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M. I. Perelló[1], A. de Maria Castro[1], A. C. Nogueira Arraes[1], S. Conte[1], D. Lacerda Pedrazzi[1], G. A. Coelho Dias[1], L. Morette Hanhoerster[2], L. C. Porto[2], F. Chigres Kuschnir[1], E. Costa[1]

Severe cutaneous adverse drug reactions: diagnostic approach and genetic study in a Brazilian case series

[1]Allergy and Immunology Service, Rio de Janeiro State University, Rio de Janeiro, Brazil
[2]Histocompatibility and Cryopreservation Laboratory, Rio de Janeiro State University, Rio de Janeiro, Brazil

Key words

Drug allergy; genetics; immunologic tests; pharmacology; pharmacogenomics.

Corresponding author
Maria Inês Perelló
Allergy and Immunology Service
Rio de Janeiro State University
Marechal Rondon Avenue 381
Rio de Janeiro, Brazil
ORCID ID: 0000-0003-4824-8294
E-mail: mariaiplf@gmail.com

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Impact statement

The study is the first case series of SCAR with HLA analysis from Brazil. It had shown the occurence of the various described alleles of risk all over the world among our mixed population. The finding of allopurinol, carbamazepine and abacavir related HLA alleles combined with patch test positivity to anticonvulsants reinforced the culprit drug.

Summary

Background. *Severe cutaneous adverse reactions (SCAR) are potentially fatal reactions. Genetic predisposition is involved in their pathogenesis related to drugs and ethnicities, however in a mixed population these relationships are still unknown. The aim of this study was to describe phenotypes, suspect drugs and HLA-alleles related to SCAR, identified by a systematized approach in a Brazilian case series.* ***Methods****. Patients who were diagnosed with SCAR between March 2011 and July 2019 at our university hospital were included. European Network for Drug Allergy (ENDA) questionnaire was used to collect clinical and laboratory data and algorithms for assessment of drug causality were applied. Socio-demographic variables included age, gender and skin color/ethnicity. Drug patch tests (DPT) and HLA-A, -B, -DRB1 typing were carried out.* ***Results****. A total of 74 patients were included: 36 (48.64%) with SJS/TEN, 32 (43.24%) DRESS/DIHS, 3 (4.05%) AGEP, 2 (2.70%) overlap (DRESS/SJS and DRESS/AGEP) and 1 (1.35%) GBFDE. The median age was 31.5 years (IQR = 14-52.25), most were female (n = 44/59.46%) and brown (n = 38/51.35%). Anticonvulsants (n = 32/43.24%) were the largest group involved and antibiotics (n = 26/35.13%) were the second most common. Two patients with DRESS died during the acute phase. Positive DPT were shown only in anticonvulsant associated DRESS. HLA related to abacavir, allopurinol and carbamazepine were identified.* ***Conclusions.*** *A systematized approach allowed the phenotypic characterization of SCAR. The HLA-A*31:01, B*57:01 and B*58:01 alleles were identified, reinforcing the causality in SCAR by CBZ, ABC and ALLO in the Brazilian population.*

Introduction

Severe cutaneous adverse reactions (SCAR) are rare, delayed type, life-threatening hypersensivity drug reactions that include four phenotypes: Stevens Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), drug reaction with eosinophilia and systemic symptoms/drug induced hypersensitivity syndrome (DRESS/DIHS), acute generalized exanthematous pustulosis (AGEP) and more recently, generalized bullous fixed drug eruption (GBFDE) (1). Incidence of SCAR reaches 2% in hospitalized patients, with mortality rate between 5 and 50% (2). The development of sequelae with variable degrees of morbidity and incapacity has a direct impact on quality of life (3). Their high morbidity and mortality highlight the importance of rapid diagnosis and immediate withdrawal of the suspect drug (4, 5). Clinical diagnosis obeys the multinational registry of SCAR (RegiSCAR) and grading system criteria, while the etiology is presumed by chronological criteria, drug notoriety and application of causality algorithms (1). The drug patch tests (DPT) are safe and may be useful to ratify the etiology when positive and intradermal skin tests (IDT) can be done in selected cases. Oral provocation test (OPT) is contraindicated (6).

In clinical practice, HLA typing is a useful tool for screening genetically susceptible individuals for only a few drugs for which cost-effectiveness studies have already been delineated (7,8). It can also be used for differential diagnosis of bedside SCAR in patients using highly related medications. However, the relevance of such alleles in the Brazilian mixed population is unknown.

