502* in the Han Chinese population is 8%, which is relatively higher than any other population of the world. From India, a 0.6% (average 2.5%) prevalence of *HLA-B*1502* indifferent communities has been reported [108]. Most of them are sub-Hindu communities, except Parsi, in which a0% prevalence is reported. Mehta's *et al* [108] series included eight patients belonging to the Hindu community, which is the biggest community of India comprising of one billion Indians. Six out of eight patients had *HLA-B*1502* while one of the 10 controls were found to be positive. This clearly indicates a significant association (Odds ratio: 71.40 [95% CI, 3.0.1698]; *P* = 0.0014) between carbamazepine-induced SJS and *HLA-B*1502* among Indians.

In our study out of 330 patients on CBZ, 54 tested positive for *HLA-B*1502* allele.

The prevalence of *HLA-B*1502* allele in healthy age and sex matched controls was 7%, which is very similar to the allele frequency in the Han Chinese.

Elizabeth J. Phillips, and Simon A. Mallal[189] in the paper '*HLA-B*1502* Screening and Toxic Effects of Carbamazepine' reported the absence of the Stevens–Johnson syndrome and its related disease, toxic epidermal necrolysis (SJS–TEN), in subjects who were screened for the *HLA-B*1502* allele and were advised not to take carbamazepine if they carried the allele. Case–control studies involving subjects of Han Chinese and Thai origin have also shown an association between SJS–TEN and the receipt of phenytoin, lamotrigine, and oxcarbazepine among *HLA-B*1502* carriers. The Food and Drug Administration has stated that "healthcare providers should consider avoiding phenytoin as alternatives for CBZ [carbamazepine] in patients who test positive for *HLA-B*1502*." Chen et al. state that lamotrigine and oxcarbazepine were among the alternative medications offered to *HLA-B*1502* carriers and did not indicate whether the investigators or treating physicians warned patients not to take phenytoin. The increasing use of *HLA-B*1502* screening to prevent carbamazepine-induced SJS–TEN will lead to the replacement of this drug with other anticonvulsants among *HLA-B*1502* carriers. Given the availability of other elective therapeutic choices, it may be prudent to advise *HLA-B*1502* carriers to avoid not only carbamazepine but also other structurally related anticonvulsants, such as phenytoin, oxcarbazepine, and possibly lamotrigine.

In our study we have avoided all the AEDs like phenytoin, oxcarbazepine and lamotrigine in patients who developed CBZ induced SJS.

Hiroko Ikeda, Yukitoshi Takahashi, Etsuko Yamazaki, Tateki Fujiwara, *et al*[190] in the paper HLA Class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions stated that the drug causes cutaneous adverse drug reactions (cADRs) that may range from mild to severe. Since the human leukocyte

antigen *HLA-B*1502* is associated with Stevens-Johnson syndrome (SJS) induced by CBZ in Han Chinese, they examined HLA class I in 15 Japanese patients who fulfilled the diagnostic criteria for CBZ-induced cADRs (mild in 10 and severe = SJS in 5). HLA-B*1518,HLA-B*5901 and HLA-C*0704 alleles showed higher relative risks (above 10.0) for severe cADRs. The haplotype (HLA-A*2402-B*5901-C*0102) had high relative risk (16.09) for severe cADRs. In patients with severe cADRs, frequencies of HLAA* 1101, HLA-A*3303, HLA-B*1501, HLA-B*4403, HLA-B*5101, HLA-B*5201, HLA-C*0702, and HLA-C*1202 alleles are relatively lower than in the Japanese population. These data may suggest that HLA-B*5901 is one of the candidate markers for CBZ-induced SJS in Japanese.

In our study, HLA-B*5901 allele was not tested.

In our study patients with MPE were not tested for *HLA-B*1502* allele. This is based on the study by Wang Q, Zhou JQ, Zhou LM, Chen ZY, *et al*[191] in the paper 'Association between *HLA-B*1502* allele and carbamazepine-induced severe cutaneous adverse reactions in Han people of southern China mainland' who mentioned that previous studies have found a strong association between *HLA-B*1502* and carbamazepine-induced Stevens-Johnson syndrome in Asian areas including Taiwan, Hong Kong and Thailand. This study explores the association between *HLA-B*1502* allele and carbamazepine-induced cutaneous adverse reactions in Han Chinese of southern China mainland, and find the genetic marker that can predict carbamazepine-induced cutaneous adverse reactions. *HLA-B*1502* allele genotyping was performed by a polymerase chain reaction-sequence specific primers (PCR-SSP) method in 48 Han Chinese subjects who had carbamazepine-induced cutaneous adverse reactions, including 9 severe cutaneous adverse

reaction patients with Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) and 39 cutaneous adverse reaction patients with maculopapular eruption (MPE). Meanwhile 80 carbamazepine-tolerant controls and 62 healthy individuals were also tested. The frequency of *HLA-B*1502* allele among SJS/TEN patients (100%) is significantly higher than carbamazepine-tolerant controls (13.75%, P<0.001) and healthy individuals (17.74%, P<0.001). But the frequency between MPE patients and carbamazepine-tolerant controls (25.64% vs.13.75%, P=0.110) did not have any significant difference. The data showed that *HLA-B*1502* allele is strongly associated with carbamazepine-induced SJS/TEN but not MPE in Han Chinese of southern China mainland.

In our study, only *HLA-B*1502* was considered and the presence of the allele was strongly correlated to the presence of SJS/TEN, with a high Positive Predictive Value, although not 100%, as in the Han Chinese population.

*HLA-B*1502* allele was detected in this study by SSP method using conventional PCR and newer methods like high resolution PCR methods were not used.

In the genome-wide association study by McCormack and colleagues, samples were obtained from 26 patients with confirmed carbamazepine hypersensitivity syndrome, 106 patients with carbamazepine associated maculopapular exanthema (rash without systemic involvement), and 12 patients who had developed SJS/TEN. These individuals were then compared with genotype data derived from the Welcome Trust Case Control Consortium, U.K. National Blood Services Collection, and the 1958 British Birth Cohort. In addition, a clinical control group (n = 257) of patients receiving carbamazepine for at least 3 months with no evidence of hypersensitivity were also compared.

For both patients with DRESS and SJS/TEN, a strong signal in the HLA-A region on chromosome 6 was seen with HLA A*3101 being most strongly associated. This variant, which has a prevalence of up to 5% in Northern Europeans, was observed in 40% of patients with either DRESS or SJS/TEN as compared with only 4 to 5 percent of control subjects. With regard to maculopapular exanthema, HLA-A*3101 was again the most strongly associated allele, being seen in 27% of patients versus 4% of controls.

Although the population of this study was of Caucasian decent, it is interesting to note that HLA-A*3101, which is found in about 9% of Japanese and 2% of Han Chinese, has also been associated with carbamazepine hypersensitivity including SJS/TEN and maculopapular exanthema in these patient populations. Taken together, these data provide compelling evidence that HLA-A*3101 is yet another important predictor of carbamazepine-associated cutaneous hypersensitivity reactions.

Confidence in these findings is enhanced when one considers the consistency of the data across several independent groups of case subjects as well as controls. Now, can we use these data to reliably screen and identify potentially at-risk patients and thereby avoid serious hypersensitivity? Perhaps yes.

McCormack and colleagues suggest that the presence of this allele increases the risk of a hypersensitivity reaction to 26%, whereas its absence lowers that risk to just fewer than 4%. If one assumes that the prevalence of carbamazepine hypersensitivity is 5% in the European Caucasian population, then about 83 patients would need to be screened to prevent one hypersensitivity reaction. Of course, the number of patients needed to be screened would fall if, in fact, the actual prevalence

of hypersensitivity were greater. For example, a carbamazepine hypersensitivity incidence of 10% would reduce the number needed to be screened to 39.

In our study, no SNP studies or linkage studies were performed.

Since all the subjects were unrelated familial association studies were not studied.

Patch testing is safe and effective in CBZSJS/TEN. With the use of patch tests in patients who suffer from severe cutaneous adverse reactions, both the clinicians and the patients may be more aware of avoiding the single culprit drug. It also offers a way for alternative antiepileptic drugs selection for patients with CBZ-induced severe cutaneous adverse reactions. Patch testing[192] was positive in Ten (62.5%) of the 16 CBZ-SJS or TEN in a series. The authors had proposed two reasons for high proportion of positive results in SJS/TEN by their: (i) Genetic factor: patients with *HLA-B*1502* may have a higher potential to show positive reaction to CBZ by patch test. A further study has shown that *HLA-B*1502* was not only a genetic marker but also had a functional role and provided a higher binding affinity to CBZ than other *HLA-B*1502* molecules. (ii) Drug antigenicity: different drug antigens might have different antigenicity, such as allopurinol was rarely reported to cause a positive reaction on patch testing in patients with allopurinol hypersensitivity. CBZ may be more antigenenic for patch testing than other drugs.

We did not carry out patch testing in our patients.

Manuela G. Neuman, Lawrence Cohen, Radu M. Nanau, and Paul A. Hwang[193] in the article on 'Genetic and immune predictors for hypersensitivity syndrome to antiepileptic drugs' discussed about hypersensitivity syndrome reactions (HSR) to antiepileptic drugs (AED) are associated with severe clinical

cutaneous adverse reactions (SCAR). They aimed to assess HSRs to AEDs using the in vitro lymphocyte toxicity assay (LTA) in patients who manifested HSRs clinically; to correlate LTA results with the clinical syndrome; to correlate LTA results with the human leukocyte antigen (HLA) allele B*1502 (*HLA-B*1502*) positivity in a Han Chinese-Canadian population; and to determine the cytokine network in this population. Patients that developed fever and cutaneous eruptions in the presence or absence of organ involvement within 8 weeks of exposure to carbamazepine (CBZ), phenytoin (PHY), or lamotrigine (LTG) were enrolled. Control patients received AEDs without presenting HSR. They investigated 10 CBZ-HSR patients (4 with Stevens-Johnson syndrome [SJS]), 24 CBZ-controls, 10 PHY-HSR patients (4 with drug-induced liver injury [DILI]), 24 PHY-controls,6 LTG-HSR patients (1 with SJS and 1 with DILI), and 24 LTG-controls. There were 30 Han Chinese individuals (14 HSR patients and 16 controls) in their cohort. LTA toxicity greater than 12.5% 6 2.5% was considered positive. Differences among groups were determined by analysis of variance. In addition, they measured cytokine secretion in the patient sera between 1 month and 3 years after the event. All Han Chinese individuals and 30% of Caucasians were genotyped for *HLA-B*1502*. A perfect correlation (r 5 0.92) was observed between positive LTA and clinical diagnosis of DILI and SJS/toxic epidermal necrolysis (TEN). *HLA-B*1502* positivity in Han Chinese is a predictor of CBZ-HSR and PHY-HSR. *HLA-B*1502*-negative Han Chinese receiving only CBZ or a combination of CBZ and PHY tolerated the drug(s) clinically, presenting negative CBZ-LTA and PHY-LTA. However, 3 patients presenting negative CBZ-LTA and PHY-LTA, as well as negative *HLA-B*1502*, showed positive LTG-LTA (38%, 28%, and 25%, respectively), implying that they should not be prescribed LTG. Three patients had LTA positive to both PHY and CBZ, and 3 others had LTA positive to

both PHY and LTG. Clinically, all 6 patients presented HSR to both drugs that they tested positive to (cross-reactivity). Patients were grouped based on the clinical presentation of their symptoms as only rash and fever or as a triad of rash, fever and DILI or SJS/TEN that characterizes ‘‘true’’ HSR. Levels of proinflammatory cytokines were significantly higher in patient sera compared with control sera. More specifically, the highest levels of tumour necrosis factor-a have been measured in patients presenting ‘‘true’’ HSR, as were the apoptotic markers Fas, caspase 8 activity, and M30. The LTA is sensitive for DILI and SJS/TEN regardless of drug or patient ethnicity. HSR prediction will prevent AED-induced morbidity. In Han Chinese, *HLA-B*1502* positivity is a predictor for CBZ-HSR and PHY-HSR. Its negativity does not predict a negative LTG-HSR. There is cross reactivity between AEDs. Additionally, T-cell cytokines and chemokines control the pathogenesis of SJS/TEN and DILI, contributing to apoptotic processes in the liver and in the skin.

Lymphocyte transformation test (LTT) is an in vitro alternative method, which measures the drug-related T-cell response to identify culprit drugs, but it is currently not sufficiently reliable. In a series, when peripheral blood mononuclear cells of the CBZSJS⁄TEN were tested with OXC by LTT, the results always showed a positive cross-activated (100%), while only15–50% patients showed positive cross-reactivity among the AEDs, which was more correlated with the real clinical experience of CBZ and OXC cross-hypersensitivity.

We did not carry out LTT in our patients.

Many viral and Mycoplasma co-infections were not studied.

Genetic studies of Toll3 receptors and incidence of this gene in the Indian population were not done.

Chung et al. first identified an association between *HLA-B*1502* and carbamazepine-induced SJS/TEN. They later showed a 100% association between the *HLA-B*1502* allele and carbamazepine-induced SJS in Han-Chinese patients, with an extremely high odds-ratio (2504) compared with those who were carbamazepine-tolerant. This strong link suggests that the product of this gene may have a direct functional role in drug hypersensitivity.

It has been proposed that the *HLA-B*1502* allele codes for a molecule that is displayed on the surface of antigen-presenting cells[194, 195].

One hypothesis proposes that carbamazepine or a metabolite combined with an unknown peptide binds to a cell surface molecule, which then activates naive CD8+ T-lymphocytes, which in turn proliferate and lead to STS/TEN.

Patients with the *HLA-B*1502* allele may also have a high risk of STS/TEN from other drugs used in the management of epilepsy. Phenytoin, Oxcarbazepine, Topiramate, and Lamotrigine have all been associated with SJS/TEN, and their use should be avoided[196].This leaves few therapeutic options and gabapentin, Pregabalin and clonazepam, are the only potentially useful medications without the associated complications.

Hung SI et al[197] reported that compared with other categories of drugs, such as antibiotics and NSAIDs, antiepileptic therapies are associated with a high incidence of SJS and TEN. They previously reported that carbamazepine (CBZ)-SJS/TEN is strongly associated with the *HLA-B*1502* in Han Chinese, which has been confirmed in other Southeast Asian countries where the allele is prevalent. They extended the study of HLA susceptibility to three different antiepileptic drugs, phenytoin (PHT), lamotrigine (LTG) and oxcarbazepine (OXC), which have structural similarity to CBZ. They found that *HLA-B*1502* was present in eight out of 26

(30.8%) PHT-SJS/TEN, two out of six (33%) LTG-SJS and three out of three (100%) OXC-SJS patients. In addition, HLA-B*1301, Cw*0801 and DRB1*1602 also showed an association with PHT-SJS/TEN (p = 0.0128-0.0281; OR: 3.0-4.3). Their r results indicate that OXC, PHT and LTG, which possess an aromatic ring just as CBZ does, when causing SJS/TEN, share a common risk allele. Aromatic antiepileptic drugs causing SJS/TEN in *HLA-B*1502* carriers may act on a similar pathogenetic mechanism, although other genetic/nongenetic factor(s) may also contribute to the pathogenic mechanism of the disease. They suggested that aromatic antiepileptic drugs, including CBZ, OXC and PHT, should be avoided in the B*1502 carrier and caution should also be exercised for LTG.

