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Optimal step doses for drug provocation tests to prove beta-lactam hypersensitivity

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β-lactams; dose; drug hypersensitivity reaction; drug provocation test; survival analysis.

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Abstract

Background: Drug provocation tests (DPT) are commonly performed as part of β-lactam (BL) allergy workup, in case of negative skin tests (ST) and in the absence of contraindications. The recommendations of learned societies have created a frame for DPT performance, but protocols vary widely between centres, generating various hypothesis-driven protocols (i.e. empirical dosing, driven by both safety concerns and practical aspects).
Methods: The primary objective of this retrospective analysis was to detect eliciting dose thresholds (reactive doses) during BL DPT, using the survival analysis method, in order to suggest optimal step doses. Our secondary objective was to evaluate the safety of our 30-min incremental 1-day protocol. The study included all the patients explored in the Allergy Unit of the University Hospital of Montpellier (France), between September 1996 and July 2015 for a suspicion of drug hypersensitivity reaction to BLs, with negative ST and positive DPT.
Results: During the study period, 182 positive DPT (accounting for 171 hypersensitive patients) were analysed. We identified eliciting thresholds, and we suggest the following steps for DPT to BLs: 5–15–30–50% of daily therapeutic dose (with additional lower steps for index reactions of anaphylaxis). We confirm the safety of 1-day protocol for immediate and mild nonimmediate reactors, for both children and adults, with a surveillance period of 2 h after the last administered dose, and a prolonged surveillance after discharge of 48 h.
Conclusion: This data-driven approach in designing DPT protocols is a step forward in improving DPT standardization, starting with the most frequently tested drugs, BL antibiotics.

A drug provocation test (DPT) is the controlled administration of a drug to confirm or rule out drug hypersensitivity reactions (DHR) (1, 2). Due to its inherent risks, it is performed at the end of a stepwise approach in the planning of a drug allergy workup, and it should be performed after individual risk–benefit evaluation. It has become over the years the gold standard of the drug allergy workup. The recommendations of learned societies have created a frame for DPT performance (1, 2), but protocols vary widely between centres, in terms of dose steps and time intervals between incremental doses (3–10), generating various hypothesis-driven protocols (i.e. empirical dosing, driven by both safety concerns and practical aspects). Several factors may account for these differences: the chronology of the index clinical reaction/history (immediate *vs* nonimmediate reactions), its severity (anaphylaxis

Abbreviations
AO, angioedema; BL, β-lactam; CI, confidence interval; DHR, drug hypersensitivity reactions; DPT, drug provocation test; D_{ther}, therapeutic daily dose; ENDA/DAIG, European Network of Drug Allergy/Drug Allergy Interest Group; IQR, interquartile range; MPE, maculopapular exanthema; NPV, negative predictive value; RD, reactive dose; R_{time}, reactive time point; ST, skin tests; U, urticaria.

vs mild reactions), the population involved (children *vs* adults), and last but not least, the various centres' experiences and infrastructure. It is generally accepted that 'one should start with a low dose, carefully increasing this and stopping as soon as the first objective symptoms occur' (1). However, when to stop or complete the protocol and declare negative result of DPT is controversial. Some groups may accept a single unit dose to declare negative result, while others prone prolonged DPT (5, 11, 12) or even full therapeutic course to ensure tolerance (10). It is generally accepted that too low a dose might cause false-negative results (1). Reaching (or getting close to) a therapeutic daily dose (D_{ther}) is desirable during DPT and has reached international consensus (2).

Drug provocation tests are commonly performed as part of β-lactam (BL) allergy workup, in case of negative skin tests (ST) (2, 11, 12). More than 10 years ago, our group published several hypothesis-driven DPT protocols, including DPT for BL (6). Our protocol was based on 30-min incremental doses for both immediate and mild nonimmediate DHR. We have confirmed a high negative predictive value (NPV) for this protocol, for both BL (13) and nonsteroidal anti-inflammatory drugs (14). Since this pioneered publication (6), we have carried out documenting all our cases prospectively. The aim of this retrospective analysis was to detect eliciting dose thresholds (reactive doses) during BL DPT, using the survival analysis method, in order to suggest optimal DPT step doses. This data-driven approach in designing the DPT protocol is a step forward in improving DPT standardization, starting with the most frequently tested drugs, *that is* BL antibiotics. Our secondary objective was to evaluate the safety of our 30-min incremental 1-day protocol.