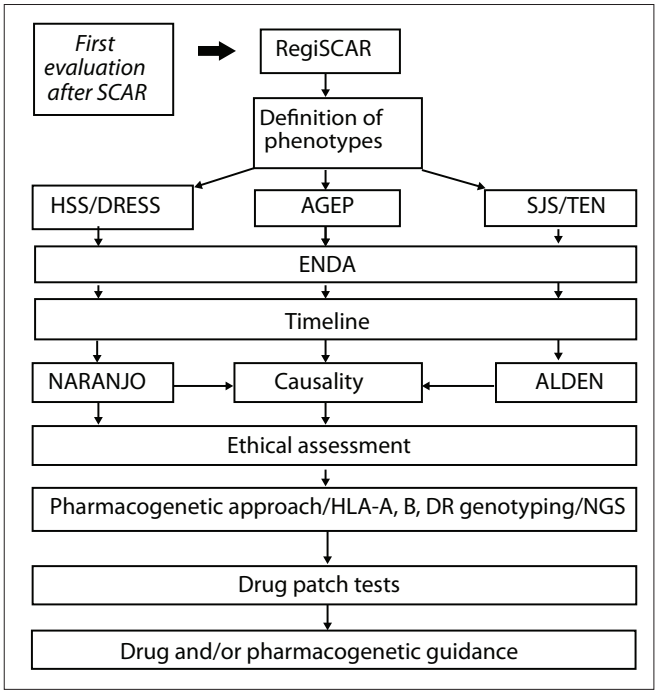
Methods

Cross-sectional, retrospective study, based on medical records of SCAR patients referred to the Adverse Drug Reaction (ADR) Ambulatory of Rio de Janeiro State University/Brazil (UERJ), between March 2011 and July 2019. In order to identify DRESS/DIHS, AGEP, SJS/TEN and GBFDE we used the RegiSCAR criteria (1). In patients diagnosed in other hospitals, a copy of the hospitalization record was requested to analyze clinical data. Demographic variables analyzed were gender, age and skin color/ethnicity, obtained by the self-attribution method (9).

Clinical approach in all cases included the application of ENDA questionnaire, adapted to Brazilian portuguese (10, 11) and a timeline to register clinical and laboratorial data. Patients suspected of DRESS/DIHS were submitted to the DRESS probability classification (12). The European Study of Severe Cutaneous Adverse Reactions (EuroSCAR) scoring system established a score for possible, probable and defined cases of AGEP (13). In SJS/NET, the degree of epidermal detachment was used to classify cases in three groups: 1-10% as SJS, 10-30% overlap SJS/TEN and > 30% as TEN (14). GBFDE was considered when well-demarcated dusky red or heavily pigmented patches with blisters and erosions involving the skin and mucosa were seen (1). Overlap forms of SCAR were suspect if the criteria for definite or probable diagnosis of, at least, 2 SCARs were filled (15). The timeline and causality algorithms were used to define culprit drugs. The clinical judgment of the attending physician was proposed based on the latency period, drug notoriety and characteristics of each phenotype (16). For all reactions the Naranjo Scale (17) was applied. For Stevens-Johnson syndrome/toxic epidermal necrolysis, the algorithm for assessment of drug causality in Stevens-Johnson syndrome/toxic epidermal necrolysis (ALDEN) was used (18). When the patient was consuming more than one suspected drug, it was considered the one that presented the highest score in algorithms and if the score was similar, both drugs were considered together.

In addition, we performed DPT, according to the European Society of Contact Dermatitis (ESCD) guidelines (19) and DNA–based typing (HLA-ABDR). A diagnostic algorithm was developed for the routine systematized approach of patients (**figure 1**). DPT were performed at least six months after complete healing of reaction and one month after discontinuation of systemic corticosteroid. Tests were conducted using diluted doses in white petrolatum up to 10% with the active ingredient and up to 30%

Figure 1 *- Diagnostic algorithm.*



with the commercialized form. Petrolatum was used as control. DPT were manufactured in authorized pharmacy. Finn Chambers on Scanpor tape (Smart Practice Phoenix, AZ, USA) were used and results were reported according to the International Contact Dermatitis Research Group (ICDRG) criteria on days 2 and 4. If the patch tests were negative on day 4, additional reading was carried out on day 7. Healthy volunteers without prior exposure to the suspected drug were tested as negative controls in all DPT and standard DPT were negative with the used vehicles. Patients were submitted to peripheral venous blood collection in our institution Clinical Pathology Service (Capsula). The material was used to HLA-A, -B, -DRB1 typing by PCR-RSSO (One Lambda, Canoga Park, CA/USA), which was performed in the majority of patients. In cases in which drug-related risk alleles were identified, AllTypeTM next generation sequencing (NGS 11_Loci Amplification Kit – One Lambda, Canoga Park, CA/USA) was performed. Pharmacogenetic advice was given for patients with well-defined risk alleles and their close relatives.