Chaichon Locharernkul, Jakrin Loplumlert, Chusak Limotai, Wiwat Korkij, *et al*[198] in their article on 'Carbamazepine and phenytoin induced Stevens-Johnson syndrome is associated with *HLA-B*1502* allele in Thai population' stated that previous studies found a strong association between *HLA-B*1502* and carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS) in Han Chinese, but not in Caucasian populations. Even in Han Chinese, the *HLA-B*1502* was not associated with CBZ-induced maculopapular eruptions (MPE). This study seeks to identify whether *HLA-B*1502* is associated with CBZ- or phenytoin (PHT)-induced SJS or MPE in a Thai population. They selected Eighty-one Thai epileptic patients between 1994 and 2007 from the Chulalongkorn Comprehensive Epilepsy Program were recruited. Thirty-one subjects had antiepileptic drug (AED)-induced SJS or MPE (6 CBZ-SJS, 4 PHT-SJS, 9 CBZMPE, 12 PHT-MPE), and 50 were AED-tolerant controls. For the first time, a strong association between *HLA-B*1502* and PHT-induced SJS was found (p = 0.005). A strong association was also found

between the *HLA-B*1502* and CBZ-induced SJS (p = 0.0005), making Thai the first non-Chinese population demonstrating such an association. Some patients, who were *HLA-B*1502* and suffered from CBZ-induced SJS, could be tolerant to PHT and vice versa. This suggests that *HLA-B*1502* may be a common attribute required for a Thai patient to develop SJS from these two AEDs; other different elements, however, are also needed for each AED. In addition, no association between HLA-B alleles and CBZ- or PHT-induced MPE was found. CBZ- and PHT-induced SJS, but not MPE, is associated with *HLA-B*1502* allele in Thai population.

Chen P, Lin JJ, Lu CS, Ong CT, *et al*[199] in their article on 'Carbamazepine-Induced Toxic Effects and *HLA-B*1502* Screening in Taiwan' observed that Carbamazepine, an anticonvulsant and a mood-stabilizing drug, is the main cause of the Stevens-Johnson syndrome (SJS) and its related disease, toxic epidermal necrolysis (TEN), in Southeast Asian countries. Carbamazepine-induced SJS-TEN is strongly associated with the *HLA-B*1502* allele. The authors sought to prevent carbamazepine-induced SJS-TEN by using *HLA-B*1502* screening to prospectively identify subjects at genetic risk for the condition. From 23 hospitals in Taiwan, they recruited 4877 candidate subjects who had not taken carbamazepine. They genotyped DNA purified from the subjects' peripheral blood to determine whether they carried the *HLA-B*1502* allele. Those testing positive for *HLA-B*1502* (7.7% of the total) were advised not to take carbamazepine and were given an alternative medication or advised to continue taking their pre-study medication; those testing negative (92.3%) were advised to take carbamazepine. They interviewed the subjects by telephone once a week for 2 months to monitor them for symptoms. They used the estimated historical incidence of SJS-TEN as a control. They

identified mild, transient rash developed in 4.3% of subjects; more widespread rash developed in 0.1% of subjects, who were hospitalized. SJS-TEN did not develop in any of the *HLA-B*1502*-negative subjects receiving carbamazepine. In contrast, the estimated historical incidence of carbamazepine-induced SJS-TEN (0.23%) would translate into approximately 10 cases among study subjects (P<0.001). The identification of subjects carrying the *HLA-B*1502* allele and the avoidance of carbamazepine therapy in these subjects was strongly associated with a decrease in the incidence of carbamazepine-induced SJS-TEN.

Chung WH and Hung SI[200] in a study entitled 'Genetic Markers and Danger Signals in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis' mention that Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening adverse reactions, which could be induced by a variety of drugs. It was proposed that human leukocyte antigen (HLA)-restricted presentation of antigens (drugs or their metabolites) to T lymphocytes initiates the immune reactions of SJS/TEN. However, the genetic susceptibility and the exact pathogenesis were not clear until the recent studies. They first identified that *HLA-B*1502* is strongly associated with carbamazepine (CBZ)-induced SJS/TEN and HLA-B*5801 with allopurinol-SJS/TEN in Han Chinese. The same associations had been validated across different human populations. For the downstream danger signals, Fas-Fas ligand (FasL) and perforin/granzyme B had been advocated as cytotoxic mediators for keratinocyte death in SJS/TEN. However, expression levels of these cytotoxic proteins from the skin lesions were too low to explain the distinct and extensive epidermal necrosis. Their recent study identified that **Granulysin**, a cytotoxic protein released from cytotoxic T cells or natural killer (NK) cells, is a key

mediator for disseminated keratinocyte death in SJS/TEN. This article aims to provide an overview of both of the genomic and immunologic perspectives of SJS/TEN. These studies give us a better understanding of the immune mechanisms, biomarkers for disease prevention and early diagnosis, as well as providing the therapeutic targets for the treatments of SJS/TEN.

Asia is a large region with 60% of world population. The region is the home of many national groups with different culture and ethnicity. Data from the WHO Uppsala Monitoring Center (WHO-UMC) and Novartis CBZ-SJS/TEN reports 2000-2006 showed that the incidence of ACDR induced by CBZ was high among some Asian countries. However, the incidence is not known in many geographical regions and ethnic groups, therefore it is important to document the incidence of ACDR induced by CBZ in these areas. Previous studies have found a strong association between *HLA-B*1502* and carbamazepine-induced Stevens-Johnson syndrome in Asian areas including Taiwan, Hong Kong and Thailand. As the importance of *HLA-B*1502* as marker of CBZ-induced SJS/TEN is being established particularly among Han Chinese, and the prevalence of *HLA-B*1502* in various ethnic groups are being determined, there are still large parts of Asia where the prevalence of *HLA-B*1502* is not known. This is for example, the Han and non-Han Chinese from different parts of China, Filipinos, various ethnic groups in Indonesia, Pakistan, Bangladesh, Myanmar, Cambodia and Lao PDR. The determination of the prevalence of *HLA-B*1502* in these ethnic groups is thus of high priority. To-date, the significance of *HLA-B*1502* as a marker for CBZ-induced SJS/TEN is only established among the Han Chinese, less so among the Malays and Thais. As *HLA-B*1502* as a marker of CBZ-induced SJS/TEN is ethnicity specific, there is also a

need to determine the relationship between *HLA-B*1502* and CBZ-induced SJS/TEN in other ethnic groups in Asia. There are many unanswered questions in CBZ-induced ACDR and *HLA-B*1502* waiting to be explored. How CBZ dosage affects the likelihood and timing of CDR is uncertain. The reason why ACDR is delayed in certain drugs such as CBZ as compared to early ACDR in antibiotic is also not known. The exact mechanism of how CBZ modulates cytotoxic activity via HLA gene is also poorly understood. Other questions to be explored include the role of other HLA-B subtypes in CBZ-induced SJS/TEN, significance of *HLA-B*1502* and other HLA subtypes in ACDR induced by other antiepileptic drugs, e.g. phenytoin and lamotrigine.

Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are severe cutaneous adverse drug reactions caused by carbamazepine (CBZ) and phenytoin. A strong association has been reported between human leukocyte antigen *HLA-B*1502* and carbamazepine-induced SJS in Han Chinese patients.

More recently, a genomewide association study has shown an association between the HLAA*3101 allele and SJS–TEN in Japanese persons, and a report in this issue of the Journal shows the association between this allele and a range of hypersensitivity reactions, including SJS–TEN, in persons of European descent. Among persons of Han Chinese descent, carbamazepine-induced SJS–TEN almost never occurs in non-carriers of the *HLA-B*1502* allele, evidence that this allele is directly involved in the pathogenesis of the condition. Carbamazepine directly binds to HLA-B molecules on antigen presenting T cells and contributes to cell death mediated by cytotoxic T cells in persons with SJS-TEN.13 *HLA-B*1502* can directly present carbamazepine to cytotoxic T cells without antigen processing. More

important, carbamazepine specific T-cell-mediated cytotoxicity is restricted to *HLA-B*1502*.The risk of carbamazepine-induced SJS–TEN is significantly higher among persons of Chinese origin who carry the *HLA-B*1502* allele than among those who do not carry the allele (odds ratio, 1357; 95% confidence interval, 193 to 8838; P = 1.6×10−41).10 If *HLA-B*1502* were used as a marker to predict carbamazepine induced SJS–TEN, the test would have a high sensitivity (98.3%) and specificity (95.8%). On the basis of an incidence of carbamazepine-induced SJS–TEN of 0.25%, this allele would have a negative predictive value of 99.9% and a positive predictive value of 5.6%. The use of *HLA-B*1502* genotyping to prevent carbamazepine-induced SJS–TEN in routine clinical practice thus seems warranted.

The US Food and Drug Administration has recommended genetic screening for the human leukocyte antigen-B (HLA-B)*1502 allele in patients of Asian ethnicity before starting carbamazepine therapy, to avoid the fatal adverse treatment-related events associated with this drug. The association between cross-reactivity to antiepileptic drugs (AEDs) and the *HLA-B*1502* allele has been only rarely reported. If the genetic association could be confirmed in larger studies, the *HLA-B*1502* allele should be tested for in any patient experiencing cADRs, to avoid cross reactivity to AEDs[201].

We agree with the US Food and Drug Administration (FDA) recommendations that patients with ancestry from areas in which *HLA-B*1502* is present should be screened for this allele before starting treatment with CBZ. If they test positive, CBZ should not be used unless the expected benefit clearly outweighs the increased risk of serious cutaneous reactions.

Unfortunately, in many areas of the world where *HLA-B*1502* has a relatively high prevalence, laboratory facilities for its determination may not be readily accessible or the cost of testing may not be affordable by most patients. Screening requirements, in any case, do not apply to patients already chronically treated with CBZ.

Because over 90% of drug-induced SJS/TEN occur within 2 months of starting treatment, patients who have been taking CBZ for at least a few months without developing severe cutaneous reactions are at low risk of developing SJS or TEN during continuation of treatment, even if they carry the *HLA-B*1502* allele.

Jean-claude Roujeau et al[202] in their paper 'Medication Use and the risk of Stevens–Johnson Syndrome or Toxic Epidermal Necrolysis, conducted a case–control study to quantify the risks associated with the use of specific drugs. Data were obtained through surveillance networks in France, Germany, Italy, and Portugal. Drug use before the onset of disease was compared in 245 people who were hospitalized because of toxic epidermal necrolysis or Stevens–Johnson syndrome and 1147 patients hospitalized for other reasons (controls). Crude relative risks were calculated and adjusted for confounding by multivariate methods when numbers were large enough. Among drugs usually used for short periods, the risks were increased for trimethoprim–sulfamethoxazole and other sulfonamide antibiotics (crude relative risk, 172; 95 percent confidence interval, 75 to 396), chlormezanone (crude relative risk, 62; 21 to 188), aminopenicillins (multivariate relative risk, 6.7; 2.5 to 18), quinolones (multivariate relative risk, 10; 2.6 to 38), and Cephalosporins (multivariate relative risk, 14; 3.2 to 59). For acetaminophen, the multivariate relative risk was 0.6 (95 percent confidence interval, 0.2 to 1.3) in France but 9.3 (3.9 to 22)

in the other countries. Among drugs usually used for months or years, the increased risk was confined largely to the first two months of treatment, when crude relative risks were as follows: carbamazepine, 90 (95 percent confidence interval, 19 to ∞); phenobarbital, 45 (19 to 108); phenytoin, 53 (11 to ∞); valproic acid, 25 (4.3 to∞); oxicam nonsteroidal anti-inflammatory drugs (NSAIDs), 72 (25 to 209); allopurinol, 52 (16 to 167); and corticosteroids, 54 (23 to 124). For many drugs, including thiazide diuretics and oral hypoglycaemic agents, there was no significant increase in risk. They concluded that the use of antibacterial sulfonamides, anticonvulsant agents, oxicam NSAIDs, allopurinol, chlormezanone, and corticosteroids is associated with large increases in the risk of Stevens–Johnson syndrome or toxic epidermal necrolysis. But for none of the drugs does the excess risk exceed five cases per million users per week.

An important perspective on Stevens–Johnson syndrome and toxic epidermal necrolysis as a public health issue is provided by the excess risks. Their results indicate that the highest risks are associated with antibacterial sulphonamides, with an excess risk of 4.5 cases per million exposed persons per week. These extremely low risks are consistent with the rarity of these diseases. Given the high morbidity and mortality associated with these conditions, however, prescribing physicians should still consider that alternative therapies have substantially lower excess risks.

We took care in the management of our patients not to expose them to any of the above drugs capable of inducing SJS: Aminopenicillins, trimethoprim–sulfamethoxazole and other sulphonamides, Quinolones and Oxicams.

Tassaneeyakul W, Tiamkao S, Jantararoungtong T, Chen P, *et al*[203] in their article on ‘Association between *HLA-B*1502* and carbamazepine-induced severe

cutaneous adverse drug reactions in a Thai population' commented that Carbamazepine (CBZ) has been reported as the most common culprit drug for Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in several Asian countries including Thailand. A strong association between *HLA-B*1502* and CBZ-induced SJS/TEN has been reported in Han Chinese but not in Caucasian and Japanese populations. A case-control study was conducted to determine whether *HLA-B*1502* is a valid pharmacogenetic test for SJS/TEN caused by CBZ in a Thai population. Among 42 CBZ-induced patients with SJS/TEN, 37 (88.10%) patients carried the *HLA-B*1502* while only 5 (11.90%) of the CBZ-tolerant controls had this allele. The risk of CBZ-induced SJS/TEN was significantly higher in the patients with *HLA-B*1502*, with an odds ratio (OR) of 54.76 [95% confidence interval (CI) 14.62-205.13, p = 2.89 x 10(-12)]. The sensitivity and specificity of *HLA-B*1502* for prediction of CBZ-induced SJS/TEN were 88.10%. By assuming a 0.27% as a prevalence rate of CBZ-induced SJS/TEN in a Thai population, the positive predictive value (PPV) and negative predictive value (NPV) of the *HLA-B*1502* were 91.92% and 99.96%. Results from this study suggest that *HLA-B*1502* may be a useful pharmacogenetic test for screening Thai individuals who may be at risk for CBZ-induced SJS and TEN.