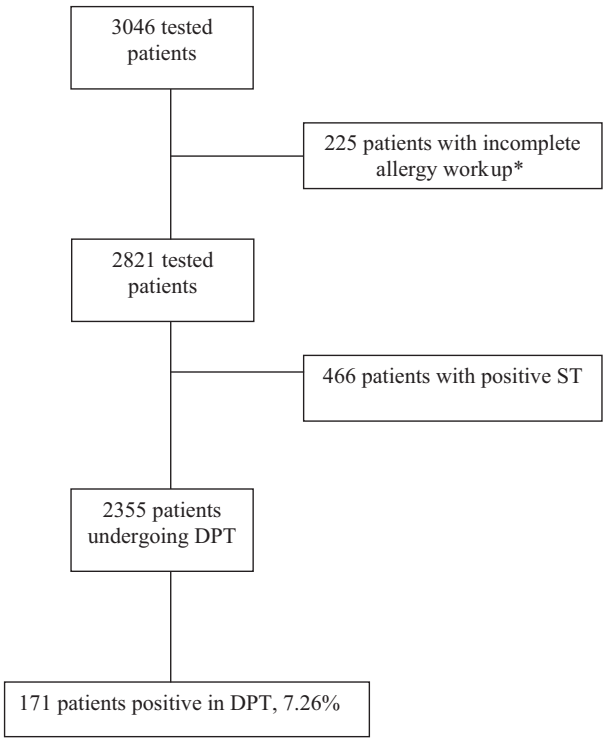

Figure 1 Flow chart of patients undergoing an allergy workup for a suspicion of drug hypersensitivity reactions to beta-lactams.
*Patients with negative ST, and no DPT performed due to:
-lost to follow-up (205 cases)
-contraindication to DPT, due to life-threatening nonimmediate reaction (20 cases).
DPT, drug provocation tests; ST, skin tests.

Methods

Study population

Patients were selected from our historico-prospective cohort, the *Drug Allergy and Hypersensitivity Database* (DAHD®). The study included all the patients explored in the Allergy Unit of the University Hospital of Montpellier (France), between September 1996 and July 2015 for a suspicion of DHR to BL, with negative ST and with positive DPT (Fig. 1). Patients who had experienced severe cutaneous adverse reactions, drug-induced autoimmune diseases or severe organ involvement were excluded as they were contraindicated to DPT (2). The patients gave their written informed consent to take part in clinical research. The local ethics committee 'Comité de Protection des Personnes Sud-Mediterranée III' approved the design of the study.

DPT protocols

Overall, the DPT protocols observed the following scheme: (0.01%)–0.1–0.5–1–5–10–20–50–100% (or 150%) of the maximum single unit dose. The precise dose escalation is shown in Table 1, for each BL eliciting positive DPT. Possible individual adaptations according to the reaction during DPT were allowed (see text below). These *a priori*, hypothesis-driven protocols are based on previous experience (6) and general European Network of Drug Allergy (ENDA)/Drug Allergy Interest Group (DAIG) of the European Academy of Allergy and Clinical Immunology recommendations for DPT performance (1, 15):

- The first dose was 1 mg for most BL, which is $< 1 : 100$ of the therapeutic daily dose (D_{ther}) for all BL. In case of clinical history of anaphylactic shock, the starting dose was 1 : 10 or 1 : 100 of this usual first dose;
- The last dose was equal to (or higher than) a maximum single dose;
- The total dose reached (if DPT was finished) was set to approach/reach a D_{ther}.

For children, the last dose was calculated according to their weight, in order to reach a total dose corresponding to their D_{ther}. Time intervals between doses were set at 30 min. Shorter intervals are considered in the drug allergy field to induce desensitization, and longer intervals with too many steps are barely compatible with one-day hospitalization. The same protocols were used for clinical histories of immediate and nonimmediate reactions.

