See related commentary on pg 2047

Cyclosporine Use in Epidermal Necrolysis Is Associated with an Important Mortality Reduction: Evidence from Three Different Approaches

Carlos González-Herrada[1], Sara Rodríguez-Martín[2], Lucía Cachafeiro[3], Victoria Lerma[2], Olga González[1], José A. Lorente[4,5,6], Antonio Rodríguez-Miguel[2], Jessica González-Ramos[7], Gaston Roustan[8], Elena Ramírez[9], Teresa Bellón[10] and Francisco J. de Abajo[2], for the PIELenRed Therapeutic Management Working Group*

Several immunomodulatory agents are used in the treatment of epidermal necrolysis, but evidence of their efficacy is limited. The Autonomous Community of Madrid has two reference burn units to which all patients with epidermal necrolysis are referred. One burn unit has mostly used cyclosporine (CsA), and the other has used non-CsA therapies (mainly high-dose intravenous immunoglobulin). The allocation of patients to one or the other burn unit was mainly based on proximity, resembling a random assignment. Thus, we took advantage of this "natural experiment" to estimate the mortality risk ratio (MRR) of CsA (n = 26) compared with non-CsA (n = 16) treatment using hospital as an instrumental variable over the period from 2001 to 2015. We also computed the observed versus expected (O/E) MRR in a case series of 49 CsA-treated patients (including 23 patients from other regions treated in Madrid), and using the Score for Toxic Epidermal Necrolysis (i.e., SCORTEN) scale to estimate the expected values. The instrumental variable-based MRR of CsA versus non-CsA was 0.09 (95% confidence interval = 0.00–0.49). The O/E analysis also showed a reduction in mortality risk (MRR_{OE} = 0.42; 95% confidence interval = 0.14–0.99). We identified five other case series of CsA-treated patients providing MRR_{OE} and meta-analyzed their results. The pooled MRR_{OE} (including from this study) was 0.41 (95% confidence interval = 0.21–0.80). All three approaches consistently show that CsA reduces the mortality in epidermal necrolysis patients.

Journal of Investigative Dermatology (2017) **137,** 2092–2100; doi:10.1016/j.jid.2017.05.022

INTRODUCTION

Epidermal necrolysis (EN), the unified denomination proposed recently for toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS), and SJS-TEN overlap, is a rare disease frequently induced by drugs (Heng et al., 2015) that has a high mortality rate (Sekula et al., 2013). The prompt withdrawal of the offending medication (García-Doval et al., 2000) and the application of supportive measures are the main therapeutic cornerstones (Palmieri et al., 2002). Various immunomodulating agents have been used to treat it (Roujeau and Bastuji-Garin, 2011;Valeyrie-Allanore et al., 2010), but the evidence of their efficacy is still limited, mostly because the studies performed thus far have been based on small case series, none of which reached statistical significance. A formal randomized clinical trial comparing these treatments would theoretically be the best approach, but it has not been implemented because of the obvious difficulties in recruiting patients.

[1]*Dermatology Department, University Hospital of Getafe, Getafe, Madrid, Spain;* [2]*Clinical Pharmacology Unit, Príncipe de Asturias University Hospital, Department of Biomedical Sciences, University of Alcalá, Institute for Health Research IRYCIS, Alcalá de Henares, Madrid, Spain;* [3]*Burn Unit, Intensive Care Unit, La Paz University Hospital, Madrid, Spain;* [4]*Burn Unit, Intensive Care Unit, University Hospital of Getafe, Getafe, Madrid, Spain;* [5]*Respiratory Diseases Networking Biomedical Research Centre (CibeRes), Madrid, Spain;* [6]*European University of Madrid, Madrid, Spain;* [7]*Dermatology Department, La Paz University Hospital, Madrid, Spain;* [8]*Dermatology Department, Puerta de Hierro University Hospital, Majadahonda, Madrid, Spain;* [9]*Clinical Pharmacology Department, La Paz University Hospital, Madrid, Spain; and* [10]*Drug Hypersensitivity Laboratory, Institute for Health Research IdiPAZ, La Paz University Hospital, Madrid, Spain*

**The PIELenRed Therapeutic Management Working Group includes the following members: Teresa Bellón, Rosario Cabañas, Lucía Cachafeiro, Abelardo García de Lorenzo, Jessica González-Ramos, Olga Hernández, Pedro Herranz, Elena Ramírez, Elena Ruíz Bravo, Yolanda Alonso, Jose Antonio Aramburu, Natalia Cámara, Olga González, Carlos González-Herrada, Olga Laosa, José A. Lorente, Amelia Moscoso, Concepción Payares, Gastón Roustan, Francisco J. de Abajo, Alicia Quesada, Victoria Lerma and Sara Rodríguez-Martín*

Correspondence: Francisco J. de Abajo, Clinical Pharmacology Unit, Príncipe de Asturias University Hospital, Carretera Alcalá-Meco s/n, 28805 Alcalá de Henares, Madrid, Spain. E-mail: francisco.abajo@uah.es

Abbreviations: AT, as treated; BSA, body surface are; BU, burn unit; CI, confidence interval; CsA, cyclosporine; EN, epidermal necrolysis; ITT, intention to treat; IV, instrumental variable; IVIg, intravenous immunoglobulin; LPUH, La Paz University Hospital; MRR, mortality risk ratio; O/E, observed versus expected; SCORTEN, Score for Toxic Epidermal Necrolysis; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis; UHG, University Hospital of Getafe

Received 31 January 2017; revised 27 April 2017; accepted 22 May 2017; accepted manuscript published online 17 June 2017

When the efficacy of two treatments is to be compared in a nonexperimental setting, the possibility exists that, despite the use of appropriate statistical techniques for adjustment, the results are biased because of unmeasured, or not perfectly measured, confounding factors. Moreover, in studies with small sample sizes and/or scarce events, adjustment methods can be inefficient. A method proposed to tackle these problems is the use of instrumental variables (IVs) (Greenland, 2000). It is accepted that an analysis based on a valid IV (as randomization itself) can help balance the confounding factors (measured and unmeasured) between comparison groups and enhance the internal validity of the study (Rassen et al., 2009). The main problem, however, is finding an appropriate IV.