In our study the positive predictive value (PPV) and negative predictive value (NPV) of the *HLA-B*1502* were 81.82% and 99.96%.

Yan Zhang, Jin Wang, Li-Mei Zhao, Wei Peng, *et al*[204] in their article on 'Strong association between *HLA-B*1502* and carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in mainland Han Chinese

patients', examine the association of *HLA-B*1502* allele with CBZ-induced SJS/TEN in the mainland Han Chinese population. *HLA-B*1502* genotyping with sequence-specific primer polymerase chain reaction (PCR-SSP) and PCR sequencing based typing (PCR-SBT) was performed on 17 CBZ-induced SJS/TEN patients, 21 CBZ-tolerant controls, and 185 healthy controls recruited during 2008–2010.*HLA-B*1502* allele was present in 94.1% (16/17) of CBZ-SJS/TEN patients, 9.5% (2/21) of CBZ-tolerant patients, and 9.2% (17/185) of healthy controls. The risk of CBZ-induced SJS/TEN was significantly higher (P<0.01) in the patients with *HLA-B*1502*. One CBZ-induced SJS patient tested negative for *HLA-B*1502*, and the test result showed HLA-B*3503/B*4601. They found a strong association between *HLA-B*1502* and CBZ-induced SJS/TEN in the Han Chinese population from central and northern China. Combined with previous studies of the southern Han Chinese subpopulation, their results suggest that *HLA-B*1502* is strongly associated with CBZ-induced SJS/TEN in the whole Han Chinese population.

Ana Alfirevic, Andrea L Jorgensen, Paula R Williamson, David W Chadwick *et al*[205] in their article on 'HLA-B locus in Caucasian patients with carbamazepine hypersensitivity' stated that a strong pharmacogenetic association has been reported in Chinese patients between human leukocyte antigen *(HLA)-B*1502* and carbamazepine (CBZ)-induced Steven-Johnson syndrome (SJS). They have genotyped the HLA-B alleles in 56 Caucasian patients with varying severities of CBZ hypersensitivity and 43 controls on CBZ without adverse effects. They found none of their patients (including two with blistering skin rashes) were positive for the *HLA-B*1502* allele. HLA-B*0702 allele may protect against severe CBZ hypersensitivity but warrants further study. Of secondary interest, the correlation

between HLA-B*0801 and HLA-DR3, DQ2 and TNF-308 alleles (on the ancestral haplotype 8.1) is consistent with their previous findings. *HLA-B*1502* does not seem to be a marker for all forms of CBZ-induced hypersensitivity in a Caucasian population. HLA-B*0801 and HLA-DR3, DQ2 and TNF-308 alleles were not tested in our study.

Hung SI, Chung WH and Chen YT[206] in their paper on 'HLA-B genotyping to detect carbamazepine induced Steven-Johnson Syndrome: implications for personalizing medicine' stated that preventing severe adverse drug reactions by identifying people at risk with a simple genetic test is the goal of many pharmacogenomic studies. Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are related, life-threatening cutaneous adverse reactions, most often caused by medication. The overall incidence and the commonly offending drugs vary among different ethnic populations. Susceptibility to such idiosyncratic reactions is thought to be genetically determined and immune mediated. Finding a strong genetic association between a particular human leukocyte antigen (HLA)-B allele and the reaction to a specific drug provides evidence that the pathogenesis of the severe cutaneous adverse drug reactions involves major histocompatibility complex-restricted presentation of a drug or its metabolites for T-cell activation. In the case of carbamazepine-induced SJS/TEN, the tight association of the *HLA-B*1502* allele (sensitivity 100%, specificity 97% and odds ratio 2504) provides a plausible basis for further development of such a test to identify individuals at risk of developing this life-threatening condition.

In our study the sensitivity 96.43%, specificity 95.95% and odds ratio 2823 provides a plausible basis for further development of such a test to identify individuals at risk of developing this life-threatening condition.

Kulkantrakorn K, Tassaneeyakul W, Tiamkao S, Jantararoungtong T, *et al*[207] in their article on '*HLA-B*1502* Strongly Predicts Carbamazepine-Induced Stevens–Johnson Syndrome and Toxic Epidermal Necrolysis in Thai Patients with Neuropathic Pain' explained that Carbamazepine (CBZ) is one of the standard pharmacological treatments for neuropathic pain. However, its serious adverse drug reactions include Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Recently, *HLA-B*1502* allele was implicated as a genetic marker of CBZ-induced SJS/TEN in some Asian epilepsy populations. They describe the clinical characteristics of SJS/TEN in Thai patients with neuropathic pain who were treated with CBZ, and to determine the association of *HLA-B*1502* in these patients, comparing with those who exposed to CBZ for at least 6 months without any cutaneous reactions. They found that Thirty-four SJS/TEN patients and 40 control patients were included in this study. Mean age of SJS/TEN patients was 47 years. SJS/TEN was developed in 10.8 ± 1.4 days after initiation of CBZ. *HLA-B*1502* allele was found in 32 of 34 SJS/TEN patients (94.1%) but it was found only in 7 of 40 control patients (17.5%). The association was very strong with an odds ratio of 75.4. Sensitivity and specificity of this *HLA-B*1502* genotype test were 94.1% and 82.5%, respectively, while the positive predictive value and negative predictive value were 1.43% and 99.98%, respectively. Positive and negative likelihood ratios were 5.37 and 0.07, respectively. They also found *HLA-B*1502* is a strong genetic marker for CBZ-induced SJS/TEN in Thai patients with neuropathic pain. Our study design and conclusions are consistent as in this study.

Celeste B.L. Man, Patrick Kwan, Larry Baum, Evelyn Yu, et al[208] in their paper on 'Association between *HLA-B*1502* Allele and Antiepileptic Drug-Induced Cutaneous Reactions in Han Chinese' reported a previous study conducted in

Taiwan that found a 100% association between *HLA-B*1502* allele and carbamazepine-induced Stevens-Johnson syndrome (SJS) in Han Chinese subjects, with an extremely high odds ratio compared with carbamazepine-tolerant subjects (odds ratio=2,504).They examined this association in 24 Hong Kong Han Chinese subjects who had cutaneous adverse reactions induced by different antiepileptic drugs (AEDs). They were matched with 48 AED-tolerant controls. HLA-B∗1502 was associated with severe cutaneous reactions (SCR) induced by AEDs, which included carbamazepine, phenytoin, and lamotrigine (p = 0.001, odds ratio = 17.6), but was not associated with maculopapular exanthema (MPE) (p=0.32). Further studies in larger samples of ethnically matched subjects should be conducted to confirm the findings. Identification of genetic polymorphisms predisposing to development of AED-induced SCR offers the possibility of avoiding these high-risk drugs in genetically susceptible individuals. We did not test HLA-B∗1502 in any patient on Lamotrigine or Lamotrigine induced SJS/TEN.

We had previously reported[209] that 17 patients (9 women and 8 men) with a mean age of 39.17 years (range: 8 - 68), who developed SJS on exposure to Carbamazepine, were all positive for *HLA–B*1502*. 43 patients (23 women and 20 men) with a mean age of 36.71 years (range: 17-58), who tolerated CBZ without SJS/TEN did not have *HLA–B*1502* allele.

In our study, the Men: Women ratio was 29:27. No differences in gender has been noted among patients with CBZ induced SJS.

Age distribution Table

0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80
2	9	17	14	7	5	0	2

The mean age of the patients who developed SJS was 32.9 years, as reported by other workers.

SJS/TEN were frequently seen the age groups of 21 – 50 years. This is also as cited in the literature.

The mean duration of Hospitalization in our study was 8 days (2 – 28 days).

A multidisciplinary team consisting of Neurologists, Dermatologists, General physicians and Ophthalmologists were involved during the In Patient care of patients.

The Mortality in our study was 2/56 = 3.57%.

Our findings suggest that screening patients for the *HLA-B*1502* allele before the initiation of carbamazepine treatment and withholding carbamazepine from *HLA-B*1502*–positive patients can reduce the incidence of carbamazepine-induced SJS–TEN.

Early withdrawal of carbamazepine does not prevent a more severe SJS–TEN or TEN-like reaction, since once patients are sensitized by carbamazepine and have early blisters or ulcers, SJS–TEN progresses, even after the withdrawal of the drug.

The incidence of carbamazepine-induced SJS–TEN in *HLA-B*1502*–negative persons is very low. Our results suggest the value of *HLA-B*1502* screening to prevent carbamazepine-induced SJS–TEN.

A similar population was studied between 1997 and 2001 by Mockenhaupt et al. in a multinational case-control study in Europe covering more than 100 million inhabitants in which special attention was given to newly marketed drugs[210]. This study identified nevirapine, lamotrigine, and sertraline, as drugs with a significantly increased risk of inducing SJS/TEN. Older drugs identified as having a

high risk of inducing SJS/TEN were sulfamethoxazole/trimethoprim (SMX/TMP), sulfonamides (sulfasalazine, sulfadiazine, sulfadoxine, sulfafurazole), allopurinol, carbamazepine, phenytoin, phenobarbital, and NSAID's of the oxicam-type (meloxicam, piroxicam, tenoxicam).

Mockenhauptet al. were able to show that almost all cases of SJS/TEN developed within 63 days of starting use of antiepileptic drugs, and that the risk of developing SJS/TEN per 10 000 new users was significantly increased for carbamazepine (1.4 cases per 10 000 users), lamotrigine (2.5), phenobarbital (8.1) and phenytoin (8.3). The incidence for valproic acid was low compared to other antiepileptic drugs with 0.4 cases per 10 000 users[211]. The incidence of SJS/TEN under treatment with valproic acid is confounded by the concomitant use of other drugs, such as lamotrigine.

Ueta et al[212] state that given the association between the onset of SJS/TEN and infections, the possibility that there is an association between SJS/TEN and a disordered innate immune response should be considered. The first line of defence against infection is comprised of evolutionarily conserved sets of molecules, the Toll–like receptors (TLRs). TLR3 recognises double-stranded RNA associated with viral infections. This Japanese single-nucleotide-polymorphism (JSNP) database reports 7 polymorphisms consisting of 7 SNPs in the human TLR3 gene; 3 of the 7 SNPs are coded in exon regions, (i.e. 293248A/G, 293391A/G and 299698T/G), and the other 4 are coded in intron regions, (i.e. 294440G/C, 294732C/T, 208036T/C and 298054C/T). These 7 SNPs were analysed in 57 Japanese patients with SJS/TEN with ocular surface complications and in 160 Japanese healthy controls. SNP 299698T/G and the genotype patterns of 293248A/A and 299698T/T were strongly

associated with SJS/TEN. The results suggest that polymorphisms in the TLR3 gene could be associated with SJS/TEN in the Japanese population.

The first line of defence against infection is comprised of evolutionarily conserved sets of molecules, the Toll-like receptors (TLRs). The triggering of TLRs results in the secretion of antibacterial peptides and proinflammatory cytokines. The inflammatory response results in the recruitment of cells of adaptive immunity to initiate clearance of the pathogens. TLR3 recognises double-stranded (ds)RNA associated with viral infections, a component of the life cycle of most viruses[213.] As functional deterioration of TLR3 can predispose individuals to increased susceptibility to viral infections, the detection of TLR3 polymorphisms could yield critical information for risk assessment regarding susceptibility to microbial infections in the context of SJS/TEN. To date, no reports are available on the genetic loci of TLR3in subjects with SJS/TEN. Therefore, the authors performed single nucleotide polymorphism (SNP) association analysis of theTLR3 gene, which maps to chromosome 4q35. The Japanese single-nucleotide polymorphism (JSNP) database reports seven polymorphisms consisting of seven SNPs in the human TLR3gene: three of the seven SNPs are coded in exon regions ie,293248A/G (rs.3775290, exon 4, silent SNP), 293391A/G(rs.3775291, exon 4, change SNP) and 299698T/G(rs.3775296, exon 2, UTR SNP), and the other four are coded in intron regions (i.e. 294440G/C (rs.3775292, intron 3),294732C/T (rs.3775293, intron 3), 208036T/C (rs.3775294,intron 2) and 298054C/T

They analysed these seven SNPs in 57 Japanese patients with SJS/TEN with ocular surface complications and in 160 healthy Japanese controls and found that SNP 299698T/G and the genotype patterns of 293248A/A and 299698T/T are strongly associated with SJS/TEN. These results suggest that polymorphisms in the

TLR3 gene could be associated with SJS/TEN in the Japanese population. They hypothesised that viral infection and/or drugs might trigger a disorder in the host innate immune response, and that this event is followed by aggravated inflammation of the mucous membranes, ocular surface and skin. Genetic and environmental factors could play a role in an integrated aetiology of SJS/TEN. Because the 299698T/G SNP, which showed a significant association with SJS/TEN, is encoded in the exon region, the authors consider it important to extend this study by performing expression and function analysis of the TLR3 protein with this SNP.

According to the International HapMap project, the299698T/G (rs.3775296) SNP exists not only in Japanese (G/G0.386, G/T 0.500, T/T 0.114) but also in Han Chinese (G/G 0.659,G/T 0.295, T/T 0.046) and Caucasian (G/G 0.719, G/T 0.263, T/T0.018) populations, indicating that it is important to examineTLR3 SNPs in non-Japanese populations. TLR3 is involved in responses to dsRNAs. As rhinoviruses are a major cause of the common cold and the acute exacerbation of chronic obstructive pulmonary disease, the functional requirement for TLR3 in the host response against infection with live viruses, especially rhinovirus infection, has been proposed[214]. The association documented here complements previous findings compatible with an unregulated innate immune response as an important pathophysiological condition in inflammatory ocular surface diseases. SJS/TEN could be the consequence of exposure of genetically susceptible individuals to specific environmental precipitants. A report from the US showed that the HLA (human leukocyte antigen)-B12 HLA-Bw44 antigen was significantly increased in Caucasian patients with SJS with ocular involvement[215]. Analyses of patients with TEN in France also disclosed an association with HLA-B12 (HLA-Bw44) [216]. In Han Chinese, there was a very strong association between carbamazepine-induced SJS

and the *HLA-B*1502* allele[217]. Elsewhere the authors have reported that, in the Japanese, HLA-A*0206 was strongly associated with SJS/TEN, with ocular surface complications[218]. These findings suggest that SJS/TEN is associated with a complex genetic-inheritance background, and that specific combinations of genes are required for disease-onset. The pathophysiological mechanisms underlying the onset of SJS/TEN have not been fully established, although the involvement of immune mechanisms and altered drug metabolism has been suggested[219–223].