Descriptive analyzes were performed using Microsoft Office Excel 2010 (Microsoft Co., WA/USA). The dichotomous variables were described by numbers and percentages, and the continuous variables by median, interquartile range (IQR) and/or mean (average; minimum-maximum).

The study was approved by the local ethical committee. Signed informed consent of controls and patients were acquired.

Results

A total of 74 patients were included: 36 SJS/TEN (48.64%), 32 DRESS/DIHS (43.24%), 3 AGEP (4.05%), 2 (2.70%) overlap cases (DRESS/SJS and DRESS/AGEP) and 1 GBFDE (1.35%). The median age was 31.5 years (IQR = 14-52.25) and the majority were female (n = 44/59.46%). Regarding skin color, 38 (51.35%) were brown, 18 (24.32%) black and 16 (21.62%) white, and only two were Asian. All patients had a probable score on Naranjo scale. The median latency period was 15 days (IQR = 8-26.25) (**table I**). Aromatic anticonvulsants (ACA), including carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB) and lamotrigine (LMT) were involved in more than half of the reactions, followed by antibiotics with predominance of beta-lactams. Analgesics/NSAID, allopurinol, among others, were the remaining culprit drugs (**table II**).

Patients with SJS/TEN spectrum presented as SJS (n = 22/61.11%), overlap SJS/TEN (n = 8/22.22%) and TEN (n = 6/16.66%). DRESS/DIHS was the second most frequent SCAR. Kardaun's classification for DRESS scored as probable (n = 19/59.37%), definite (n = 12/37.5%) and possible case (n = 1/3.12%). All of them presented hepatic involvement and significant eosinophilia was the most common haematological finding. Four patients presented disease reactivation and were readmitted to the hospital after apparent control of the clinical picture. Three patients with AGEP were identified. All were female, had a probable EuroSCAR score for AGEP and good response to systemic corticosteroids. A 21-year-old male had GBFDE (1.35%). He presented 3 episodes of pharmacodermia after use of dipyrone, with reactivation of residual hyperpigmented lesions and appearance of new lesions on the face, trunk, oral mucosa and genitalia.

Overlap SCAR probability criteria were identified in a 50-year-old female with definitive diagnosis of allopurinol (ALLO) induced DRESS and probable AGEP according to RegiSCAR/EuroSCAR score. Another case of SCAR overlap was identified in a SJS/TEN 82 years old black woman using naproxen for 10 days. ALDEN scored very probable, but she also had fever, eosinophilia and liver involvement making DRESS a probable diagnosis by RegiSCAR.

Only 30 patients authorized the DPT: DRESS and DRESS/AGEP overlap (n = 14), AGEP (n = 2) and SJS/TEN (n = 14). Most of them were done with the commercialized form (n = 22/73.33%) and the remaining with pure substances provided by hospital pharmacy. Positive DPT were seen only in anticonvulsants-induced DRESS/DIHS: five with CBZ up to 10% dilution and 1 with 30% dilution of PB. The positivity of DPT for CBZ in DRESS/DIHS patients was 85.33% (**table III**). A CBZ-induced DRESS patient with negative DPT at 5% had rash and fever without organ involvement and the investigation was not continued. None of the other patients presented reactions related to tests.

The HLA-A, -B, -DRB1 typing was performed in 67 patients (90.54%). Risk alleles related to abacavir (ABC), ALLO and CBZ have been identified in eight (11.94%) out of sixty-seven patients who underwent genetic testing (**table IV**).

Discussion

In our series, as in other publications of SCAR, women were more affected (19, 20). Most of SCAR patients presented the SJS/TEN spectrum. The median age was lower than in other studies and reflects the characteristic of our Service, where we care adults and children. The predominance of brown skin color reflects the characteristic miscegenation of Brazilian population. Diagnosis of SCAR is eminently clinical and represents a challenge since it includes a variety of differential diagnoses. The use of criteria established by Euro/RegiSCAR is of paramount importance. The ENDA questionnaire and the timeline were valuable auxiliary tools in the SCAR patient approach (10). The visualization of the chronology of the clinical-laboratory data and use of the suspicious drugs allowed a better characterization of SCAR.

The skin rash biopsy may be helpful because it presents typical findings in the cases of SJS/TEN and AGEP, however it is non-specific in DRESS/DIHS (21). In this study, it was useful in an overlapping phenotype (DRESS/AGEP) in a patient with DRESS criteria. Although overlapping of different skin lesion patterns is not uncommon among phenotypes, true cases of SCAR overlap seem to be rare (15).