The Autonomous Community of Madrid (hereafter referred to as *Madrid*), a region of approximately 6 million inhabitants, has two burn units (BUs) located in the north (La Paz University Hospital [LPUH]) and south (University Hospital of Getafe [UHG]), respectively, which treat patients with EN, mostly from the region but also from other parts of Spain lacking these facilities. The allocation of patients, particularly those from Madrid, to one or another BU is a random-like process based on proximity and is not linked to any clinical aspect of the disease such as severity. UHG has been using cyclosporine (CsA) (also spelled as "ciclosporin") in EN patients for more than 20 years and was one of the pioneering centers in the world to use this treatment (Arévalo et al., 2000), whereas LPUH has mostly used non-CsA treatments (mainly intravenous immunoglobulin [IVIg]). This situation can be regarded as a "natural experiment," in which hospital assignment is acting as an IV. Thus, we exploited such a situation to perform an IV analysis over the study period from 2001 to 2015 with the aim to assess the effect of CsA on mortality compared with non-CsA therapies. We also evaluated the effect of CsA on mortality through two other approaches: (i) a traditional observed versus expected (O/E) mortality analysis using Score for Toxic Epidermal Necrolysis (SCORTEN) to estimate the expected deaths (Bastuji-Garin et al., 2000) and (ii) a systematic review with a meta-analysis of all published case series that used an O/E approach.

RESULTS

Out of 91 potential patients identified, 71 were validated patients with a diagnosis of EN (see case validation results in Supplementary Table S1 online) who were admitted to either of the two BUs (45 to UHG and 26 to LPUH). Overall, 42 patients were from Madrid and 29 from other regions (Figure 1).

The "natural experiment"

This study was restricted to 42 patients diagnosed with EN and living in Madrid (UHG = 23 and LPUH = 19). All patients in UHG except one (96%) were treated with CsA, and four patients in LPUH (21%) were treated with CsA. (Two of them initially received IVIg.) With the exception of sex, no statistically significant differences in baseline characteristics were observed either between hospitals (see Supplementary Table S2 online) or between treatments (CsA vs. non-CsA) (Table 1), including prognostic factors such as age, days from start of symptoms to admission, percentage of mucosal involvement, total body surface area involved, clinical entity (SJS, TEN, overlap) or pre-existing diseases. The average SCORTEN 24-hour values were comparable in both hospitals (UHG = 2.4 vs. LPUH = 2.5, $P = 0.70$) and in both treatment groups (CsA = 2.4 vs. non-CsA = 2.5; $P = 0.82$) (Table 1).

Only one patient died in UHG (out of n = 23, 4.3%) and six in LPUH (out of n = 19, 31.6%) yielding a mortality risk ratio (MRR) of 0.14 (95% confidence interval [CI] = 0.02−0.94). By analogy with clinical trials, this approach can be called "intention to treat" (ITT) analysis: patients were analyzed in the group they were assigned to. However, the ITT analysis underestimates the treatment effect when there is incomplete adherence to treatment. To adjust for this, we used an IV analysis, giving rise to an MRR_{IV} of 0.09 (95% CI = 0.00−0.49) (Table 2).

Grouping patients by the actual treatment received (the conventional "as treated" (AT) approach) we found that two patients died in the CsA group (out of n = 26; 7.7%) and that five died in the non-CsA group (out of n = 16; 31.3%), yielding a crude MRR_{AT} of 0.25 (95% CI = 0.04−1.06). After adjusting for SCORTEN and total body surface area (BSA) involved, we obtained an MRR_{AT} of 0.13 (95% CI = 0.01−0.93) (Table 2). When we compared CsA versus IVIg, the adjusted MRR_{AT} was 0.09 (95% CI = 0.01−0.83). The inclusion of all 71 patients (from Madrid and other regions) also yielded an important mortality reduction in the IV analysis (MRR_{IV} = 0.19, 95% CI = 0.03−0.72) and in the ITT and the AT analyses (see Supplementary Table S3 online).

The O/E approach

The O/E approach was performed in all 71 patients with a validated diagnosis of EN. Forty-nine patients were treated with CsA; five of them died (10.2%), although 11.8 (24.1%) deaths were expected according to SCORTEN. Twenty-two patients were treated with non-CsA therapies, seven of whom died (31.8%), although 6.4 deaths (29.1%) were expected. The resulting MRR_{OE} was 0.42 (95% CI = 0.14−0.99) for CsA and 1.09 (95% CI = 0.44−2.25) for non-CsA (see Table 3 for specific treatment results). When we used the auxiliary score instead of SCORTEN, the results were almost identical (Table 3). Also, when the analysis was restricted to patients with complete data for SCORTEN (n = 22 for CsA and n = 17 for non-CsA groups), we obtained an MRR_{OE} of 0.32 (95% = 0.04−1.15) for CsA and an MRR_{OE} of 1.06 (95% CI = 0.35−2.48) for non-CsA. When the O/E analysis was restricted to patients from Madrid, the point estimate did not materially change (CsA MRR_{OE} = 0.31, 95% CI = 0.04−1.11; non-CsA MRR_{OE} = 1.09, 95% CI = 0.35−2.54). Twenty-eight patients treated with CsA (57.1%) received steroids before admission, but a similar effect of CsA was observed in both patients receiving steroids (MRR_{OE} = 0.37, 95% CI = 0.04−1.34) and patients not receiving steroids (MRR_{OE} = 0.47, 95% CI = 0.10−1.38). Deaths were evenly distributed over time (see Supplementary Figure S1 online), and no statistically significant interaction with time (a period effect) was detected.

Secondary efficacy variables

We collected accurate data from only 40 patients with nonfatal EN who were treated with CsA (n = 38 from UHG