A strong association between *HLA-B*1502* and carbamazepine-induced SJS–TEN has been found in Asian populations other than the Han Chinese, including Malay, Thai, and South Asian Indians. In Malaysia, Thailand and India, studies have shown that carbamazepine was the major cause of drug-induced SJS–TEN. Since the contribution of *HLA-B*1502* to carbamazepine-induced SJS–TEN has been proved to be causal, we emphasize that in these countries, in which *HLA-B*1502* is relatively prevalent, *HLA-B*1502* screening could provide a benefit.

Ming Ta Michael Lee, Shuen-Iu Hung, Chun-Yu Wei and Yuan-Tsong Chen[224] in their paper on 'Pharmacogenetics of toxic epidermal necrolysis' comment on the importance of this field: Advances in genome technologies have allowed researchers to identify genetic markers associated with this drug-associated event and these have provided a potential tool for prevention. Current updates of genetic biomarkers that have been identified as being associated with TEN/SJS induced by several drugs, and the associations of these markers in different populations, are discussed. The strong association of *HLA-B*1502* and carbamazepine (CBZ)-induced TEN/SJS have been reported by several independent studies. This association was mostly observed in patients of Southeast Asian ancestry; it was not observed in populations with low *HLA-B*1502* allele frequency. Studies also suggest that drugs with a similar

chemical structure to CBZ might also induce TEN/SJS in patients with *HLA-B*1502*. In addition to CBZ, HLA-B*5801 was also found to associate with allopurinol-induced TEN/SJS. This strongly suggests that the associations of these markers with TEN/SJS are drug specific. The strong association between CBZ and *HLA-B*1502* has prompted the US Food and Drug Administration to update the label for CBZ to include genetic information and to recommend genetic testing before prescribing CBZ. Patients with Asian ancestry or who are from regions prevalent in *HLA-B*1502* should be screened before CBZ treatment.

Hu FY, Wu XT, An DM, Yan B, *et al*[225] in their article on 'Pilot association study of oxcarbazepine-induced mild cutaneous adverse reactions with *HLA-B*1502* allele in Chinese Han population' state that recent study demonstrated that *HLA-B*1502* was a common risk allele in aromatic antiepileptic drugs (AEDs) induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese. However, the association of AEDs-induced mild maculopapular eruption (MPE) with *HLA-B*1502* remains unclear until recently. In the present study, they conducted a pilot study to detect a possible association of oxcarbazepine (OXC)-induced MPE with *HLA-B*1502* allele in Chinese Han population. They enrolled 90 subjects involving 9 patients with OXC-induced MPE and two groups of controls, 9 OXC-tolerant and 72 normal controls. High-resolution HLA genotyping was performed by specific kit. The results of HLA genotyping are expressed as positive or negative for *HLA-B*1502* allele. Differences in genotype frequencies between groups were assessed by the Fisher's exact test. Four cases were detected as positive for *HLA-B*1502* amongst 9 patients. However, only 1 subject was positive amongst 9 tolerant controls, and 6 subjects were positive amongst 72 normal controls. The difference in

*HLA-B*1502* allele frequencies between the MPE group and normal controls was statistically significant (OR: 8.8; 95% CI: 1.853-41.790; P=0.011). In addition, they also observed an increased frequency of *HLA-B*1502* allele in patients (44.44%) compared with tolerant controls (11.11%), although it failed to reach statistical significance (P=0.294).Their findings indicate that *HLA-B*1502* allele may contribute to the genetic susceptibility to OXC-induced MPE in Chinese Han population. In order to safer AEDs use, they recommend that *HLA-B*1502* allele should be tested for patients with OXC-induced MPE before changing to other AEDs, and AEDs with similar chemical structure should be avoided in individuals who test positive for *HLA-B*1502* allele. It should be pointed out that, their results may well be just be by chance owing to the small sample size and should be further confirmed in future studies.

Locharernkul C, Shotelersuk V and Hirankarn N[226] in their report on 'Pharmacogenetic screening of carbamazepine-induced severe cutaneous allergic reactions' state that recent studies associated the *HLA-B*1502* allele with carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) in patients from China, Thailand and Malaysia. No association has been found in patients from Europe or Japan. Linkage summary reports from East and South-east Asia predict a highly significant odds ratio (OR) of 84.75 (95% confidence interval [CI]=42.53-168.91; p=8.96×10[-15]) with sensitivity and negative predictive values of 92% and 98%, respectively. The higher prevalence of *HLA-B*1502* allele among certain Asian populations (10-15%) compared to Caucasians (1-2%) may explain a 10-fold to 25-fold higher incidence of CBZ-SJS/TEN in patients from Asia. Screening for *HLA-B*1502* before using CBZ can prevent SJS/TEN in certain populations, but screening may be less beneficial in populations with low

*HLA-B*1502* allele frequency and in patients exposed to CBZ for more than 2 months. A retrospective study demonstrated that the costs of *HLA-B*1502* screening were less than those of SJS treatment. This article reviews possible benefits and concerns of *HLA-B*1502* screening in clinical practice.

Choong-Chor Chang, Chun-Lai Too, Shahnaz Murad and Suraiya Hani Hussein[227] in their article on 'Association of *HLA-B*1502* allele with carbamazepine induced toxic epidermal necrolysis and Stevens–Johnson syndrome in the multi-ethnic Malaysian population' described that Carbamazepine (CBZ), a frequently used anticonvulsant drug, is one of the most common causes of life-threatening cutaneous adverse drug reactions such as toxic epidermal necrolysis (TEN) and Stevens–Johnson syndrome (SJS). Recent studies have revealed a strong association between *HLA-B*1502* and CBZ-induced TEN/SJS in the Taiwan Han Chinese population. This study is aimed to investigate the association between human leukocyte antigens (HLA) and CBZ-induced TEN/SJS in the multi-ethnic Malaysian population. A sample of 21 unrelated patients with CBZ-induced TEN/SJS and 300 race matched, healthy controls were genotyped for HLA-A, -B and -DR using polymerase chain reaction (PCR). Allele frequencies were compared.

Kheng Seang Lim, Patrick Kwan, and Chong Tin Tan[228] in their paper on 'Association of *HLA-B*1502* allele and carbamazepine-induced severe adverse cutaneous drug reaction among Asians, a review' showed strong association between *HLA-B*1502* and carbamazepine-induced Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) was demonstrated among Han Chinese in 2004. Studies from Europe showed that the *HLA-B*1502* is not a universal marker for SJS/TEN, but is ethnicity specific for Asians. Reports across Asia has shown that the prevalence of *HLA-B*1502* is high among Han Chinese (5-15%), Malays (12-

15%), and Thais (8-27%), but low among Japan, Korea, Sri Lanka, and most ethnic groups in India. Other than Han Chinese, the association between *HLA-B*1502* and carbamazepine-induced SJS-TEN is also seen among the Thais and Malay. There is urgent need for further studies to determine the prevalence of SJS/TEN, and *HLA-B*1502* in the various ethnic groups in Asia, and its association with carbamazepine-induced SJS-TEN in each of these ethnic groups. In view of the significant morbidity and mortality in SJS-TEN, facilities should be developed to allow for screening of *HLA-B*1502* before carbamazepine is prescribed to the Hans Chinese, Malays and Thais. For those who experience no adverse cutaneous reaction after 3 months use of carbamazepine, the risk of SJS/TEN is low, and the drugs can be continued.

Wen-Hung Chung, Shuen-Iu Hung and Yuan-Tsong Chen[229] in their article on 'Human leukocyte antigens and drug hypersensitivity' review the recent literature on the identification of human leukocyte antigen (HLA) alleles as major susceptible genes for drug hypersensitivity and discuss the clinical implications. Several recent studies have reported strong genetic associations between HLA alleles and susceptibility to drug hypersensitivity. The genetic associations can be drug specific, such as *HLA-B*1502* being associated with carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN), HLA-B*5701 with abacavir hypersensitivity and HLA-B*5801 with allopurinol induced severe cutaneous adverse reactions. A genetic association can also be phenotype-specific, as B*1502 is associated solely with carbamazepine-SJS/TEN, and not with either maculopapular eruption or hypersensitivity syndrome. Furthermore, a genetic association can also be ethnicity specific; carbamazepine-SJS/TEN associated with B1502 is seen in south-east Asians but not in whites, which may be explained by the

different allele frequencies. The strong genetic association suggests a direct involvement of HLA in the pathogenesis of drug hypersensitivity when the HLA molecule presents an antigenic drug for T cell activation. The high sensitivity/specificity of some markers provides a plausible basis for developing tests to identify individuals at risk for drug hypersensitivity. Application of *HLA-B*1502* genotyping as a screening tool before prescribing carbamazepine could be a valuable tool in preventing carbamazepine-induced SJS/TEN in south-east Asian countries.

Hung SI, Chung WH, Liu ZS, Chen CH, *et al*[230] in their paper on 'Common risk allele in aromatic antiepileptic-drug induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Han Chinese' stated that compared with other categories of drugs, such as antibiotics and NSAIDs, antiepileptic therapies are associated with a high incidence of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). They previously reported that carbamazepine (CBZ)-SJS/TEN is strongly associated with the *HLA-B*1502* in Han Chinese, which has been confirmed in other Southeast Asian countries where the allele is prevalent. Here, they extend the study of HLA susceptibility to three different antiepileptic drugs, phenytoin (PHT), lamotrigine (LTG) and oxcarbazepine (OXC), which have structure similarity to CBZ. They carried out a case-control association study. They enrolled 26 PHT-, six LTG- and three OXC-induced SJS/TEN patients, 113 PHT-tolerant and 67 LTG-tolerant subjects who were on the drug, respectively, for more than 3 months without the adverse reactions, and 93 normal subjects from the general population. The HLA-A, B, C and DRB1 genotypes were determined.

Shuen-Iu Hung, Wen-Hung Chung, Shiou-Hwa Jee, Wen-Chieh Chen, *et al*[231] in their article 'Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions' explained the anticonvulsant carbamazepine (CBZ) frequently causes cutaneous adverse drug reactions (cADRs).They reported that SJS/TEN caused by CBZ is strongly associated with the *HLA-B*1502* gene in Han Chinese. Here, they extended their genetic study to different types of CBZ-cADRs (91 patients, including 60 patients with SJS/TEN, 13 patients with hypersensitivity syndrome and 18 with maculopapular exanthema versus 144 tolerant controls). They used MALDI-TOF mass spectrometry to screen the genetic association of 278 single nucleotide polymorphisms (SNPs), which cover the major histocompatibility complex (MHC) region, tumour necrosis factor-alpha, heat shock protein and CBZ-metabolic enzymes, including CYP3A4, 2B6, 2C8, 2C9, 1A2 and epoxide hydrolase 1. In addition they genotyped 20 microsatellites in the MHC region and performed HLA-typing to construct the recombinant map. They narrowed the susceptibility locus for CBZ-SJS/TEN to within 86 kb flanking the HLA-B gene on the extended B*1502 haplotype, and confirmed the association of B*1502 with SJS/TEN [Pc = 1.6 10–41, odds ratio (OR)= 1357; 95% confidence interval (CI) =193.4–8838.3]. By contrast to CBZ-SJS/TEN, *HLA-B*1502* association was not observed in the MPE or HSS groups: MPE was associated with SNPs in the HLA-E region and a nearby allele, HLA-A*3101 (Pc =2.2 10–3, OR= 17.5; 95% CI= 4.6–66.5), and HSS with SNPs in the motilin gene (Pc = 0.0064, OR= 7.11; 95% CI= 3.1–16.5) located terminal to the MHC class II genes. No SNPs in genes involved in CBZ metabolism were associated with CBZ-induced cADRs. Their data suggest that *HLA-B*1502* could contribute to the pathogenesis of CBZ-SJS/TEN, and that genetic susceptibility to CBZ-induced cADRs is phenotype specific.

X.T. Wu, F.Y. Hu, D.M. An, B. Yan, *et al*[232] in their article on 'Association between carbamazepine-induced cutaneous adverse drug reactions and the *HLA-B*1502* allele among patients in central China' investigated the association between carbamazepine (CBZ)-induced cutaneous adverse drug reactions (cADRs) and the *HLA-B*1502* allele among patients from central China. Eight patients with Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN), 28 with mild maculopapular eruptions (MPEs), 50 CBZ-tolerant controls, and 71 healthy volunteers were recruited. HLA genotyping was performed using the polymerase chain reaction sequence-based typing (SBT)method. As a result, the *HLA-B*1502* allele was observed at the following rates: (1) 100% (8/8) among those with CBZ-induced SJS/TEN, (2) 10.7% (3/28) among those with CBZ-induced MPEs; (3) 8.0% (4/50) among CBZ-tolerant controls; (4) 8.5% (6/71) among healthy volunteers. The eight patients with SJS/TEN positive for the *HLA-B*1502* allele had an odds ratio (OR) of 184 compared with CBZ-tolerant controls. There was no significant difference in frequency between patients with MPEs and CBZ-tolerant controls (PN0.05). Thus, CBZ-induced SJS/TEN, but not MPEs, is strongly associated with *HLA-B*1502*. Testing for *HLA-B*1502* should be recommended for patients from central China prior to initial CBZ treatment.

C Lonjou, L Thomas, N Borot, N Ledger, *et al*[233] in their paper 'A marker for Stevens-Johnson syndrome: ethnicity matters' reported Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but severe cutaneous adverse drug reactions, which can be caused by a certain number of specific drugs among which is carbamazepine, an antiepileptic agent. A very strong association of

carbamazepine-induced SJS with *HLA-B*1502* has recently been described in the Han Chinese population. Here in, they report preliminary results from a European study (RegiSCAR) of 12 carbamazepine-induced SJS/TEN cases (nine French and three German). Among these only four had a *HLA-B*1502* allele. Remarkably, these four patients had an Asian ancestry, whereas the others did not as far as they have ascertained. This shows that although the HLA region may contain important genes for SJS, the *HLA-B*1502* allele is not a universal marker for this disease and that ethnicity matters.