The high index of suspicion for drug involvement is crucial because the immediate suspension of suspected and non-essential drugs is a determining factor for prognosis (22, 23). A long latency period delays the suspension of the culprit drug and causality can be falsely attributed to drugs used to treat reaction prodromes, on the other hand, drugs used for long time should not be forgotten especially if used irregularly or intermittently (16, 24, 25). In this study, one patient with ALLO associated SJS/TEN and another PB related DRESS patient presented SCAR after one year of irregular drug use.

Causality algorithms were applied in the approach of all patients. Naranjo's criteria (17) cover general aspects of drug reactions, and, in this study, it often did not reach high or low imputability scores, while ALDEN (18), developed for epidermal necrolysis, allowed us to identify and/or exclude some medications as an etiological factor in most patients.

As in the majority of studies, anticonvulsants, antibiotics, NSAID and ALLO were the main classes of drugs involved (16, 20, 26, 27). The group of ACA was involved in most of our cases of DRESS/DIHS. Among ACA, CBZ followed by PHT was the major involved in DRESS. In SJS/NET group, anticonvulsants were also more involved in the etiology of the disease, but antimicrobials (especially beta-lactams) represented the second largest group. The significant higher risk associated with concomitant use of valproic acid and lamotrigine, due to increased half-life of lamotrigine elimination, was identified in an overlap SJS/NET case (28).

Table I *- Demographic, clinical, and laboratory characteristics of patients.*

Characteristic	SJS/TEN (n = 36)	DRESS (n = 32)	AGEP (n = 3)	GBFDE (n = 1)	Overlap (n = 2)
Age (years)				21	
median (IQR)	29.5(13.75-46)	31 (12.75-53.75)	-	-	-
average (min-max)	-	-	42 (16-55)	-	66 (50-82)
Skin color/ethnicity n (%)					
Brown	16 (44.44)	19 (59.37)	2 (66.66)	1 (100)	-
White	8 (22.22)	8 (28.12)	1 (33.34)	-	-
Black	11 (30.55)	3 (9.37)	-	-	2 (100)
Yellow	1 (2.77)	1 (3.12)	-	-	-
Sex n (%)					
Female	21 (58.33)	14 (43.75)	3 (100)	-	2 (100)
Male	15 (41.66)	18 (56.25)	-	1 (100)	-
Risk fator n (%)					
Autoimmunity	4 (11.11)	3 (9.37)	1 (33.33)	-	1 (50)
HIV serology	2 (5.55)	2 (6.25)	-	-	-
HLA risk allele presence	4 (11.11)	3 (9.37)	-	-	1 (50)
Latency period (days)	-			2	
median (IQR)	15 (6.75-22.5)	16.5 (15-28.5)		-	
average (min-max)	-		12.5 (2-15)		15 (10-20)
Clinical findings n (%)					
Skin involvement	36 (100)	32 (100)	3 (100)	1 (100)	2 (100)
Mucosal involvement	36 (100)	6 (18.75)	-	1(100)	1 (50)
Lymphadenopathy	3 (8.30)	19 (59.37)	1 (33.33)	-	1 (50)
Fever ≥ 38 °C	33 (91.66)	31 (96.87)	2 (67.70)	1 (100)	2 (100)
Hematologic findings, n (%)					
Eosinophilia	-	25 (78.12)	-	-	2 (100)
Neutrophilia (> 7,000)	-	-	3 (100)	-	1 (50)
Involved organs n (%)					
Liver	6 (16.66)	31 (96.87)	-	-	2 (100)
Gastrointestinal	1 (2.77)	5 (15.62)	-	-	-
Kidney	6 (16.66)	6 (18.75)	-	-	-
Lung	5 (13.88)	8 (32.0)	1 (33.33)	-	1 (50)
Heart	5 (13.88)	6 (18.75)	-	-	-
SNC	2 (5.55)	3 (9.37)	-	-	-
Treatment n (%)					
Systemic corticosteroid	24 (66.66)	27 (84.37)	3 (100.0)	1 (100)	2 (100)
Systemic corticosteroid + IGIV	6 (16.66)	3 (9.37)	-	-	-
IGIV	4 (11.11)	-	-	-	-
Supportive care only	2 (5.55)	-	-	-	-