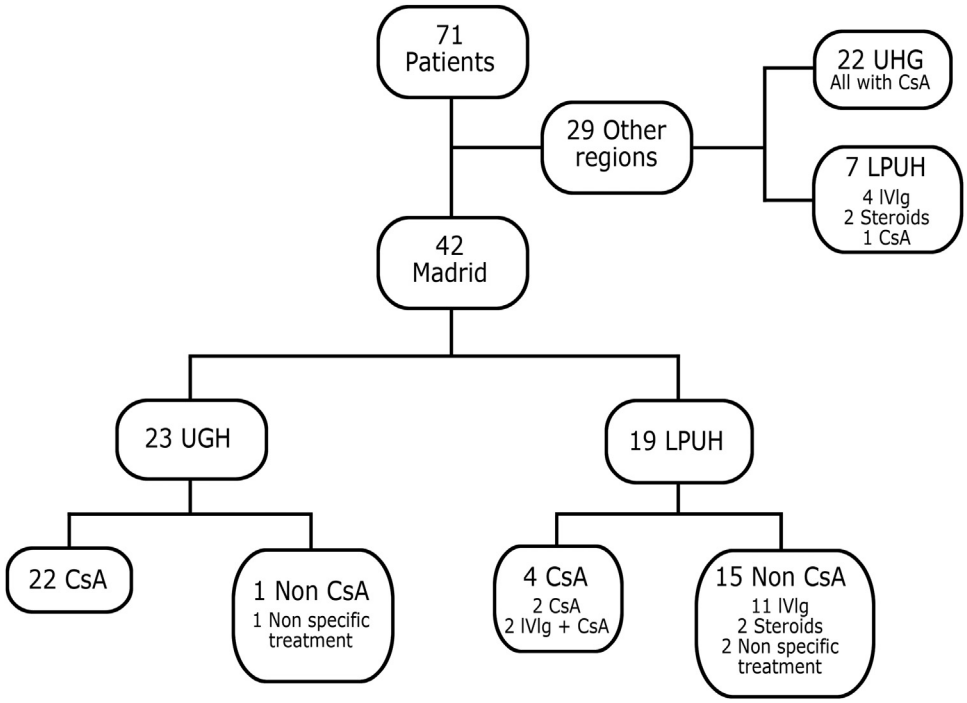

Figure 1. Flow chart of epidermal necrolysis patients included in the study by hospital and treatment. A total of 71 patients with a validated diagnosis of epidermal necrolysis were admitted to either of the participating burn units, University Hospital of Getafe (UHG) and La Paz University Hospital (LPUH). Patients from the region of Madrid (n = 42) were selected to take part in the "natural experiment". All 71 patients were included in the observed versus expected analysis. CsA, cyclosporine; IVIg, intravenous immunoglobulin.

and n = 2 from LPUH). BSA stabilization was reached at a mean ± standard deviation of 2.4 ± 1.5 days from the day CsA was started, and 87% of patients reached this goal within the first 3 days. The re-epithelialization process began after an average of 3.7 ± 1.9 days, and complete re-epithelialization was reached after a mean of 9.2 ± 4.2 days (median = 8 days, range = 3–24 days). In 34 patients (85%), the re-epithelialization process was complete within the first 12 days. No record on secondary efficacy variables was available from non-CsA–treated patients (all from LPUH), and the comparison between treatments was not feasible.

Systematic review and meta-analysis

The automatic search retrieved 126 articles, although only five met the inclusion criteria (see Supplementary Figure S2 online). The characteristics of these five studies are shown in Table 4. The pooled MRR of observed deaths with respect to the SCORTEN-based predicted deaths (MRR_{OE}) of these five studies was 0.40 (95% CI = 0.16–1.01, $P = 0.052$). The inclusion of our study yielded a pooled MRR_{OE} of 0.41 (95% CI = 0.21–0.80) (Figure 2). No heterogeneity was observed across studies ($I^2 = 0.0\%$), nor was evidence of a publication bias (Egger test, $P = 0.50$) (see funnel plot in Supplementary Figure S3 online).

In patients with an expected mortality risk of 30%, an MRR of 0.41 would yield an absolute risk reduction attributed to CsA of 17.7% and a number needed to treat of 5.6 (see Supplementary Tables S4 and S5 online for other scenarios).

DISCUSSION

The results of the three different methodological approaches performed in this study (a "natural experiment", an O/E analysis, and a meta-analysis) consistently suggest that the use of CsA in patients with EN is associated with an important reduction in all-cause mortality during hospitalization compared with other treatments, in particular compared with IVIg (a risk reduction of around 90%), and also compared with the SCORTEN-predicted mortality (a reduction of around 60%). The pooled MRR_{OE} using all published studies (including or not including this study) was also consistent, with a 60% mortality reduction.

In the "natural experiment," we took advantage of the seemingly random allocation of patients to one or the other BU. Hospital assignment fulfils, in our view, the three conditions of a valid IV (Rassen et al., 2009). First, it was strongly associated with the treatment patients actually received (96% patients in UHG received CsA and 79% patients in LPUH received non-CsA treatments). Second, hospital allocation was not related to confounding factors, because the reason to send a patient with EN was based on proximity and not on disease severity. To ensure this condition, we restricted the IV analysis to patients living within the region, because the allocation of patients from other regions might relate to factors other than proximity; we checked this second condition empirically and showed that there were no significant differences between both hospitals in prognostic factors. Third, hospital allocation had no direct influence on mortality; although this condition is fundamentally unverifiable in the IV analysis (Rassen et al., 2009), both BUs are National Reference Centers (NRCs) certified by the Ministry of Health, and therefore we can assume that supportive care provided by both BUs is similar. The comparison of crude mortality rates in burn patients as a proxy for mortality in EN patients was ruled out because of the important differences between both type of patients and their management. This unverifiable assumption,

Table 1. Patient characteristics for each treatment group (Madrid region)

Characteristics	CsA n = 26	Non-CsA			
		IVIg n = 11	Steroids n = 2	None n = 3	Total n = 16
Hospital, n (%)					
UHG	22 (84.6)	0	0	1 (33.3)	1 (6.3)
LPUH	4 (15.4)	11 (100)	2 (100)	2 (66.7)	15 (93.8)[1]
Clinical entity, n (%)					
SJS	3 (11.5)	2 (18.2)	0	1 (33.3)	3 (18.8)
SJS/TEN overlap	8 (30.8)	6 (54.6)	2 (100)	0	8 (50.0)
TEN	15 (57.7)	3 (27.3)	0	2 (66.7)	5 (31.3)
Age in years, n (%)					
15–40	10 (38.5)	4 (36.4)	1 (50.0)	1 (33.3)	6 (37.5)
41–65	12 (46.2)	4 (36.4)	1 (50.0)	1 (33.3)	6 (37.5)
>65	4 (15.4)	3 (27.3)	0 (0.0)	1 (33.33)	4 (25.0)
Mean ± SD in years	47.0 ± 17.2	55.0 ± 20.8	48.5 ± 19.1	60.3 ± 33.0	55.2 ± 21.7
Median (range) in years	45 (16–83)	50 (30–87)	48.5 (35–62)	61 (27–93)	54 (27–93)
Women, n (%)	20 (76.9)	2 (18.2)	0 (0.0)	2 (66.7)	4 (25.0)[1]
Steroids treatment before BU admission, n (%)	15 (57.7)	4 (36.4)	1 (50.0)	1 (33.3)	6 (37.5)
Days to admission					
Mean ± SD	3.5 ± 3.5	3.2 ± 3.8	5.5 ± 3.5	2.7 ± 1.2	3.4 ± 3.4
Median (range)	3 (0–17)	1 (0–13)	5.5 (3–8)	2 (2–4)	2.5 (0–13)
Mucosal involvement, n (%)	24 (92.3)	8 (72.7)	2 (100)	3 (100)	13 (81.3)
% Total BSA involvement at admission					
Mean ± SD	39.3 ± 25.8	29.9 ± 26.2	18.0 ± 9.9	46.3 ± 37.4	31.5 ± 26.9
Median (range)	37.5 (5–80)	22 (8–80)	18 (11–25)	60 (4–75)	25 (4–80)
Pre-existing diseases, n (%)	18 (69.2)	9 (81.8)	1 (50.0)	1 (33.3)	11 (68.8)
SCORTEN-24h, n (%)[2]					
0	1 (3.9)	1 (9.1)	0 (0.0)	0 (0.0)	1 (6.3)
1	3 (11.5)	1 (9.1)	1 (50.0)	1 (33.3)	3 (18.8)
2	11 (42.3)	1 (9.1)	0 (0.0)	1 (33.3)	2 (12.5)
3	6 (23.1)	5 (45.5)	1 (50.0)	1 (33.3)	7 (43.8)
4	5 (19.2)	3 (27.3)	0 (0.0)	0 (0.0)	3 (18.8)
Mean ± SD SCORTEN	2.42 ± 1.06	2.73 ± 1.27	2.00 ± 1.41	2.00 ± 1.00	2.50 ± 1.21
Median (range) SCORTEN	2 (0–4)	3 (0–4)	2 (1–3)	2 (1–3)	3 (0–4)
Expected deaths, n (%)	6.5 (25.0)	3.7 (33.6)	0.4 (20.0)	0.5 (16.7)	4.6 (28.8)
Observed deaths, n (%)	2 (7.7)	5 (45.5)	0	0	5 (31.3)