Yi-Wu Shi, Fu-Li Min, Bin Qin, Xin Zou, *et al*[234] in their article on 'Association between HLA and Stevens–Johnson Syndrome Induced by Carbamazepine in Southern Han Chinese: Genetic Markers besides B*1502' mentioned that previous studies have demonstrated a strong association between carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (CBZ-induced SJS/TEN) and *HLA-B*1502* in Chinese, and HLA-A*3101 but not *HLA-B*1502* in Caucasians and Japanese. Cases with CBZ-induced SJS/TEN negative for *HLA-B*1502* were reported recently in Southeast Asia. Negative correlations between CBZ-induced SJS/TEN and B*0702 or B*4001 have also been reported, suggesting a possible protective role. Here, they genotyped HLA-B and HLA-A in 18 cases with CBZ-induced SJS/TEN, in comparison with CBZ tolerant and normal controls in Southern Han Chinese. A strong association between *HLA-B*1502* and CBZ-induced SJS/TEN was found, with 72.2% sensitivity and 87.1% specificity. However, they also found five patients with SJS (5/18, 27.78%) who were negative for *HLA-B*1502*. HLA-A*2402 was present in nine of 16 cases with SJS (56.25%, including three of five cases negative for *HLA-B*1502*), which was significantly more frequent than that

of CBZ-tolerant controls or the general southern population. Only one case with SJS carried HLA-A*3101. No statistical difference in the mean age, sex ratio and CBZ usage was found between the CBZ-induced SJS/TEN group and the CBZ-tolerant group. In search for possible protective genetic markers in *HLA-B*1502*-positive but CBZ-tolerant patients, they failed to find any significant factors in the HLA alleles observed. Given the association between *HLA-B*1502* and CBZ-induced SJS/TEN, genetic testing before initiating CBZ therapy is suggested in Han Chinese population. However, physicians should also be vigilant about SJS/TEN in those negative for *HLA-B*1502*. Other factors for the development of CBZ-induced SJS/TEN in *HLA-B*1502*-negative patients and protective factors in CBZ-tolerant patients should be investigated further.

Sae-Hoon Kima, Kyung Wha Leed, Woo-Jung Songa, Sang-Heon Kime, *et al*[235] in their article Carbamazepine-induced severe cutaneous adverse reactions and HLA genotypes in Koreans stated although the US FDA recommends screening for *HLA-B*1502* allele in most of Asian ancestry before initiating carbamazepine therapy, the HLA associations with carbamazepine hypersensitivity in non-Chinese Asian populations remain unclear. This study investigated the association between the HLA class I genotype and carbamazepine-induced severe cutaneous adverse reaction (SCAR) in Koreans. Twenty-four patients who had developed carbamazepine-induced SCAR (7 Stevens—Johnson syndrome (SJS), 17 drug hypersensitivity syndrome (HSS)), 50 carbamazepine tolerant controls from the Korean Pharmacogenetic Adverse Drug Reaction Research Network and data of 485 Korean general population from a previously published study were recruited. HLA-A, -B, and -C genotyping was performed by direct DNA sequence analysis. Only one of the seven SJS patients was positive for the B*1502 allele, but the

frequency of B*1511was much higher in the patients with CBZ-SJS than in the CBZ-tolerant control patients (P = 0.011, Pc = not significant; OR = 18.0(2.3—141.2)). The frequencies of A*3101 in carbamazepine-induced HSS and SCAR were significantly higher than those in carbamazepine-tolerant controls (Pc = 0.011, OR = 8.8(2.5—30.7) and Pc = 0.013, OR = 7.3(2.3—22.5), respectively). The frequencies of B*1511 in carbamazepine-SJS and A*3101 in carbamazepine-HSS/SCAR were significantly higher than those in the general population. *HLA-B*1502* does not seem to be an effective predictive marker for carbamazepine induced SCAR, while HLA-B*1511 and A*3101 was associated with carbamazepine-induced SJS and HSS/SCAR respectively in the Korean population.

Y-C Chen, C-Y Chu and C-H Hsiao[236] in the paper on Oxcarbazepine-induced Stevens–Johnson syndrome in a patient with *HLA-B*1502* genotype quoted that a strong association between *HLA-B*1502*allele and carbamazepine-induced SJS (CBZSJS) has been documented in Han Chinese in one previous study. Oxcarbazepine (OXC), a 10-keto analogue of CBZ, is considered to be much safer than CBZ due to its different metabolic pathway. Herein, they describe a Chinese male diagnosed as having oxcarbazepine-induced SJS (OXC-SJS) with also positive HLAB *1502allele. They also estimated the incidence of developing SJS/ TEN in patients receiving CBZ and OXC. SJS and TEN are characterized by rapidly expanding maculopapular exanthema with mucosal involvement, constitutional symptoms and variable internal organ involvement. The estimated incidence of SJS/TEN is much higher in Han Chinese. Carbamazepine is the most common offending drug in Taiwan, and the genetic marker *HLA-B*1502* has been documented for CBZ-SJS in Han Chinese of Taiwan OXC is considered to be much

safer and well tolerated than CBZ. Clinically, OXC-SJS are quite rare compared with the frequency of CBZ-SJS, and the presented patient herein is the first reported case of OXC-SJS in Taiwan. They cumulated the estimated patient-years from 2004 to 2007 according to the total prescription amounts of CBZ and OXC in Taiwan. The total exposures of CBZ and OXC are 71 391 and 11 961 patient-years, respectively. The average incidence of SJS in Han Chinese has been calculated as 8 cases per million person-years and that of CBZ-SJS accounts for 25%–33% of these cases. They estimated the case number of CBZSJS to be 182.6–243.5 in Taiwan during the year 2004–2007. Thus, the incidence of SJS in patients receiving CBZ in Taiwan would be 2.6–3.4 cases per thousand person-years. On the other hand, there was only one case of OXC-SJS in Taiwan during this period. Therefore, the incidence of SJS in patients receiving OXC was only 0.08 cases per thousand person-years. The estimated relative risk of OXC-SJS was 30- to 40-fold lower than that of CBZ-SJS in Han Chinese in Taiwan. Further studies of enrolling more OXC-tolerant cases to survey the prevalence of *HLA-B*1502* allele in this patient group would be helpful to elucidate the role of *HLA-B*1502* in OXC-SJS.

Yi-Wu Shi, Fu-Li Min, Xiao-Rong Liu, Li-Xuan Zan *et al*[237] in their article 'HLA-B Alleles and Lamotrigine-Induced Cutaneous Adverse Drug Reactions in the Han Chinese Population' explained that Lamotrigine (LTG) is a commonly used antiepileptic drug. However, the use of LTG is limited because of its cutaneous adverse drug reactions (cADRs) ranging from mild maculopapular eruption (MPE) to severe Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). A strong association between *HLA-B*1502* and carbamazepine-induced SJS/TEN has been identified in Chinese and Thai. Although three of seven cases with *HLA-B*1502*

have been reported in LTG-induced SJS/TEN so far, the relationship between *HLA-B*1502* and LTG-induced SJS／TEN needs further investigation. It is also unclear whether there is a specific genetic marker associated with LTG-induced MPE in Chinese. In this study, they genotyped 43 Han Chinese patients treated with LTG (14 cases with LTG-induced cADRs and 29 LTG-tolerant controls), using PCR-SSP for *HLA-B*1502* testing and low-resolution genotyping, as well as sequencing for four-digit genotyping. The two cases with SJS were negative for *HLA-B*1502*, with B1301／1301 and 4601／5610, respectively. Combining the data with previous studies, there was no significant difference in the frequency of subjects with *HLA-B*1502* between the LTG-induced SJS/TEN group and the LTG-tolerant group (p = 0.08, OR 4.23, 95% CI 0.94–18.97). In the MPE group, only one was positive for *HLA-B*1502*. There was no significant difference in the frequency of a specific HLA-B allele between the MPE group and the LTG-tolerant group either. In this study, no significant association between *HLA-B*1502* and LTG-induced SJS or MPE was found. Given the small sample size and only HLA-B locus genotyping, further large-scale studies are required to explore genetic associations with LTG-induced cADRs.

An DM, Wu XT, Hu FY, Yan B, *et al*[238] in their article on 'Association study of lamotrigine-induced cutaneous adverse reactions and *HLA-B*1502* in a Han Chinese population' emphasized the antiepileptic drugs including lamotrigine (LTG) and carbamazepine (CBZ) are among the most common causes of cutaneous adverse reactions (cADRs). Human leukocyte antigen *(HLA)-B*1502* has been strongly associated with CBZ-induced Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). To investigate this relationship, they performed high-resolution HLA genotyping on LTG-tolerant controls, healthy volunteers, and patients affected by

LTG-induced cADRs, ranging from maculopapular exanthema (MPE) to SJS/TEN. Patients with LTG-induced cADRs (n=25, including three with SJS/TEN and 22 with MPE), 21 LTG-tolerant controls, and 71 healthy volunteers were enrolled. The differences in the starting dosage of LTG among the SJS/TEN, MPE, and LTG-tolerant control groups were not statistically significant. *HLA-B*1502* frequency was 33.3% (1/3; LTG-induced SJS/TEN group), 9.1% (2/22; LTG-induced MPE group), 4.8% (1/21; LTG-tolerant group), and 8.5% (6/71; healthy volunteers). There was no significant difference in the frequency of subjects with the *HLA-B*1502* allele between the SJS/TEN group and LTG-tolerant group (p=0.239, OR=10.0, 95% CI 0.44-228.7), and healthy volunteers (p=0.26, OR=5.42, 95% CI 0.43-68.8), MPE and LTG-tolerant groups (p=1.0, OR=1.08, 95% CI 0.20-5.8), and healthy volunteers (p=1.0, OR=2.0, 95% CI 0.17-23.9). None of the HLA alleles detected were associated with LTG-induced cADRs. In conclusion, *HLA-B*1502* and other HLA alleles are not directly associated with LTG-induced MPE. The possibility that *HLA-B*1502* is associated with an increased risk of LTG-induced SJS/TEN could not be excluded.

The main biological function of HLA molecules is to bind short peptides derived from the processing of intracellular and extracellular proteins, and to present them on the cell surface to specific T cells. The peptide specificity of a given HLA molecule resides in its binding groove, lined with different pockets that engage specific side chains of the peptide ligands. HLA genes are extremely polymorphic and they encode proteins which play a crucial role in immunity. However, not all genetically different molecules are functionally different, and it is possible to group them into supertypes on the basis of their functional binding properties. The *HLA-*

*B*1502* allele is part of the HLA-B62 super type. However, the role of supertypes may not be relevant for immune response to drugs, which are usually very small nonpeptide molecules. In some cases there may be haptenization of self-peptides by a reactive metabolite, but it has been demonstrated that medications are often presented through noncovalent binding with MHC Class I molecules. One may hypothesize that rare and severe reactions to drugs, such as SJS or TEN are dependent on infrequent HLA alleles, each being capable of binding to a 'specific' drug.

Our data are very intriguing and our results clearly exclude the hypothesis that the 100%association observed in Taiwan with B*1502 is universal. As the odds ratio for this allele is extremely high in the Taiwanese study (odds ratio ¼ 895 for CBZ-SJS/normal with a 95% CI¼ 50–15,869),

Our present study with 352 cases was of sufficient size to detect such a strong effect.

Abdelbaset A Elzagallaai, Facundo Garcia-Bournissen, Yaron Finkelstein, John R Bend, et al[239] in their article 'Severe bullous hypersensitivity reactions after exposure to carbamazepine in a Han-Chinese child with a positive HLAB* 1502 and negative in vitro toxicity assays' stated that there are evidence for different pathophysiological mechanisms in drug hypersensitivity syndrome (DHS) which can present in several clinical forms ranging from simple maculopapular skin rash to severe bullous reactions and multi-system dysfunction. Genetic analysis of DHS patients has revealed a striking association between carbamazepine (CBZ)-induced severe bullous reactions, such as Steven-Johnson Syndrome, and toxic epidermal necrolysis in individuals from Southeast Asia who carry a specific HLA allele (*HLA*-

*B*1502*). This ethnic-specific relationship with a disease phenotype has raised the question of the commonality of the pathogenesis mechanisms of these diseases. The aim of this study was to investigate the genetic and metabolic bases of DHS development to help predict patient susceptibility. A case of carbamazepine-induced Steven-Johnson Syndrome reaction in a *HLA-B*1502* positive child of Han Chinese origin, a carbamazepine-induced DHS case in a Caucasian patient and 3 healthy controls were investigated. They performed two types of in vitro toxicity assay, the lymphocyte toxicity assay (LTA) and the novel in vitro platelet toxicity assay (iPTA) on cells taken from the Chinese child 3 and 9 months after recovery from the reaction and from two healthy volunteers. They also tested the Caucasian patient, who developed CBZ-induced DHS, 3 months after the reaction. Both LTA and iPTA tests were negative 3 and 9 months after the reaction on samples from the Chinese child whereas the tests were positive in the Caucasian patient. These results strongly suggest more than one mechanistic pathway for different CBZ-induced hypersensitivity reactions in patients with different ethnic backgrounds.

In our study, both LTA and iPTA tests were not done.

McCormack M, Alfirevic A, Bourgeois S, Farrell JJ, et al[55] in their article on 'Carbamazepine Hypersensitivity: Progress Toward Predicting the Unpredictable HLA-A*3101 and Carbamazepine-Induced Hypersensitivity Reactions in Europeans' state that Carbamazepine causes various forms of hypersensitivity reactions, ranging from maculopapular exanthema to severe blistering reactions. The *HLA-B*1502* allele has been shown to be strongly correlated with carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS-TEN) in the Han Chinese and other Asian populations but not in European populations. They

performed a genome wide association study of samples obtained from 22 subjects with carbamazepine-induced hypersensitivity syndrome, 43 subjects with carbamazepine-induced maculopapular exanthema, and 3987 control subjects, all of European descent. They tested for an association between disease and HLA alleles through proxy single-nucleotide polymorphisms and imputation, confirming associations by high-resolution sequence-based HLA typing. They replicated the associations in samples from 145 subjects with carbamazepine-induced hypersensitivity reactions. The HLA-A*3101 allele, which has a prevalence of 2 to 5%in Northern European populations, was significantly associated with the hypersensitivity syndrome (P=3.5×10(-8)). An independent genome wide association study of samples from subjects with maculopapular exanthema also showed an association with the HLA-A*3101 allele (P=1.1×10(-6)). Follow-up genotyping confirmed the variant as a risk factor for the hypersensitivity syndrome (odds ratio, 12.41; 95% confidence interval [CI], 1.27 to 121.03), maculopapular exanthema(odds ratio, 8.33; 95% CI, 3.59 to 19.36), and SJS-TEN (odds ratio, 25.93; 95% CI, 4.93 to 116.18). They concluded that the presence of the HLA-A*3101 allele was associated with carbamazepine-induced hypersensitivity reactions among subjects of Northern European ancestry. The presence of the allele increased the risk from 5.0% to 26.0%, whereas its absence reduced the risk from 5.0% to 3.8%.The association with HLA-A*3101 is seen in more diverse ethnic groups, and predisposes to mild as well more severe cutaneous reactions associated with carbamazepine[240].

In our study, the association of SJS/TEN with HLA-A*3101were not studied.

In this study, most of the patients were given alternative nonaromatic AEDs, such as Topiramate, Gabapentin, Pregabalin, Zonisamide and Lacosamide after their SJS/TEN episodes and they all showed tolerance to these nonaromatic AEDs with good control of epilepsy.