→

Characteristic	SJS/TEN (n = 36)	DRESS (n = 32)	AGEP (n = 3)	GBFDE (n = 1)	Overlap (n = 2)
Treatment time (days)			-	18	-
median (IQR)	24 (5-52.5)	120 (30 -180)	-	-	-
average (min-max)	-	-	36 (4-90)	-	55 (20-90)
Inpatient stay (days)	-	-	-	10	-
median (IQR)	19.5 (13-29.5)	15 (12-30)	-	-	-
average (min-max)	-		2.5 (1-4)		16.5 (12-21)
Death n (%)	-	2 (6.25)	-	-	-

SJS: Stevens-Johnson Syndrome; TEN: Toxic Epidermal Necrolysis; DRESS: Drug Reaction with Eosinophilia and Systemic Symptoms; AGEP: Acute Generalized Exanthematous Pustulosis; GBFDE: Generalized Bullous Fixed Drug Eruption; IQR: interquartile range; HLA: human leukocyte antigen; IGIV: intravenous immunoglobulin.

Table II - *Etiology of SCAR.*

Drugs	DRESS/DIHS n = 32 (%)	SJS/TEN n = 36 (%)	AGEP n = 3 (%)	GBFDE n = 1 (%)	Overlap n = 2 (%)	Total n = 74 (%)
Aromatic anticonvulsants	20 (62.5)	23 (63.88)	0 (0.00)	0 (0.00)	0 (0.00)	43 (58.11)
Carbamazepine	8	5	0	0	0	13 (17.57)
Lamotrigine	1	5	0	0	0	6 (8.10)
Oxcarbazepine	1	0	0	0	0	1 (1.35)
Phenobarbital	2	7	0	0	0	9 (12.16)
Phenytoin	8	6	0	0	0	14 (18.92)
Antibiotics	5 (15.62)	22 (61.11)	1 (33.33)	0 (0.00)	0 (0.00)	28 (37.83)
Azithromycin	0	1	0	0	0	1 (1.35)
Amoxicilin	0	3	1	0	0	4 (5.40)
Ampicilin	0	1	0	0	0	1 (1.35)
Benzathin penicilina	0	1	0	0	0	1 (1.35)
Meropenem	0	3	0	0	0	3 (4.05)
Cefaclor	0	1	0	0	0	1 (1.35)
Cefalexine	1	0	0	0	0	1 (1.35)
Cefepime	0	1	0	0	0	1 (1.35)
Ceftriaxone	1	1	0	0	0	2 (2.70)
Chloramphenicol	0	1	0	0	0	1 (1.35)
Gentamicina	0	1	0	0	0	1(1.35)
Quinolone	0	2	0	0	0	2 (2.70)
Sulfamethoxazole	2	3	0	0	0	5 (6.75)
Tetracycline	0	1	0	0	0	1 (1.35)
Vancomycin	1	2	0	0	0	3 (4.05)
Antiviral drugs	1 (3.12)	2 (5.55)	0 (0.00)	0 (0.00)	0 (0.0)	3 (4.05)
Abacavir	1	0	0	0	0	1 (1.35)
Nevirapine	0	2	0	0	0	2 (2.07)

Drugs	DRESS/DIHS n = 32 (%)	SJS/TEN n = 36 (%)	AGEP n = 3 (%)	GBFDE n = 1 (%)	Overlap n = 2 (%)	Total n = 74 (%)
Allopurinol	2 (6.25)	7 (19.44)	1 (33.33)	0 (0.00)	1(50.0)	11 (14.86)
Analgesic/anti-inflammatory	5 (15.62)	10 (27.77)	2 (66.6)	1 (100.0)	2 (100.0)	18 (24.32)
Diclofenac	0	3	1	0	0	4 (5.40)
Ibuprofen	0	3	0	0	0	3 (4.05)
Naproxen	0	1	0	0	1	2 (2.70)
Nimesulide	0	2	0	0	0	2 (2.70)
Tenoxican	0	1	0	0	0	1 (1.35)
Dipyrone	4	3	0	1	1	9 (12.16)
Acetominophen	1	0	1	0	0	2 (2.70)
Non-antimicrobial sulfonamides	5 (15.62)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	5 (6.75)
Dapsone	2	0	0	0	0	2 (3.07)
Sulfasalazine	2	0	0	0	0	2 (3.07)
Sulfadiazine	1	0	0	0	0	1 (1.35)
Dexamethasone	0	0	1	0	0	1 (1.35)

SJS: Stevens-Johnson Syndrome; TEN: Toxic Epidermal Necrolysis; DRESS: Drug Reaction with Eosinophilia and Systemic Symptoms; AGEP: Acute Generalized Exanthematous Pustulosis; GBFDE: Generalized Bullous Fixed Drug Eruption.