Abbreviations: BSA, body surface area; BU, burn unit; CsA, cyclosporine; IVIg, intravenous immunoglobulin; LPUH, La Paz University Hospital; SCORTEN, Score of Toxic Epidermal Necrosis; SCORTEN-24h, Score of Toxic Epidermal Necrosis at admission; SJS, Stevens-Johnson syndrome; SD, standard deviation; TEN, toxic epidermal necrolysis; UHG, University Hospital of Getafe.

[1]CsA compared with non-CsA treatment, $P < 0.05$.

[2]Including patients with imputed values.

however, must be considered as a limitation of the IV analysis. The result from the AT analysis adjusted for potential confounding factors was close to that of the IV analysis, which reinforces its validity.

The O/E analysis was also consistent with a dramatic reduction in mortality. This analysis was based on a large series of CsA-treated patients showing a statistically significant result that is, to the best of our knowledge, previously unreported. Other studies (Valeyrie-Allanore et al., 2010; Kirchhof et al., 2014), however, suggested this mortality benefit before. Our systematic review allowed us to identify five other case series with a minimum number of patients and enough information to estimate the $MRR_{O/E}$. All of them showed a trend toward a beneficial effect of CsA, although none reached a statistically significant level. When we pooled the results of these five studies, the overall MRR_{OE} showed a 60% reduction at the edge of statistical significance. Once we added our study, the pooled MRR_{OE} clearly crossed the significance level (see cumulative meta-analysis in Supplementary Figure S4 online). In three studies, no death was observed; thus, we artificially added one death to both the observed and expected numbers to estimate a meaningful risk ratio and confidence interval. Because this maneuver may distort the risk ratio to the null hypothesis, it is likely that the true-pooled mortality risk reduction is even higher.

The evidence of a mortality benefit with other treatments has been much less compelling. For instance, Roujeau and Bastuji-Garin (2011), in a meta-analysis of eight and three studies with IVIg and corticosteroids, respectively, using the O/E methodology, concluded that neither of the two treatments had a relevant or significant reduction in the risk of dying. In another meta-analysis of 13 studies, Barron et al. (2015) estimated a nonsignificant O/E ratio of 0.81

Table 2. Mortality results of cyclosporine (CsA) compared with non-cyclosporine (non-CsA) therapies in the "natural experiment" applying three different analyses (Madrid region)

ITT analysis	**UHG (n = 23)**	**LPUH (n = 19)**		**MRR_{ITT} (95% CI)**
	1 (4.3%)	6 (31.6%)		0.14 (0.02–0.94)
AT analysis	**CsA (n = 26)**	**Non-CsA (n = 16)**	**Crude MRR_{AT} (95% CI)**	**Adjusted[1] MRR_{AT} (95% CI)**
	2 (7.7%)	5 (31.3%)	0.25 (0.04–1.06)	0.13 (0.01–0.93)
IV analysis	**CsA (n = 26)**	**Non-CsA (n = 16)**		**MRR_{IV} (95% CI)**
	2 (7.7%)	5 (31.3%)		0.09 (0.00–0.49)

Abbreviations: AT, as treated; CI, confidence interval; CsA, cyclosporine; ITT, intention to treat; IV, instrumental variable; LPUH, La Paz University Hospital; MRR, mortality risk ratio; SCORTEN, Score of Toxic Epidermal Necrosis; UHG, University Hospital of Getafe.

[1]Adjusted for SCORTEN and percentage of total body surface area involved at admission (both as continuous variables). Sex, age, time from first symptoms to admission, steroid use before admission, and year of reaction (in three periods) hardly changed the main association and were not retained in the final model.

(95% CI = 0.62–1.08) with IVIg, although they found a significant inverse correlation between the risk ratio and the total IVIg dosage used, with better results in those studies using a total dose greater than 2 g/kg. (In our study, the dose used exceeded this level, which would not explain the poor results found.) Finally, Huang et al. (2012) reviewed the results from six studies in which IVIg was compared with supportive care and found no beneficial effect (odds ratio = 1.00; 95% CI = 0.58–1.75). Recently, the use of steroid pulse therapy has been proposed with encouraging results (Hirahara et al., 2013; Kardaum and Jonkman, 2007), but experience is still limited.

Our data on secondary efficacy variables suggest a prompt biological effect of CsA, with most patients reaching BSA stabilization within the first 3 days of treatment and a complete re-epithelialization within the first 12 days. This observation supports the idea that the benefit of CsA stems from its ability to arrest the disease progression (Arévalo et al., 2000), which ultimately would prevent organ dysfunction. The precise mechanism of action of CsA to explain the important benefit shown in this study is unknown. The important role of granulysin in EN (Chung et al., 2008) makes the hypothesis of CsA acting through this mediator attractive, but as far as we know, there is no conclusive evidence of a direct effect (Sarwal et al., 2001). It has been reported that the acquisition of effector functions by cytotoxic lymphocytes is largely dependent on IL-2 (Pipkin et al., 2010) and that perforin is necessary to cause apoptosis of target cells in the presence of granulysin (Saini et al., 2011). Both IL-2 and perforin expressions on T cells are highly sensitive to CsA treatment (Glimcher et al., 2004), which means that indirectly CsA could be acting on granulysin. Of note, the dosage schedules of CsA reported in the case series examined were different, although the magnitude of the benefit was rather similar. The common pattern, however, seems to be a dose of 3–5 mg/kg/day for at least 5–7 days. Although this short course might be enough, the major series reported the use of CsA for longer periods, and we recommend following one of those published dosing schedules. (In a personal communication from 8 March 2017, the French Reference Center reported to us the use of 3 mg/kg/day for 7–10 days without tapering off).