Tai-Ming Ko, Wen-Hung Chung, Chun-Yu Wei, Han-Yu Shih, *et al*[241] in the article on Shared and restricted T-cell receptor use is crucial for carbamazepine-induced Stevens-Johnson syndrome demonstrated Stevens-Johnson syndrome (SJS) and its related disease, toxic epidermal necrolysis (TEN), are life-threatening drug hypersensitivities with robust immune responses to drugs. Despite the strong HLA predisposition to drug hypersensitivities, such as *HLA-B*1502* to carbamazepine (CBZ)–induced SJS/TEN, it remains unknown whether particular T-cell receptors (TCRs) participate in recognition of small drug/peptide–HLA complexes. Using the strong HLA predisposition in patients with CBZ-induced SJS/TEN as a model, they aimed to study the use of TCR repertoire in patients with drug hypersensitivity. They enrolled patients with CBZ-SJS/TEN, tolerant control subjects, and healthy subjects who had no history of CBZ exposure. They isolated PBMCs from the subjects, cultured CBZ-specific T cells, and globally investigated the expression level and third complementarily-determining region length distribution of the TCR profile. They further assessed the pathogenic role of the disease-specific clonotype using real-time PCR–based tests and functional analysis. On drug stimulation, CBZ-specific CD81 T cells were expanded in vitro and activated to release granulysin. Notably, VB-11-ISGSY was identified as the most predominant clonotype and shared among different subjects. This clonotype was present in 16 (84%) of 19 patients with SJS/TEN, absent in all 17 tolerant patients, and present at

a low frequency in healthy subjects (4/29 [14%]). CBZ-specific cytotoxicity could be primed in vitro in the PBMCs of healthy subjects who are carriers of *HLA-B*1502* and VB-11-ISGSY; this cytotoxicity could be blocked by an anti–TCR-VB-11 antibody. Furthermore, a single T-cell clone expressing VA-22-FISGTY/VB-11-ISGSY showed significant cytotoxicity against *HLA-B*1502*–positive antigen-presenting cells and CBZ. This study establishes the key role of the TCR in the pathogenic mechanism of SJS/TEN, explains why some *HLA-B*1502* carriers are tolerant to CBZ, and provides a biomarker profile for drug hypersensitivity.

In our study, we did not carry out any TCR assays.

Umapathy Shankarkumar, Krishnakumar N. and Shah KanjakshaGhosh[242] in the paper *HLA-B*1502* allele association with oxcarbamazepine-induced skin reactions in epilepsy patient from India discussed about serious allergic cutaneous reaction, especially Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are the most feared complications of antiepileptic drug (AED) therapy. SJS/TEN is reported to be two to three times more prevalent in Han Chinese than Caucasians (Chung et al., 2004), with carbamazepine use associated with 25–33% of cases for Asians (Kamaliah et al.,1998) compared to 5–6% for Europeans (Roujeau et al.,1995). Oxcarbamazepine is a 10-keto analog of carbamazepine, which acts by blocking voltage-gated sodium channels and exhibits a linear pharmacokinetic property. It does not induce its own metabolism, as it is not dependent on cytochrome P450 isozymes. The epoxide generation seen in carbamazepine, which is responsible for various cutaneous side effects, is unknown for this drug? Recently a study conducted in Taiwan found 100% association

between the *HLA-B*1502* allele and carbamazepine induced SJS in Han Chinese individuals.

In our study no patient with CBZ induced SJS was exposed to Oxcarbamazepine.

Shen Y, Nicoletti P, Floratos A, Pirmohamed M, *et al*[243] in their observation in 'Genome-wide association study of serious blistering skin rash caused by drugs' noted that SJS and TEN are rare but severe, potentially life threatening adverse drug reactions characterized by skin blistering. Previous studies have identified drug-specific and population-specific genetic risk factors with large effects. In this study, they report the first genome-wide association study (GWAS) of SJS/TEN induced by a variety of drugs. Their aim was to identify common genetic risk factors with large effects on SJS/TEN risk. They conducted a genome-wide analysis of 96 retrospective cases and 198 controls with a panel of over one million single-nucleotide polymorphisms (SNPs). They further improved power with about 4000 additional controls from publicly available datasets. No genome-wide significant associations with SNPs or copy number variants were observed, although several genomic regions were suggested that may have a role in predisposing to drug-induced SJS/TEN. Their GWAS did not find common, highly penetrant genetic risk factors responsible for SJS/TEN events in the cases selected.

Recommendations for Diagnosis and Treatment of Stevens-Johnson Syndrome

Diagnosis:

1. Identify and discontinue potential causative drug(s); common offending agents include antibiotics, anticonvulsants, NSAIDs, and allopurinol.

2. Perform skin biopsy (e.g. lesional biopsy for routine microscopy with hematoxylin-eosin staining and perilesional biopsy for direct immunofluorescence microscopy) to confirm diagnosis and rule out other conditions, including pemphigus vulgaris, bullous pemphigoid, paraneoplastic pemphigus, linear IgA bullous dermatosis, staphylococcal scalded skin syndrome, acute graft vs-host disease, acute generalized exanthematous pustulosis, and virus infection.
3. In patients with cough, fever, and constitutional symptoms, perform chest radiography and serologic studies (e.g. IgM and IgG) for *Mycoplasma pneumoniae* infection.
4. In patients with mucosal erosions and crust, consider swab culture for herpes simplex virus infection.
5. If concerned about internal organ involvement and/or sepsis, perform laboratory studies (e.g. complete blood cell count, electrolytes, glucose, creatinine, and liver function tests) and obtain cultures (e.g. skin, urine, blood, and intravascular lines), as appropriate.

Treatment:

1. Providing supportive care is the most important measure.
2. Monitor fluids, electrolytes, and body temperature and watch for signs of infection/sepsis.
3. Obtain ophthalmology consultation for recommendations (e.g. antiseptic eye drops) and measures to decrease long-term sequelae (e.g. scarring and vision loss).
4. Provide saline to involved mucosal surfaces and orifices (e.g. oral and nasal).

5. Suggest analgesia (e.g. oral “swish-and-spit” solutions containing lidocaine, diphenhydramine, and Maalox).
6. Apply white petrolatum jelly (Vaseline) to denuded skin.
7. Consider tap water wet dressings with topical corticosteroids for areas of active inflammation (e.g. red and itchy).
8. Give antibiotics (e.g. azithromycin) in cases due to *M pneumonia* infection; otherwise, antibiotic therapy should be initiated only if sepsis or other infection occurs and should not be used for prophylaxis.
9. Avoid skin trauma.
10. Provide management in the intensive care unit if the following are needed/noted: extensive wound care, clinically important medical comorbid conditions (e.g. cardiac, pulmonary, or renal), internal organ involvement, sepsis, and hemodynamic or electrolyte instability.
11. Consider IVIG (total dose of 3 g/kg in divided doses over 3 d) in severe cases (need to check IgA level because anaphylaxis can occur in IgA-deficient patients).
12. Giving systemic corticosteroid therapy is controversial and should be considered only in severe cases early in the disease course (i.e. before clinically important epithelial sloughing has occurred) because it could promote infectious complications and sepsis; if used, intravenous methylprednisolone 2 to 2.5 mg/kg/d in divided doses for a few days can be considered.
13. At hospital discharge, perform close ophthalmology follow-up and monitor other mucosal surfaces (e.g. genitourinary and gastrointestinal) for chronic sequelae such as strictures, with referrals to specialists as appropriate.

Barvaliya M et al[244] reported an audit on the causative drugs, clinical outcome, and cost of management in SJS, TEN, and SJS-TEN overlap in Tertiary care hospitals-based multicentric retrospective case series study. Indoor case papers of SJS, TEN, and SJS-TEN overlap admitted between January 2006 and December 2009 in four tertiary care hospitals of Gujarat were scrutinized. Data were collected for demographic information, causative drugs, investigations, treatment given, duration of hospital stay, time interval between onset of symptoms and drug intake, clinical outcome, and complications. Data were analyzed to find out proportion of individual drugs responsible, major complications, and clinical outcome in SJS, TEN, and SJS-TEN overlap. Total cost of management was calculated by using cost of drugs, investigations, and consumables used during entire hospital stay. One-way Analysis of Variance followed by Tukey-Kramer multiple comparison test was used for comparison of incubation period, duration of hospital stay, and cost of management. The study revealed that Antimicrobials (50%), nonsteroidal anti-inflammatory drugs (22.41%), and anti-seizure drugs (18.96%) were the most commonly associated groups. Nevirapine (28.12%) was the most common drug. Anti-seizure drugs were more often associated with serious form of adverse reaction (TEN: 81.8%) than other drugs. Duration of hospital stay (20.6 vs 9.7 days) and cost of management (Rupees 7910/- Vs Rs 2460/-) were significantly higher in TEN than SJS (P=0.020 and P<0.001, respectively). Time duration between drug intake and onset of symptoms (17.7 vs 27.5 days) was insignificantly lower in TEN as compared with SJS. Secondary infection (28.12%) was the most common complication noted. Mortality rate was 15.6% among all cases; 9% in SJS and 26.7% in TEN. They

concluded that antimicrobial drugs are the most commonly implicated drugs and cost of managing these adverse drug reactions is higher than other serious ADRs.

Management and Therapy

Stevens-Johnson syndrome is both a physically and psychologically devastating disease. One should deal with the physiological complications of the disease process, but the psychological trauma often associated with such an initially disfiguring disease leaves wounds that are not visible. Constant support of both the patient and the nursing staff is necessary to relieve some of the anxiety associated with this syndrome. Education and reassurance should be as much a part of the treatment process as drug therapy.

Management of patients with Stevens-Johnson syndrome is usually provided in intensive care units or burn centers.

No specific treatment of Stevens-Johnson syndrome is noted; therefore, most patients are treated symptomatically. In principle, the symptomatic treatment of patients with Stevens-Johnson syndrome does not differ from the treatment of patients with extensive burns.

Treatment in acute stage

Management in the acute stage involves sequentially evaluating the severity and prognosis of disease, prompt identification and withdrawal of the culprit drug(s), rapidly initiating supportive care in an appropriate setting, and eventual "specific" drug therapy as described in detail below.

Rapid evaluation of severity and prognosis

As soon as the diagnosis of SJS or TEN has been established, the severity and prognosis of the disease should be determined so as to define the appropriate medical setting for further management. In order to evaluate prognosis in patients with SJS/TEN, the validated SCORTEN disease severity scoring system can be used.

Patients with a SCORTEN score of 3 or above should be managed in an intensive care unit if possible.

Prompt withdrawal of culprit drug(s)

Prompt withdrawal of causative drugs should be a priority when blisters or erosions appear in the course of a drug eruption.

Garcia-Doval et al. have shown that the earlier the causative drug is withdrawn, the better the prognosis, and that patients exposed to causative drugs with long half-lives have an increased risk of dying[245].

In order to identify the culprit drug(s) it is important to consider the chronology of administration of the drug and the reported ability of the drug to induce SJS/TEN. The chronology of administration of a culprit drug, or time between first administration and development of SJS/TEN, is between 1 and 4 weeks in the majority of cases.

Supportive Care

Fluid and Electrolyte Losses

Fluid losses are not as severe as with thermal burns of equal surface area, however, fluid-replacement therapy is needed. Factors that contribute to massive fluid shifts are evaporation from skin lesions and leakage from capillaries. Both result in the loss of blood-borne proteins. Such fluid shifts may be prevented by early covering of the area with xenografts. As with burns, aggressive fluid replacement may prevent some of the complications of volume depletion.

Fluid management is provided by macromolecules and saline solutions during the first 24 hours. Phosphate salts are necessary in the presence of hypophosphatemia. Appropriate early and aggressive replacement therapy is required in case of hyponatraemia, hypokalaemia or hypophosphatemia which quite frequently occur. The amount of fluids required in patients with Stevens-Johnson syndrome is usually less than in those patients with burns covering the same body surface area. A critical element of supportive care is the management of fluid and electrolyte requirements. Intravenous fluid should be given to maintain urine output of 50 - 80 mL per hour with 0.9% NaCl supplemented with 20 mEq of KCl.

After the second day of hospitalization, oral intake of fluids provided by nasogastric tube is often begun, so that intravenous fluids can be tapered progressively and discontinued, usually in 2 weeks.

Massive parenteral nutrition is necessary as soon as possible to replace the protein loss and to promote healing of cutaneous lesions. Intravenous insulin therapy may be required because of impaired glycoregulation.

Environmental temperature raised to 30-32°C reduces caloric loss through the skin. Fluidized air beds are recommended if a large portion of the skin on the patient's backside is involved. Heat shields and infrared lamps are used to help reduce heat loss.

Respiratory Complications

Pulmonary support is critical to the survival of the patient. Death is often attributable to respiratory failure. Respiratory failure results from a combination of factors. These include sloughing of the tracheobronchial mucous membrane, retention of mucus, and possible immune deficiency, which makes these patients more susceptible to infections. Pulmonary management includes ultrasonic nebulation, incentive spirometry, postural drainage, suctioning, physiotherapy, and appropriate treatment of infection. Arterial blood gases should be closely followed for signs of respiratory failure. At the first signs of failure, intubation with ventilatory support should be seriously considered.

Anticoagulation with heparin for the duration of hospitalization is recommended. Antacids reduce the incidence of gastric bleeding.

Pulmonary care includes aerosols, bronchial aspiration, and physical therapy. Tranquilizers are used to the extent limited by respiratory status.

SJS/TEN is a life threatening condition and therefore supportive care is an essential part of the therapeutic approach[246]. A multicenter study conducted in the USA[247], and including 15 regional burn centers with 199 admitted patients, showed that survival rate - independent of the severity of disease (APACHE-score and TBSA = Total body surface area) - was significantly higher in patients who were transferred to a burn unit within 7 days after disease-onset compared with patients admitted after 7 days (29.8% vs 51.4% ($p < 0.05$)). This positive association of early referral and survival has been confirmed in other studies[248, 249].

A single center retrospective study on the outcome of patients after admission to a burn center identified sepsis at the time of admission as the most important negative prognostic factor, followed by age, and to a lesser extent the percentage of

total body surface area involved. Co-morbidities and the use of steroids may be important on an individual basis, but lose significance in presence of other factors[250].

Wounds should be treated conservatively, without skin debridement which is often performed in burn units, as blistered skin acts as a natural biological dressing which likely favours re-epithelialisation. Non-adhesive wound dressings are used where required, and topical sulfa containing medications should be avoided.

Infection Control

Patients with Stevens-Johnson syndrome are at a high risk of infection. Sterile handling and/or reverse-isolation nursing techniques are essential to decrease the risk of nosocomial infection. Cultures of blood, catheters, gastric tubes, and urinary tubes must be performed regularly.