In this series, asymptomatic hyperuricemia was the main motivation for the treatment with allopurinol (80% of patients) in ALLO group. High doses of ALLO associated use of diuretics and renal failure increase SCAR risk besides genetic factors (29). As not all patients with hyperuricemia develop gout or renal disease, ALLO therapy for asymptomatic hyperuricemia should be discouraged (30). The 2012 American College of Rheumatology Guidelines for Management of Gout recommend HLA genotyping in selected subpopulations (individuals of Korean descent with stage 3 or worse chronic kidney disease and those of Han-Chinese or Thai descent) prior starting ALLO treatment (31).

In our DRESS/DIHS cases, clinical-laboratory findings were similar to those previously described in literature (32) and corticosteroids were the mainstay of treatment in all patients. IVIG was also used in 2 of them with good clinical evolution (33). Corticosteroids were used in the majority of patients in the SJS/TEN group. Although it is classically related to higher mortality rates (34), there were no deaths in this group, maybe due to the greater frequency of patients with cutaneous detachment < 10%, since the main cause of death is septic shock, which is directly related to the extent of detachment (35). Patients with AGEP, GBFDE and overlap DRESS/AGEP and DRESS/SJS also received corticotherapy, without complications.

Two patients died of DRESS. Although the most common cause of death n DRESS is liver failure (22) all patients in our series recovered liver function, except for a CBZ-DRESS patient who died of multiple organ failure. A vancomycin-DRESS patient died with suspected diagnosis of acute necrotizing eosinophilic myocarditis. He developed cardiogenic shock in the first week of disease. Cardiac involvement is a rare and potentially fatal complication that can range from up to 4 months or evolve to long-term heart failure (36). Milder forms (hypersensitivity myocarditis) are probably underdiagnosed due to self-limited nature, nonspecific symptoms and should always be tracked for their potential severity (37). Although endomyocardial biopsy is considered a gold standard, in view of the dramatic evolution and critical clinical status of our patient, the diagnosis was made only in clinical grounds.

DRESS has a prolonged course and clinical reactivations can occur during the acute phase of reaction related to sequential latent viral reactivations of *Herpesviridae* family (HHV6, HHV7, EBV and CMV), flare-up reaction after introduction of new drugs or immune reconstitution syndrome by decrease of corticotherapy (38-41). Although we cannot rule out the possibility of viral reactivation in the presented cases, since it was not possible to confirm them by serology or polymerase chain reaction, it is possible that tapering of corticotherapy was the main cause of clinical reactivations in some of them.

Considering all types of SCAR, AGEP presents a lower chance of complications since it generally does not evolve with systemic involvement and tends to resolve faster without complications. The observed mean of latency period was slightly higher than observed in other series. Although antibiotics are classically con-

Table III *- Drug Patch Test (SCAR).*

Case	Drug	SCAR	C (%)	T (years)	Results
1	**CBZ**	**DRESS**	**5**	**< 1**	**Positive**
2	**CBZ**	**DRESS**	**10**	**1-2**	**Positive**
3	**CBZ**	**DRESS**	**10**	**< 1**	**Positive**
4	**CBZ**	**DRESS**	**10**	**1-2**	**Positive**
5	**CBZ**	**DRESS**	**5**	**< 1**	**Positive**
6	**PB**	**DRESS**	**30**	**< 1**	**Positive**
7	CBZ	DRESS	5	1-2	Negative
8	PHT	DRESS	30	4-5	Negative
9	PHT	DRESS	10	3-4	Negative
10	SZ	DRESS	10**	3-4	Negative
11	DAP	DRESS	10**	1-2	Negative
12	CEF	DRESS	30	2-3	Negative
13	AC/DIP	DRESS	10**	5-6	Negative
14	ALLO	DRESS/AGEP	30	1-2	Negative
15	Multiple*	SJS/TEN	10	1-2	Negative
16	LMT	SJS/TEN	30	1-2	Negative
17	LMT	SJS/TEN	10**	1-2	Negative
18	PB	SJS/TEN	30	1-2	Negative
19	PB	SJS/TEN	30	2	Negative
20	PB	SJS/TEN	30	3-4	Negative
21	CBZ	SJS/TEN	30	2-3	Negative
22	CBZ	SJS/TEN	30	1-2	Negative
23	CBZ	SJS/TEN	30	3-4	Negative
24	CBZ	SJS/TEN	30	6-7	Negative
25	LEVO	SJS/TEN	10**	< 1	Negative
26	AZI/DIP	SJS/TEN	30/10**	< 1	Negatve
27	ALLO	SJS/TEN	10**	5-6	Negative
28	ALLO	SJS/TEN	10**	1-2	Negative
29	AMX/CT	AGEP	30/1	< 1	Negative
30	NSAID	AGEP	10**	< 1	Negative