Assuming that the 60% mortality reduction derived from the meta-analysis is a good estimate of the causal effect of CsA, this would imply that for every six patients with an average mortality risk of 30%, CsA would prevent an additional death if all six patients were treated with CsA, a very important benefit.

The major strength of this study is that with three different methodological approaches we obtained highly consistent results, all showing an important and statistically significant reduction in mortality among patients treated with CsA. The main limitation is that all approaches are observational in nature and therefore still subject to bias. A selection bias may have occurred if patients included in our study (in particular those treated with CsA) were different from the overall EN population. We tried to avoid this bias by including all consecutive patients with a valid diagnosis of EN who were admitted to the two BUs over a long period, with no exclusions. However, patients treated in BUs may present more severe forms of EN than those treated in other hospital units, and thus it is uncertain whether or not the CsA effect identified in this study could be extrapolated to less severe forms.

A misclassification of the disease might have occurred; however, we used published standardized procedures to minimize this potential error and, when the analyses were restricted to patients with a definitive or probable diagnosis, the results did not materially change (data not shown).

As in any observational study, residual confounding is still possible. For instance, in the IV analysis there may be factors linked to the hospital that can partly contribute to

Table 3. Results from the observed versus expected (O/E) analysis for each treatment[1]

Treatment	Observed Deaths, n (%)	Expected Deaths, n (%)		MRR_{OE} (95% CI)
CsA (n = 49)	5 (10.2)	SCORTEN	11.8 (24.1)	0.42 (0.14–0.99)
		AS	12.5 (25.5)	0.40 (0.13–0.93)
Non-CsA (n = 22)	7 (31.8)	SCORTEN	6.4 (29.1)	1.09 (0.44–2.25)
		AS	6.6 (30.0)	1.06 (0.43–2.19)
IVIg (n = 15)[2]	7 (46.7)	SCORTEN	5.0 (33.3)	1.40 (0.56–2.88)
		AS	4.3 (28.7)	1.62 (0.65–3.35)

Abbreviations: AS, auxiliary score; CI, confidence interval; CsA, cyclosporine; IVIg, intravenous immunoglobulin; MRR, mortality risk ratio; O/E, observed versus expected; SCORTEN, Score of Toxic Epidermal Necrosis.

[1]All patients treated in both burn units were considered.

[2]These patients are also included among non-CsA.

Table 4. Characteristics of the studies included in the meta-analysis[1]

	Valeyrie-Allanore et al., 2010	Singh et al., 2013	Kirchhof et al., 2014	McKenzie, 2016	Lee et al., 2017	González-Herrada et al., 2017 (This Study)
Study period	2005–2007	2011–2012	2001–2011	NR	2011–2014	2001–2015
Directionality	Prospective	Prospective	Retrospective	Prospective	Retrospective	Ambispective
Exclusion criteria	Yes[2]	Yes[3]	None	NR	Yes[4]	None
Patients receiving CsA, n	29	11	17	4	24	49
Mean age ± SD, years	34.2 ± 14.1	32.1 ± 16.2	53.2 (NR)	48.3 (NR)	50 ± 21	49.2 ± 16.9
Male, n (%)	12 (41.4)	6 (54.5)	7 (41.2)	2 (50.0)	15 (62.5)	20 (40.8)
Clinical entity, n (%)				NR		
SJS	10 (34.5)	5 (45.5)	11 (29.7)		8 (33.3)	5 (10.2)
SJS/TEN overlap	12 (41.4)	3 (27.3)	12 (32.4)		9 (37.5)	14 (28.6)
TEN	7 (24.1)	3 (27.3)	8 (21.6)		7 (29.2)	30 (61.2)
Mean Total BSA involvement at admission (%)	12.2 (±8.2)	23.4 (±16.3)	28.7 (±26.6)	NR	26 (±20)	43.5 (±26.9)
Mean ± SD time from onset to admission, days	2.8 ± 1.8	2.6 ± 0.7	4.3 ± 5.9	NR	1.8 ± 1.7	4.3 ± 3.5
CsA dosing schedule (oral route)	3 mg/kg/day (10 days) 2 mg/kg/day (10 days) 1 mg/kg/day (10 days)	3 mg/kg/day (7 days) 2 mg/kg/day (7 days)	3–5 mg/kg/day (on average 7 days)	3–5 mg/kg/day (4–5 days)	3 mg/kg/day (10 days) 2 mg/kg/day (10 days) 1 mg/kg/day (10 days)	3 mg/kg/day (until complete re-epithelialization) and then 10-mg/day reduction every 48 hours
Prior steroid treatment	None	None	47%	NR	21%	57.1%
Mean ± SD time to complete re-epithelialization, days	12.4 ± 7.7	14.5 ± 4.1	NR	NR	NR	9.1 ± 4.2
Mean ± SD SCORTEN	1.27 (NR)	1.45 (NR)	1.65 ± 1.23	2.75 (NR)	2.5 (NR)	2.39 ± 1.17
Observed mortality	0	0	1	0	3	5
Expected mortality	2.75	1.11	2.40	1.54	7.18	11.8
O/E ratio (95% CI)	0.27 (0.01–1.49)[5]	0.47 (0.01–2.64)[5]	0.42 (0.01–2.32)	0.39 (0.01–2.19)[5]	0.42 (0.09–1.22)	0.42 (0.14–0.99)

Abbreviations: BSA, body surface area; CI, confidence interval; CsA, cyclosporine; NR, not reported; O/E, observed versus expected ratio; SCORTEN, Score of Toxic Epidermal Necrosis; SD, standard deviation; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

[1]All patients were treated with CsA. Observed mortality was compared with the expected mortality according to SCORTEN.

[2]Exclusion criteria: creatinine clearance < 60 ml/minute according to Cockroft formula, diastolic blood pressure > 110 mmHg, previous intolerance to CsA, pregnancy, uncontrolled diabetes mellitus, immunodeficiency with CD4 cells < 200 mm^{-3}, and prior treatment of the reaction with intravenous immunoglobulin, immunosuppressive agent, or more than one dose of corticosteroids. The inclusion criteria were age older than 15 years, admission less than 7 days after onset of the reaction, disease progression within the 24 hours preceding admission, possible follow-up during 2 months, and signed informed consent.

[3]Exclusion criteria: prior treatment with any other immunosuppressive drugs, history of intolerance to CsA, uncontrolled diabetes mellitus, HIV positivity, and multi-organ failure and sepsis. CsA was stopped if diastolic blood pressure was greater than 110 mmHg and creatinine level was greater than 150% of initial value.

[4]Exclusion criteria: renal impairment (unless receiving long-term renal replacement therapy), uncontrolled hypertension, severe infection, active malignancy, HIV infection, or pregnancy. The inclusion criteria were age older than 18 years, admission no later than 7 days after the onset of blistering, and progressive disease activity.