Because of the association between Stevens-Johnson syndrome and sulfonamides, avoid the use of silver sulfadiazine, which is commonly used in burn units. Instead, use another antiseptic, such as 0.5% silver nitrate or 0.05% chlorhexidine, to paint and bathe the affected skin areas.

Prophylactic systemic antibiotics are not recommended. Antimicrobials are indicated in cases of urinary tract or cutaneous infections, either of which may lead to bacteraemia.

The diagnosis of sepsis is difficult. One should carefully consider the decision to administer systemic antibiotics. The first signs of infection are an increase in the number of bacteria cultured from the skin, a sudden drop in fever, and deterioration of the patient's condition, indicating the need for antibiotic therapy.

The choice of antibiotic is usually based on the bacteria present on the skin. Because of impaired pharmacokinetics, similar to that present in burn patients, the

administration of high doses may be required to reach therapeutic levels. Monitoring the serum levels is necessary to adjust the dosage.

Skin Care

Several skin care approaches have been described.

Extensive debridement of nonviable epidermis followed by immediate cover with biologic dressings are among the recommended treatments.

Biologic dressings may include the following:

- Porcine cutaneous xenografts
- Cryopreserved cutaneous allografts
- Amnion-based skin substitutes
- Collagen-based skin substitutes

The ophthalmology literature supports concurrent coverage of the involved eye(s) with amniotic membrane[251].

Leaving the involved epidermis that has not yet peeled off in place and using biologic dressings only on raw dermis also has been recommended.

Skin allotransplantation reduces pain, minimizes fluid loss, improves heat control, and prevents bacterial infection.

Hyperbaric oxygen can also improve healing.

Drug Therapy

Due to the high risk of mortality, management of patients with SJS/TEN requires rapid diagnosis, evaluation of the prognosis using SCORTEN, rapid identification and interruption of the culprit drug, specialized supportive care ideally in

an intensive care unit, and the consideration of immunomodulatory agents such as high-dose intravenous immunoglobulin[252].

Acronym for prompt treatment of SJS/TEN: TREAT

Test for *HLA-B*1502*

Rapid initiation of Steroids &Iv Ig

Extended stay in ICU/ Burns Unit

Action for prevention of Relapses/ Infections/ alternative AEDs

Treatment with I & II line Immunosuppressive agents, if required

To date, a specific therapy for SJS/TEN that has shown efficacy in controlled clinical trials unfortunately does not exist.

Stevens-Johnson syndrome is a rare disorder with relatively high mortality and morbidity rates. To date, because of a lack of consensus on the proposed therapeutic modalities, intensive supportive management and withdrawal of the offending drug remain the criterion standard.

For any intervention, a prospective randomized controlled trial would be the most appropriate step to validate its use. However, a large number of patients are required to reach statistical significance. Furthermore, for ethical reasons, withdrawal of a potentially life-saving therapy for the sake of randomization with a placebo control is not possible.

A large European study designed to evaluate the efficacy of various treatments, the EuroSCAR Study, "found no sufficient evidence of a benefit for any specific treatment[253]."The group looked at mortality in patients treated with IVIG and corticosteroids. However, in a letter to the editor, Pehr disagreed with the findings in

the EuroSCAR study citing inadequate doses of IVIG and corticosteroids in that study[254].

Few studies have addressed the effect of systemic steroids or IVIG on either the development or the outcome of ocular manifestations in Stevens-Johnson syndrome and toxic epidermal necrolysis (TEN). Neither treatment appeared to have an effect on the ocular outcome in patients in two reports[255].

Several treatment modalities given in addition to supportive care are reported in the literature and these are discussed below:

1. The use of systemic steroids remains controversial. Some authors believe that they are contraindicated, especially because there may be some question about the diagnosis. Patients with infection-induced erythema multiforme do worse when steroids are given. (Note that the differentiation between Stevens-Johnson syndrome and erythema multiforme should be possible even in the acute stage.)[256] Prolonged treatment with systemic steroids has been associated with an increased prevalence of complications.

   However, concerns about the safety of systemic corticosteroids in the treatment of Stevens-Johnson syndrome are based on a few case series. In those reports, systemic corticosteroids were administered too late in the course of the disease, in inappropriately low doses, and for a very long duration that actually impaired the healing process and increased the risk of sepsis. The currently advocated approach for corticosteroid use suggests the early use of short-term (4-7 days), high-dose intravenous corticosteroids[257,258]. The ophthalmology literature contains several papers that advocate systemic and topical steroids to minimize ocular morbidity[259]. Authors have cited

salvage of vision when pulse steroid therapy has been given[260]. Others have concluded that IV steroids and immunoglobulins do not improve outcome[261]. Systemic steroids were the standard treatment until the early 1990's, although no benefit has been proven in controlled trials.

In the absence of strong evidence of efficacy, and due to the confusion resulting from the numerous steroid treatment regimens reported (treatment of short versus long duration, various dose regimens), their use has become increasingly disputed.

A recent retrospective monocenter study suggests that a short course "pulse" of high dose corticosteroids (dexamethasone) may be of benefit[262]. On the other hand, a recent retrospective case-control study conducted by Schneck et al. in France and Germany concluded that corticosteroids did not show a significant effect on mortality in comparison with supportive care only[263].

2. Thalidomide, a medication with known anti-TNFα activity that is immunomodulatory and anti-angiogenetic has been evaluated for the treatment of TEN[264, 265]. Unfortunately, in a double-blind, randomized, placebo-controlled study higher mortality was observed in the thalidomide-treated group suggesting that thalidomide is detrimental in TEN.
3. High-dose intravenous immunoglobulins: As a consequence of the discovery of the anti-Fas potential of pooled human intravenous immunoglobulins (IVIG) in vitro, IVIG have been tested for the treatment of TEN, and their effect reported in different non-controlled studies. To date, numerous case reports and 12 non-controlled clinical studies containing 10 or more patients have analyzed the therapeutic effect of IVIG in TEN. All except one study[266],

confirm the known excellent tolerability and a low toxic potential of IVIG when used with appropriate precaution in patients with potential risk factors (renal insufficiency, cardiac insufficiency, IgA deficiency, thrombo-embolic risk) [267]. Taken together, although each study has its potential biases and the 12 studies are not directly comparable, 9 of the 12 studies suggest that there may be a benefit of high-dose IVIG on the mortality associated with TEN. Analysis of studies published suggests that total IVIG doses of more than 2 g/kg may be of greater benefit than doses of 2 g/Kg or less. To determine if a dose response relationship exists, Trent et al. analyzed the published literature between 1992 and 2006, selected all studies performed in adults in which the dose of IVIG administered was reported for each patient, excluded cases appearing as duplicates in separate publications where possible, and performed a multivariate logistic regression analysis to evaluate mortality and total IVIG dose after controlling for age and affected body surface area[268].

Although this study has limitations stated by the authors and including publication bias, heterogeneous diagnostic definitions and methods of each study, as well as the exclusion of 2 studies owing to lack of individual IVIG dosing data, logistic regression results showed that, with each 1 g/Kg increase in IVIG dose, there was a 4.2-fold increase in TEN patient survival, which was statistically significant. Patients treated with high doses of IVIG had a significantly lower mortality compared with those treated with lower doses, and notably the mortality was zero percent in the subset of 30 patients treated with more than 3 g/kg total dose of IVIG. Given the favorable side-effect profile of IVIG and the data existing to date, in the authors' opinion early administration of high-dose immunoglobulin (3 g/kg total dose given over 3-4

days) should be considered alongside supportive care for the treatment of toxic epidermal necrolysis, given the absence of other validated specific therapeutic alternatives.

Prophylactic use of IVIG has also been reported. One group used IVIG in a patient who underwent cardiac catheterization but who had 4 previous Stevens-Johnson syndrome episodes after intravenous contrast injection[269].

4. The concomitant administration of corticosteroids or immunosuppressive agents remains controversial.

   IVIG has also been applied in a few children with SJS/TEN, and two non-controlled studies suggest a possible benefit[270 271].

5. Cyclosporine (CsA). CsA, a calcineurin-inhibitor, is an efficient drug in transplantation and autoimmune diseases. Arevalo et al. have performed a study as a case series with two treatment arms: CsA alone versus cyclophosphamide in combination with corticosteroids. Patients treated with CsA had significantly shorter time to complete re-epithelialisation, and fewer patients with multi-organ failure and death were observed[272]. A small case series with three TEN patients treated initially with high-doses of intravenous dexamethasone followed by CsA showed a stop in disease progression within 72 h[273]. Other single case reports also reported a positive effect of the use of CsA in TEN[274, 275].

   Recently, Valeyrie-Allanore L conducted an open, phase II trial to determine the safety and possible benefit of cyclosporin[276]. Twenty-nine patients were included in the trial (10 SJS, 12 SJS-TEN overlap and 7 TEN), and 26 completed the treatment with CsA administered orally (3 mg/kg/d for 10 days) and tapered over a month. The prognostic score predicted 2.75

deaths and none occurred (p = 0.1), suggesting that, although not statistically significant, cyclosporine may be useful for the treatment of TEN.

6. TNF antagonists. A new therapeutic approach targeting the proinflammatory cytokine TNFα has been proposed by Hunger et al. They treated one patient with a single dose of the chimeric anti-TNFα antibody (infliximab 5 mg/kg) and reported that disease progression stopped within 24 hours followed by a complete re-epithelialization within 5 days[277]. Meiss et al. report three cases with an overlap of acute generalized exanthematous pustulosis and TEN and treatment response to infliximab[278].

   Administration of the soluble TNFα Receptor Etanercept 25 mg on days 4 and 8 after onset of TEN in a single case resulted in cessation of epidermal detachment within 24 hours but subsequent death of the patient. The published data is currently insufficient to draw a conclusion on the therapeutic potential of TNF antagonists in TEN.

7. Plasmapheresis/plasma exchange (PE). PE has also been tried in SJS/TEN, but the current data does not allow a conclusion as to the potential of this approach to be drawn due to the small number of patients treated, the frequent confounding factors including different or combined treatments, and other potential biases[279-281]. Furthermore, a small single retrospective study using PE by Furubacke et al., which compared their case series with two published case series serving as controls, showed no difference in terms of mortalit[282].

8. Cyclophosphamide (CPP). CPP has been studied in small case series, either in combination with other treatments such as CsA, in conjunction with high-dose corticosteroids[283], or alone[284]. Although a beneficial effect of CPP is

suggested by the authors of these small trials, larger studies are needed to clarify these preliminary results with a special attention to potential side effects.

Treatment of sequelae

Due to the often combined involvement of the skin, eyes and mucous membranes (oral, gastrointestinal, pulmonary, genital, as well as urinary), the follow up and treatment of sequelae should be interdisciplinary.

Special attention should be given to the prevention of ocular complications. Early referral to an ophthalmologist is mandatory for assessment of the extent of eye involvement and prompt treatment with topical steroids.

Visual outcome is reported to be significantly better in patients who receive specific ophthalmological treatment during the first week of disease. Some of the ocular complications have an inflammatory background and have to be treated occasionally with ophthalmic steroids and/or extensive lubrication of the eye in order to prevent progression leading ultimately to the need for corneal transplantation.

A small single retrospective study with IVIG showed no significant effect on ocular complications in frequency and severity, but the power of the study was weak[285]. The benefit of local antibiotic treatment (ointments) is not clear. Yip et al. have reported that the use of local antibiotic treatment leads to more late complications, including, for example, dryness of the eyes.

Hypopharyngeal stenosis combined with dysphagia and oesophageal strictures are long-term complications which are difficult to treat[286, 287] and may require laryngectomy.

Treatment of Acute Ocular Manifestations

Treatment of acute ocular manifestations usually begins with aggressive lubrication of the ocular surface. As inflammation and cicatricial changes ensue, most ophthalmologists use topical steroids, antibiotics, and symblepharon lysis. In the case of mild chronic superficial keratopathy, long-term lubrication may be sufficient. In addition to lubrication, some patients may require a cosmetically acceptable long-term lateral tarsorrhaphy. In case of exposure, keratopathy and tarsorrhaphy may be required.

Maintenance of ocular integrity can be achieved through the use of amniotic membrane grafting, adhesive glues, lamellar grafts, and penetrating keratoplasty, either in the acute phase or in subsequent follow-up care.

Visual rehabilitation in patients with visual impairment can be considered once the eye has been quiet for at least 3 months. The visual rehabilitation in patients with severe ocular involvement, resulting in profound dry eye syndrome with posterior lid margin keratinization, limbal stem cell deficiency, persistent epithelial defects with subsequent corneal neovascularization, and frank corneal opacity with surface conjunctivalization and keratinization, is difficult and often frustrating for both the patient and the physician. A close, usually long-term, relationship between the patient and the ophthalmologist needs to be established to achieve the best possible result.

The removal of keratinized plaques from the posterior lid margins, along with mucous membrane grafting and/or amniotic membrane grafting, is usually the first step and one of the most important determining factors in the future success of corneal surgeries. Preferably, a skilled oculoplastic surgeon with specific experience on patients with Stevens-Johnson syndrome should perform this procedure.

Subsequently, limbal stem cell transplantation and amniotic membrane grafting with superficial keratectomy removing conjunctivalized or keratinized ocular surface can follow. Patients with persistent corneal opacity require lamellar or penetrating keratoplasty in the next step, but each exposure to alloantigenic material increases the odds of tissue rejection. Therefore, the author's advice is to strive for major, if not perfect, resurrection of the useful vision, rather than perform allografts of both eyes and keratoplasties.

To preserve corneal clarity after the visual reconstruction, the long-term use of gas-permeable scleral contact lenses may be necessary to protect the ocular surface. Long-term management frequently involves the treatment of trichitic lashes and/or eyelid margin repair for distichiasis or entropion. If the ocular surface repeatedly fails to heal after multiple surgical interventions, keratoprosthesis may be considered as a last resort.

Consultations and Long-Term Monitoring

Internal medicine, critical care, or paediatrics consultants direct inpatient care.

A dermatologist is the most likely clinician to establish the diagnosis, with or without biopsy.

Severe cases may require the involvement of a burn specialist or plastic surgery specialist.

Ophthalmology consultation is mandatory for those with ocular involvement.

Depending on organ system involvement, consultations with a gastroenterologist, pulmonologist, and nephrologists may be helpful.

Patients with SJS require regular monitoring of their medications and status.

Although patients with erythema multiforme minor may be treated as

outpatients with topical steroids, those with erythema multiforme major (i.e. Stevens-Johnson syndrome) must be hospitalized. Cases of erythema multiforme minor must be followed closely. Some authors recommend daily follow-up.