Table 1 Protocols used at present for DPTs

Antibiotics	Dose escalation*	Total DPT dose (mg)†	Unit dose/tablet, vial (mg)	Therapeutic daily dose, D_{ther}‡	
				Adults	Children
Amoxicillin§	1, 5, 10, 50, 250, 500, 1500	2316	500, 1000	2000–3000 mg¶	50 (100) mg/kg
Ampicillin	1, 5, 10, 50, 100, 500, 1500	2316	500, 1000	2000 (12 000) mg	50 (100) mg/kg
Cefaclor	1, 5, 10, 50, 250, 500	816	250, 500	750 (1500) mg	20 (40) mg/kg
Cefamandole	1, 5, 10, 50, 250, 500, 750	1566	750	1500 (3000) mg	50 mg/kg
Cefatrizine	1, 5, 10, 50, 250, 500	816	500	1000 mg	15–35 mg/kg
Cefazolin	1, 5, 10, 50, 250, 500, 1000	1816	1000	2000 (4000) mg	25–50 mg/kg
Cefixime	2, 10, 20, 40, 100, 200	372	100	400 mg	8 mg/kg
Cefotiam	2, 10, 20, 40, 100, 200	372	200	400 (800) mg	No data
Cefotaxime	1, 5, 10, 50, 250, 500, 1000, 1000	2816	500, 1000, 2000	3000 (12 000) mg	50 (200) mg/kg
Cefpodoxime	2, 10, 20, 40, 100, 200	372	100	200–400 mg	8 mg/kg
Cefradine	1, 10, 50, 250, 500, 1000	1816	500	2000 mg	50 (100) mg/kg
Ceftazidime	1, 5, 10, 50, 250, 500, 1000, 1000	2816	1000	3000 (6000) mg	50 (200) mg/kg
Ceftriaxone	1, 5, 10, 50, 250, 500, 1000	1816	1000	2000 (6000) mg	50 (100) mg/kg
Cefuroxime	1, 5, 10, 50, 250, 500	816	250, 500	500–1000 mg	30 mg/kg

DPT, drug provocation test.

*During dose escalation, the usual first dose was 1 (or 2) mg, but lower starting dose, *that is* 1 : 10 or 1 : 100 of this dose, was used if clinical history of severe anaphylactic reaction. For children, the last dose was calculated according to their weight to reach cumulative dose corresponding to D_{ther}.

†Total dose reached if DPT was completed according to the established dose escalation.

‡Higher doses (mentioned in brackets, for each drug, if applicable) than usual D_{ther} were administered in specific indications, such as in patient with cystic fibrosis.

§Including the association amoxicillin/clavulanic acid.

¶D_{ther} of amoxicillin is 2000 mg daily for most countries, but in France, the higher dose of 3000 mg daily is widely accepted.

Definitions used in the study

Collected data

Demographic characteristics, clinical history and the results of allergy workup were retrieved and analysed. Children were defined as patients younger than 18 years at the time of DPT. Immediate reactors were patients with the onset of index clinical reaction/history within 1 h after the last drug intake (2). Otherwise, patients were considered nonimmediate reactors. Urticaria and angioedema (U/AO) were considered as a single entity. Anaphylaxis was defined according to Sampson et al. (16).

Positive DPT

The DPT was considered positive if objective signs occurred during BL administration. In case of occurrence of subjective symptoms during DPT, the supervision physician could decide either to repeat the last step or to divide the next dose into two steps. When subjective symptoms occurred but the patient could complete the DPT without further objective signs or symptoms, the DPT was considered negative. When a reaction considered positive occurred at any time during DPT, the DPT was stopped and the reaction was immediately treated accordingly, as described previously (6).

DPT event

Because the purpose of the study was to define dose steps for DPT, we analysed the DPT events, rather than the patients. Thus, each DPT event was considered as a distinct entity. One DPT event ('case') was composed of one index reaction and the corresponding positive DPT result for one tested BL. The index reaction was defined as the history of DHR associated with the intake of BL. When a patient reported several histories of DHR to BL, the last clinical history was considered for the index reaction clinical presentation, assuming that the last reaction was at least as severe as the previous ones. Otherwise, the most recent severe reaction was chosen. When a patient had a positive DPT for the BL involved in the index reaction and DPT for the identification of an alternative BL were performed (and were positive), the same index reaction was attached to each DPT event, separately.