[5]Because zero deaths were observed, we added a continuity correction factor of 1 to both the observed and expected deaths to estimate the O/E mortality risk ratio and the 95% confidence interval.

the difference observed in mortality. The O/E analysis should be less exposed to confounding, because observed mortality is compared with the expected mortality in the same group of patients if they had followed the mortality rates of the population from which SCORTEN was derived, but this approach depends critically on the validity of SCORTEN, and two limitations should be mentioned. First, in a number of retrospective cases we lacked the data necessary to calculate SCORTEN; thus, we used imputed values derived from multiple imputation. Although this approach is highly recognized as the best method to address missing data in epidemiology (Greenland and Rothman, 2008), it is not infallible, and some true values may be different from the imputed ones. Notwithstanding, the O/E analysis performed in patients with complete data or with the simplified AS method yielded very similar MRR_{OE} point estimates, which suggests that a bias due to missing data cannot, by itself, explain the results. Secondly, SCORTEN was designed in 2000 and has not been updated since; advances in supportive care are not taken into account, and SCORTEN could have somewhat overrated the current expected deaths, artificially lowering

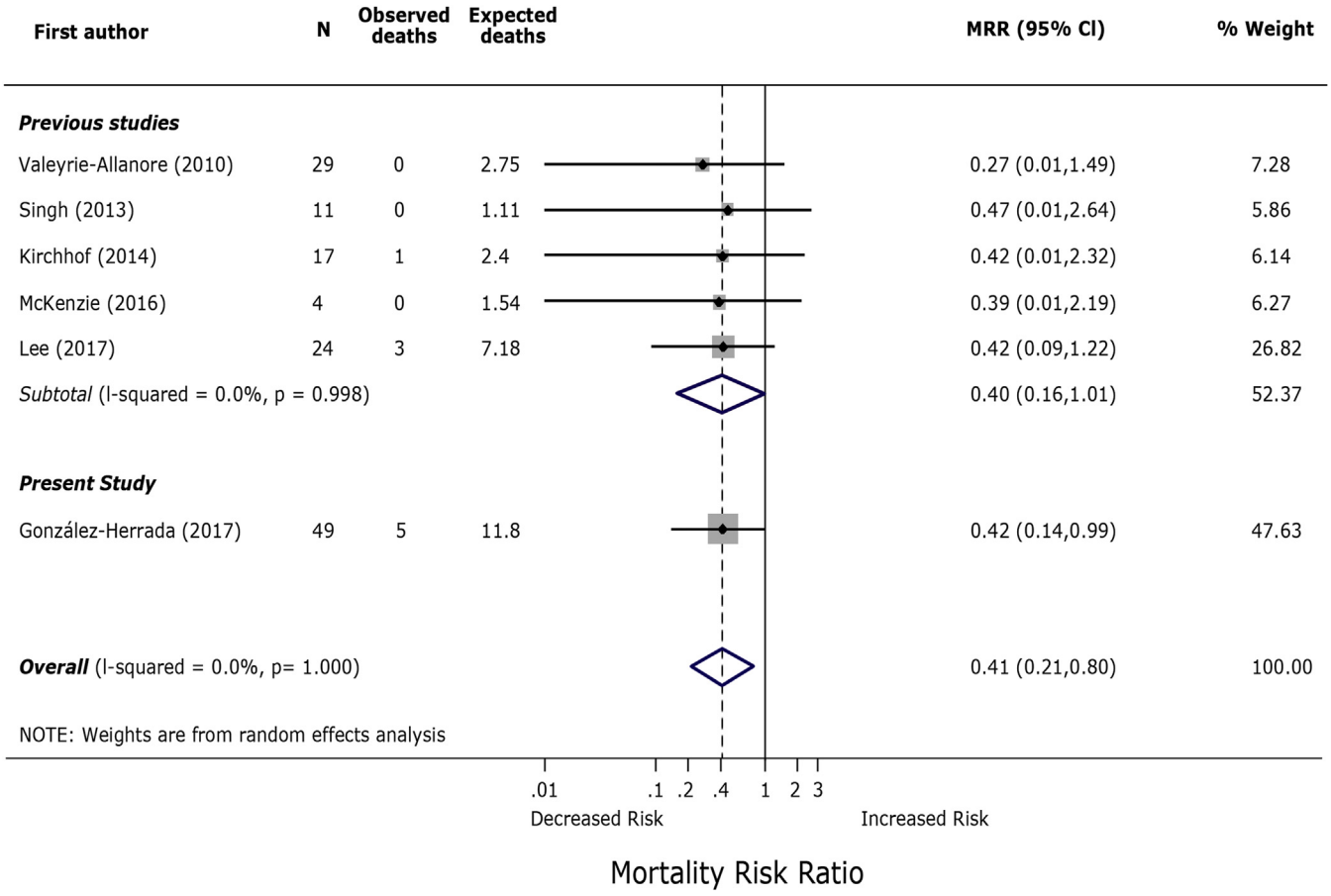

Figure 2. Forest plot of the meta-analysis. The observed and expected deaths reported by each study among patients treated with cyclosporine and the MRR (dark square) with 95% confidence interval (horizontal line) are shown. Studies are identified by first author and year of publication. In studies with zero observed deaths, a continuity correction factor of 1 was added to both the observed and the expected deaths (data not shown).The pooled results are shown in two strata: (i) the five studies found in the literature and (ii) all studies, including ours. The continuous vertical line represents the null value of the MRR; values located on the left are compatible with a mortality risk reduction and those located on the right with an increased risk. CI, confidence interval; MRR, mortality risk ratio.

the MRR_{OE}. However, there are two arguments against this as an explanation of our results: (i) recent published studies have confirmed the predictive accuracy of SCORTEN (Firoz et al., 2012; Sekula et al., 2011) and (ii) there is no reason for this potential problem to selectively affect CsA-treated patients.

In conclusion, this observational study supports and the meta-analysis of available studies confirms the hypothesis that CsA use in EN patients strongly reduces their mortality. As far as we know, no other drug has accumulated the amount of favorable evidence that CsA has. Therefore, given the difficulty in acquiring evidence from a randomized clinical trial in this field, these results should lead to serious consideration of CsA as the standard treatment for EN.

PATIENTS AND METHODS

We included all consecutive patients older than 14 years diagnosed with TEN, SJS, or SJS-TEN overlap who had been admitted to LPUH or UHG BUs during 2001–2015. Part of this study period was retrospective (2001–2010), and part of it was prospective (2011–2015). The study received the approval of the Research Ethics Committee of Príncipe de Asturias University Hospital (the coordinating center of the PielenRed Consortium). Prospective patients or their legal representatives gave written informed consent. For retrospective patients the Committee granted a waiver to the informed consent under the commitment that data were fully anonymized once extracted from clinical records. PIELenRed joined the RegiSCAR group (RegiSCAR, 2017) in 2011, and some of the patients included in this study are recorded in this international registry (see codes in Supplementary Table S6 online).