AC: acetaminophen; ALLO: allopurinol; CBZ: carbamazepine; C: concentration; CEF: ceftriaxone AP: dapsone; DIP: dipyrone; PB: phenobarbital; PHT: phenytoin; SZ: sulfasalazine; Multiple*: (SMX:S ulfamethoxazole; NVP: nevirapine; LMT: lamotrigine; PHT: phenytoin; TNX: tenoxican); 10**: pure substance a 10%; SJS: Stevens-Johnson syndrome; TEN: toxic epidermal necrolysis; T: time; DRESS: drug reaction with eosinophilia and systemic symptoms; AGEP: acute generalized exanthematous pustulosis: GBFD: generalized bullous fixed drug eruption.

sidered the most common cause, this was the suspected cause in only one of our cases, which acute localized exanthematous pustulosis (ALEP) (42, 43).

The patient with a diagnosis of GBFDE initially had a diagnosis of SJS/TEN. However, the retrospective evaluation of the history of 3 episodes with additional and progressive involvement of other skin areas after repeated expositions suggested a reassessment of the diagnostic hypothesis. GBFDE is a common and frequent mimicker of SJS/TEN cases (34). Some clues such as short latency, less constitutional symptoms and less mucosal involvement help the differential diagnosis (35).

The pharmacogenetic approach in SCAR patients is based on scientific evidence linking genetic factors such as HLA alleles and/or polymorphisms in drug-metabolizing genes related to increased susceptibility to SCAR by specific drugs and phenotypes (44, 45). Based on cost-effectiveness studies, international regulatory agencies such as Food and Drug Administration (FDA) and the European Medicine Agency (EMA) included specific drug-label information on the utility of screening patients who are candidates for use of ABC (B*57:01) and CBZ (B*15:02), regardless of ethnicity in the former and in patients with southeast Asian origin in the latter (46). Although in some groups as Asian and European people, respectively for ALLO (B*58:01) and CBZ (A*31:01), studies of cost-effectiveness have already been delineated, there is no recommendation from that agencies for routine screening (47, 48). However, in the presence of at least one copy of the HLA risk allele related to ABC, ALLO or CBZ, their use should be avoided (49-51). Risk alleles such as HLA-A*31:01 (CBZ), B*57:01 (ABC) and B*58:01 (ALLO), were identified in this study and helped to reinforce causality in eight SCAR patients. According to updated data from the Brazilian Bone Marrow Donor Network (Portuguese acronym: REDOME), the estimated frequency of alleles in the Brazilian population shows variation between the different skin colour and skin race group from 2.1 to 4%, from 2.7 to 2.9%, and 4.5 to 5.3% respectively for the B*58, B*57 and A*31 alleles (52). In our study, the self-attributed skin color referred to ancestry up to great-grand parents was investigated. Although skin color is determined by several factors and its relation to ancestry is inconsistent, this has been the main form of stratification used in scientific studies (53, 54). The genetic diversity of the Brazilian population determined by centuries of interbreeding of races was confirmed in this group by the majority of the self-attributed brown color. It is emphasized that the distance of the historical events that characterized the miscegenation of the Brazilian population compromised the knowledge of the ancestral roots in most cases.

To evaluate the risk and benefit for the use of drugs highly implicated in SCAR, the pharmacogenetic evaluation of ABC, CBZ and ALLO in selected cases for both diagnostic evaluation and case prevention, besides other non-genetic risk factors like drug interactions and renal failure, is useful. The value of the routine genetic test and the possibility of new risk haplotypes are still unknown and there is a lack of cost-effectiveness studies in Brazilian population.

In vitro tests to confirm the culprit drug is limited by lack of validation. Lymphocyte transformation test (LTT), enzyme linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISPOT) assay are available only at some research centers. Recently combined cytokine and cytotoxicity assays

Table IV *- HLA-A, -B and -DRB1 alleles in DRESS, SJS/TEN and DRESS/AGEP samples with HLA-A*31, -B*57 or -B*58.*