In our study, the above treatment guidelines were followed. As soon, as a patient reported with fever and skin rash, the patient was admitted as an in patient under a multidisciplinary team comprising of General physician, neurologist, dermatologist and ophthalmologist.

Carbamazepine was stopped immediately and another AED like Levetiracetam or clonazepam which have not been reported to include SJS was used for the control of seizures for the next three years.

All patients were treated with fluid and electrolyte replacement, short doses of steroids for 3-5 days and supportive care. Antibiotics were used based on culture and sensitivity reports. Ophthalmologic and dermatology consultations were obtained in all the patients and their advised followed. After discharge, the patients were followed up by these specialists and appropriate treatment was given.

Two patients with extensive TEN were admitted in the burns unit and later shifted to the intensive care unit for ventilatory support. Both these patients expired.

After discharge the remaining 54 patients who developed Carbamazepine induced SJS were followed up periodically every six months for the next three years.

POSSIBLE MECHANISM FOR CARBAMAZEPINE-INDUCED ADVERSE CUTANEOUS DRUG REACTION

The mechanism by which CBZ causes adverse cutaneous drug reactions (ACDR) is not well understood. Potential defects in the enzymes responsible for bioactivation and detoxification of CBZ have been proposed. CBZ is bioactivated by hepatic cytochrome P450 enzymes, which generate various potentially reactive metabolites, such as CBZ 10,11-epoxide, 3-hydroxy CBZ, 2-hydroxy CBZ, and CBZ 2,3-epoxide. Most of the reactive epoxides are detoxified to nontoxic dihydrodiols by liver microsomal epoxide hydrolase 1 (EPHX1) or to glutathione conjugates by glutathione transferase. ACDR triggered by CBZ is postulated to immune related because infiltrating inflammatory cells can be detected in the skin lesions. CD8+T-cell-mediated cytotoxic responses appear to be the major event in SJS/TEN. There is evidence supporting the view that ACDR involve major histocompatibility complex (MHC)-dependent presentation of its metabolites for T cell activation. The HLA-B allele can elicit immune responses by presenting endogenous antigens to the cytotoxic T cells, resulting in proliferation of the cytotoxic cells. Cross-reactivity among the aromatic antiepileptic drugs (CBZ, phenytoin, phenobarbital) in inducing ACDR is recognized, but has not been observed between aromatic antiepileptic drugs and lamotrigine. However, in addition to CBZ, *HLA-B*1502* allele was found in patients with lamotrigine and phenytoin-induced SJS/TEN as well, though the number were small[288]. HLA-B has been demonstrated to be significantly related to various drug related SJS/TEN, e.g. HLA-B*5701 in abacavir hypersensitivity, HLA B*5801 in allopurinol and B1513 in phenytoin. This implies the important role of HLA-B in the pathogenesis of ACDR.

SUMMARY

Asia is a large region with 60% of world population. The region is the home of many national groups with different culture and ethnicity. Data from the WHO Uppsala Monitoring Center (WHO-UMC)and Novartis CBZ-SJS/TEN reports 2000-2006showed that the incidence of ACDR induced by CBZ was high among some Asian countries. The incidence is not known in many geographical regions and ethnic groups. Therefore it is important to document the incidence of ACDR induced by CBZ in these areas. The importance of *HLA-B*1502* as marker of CBZ-induced SJS/TEN is being established particularly among Han Chinese, and the prevalence of *HLA-B*1502* in various ethnic groups are being determined, there are still large parts of Asia where the prevalence of *HLA-B*1502* is not known. This is for example, the Han and non-Han Chinese from different parts of China, Filipinos, various ethnic groups in Indonesia, Pakistan, Bangladesh, Myanmar, Cambodia and Lao PDR. The determination of the prevalence of *HLA-B*1502* in these ethnic groups is thus of high priority.

The significance of *HLA-B*1502* as a marker for CBZ-induced SJS/TEN is only established among the Han Chinese, less so among the Malays and Thais. As *HLA-B*1502* as a marker of CBZ-induced SJS/TEN is ethnicity specific, the present study enlightens our knowledge about the relationship between *HLA-B*1502* and CBZ-induced SJS/TEN in South India.

There are many unanswered questions in CBZ induced ACDR and *HLA-B*1502* waiting to be explored like the role of other HLA-B subtypes in CBZ-induced SJS/TEN, significance of *HLA-B*1502* and other HLA subtypes in ACDR induced by other antiepileptic drugs, e.g. phenytoin and lamotrigine.

CONCLUSIONS

There is a high prevalence *HLA-B*1502* allele in South Indian epileptic population on Carbamazepine (18.20%) when compared to the non-epileptic population (7%). This is being reported for the first time in the world literature and further studies have to be done in other centers in India and in other countries in different races to look for this difference. The mechanisms underlying this difference have to be elucidated.

In our study, the presence of the *HLA-B*1502* allele was strongly correlated to the development of SJS/TEN. The Positive Predictive Value for development of SJS/TEN on exposure to CBZ was 81.82 % with a very high Negative Predictive Value of 99.30%. The Sensitivity of *HLA-B*1502* Test was 96.43% with a Specificity of 95.95%.

There is a strong association (100%) of *HLA–B*1502* allele and the development of SJS in South Asians. This observation is similar to that reported in Chinese, Malay and Thai populations in South East Asia.

In conclusion, *HLA-B*1502* has recently been linked to CBZ-induced SJS/TEN in some Asian groups. Screening of *HLA-B*1502* in these ethnic groups before use of CBZ is recommended.

Determination of prevalence of *HLA-B*1502* allele and its association with CBZ-induced SJS/TEN in the different ethnic groups is thus of high priority for epilepsy care in India and South East Asia.

Benefit to the Community:

Many unexpected deaths and mortality due to SJS/TEN can be prevented by prior genetic testing, before exposure to carbamazepine. This will be a great service to the community. It is mandatory to test for *HLA-B*1502* allele, prior to initiation of carbamazepine therapy in the above populations.

This will also be a pioneering study, since similar genetic testing may become mandatory in the near future.

Benefit to the Patient:

Since *HLA-B*1502* testing will be performed prior to the administration of Carbamazepine to the patient, the chances of fatal SJS/TEN can be avoided, thus preventing the morbidity and mortality associated with the above severe cutaneous adverse reactions.

IMPACT OF THE STUDY

The present study is the largest study reported from India.

The study clearly establishes a firm link between the presence of *HLA-B*1502* allele and Carbamazepine induced SJS/TEN with high Positive and Negative Predictive Values.

It is imperative to test for *HLA-B*1502* allele, prior to starting Carbamazepine and the drug should not started for patients testing positive for *HLA-B*1502* allele.

Once a patient has developed SJS/TEN on exposure to CBZ, he should not be rechallenged with CBZ since this would trigger SJS/TEN with increased severity.

Patients testing Positive for *HLA-B*1502* allele, should not only avoid CBZ, but also Oxcarbamazepine, Phenytoin, Phosphenytoin, Phenobarbitone, Sodium Valproate and Lamotrigine.

This thesis will change the way antiepileptic medications are prescribed in India. Ideally prescription for AED should be started only after testing for *HLA-B*1502* allele.

If *HLA-B*1502* allele is detected in an epileptic patient, only AEDs like Gabapentin, Pregabalin, Zonisamide, Lacosamide and Clonazepam should be initiated.

During serial seizures or status epilepticus, Midazolam or Propofol should be initiated and Phosphenytoin should be started only if the *HLA-B*1502* allele is not detected. The time for performing the test in emergency situations should take less than 6 hours.

The *HLA-B*1502* allele is well standardized and easy to perform. The cost of the test, around Rupees fifteen hundred is affordable. This test should be performed in all clinical settings, prior to the initiation of AED therapy.

Failure to test *HLA-B*1502* allele, prior to starting one of the offending AED may lead to SJS/TEN which are associated with high mortality rates of 5- 30 % and severe mobididity and sequelae affecting the skin and mucous membranes.

RECOMMENDATION FOR USE OF CARBAMAZEPINE

As CBZ is an effective, safe and cost effective antiepileptic drug, the recent finding of HLAB* 1502 as marker of CBZ-induced SJS/TEN indicates that it is of high priority to develop facilities for testing of *HLA-B*1502* in clinical practice involving Indians, Han Chinese, Malays and Thai patients. Patients of these ethnic groups should be screened for the *HLA-B*1502* allele before starting treatment with CBZ. If tested positive, CBZ should be avoided. However, it should be noted that patients who are tested positive for *HLA-B*1502* may be at increased risk of SJS/TEN from other antiepileptic drugs e.g. lamotrigine, phenytoin and phenobarbital. It is probably advisable to avoid other antiepileptic drugs also known to cause SJS/TEN, namely lamotrigine, phenytoin and phenobarbital[289]. The median duration of developing CBZ-induced SJS/TEN is 25 to 90 days[290]. Patients who have been taking CBZ for more than 3 months without developing skin reactions are at low risk of CBZ-induced ACDR. Therefore, use of CBZ can be continued without screening.

LIMITATION OF THE STUDY

Minor Maculopapular rashes and its association with HLA allele were not considered in the present study since these are not associated with *HLA-B*1502* allele.

Other HLA alleles like HLA-A*3101 reported to be associated with carbamazepine induced SJS/TEN in some races were not tested.

This is a single site study and the patient population consisted of South Indians, predominantly from Tamilnadu. Since India is a vast nation with diverse ethnicity and many genetic races, like Aryans (North India) and Dravidians (South India), sampling of samples from the rest of the nation will give a larger picture.

Genone Wide Association Studies were not performed. This may yield additional useful information.

FUTURE DIRECTIONS

In the future, newer genetic association of drug hypersensitivity will be explored, focusing on their pathogenesis. However, to translate these research findings into clinical application, several points require stringent persuasion:

1. Different immunological hypotheses of drug hypersensitivity need to be verified, unified and rejected by practical experiments.

2. The phenotypic diagnostic criteria of individual drug hypersensitivity involving the skin (MPR, SJS/TEN and DHS) must be clearly delineated and the genetic association of each of them separately explored.

3. To add statistical strength to these studies, a large number of cases should be included. Considering the rarity of certain drug hypersensitivities, this may necessitate multicenter and even multinational study.

4. Genetic susceptibility to individual drugs should be re-evaluated in the light of ethnic backgrounds.

5. Availability and cost-effectiveness of the screening test. Designing easier, quicker and cheaper tests is essential in this regard.

6. Experimental bias toward immune genes should be avoided. Genetic susceptibility may be conferred by “metabolic” genes and novel “nonimmune” genes[291]as well.

7. Newer method of detecting the allele

   In USA and Canada, many commercial laboratories now perform high-resolution genetic testing with sequence specific primer (SSP) using patient's

blood or buccal swab[292].These methods are costly and time-consuming (take around 1 week). Recently, a new (loop-mediated isothermal amplification or LAMP) method for detecting *HLA-B*1502* genotype has been shown to be accurate (100% concordant with SSP), yet inexpensive, rapid and simple[293]. They may be useful in future in the clinical practice.

In Summary, future research will probably lead to discovery of additional genetic predictors of susceptibility to adverse reactions to antiepileptic drugs. Identification of genetic markers should, in turn, allow unraveling of the molecular mechanisms underlying these reactions[294]. Ultimately, these advances should lead not only to improved personalization of therapy but also to development of safer drugs. Future research is likely to identify other genetic predictors of adverse reactions, and the mechanisms underlying these reactions. Ultimately, this should lead to improved personalization of therapy and development of safer drugs.

Department of Experimental Medicine

The TN Dr. MGR Medical University

Questionnaire:

S. No:

Name:

Age:

Sex: M F

Address:

Occupation:

Type of Seizure:

Past AEDs taken:

Family H/o Allergy to Carbamazepine or other AED:

Past H/o Allergy:

AED to be started:

General Examination:

Height: Weight:

General Examination:

Skin and Mucous Membrane examination:

Eyes:

Respiratory system examination:

Cardiovascular system examination:

Central Nervous System examination:

Abdominal Examination:

Lab Investigation ;

MRI/ CT Scan of Brain

EEG/Videotelemetry

CBC Blood Sugar: Urea: Creatinine: Urine RE:

Consent form for testing Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions

Date:

Persons full name.....

Date of birth.....

I hereby consent to give 7 ml. of blood sample to allow genetic testing to be performed on my blood. I understand that this testing will be only for the purpose of determining Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions.

The sample will not be used for any other purpose. The testing will be performed in the Department of Experimental medicine at the Tamilnadu Dr. MGR Medical University, 69, Anna Salai, Guindy, Chennai – 600 032.

The details of the testing have been explained by .....

Signed .....

Consent form for testing Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions, in a child less than 18 years old.

Consent by parent or guardian for genetic testing for the purpose of determining Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions, in a child less than 18 years old.

Date:

Childs full name.....

Date of birth.....

Parent or guardian's full name.....

I have given permission for 7 ml. of blood sample to be taken from my child to allow genetic testing to be performed. I understand that this testing will be only for the purpose of determining Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. The sample will not be used for any other purpose. The testing will be performed in the Department of Experimental medicine, at the Tamilnadu Dr. MGR Medical University, 69, Anna Salai, Guindy, Chennai – 600 032.

The details of the testing have been explained by .....

Signed .....

ஒப்புதல் படிவம்

மரபியல் உணர்திறன் கொண்ட **கார்பமசெபின்** மருந்து உட்கொள்வதால் தோலில் இந்த மருந்தினால் ஏற்படும் எதிர் விளைவுகள் பற்றிய ஆய்வு

பெயர் : ..... தேதி .....

பிறந்த தேதி : .....

என்னிடமிருந்து 7 மில்லி லிட்டர் இரத்தம் எடுத்து மரபியல் ஆய்வுகள் மேற்கொள்ள ஒப்புதல் அளிக்கிறேன். நான் மரபியல் உணர்திறன் கொண்ட மருந்து கார்பமசெபின் சாப்பிட்டு அதனால் தோலில் ஏற்படும் மருந்துக்கு எதிரான விளைவுகள் என்ன என்பதை கண்டறியும் சோதனைக்குதான் என்பதையும் அறிவேன்.

என்னிடம் பெறப்படும் இரத்தம் இந்த ஆய்வுக்கு மட்டுமே பயன்படுத்தப்படும் என்பதையும் அறிவேன். இந்த ஆய்வுகள் தமிழ்நாடு டாக்டர் எம்.ஜி.ஆர். பல்கலைக்கழகத்திலுள்ள சோதனை மருத்துவத் துறையில் மேற்கொள்ளப்படும் என்பதையும் அறிவேன்.

குறிப்பு: ஆய்வுகள் பற்றிய எல்லா விளக்கங்களும் என்னிடம் கூறப்பட்டது.

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