Information collected

For retrospective cases, we collected the data from hospital charts. For prospective cases, we retrieved the information from direct interviews with patients and/or relatives as well as from hospital charts. The information was included in standardized forms (PIELenRed, 2015). For this study, we collected information on demographic characteristics, clinical and biochemical parameters at baseline (particularly those necessary to calculate SCORTEN), comorbidities, treatment received during hospitalization, and information on efficacy variables.

Case validation

Clinical data, pictures, and histological examinations for each patient were reviewed and validated by an expert committee (CGH, OG, JGR, TB, VL) to establish the final diagnosis according to the current classification criteria (Bastuji-Garin et al., 1993).

Drug treatments

Once admitted to the BU, patients received CsA, IVIg, steroids, or no specific treatment, according to the therapeutic protocol in each hospital, in addition to the usual supportive care (Arévalo et al., 2000). CsA was administered in UHG at an oral dose of 3 mg/kg/day, or its equivalent by intravenous route (1 mg/kg/day), until complete re-epithelialization and was then tapered off (10 mg/day reduction every 48 hours). If no recurrence occurred, the drug was then discontinued. Drug levels were not systematically monitored. CsA was not omitted or discontinued in any patient, not even in cases with suspected infection or secondary sepsis. IVIg was administered in LPUH by continuous infusion at a dose of 0.75g/kg/day for 4 days (total dose = 3 g/kg) in patients with normal renal function. A lower dose was used in patients with renal insufficiency. A few patients in LPUH received steroid-only therapy with prednisone-equivalent daily doses ranging from 37.5−100 mg for 9−12 days.

Efficacy variables

The principal efficacy variable was all-cause mortality during hospitalization. We estimated the expected death rate for every patient using SCORTEN at time of admission. For patients with missing data, we applied a multiple imputation by chained equations models to obtain imputed values (a full description of this method can be found in the Supplementary Methods, including Supplementary Table S7 and Supplementary Figures S5, S6 online). For each missing value, we computed the average value of 20 imputations. Additionally, we used the auxiliary score, a simplified method proposed by Sekula et al. (2013) when laboratory data are missing.

We also gathered information on three secondary efficacy variables: (i) time to stabilization of BSA involvement, (ii) time to re-epithelialization start, and (iii) time to complete re-epithelialization (≥90% of total BSA).

Statistical analysis

We first performed an ITT analysis in which we compared the mortality risk of the UHG group with respect to that of LPUH group and calculated its MRR_{ITT}. Then, we used hospital assignment as an IV to estimate the effect of CsA on all-cause mortality as compared with non-CsA therapies (IV analysis). This analysis keeps the advantages of ITT and additionally allows adjusting for incomplete adherence to treatment. Both analyses (ITT and IV) were restricted to patients living in Madrid, although we also performed a sensitivity analysis including all patients. Because both outcome (all-cause mortality) and exposure (CsA vs. non-CsA) were binary variables, we used a bivariate probit model (Rassen et al., 2008) for the IV analysis and calculated the marginal odds ratio (OR_{IV}) (Vittinghoff et al., 2012). The 95% CI of the OR_{IV} was obtained by bias-corrected bootstrap resampling with 1,000 repetitions (Vittinghoff et al., 2012). The OR_{IV}s and their respective 95% CIs were then converted into MRR_{IV}s (Hilbe, 2008). We also performed an AT analysis, in which patients treated with CsA (regardless of the hospital) were compared with patients treated with non-CsA therapies to estimate the crude OR_{AT}. Additionally, we built a logistic regression model and assessed in a step-forward manner the following variables as potential confounding factors: sex, age, SCORTEN, time from first symptoms to admission, total BSA involved, steroid use before admission, and year of reaction (in three periods). We retained in the model those factors that changed the association between treatment and mortality by more than 10%. The crude and adjusted OR_{AT} were transformed into MRR_{AT} (Hilbe, 2008). In a subanalysis, CsA was compared with IVIg.

For the O/E analysis, all patients treated in the two BUs over the study period were considered, regardless of the region they were transferred from. For each treatment group we computed the MRR_{OE}. The 95% CI of this measure was derived using the Fisher exact test, similarly as for the standardized mortality ratio (Soe et al., 2016). In cases of missing data for SCORTEN, we used imputed values, although a sensitivity analysis was done with only patients with complete data.

Results from secondary efficacy variables were expressed as mean time ± standard deviation and as the percentage of patients reaching the goal on days 3 or 12 from the initiation of specific treatment.

Systematic review and meta-analysis

We performed an automatic search in PubMed and Embase for all studies published up to October 2016, without language restriction, using the following terms: [*ciclosporin* OR *cyclosporin* OR *ciclosporine* OR *cyclosporine*] AND [*toxic epidermal necrolysis* OR *Stevens-Johnson Syndrome* OR *SJS* OR *epidermal necrolysis*]. Additionally, we performed a manual search of the references provided in each article. We selected all studies that fulfilled the following inclusion criteria: (i) report of at least four patients treated with CsA, (ii) provide both the all-cause observed mortality and the predicted mortality using SCORTEN at time of admission (or, alternatively, provide SCORTEN at time of admission for all patients), and (iii) have at least one expected death. For each study, we calculated the MRR_{OE} and its 95% CI. In studies with zero observed deaths, we added a continuity correction factor of 1 to both the observed and expected deaths to calculate a meaningful MRR_{OE} and its 95% CI. Then, the pooled MRR_{OE} was estimated using a random effects model. Heterogeneity was measured through the statistic I^2. All results were graphically presented in a forest plot. Publication bias was evaluated using both a funnel plot and the Egger test. The results were reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Liberati et al., 2009). For different scenarios of expected mortality, we translated the pooled MRR into absolute risk reductions (absolute risk reduction = expected mortality − [expected mortality × MRR]) and then calculated the number needed to treat (number needed to treat = 1/absolute risk reduction).

All analyses were performed using Stata12 (StataCorp, College Station, TX).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to all members of the PIELenRed Consortium and all patients who took part in this study. We would also like to thank José Domínguez Pallás (clinical photographer) for his contribution to case validation and Antonio González-Pérez for his helpful methodological comments.

This study was supported by a research grant from the Instituto de Salud Carlos III−Ministerio de Economía, Industria y Competitividad (#PI12/02267), cofounded by FEDER. The Spanish Agency of Medicinal Products and Medical Devices supports the management of the registry and database.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.05.022.