DRUG	SCAR	HLA-A*	HLA-B*	HLA-DRB1*
CBZ	DRESS	24:02:01:01	40:02:01:01	04:05:01:01
		31:01:02:01	51:01:01:01	04:05:01:03
CBZ	DRESS	**31:01:02:01**	39:03:01:01	13:02:01:01
		34:02:01	81:01:01	14:02:01:02
ABC	DRESS	01:01:01:01	38:01:01:01	04:02:01
		26:01:01:01	**57:01:01:01**	13:01:01:02
ALLO	SJS/TEN	02:01:01	**58:01:01**	11:02:01
		32:01:01	40:02:01	16:02:01
ALLO	SJS/TEN	23:17:01:01	42:02:01:02	07:01:01:01
		30:01:01:01	**58:01:01:01**	12:01:01:01
ALLO	SJS/TEN	03:01:01:01	35:01:01:05	03:01:01:01
		30:02:01:01	**58:01:01:01**	11:04:01
ALLO	DRESS/AGEP	02:02:01:01	53:01:01	13:02:01:03
		33:03:01:01	**58:01:01:03**	15:03:01:02
ALLO	SJS/TEN	01:01:01:01	35:01:01:05	01:01:01
		03:01:01:01	**58:01:01:01**	07:01:01:01

ALLO: Allopurinol; ABC: Abacavir; CBZ: Carbamazepine; AGEP: Acute Generalized Exanthematous Pustulosis; DRESS: Drug Reaction with Eosinophilia and Systemic Symptoms; SJS: Stevens-Johnson Syndrome; TEN: Toxic Epidermal Necrolysis. HLA Typing were performed with massive parallel sequencing.

(Cyto-LTT) has improved the sensitivity for identification of the drug in the resolution phase of SJS/TEN. They were not carried out in this study (55).

Skin tests also need validation and sensitivity varies with the drug, phenotype and the time since the reaction. Faced with the lack of knowledge about how long it can remain positive, it is suggested to perform them during the first year after resolution of SCAR to avoid false negative results (56, 57).

The value of DPT in DRESS has been described with sensitivity ranging from 32-70% (6, 58, 59). Regarding CBZ, the DPT sensitivity in patients with DRESS/DIHS ranged from 60 to 84.6% (59,6). It was confirmed in our study since five out of six CBZ-DRESS had positive DPT, besides one PB-DRESS. Considering all DPT applied in DRESS/DIHS in our cases, the sensitivity of the method was 42.85%, with sensitivity of 83.33% in DRESS/DIHS by CBZ, even in low concentrations (1-10%). The low sensitivity of DPT in SJS/TEN has been verified in most of published series, varying from 9-23% (6, 60). In present study, none of SJS/TEN patients, including CBZ-SJS/TEN cases had positive DPT, which confirms the low sensitivity of the method in this phenotype.

DPT are safe in the SCAR investigation, however test-induced reactivities are described especially for CBZ and acyclovir (56). In our series, one CBZ-induced DRESS patient presented rash and fever without organ involvement 24 hour after a negative 96 hour-reading DPT. Relapses in AGEP and DRESS have been described even though their DPT results were negative (6, 56). None of the other patients presented reactions related to DPT application. Given the impossibility of validation by a standard test, negative skin tests do not exclude the involvement of the suspected drug (61).

Restriction of involved drug groups were based on analysis of their culpability started at least eight weeks prior to the index reaction day. After application of causality algorithms, the drugs that showed a possible or defined score were defined as suspect, whether or not they were reinforced by DPT and/or genetic test. There are limitations in our study, such as the absence of a systematic biopsy in all patients, which is not necessary for clinical purposes, as well the non-accomplishment of *in vitro* methods. The use of a medium resolution method for HLA genotyping did not allow us the identification of four digits HLA alleles in all patients. It is known that patients tolerant to a particular drug may carry risk alleles for that same drug. However, the identification of alleles related to CBZ and ABC in patients with DRESS and ALLO in patients with SJS/TEN strongly reinforced causality and contraindicated their later use and additionally allowed pharmacogenetic guidance for patients and their relatives.

To our knowledge this is the first published case series of SCAR with documented HLA alleles and DPT in Brazil. It can be useful to other researchers and for clinical physicians working with SCAR patients in Brazil and in other middle-income countries. Moreover, multicentric studies in mixed Brazilian population may be helpful in the search for new related risk alleles. The high genetic admixture makes up a mosaic of genome ancestry with unique proportions in each Brazilian making the cost-effectiveness for genetic screening difficult. However, the possibility of

the existence of new haplotypes in an admixed population and their correlations with SCAR are still unknown and must be considered in the Brazilian population.

Conclusions

In conclusion, the systematic approach using tools for phenotyping and causality identification of SCAR are essential for the study of correlation with *in vitro* and *in vivo* methods including HLA typing and DPT. Despite the lack of validation, DPT were safe and effective to establish the diagnosis in CBZ-induced DRESS. Through HLA genotyping, the HLA-A*31:01, B*57:01 and B*58:01 alleles were identified, reinforcing its implication in SCAR induced by CBZ, ABC and ALLO, among the Brazilian population.

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Conflict of interests

The authors declare that they have no conflict of interests.

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