REFERENCES

Arévalo JM, Lorente JA, González-Herrada C, Jiménez-Reyes J. Treatment of toxic epidermal necrolysis with cyclosporin A. J Trauma Acute Care Surg 2000;48:473−81.

Barron SJ, Del Vecchio MT, Aronoff SC. Intravenous immunoglobulin in the treatment of Stevens–Johnson syndrome and toxic epidermal necrolysis: a meta-analysis with meta-regression of observational studies. Int J Dermatol 2015;54:108–23.

Bastuji-Garin S, Fouchard N, Bertocchi M, Roujeau JC, Revuz J, Wolkenstein P. SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. J Invest Dermatol 2000;115:149–53.

Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. Arch Dermatol 1993;129:92–6.

Chung WH, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, et al. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. Nat Med 2008;14:1343–50.

Firoz BH, Henning JS, Zarzabal LA, Pollock BH. Toxic epidermal necrolysis: five years of treatment experience from a burn unit. J Am Acad Dermatol 2012;67:630–5.

Garcia-Doval I, LeCleach L, Bocquet H, Otero X-L, Roujeau JC. Toxic epidermal necrolysis and Stevens-Johnson syndrome: does early withdrawal of causative drugs decrease the risk of death? Arch Dermatol 2000;136:323–30.

Glimcher LH, Townsend MJ, Sullivan BM, Lord GM. Recent developments in the transcriptional regulation of cytolytic effector cells. Nat Rev Immunol 2004;4:900–11.

Greenland S. An introduction to instrumental variables for epidemiologists. Int J Epidemiol 2000;29:722–31.

Greenland S, Rothman K. Fundamentals of epidemiologic data analysis. In: Rothman K, Greenland S, Lash TL, editors. Modern epidemiology, 3rd ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins; 2008. p. 213–7.

Heng Y, Lee H, Roujeau JC. Epidermal necrolysis: 60 years of errors and advances. Br J Dermatol 2015;173:1250–4.

Hilbe J. STATA help for odds risk. Conversion from logistic odds ratios to risk ratios, http://fmwww.bc.edu/repec/bocode/o/oddsrisk.hlp; 2008 (accessed 20 December 2016).

Hirahara K, Kano Y, Sato Y, Horie C, Okazaki A, Ishida T, et al. Methylprednisolone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis: clinical evaluation and analysis of biomarkers. J Am Acad Dermatol 2013;69:496–504.

Huang YC, Li YC, Chen TJ. The efficacy of intravenous immunoglobulin for the treatment of toxic epidermal necrolysis: a systematic review and meta-analysis. Br J Dermatol 2012;167:424–32.

Kardaun SH, Jonkman MF. Dexamethasone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis. Acta Derm Venereol 2007;87:144–52.

Kirchhof MG, Miliszewski MA, Sikora S, Papp A, Dutz JP. Retrospective review of Stevens-Johnson syndrome/toxic epidermal necrolysis treatment comparing intravenous immunoglobulin with cyclosporine. J Am Acad Dermatol 2014;71:941–7.

Lee HY, Fook-Chong S, Koh HY, Thirumoorthy T, Pang SM. Cyclosporine treatment for Stevens-Johnson syndrome/toxic epidermal necrolysis: retrospective analysis of a cohort treated in a specialized referral center. J Am Acad Dermatol 2017;76:106–13.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. BMJ 2009;339:b2700.

McKenzie. The use of cyclosporine for Stevens–Johnson syndrome and toxic epidermal necrolysis: The University of Louisville experience. J Am Acad Dermatol 2016;74:AB175.

Palmieri TL, Greenhalgh DG, Saffle J, Spence R, Peck M, Jeng J, et al. A multicenter review of toxic epidermal necrolysis treated in US burn centers at the end of the twentieth century. J Burn Care Res 2002;23:87–96.

PIELenRed. Consorcio PIELenRed, http://pielenred.hol.es/PIELenRed/; 2015 (accessed 20 December 2016).

Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A. Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. Immunity 2010;32:79–90.

Rassen JA, Brookhart MA, Glynn RJ, Mittleman MA, Schneeweiss S. Instrumental variables I: instrumental variables exploit natural variation in nonexperimental data to estimate causal relationships. J Clin Epidemiol 2009;62:1226–32.

Rassen JA, Schneeweiss S, Glynn RJ, Mittleman MA, Brookhart MA. Instrumental variable analysis for estimation of treatment effects with dichotomous outcomes. Am J Epidemiol 2008;169:273–84.

RegiSCAR. The RegiSCAR Project, www.regiscar.org; 2017 (accessed 9 March 2017).

Roujeau JC, Bastuji-Garin S. Systematic review of treatments for Stevens–Johnson syndrome and toxic epidermal necrolysis using the SCORTEN score as a tool for evaluating mortality. Ther Adv Drug Saf 2011;2:87–94.

Saini RV, Wilson C, Finn MW, Wang T, Krensky AM, Clayberger C. Granulysin delivered by cytotoxic cells damages endoplasmic reticulum and activates caspase-7 in target cells. J Immunol 2011;186:3497–504.

Sarwal MM, Jani A, Chang S, Huie P, Wang Z, Salvatierra O Jr, et al. Granulysin expression is a marker for acute rejection and steroid resistance in human renal transplantation. Hum Immunol 2001;62:21–31.

Sekula P, Liss Y, Davidovici B, Dunant A, Roujeau JC, Kardaun S, et al. Evaluation of SCORTEN on a cohort of patients with Stevens-Johnson syndrome and toxic epidermal necrolysis included in the RegiSCAR study. J Burn Care Res 2011;32:237–45.

Sekula P, Dunant A, Mockenhaupt M, Naldi L, Bouwes-Bavinck JN, Halevy S, et al. Comprehensive survival analysis of a cohort of patients with Stevens-Johnson syndrome and toxic epidermal necrolysis. J Invest Dermatol 2013;133:1197–204.

Singh G, Chatterjee M, Verma R. Cyclosporine in Stevens Johnson syndrome and toxic epidermal necrolysis and retrospective comparison with systemic corticosteroid. Indian J Dermatol Venereol Leprol 2013;79:686–92.

Soe MM, Sullivan KM, Dean AG, Mir R. Standardized mortality ratio. In: Dean AG, Sullivan KM, Soe MM, editors. Open Epi: Open source epidemiologic statistics for public health, www.OpenEpi.com; 2006 (accessed 11 January 2017).

Valeyrie-Allanore L, Wolkenstein P, Brochard L, Ortonne N, Maitre B, Revuz J, et al. Open trial of ciclosporin treatment for Stevens–Johnson syndrome and toxic epidermal necrolysis. Br J Dermatol 2010;163:847–53.

Vittinghoff E, Glidden DV, Shiboski SC. Regression methods in biostatistics. 2nd ed. New York, NY: Springer; 2012.