The hypersensitivity reaction elicited during the DPT event was classified as *similar to*, *less* or *more severe* than the index reaction according to semiological criteria. The DPT events with different semiology from index reaction/history were also noted. Isolated benign cutaneous reactions, such as U/AO and maculopapular exanthema (MPE), were considered less severe than anaphylactic shock or isolated respiratory symptoms involving bronchospasm. For nonimmediate reactions, MPE with general signs and symptoms (systemic involvement) was considered more severe than a cutaneous reaction with no such associated manifestations.

Reactive dose

The reactive dose (RD) was calculated from the total cumulative dose (mg) reached when the DPT was considered positive. Due to the complexity and multitude of DPT protocols (D_{ther} varying according to the tested BL and the age of the patient), we chose to define the RD as a ratio between the

cumulative RD (mg) and the total DPT dose (i.e. the dose reached if DPT was to be completed; see Table 1). The RD was expressed in percentage, varying from 0.01% to 100%.

Reactive time point

The reactive time point (R_{time}) was the delay between the DHR elicited by DPT and the last administered dose during DPT. It was analysed according to two chronologies: the day of DPT (i.e. within 24, 48 and 72 h), and according to the delay after the last administered dose (i.e. within 1 or >1 h).

Surveillance period

Patients were kept under medical surveillance for 2 h after the last administered dose. If no reaction occurred, they were discharged with a corticosteroid and antihistaminic prescription, a result, a telephone number and an email contact. Patients were educated to observe their reaction after discharge (in case of index clinical/history of nonimmediate reaction), contact the staff immediately and treat accordingly if the reaction occurred. If an immediate reaction occurred during the DPT, the patient was properly treated and released only when all symptoms had disappeared (typically 2–3 h after the reaction).

Technical aspects (miscellaneous)

We followed the general ENDA/DAIG recommendations for DPT indications, contraindications, prohibited co-medication and enhanced safety measures (e.g. intravenous catheter) in case of anaphylaxis during clinical history (1, 2). Uniformed capsules/preparations, delivered in specified doses prepared by the hospital pharmacy, were used for DPT. The oral route was chosen systematically, except for drugs with only intravenous preparations. All DPT were performed during one-day hospitalization.

Statistical methods

Qualitative variables were expressed as numbers and percentages. Quantitative data were expressed as medians with interquartile range (IQR) with 25th and 75th percentiles (Q25–Q75 IQR, not normally distributed data assessed with a Shapiro–Wilk test). Comparisons for the qualitative data were carried out between groups using chi-square or Fisher's exact test for small samples. Comparisons of quantitative variables were made using the Wilcoxon–Mann–Whitney tests. Clinical risk factors were searched using univariate and multivariate logistic regression, and odds ratios (ORs) were expressed with 95% confidence intervals (CIs).

Reactive dose and R_{time} were considered as time-dependent data and were analysed with survival data analysis methods.

Results

Patient characteristics and DPT events

During the study period, 171 patients had positive DPT, accounting for 182 positive DPT events (Table 2). Median age at DPT was 42 years (31-55). There were 22 (12.8%) children having 24 DPT events. The culprit BL was penicillin (either known or unknown precise trade mark) in most cases (136, 74.7%). More than two-thirds of DPT events (130 DPT events, 71.4%) were performed for an index reaction of non-immediate or of unknown chronology. However, fifty per cent of index reaction/history occurred within 1 day of drug intake. The most frequent index reaction was U/AO, followed by MPE and anaphylaxis with or w/o shock. Overall, a history of anaphylaxis was present in 53 cases (29.1%). The delay between index reaction and DPT was ranging from as early as 1 month and up to 50 years with a median of 13.5 months (4–120). The median DPT time was 3 h (2–3).

Table 2 Descriptive analysis of the DPT events

Clinical characteristics	Number (%)
Sex*	
Female	131 (76.6)
Antibiotic	
Penicillin	111 (61)
Cephalosporin	46 (25.2)
Unspecified penicillin	25 (13.7)
Chronology of the index reaction	
1 h†	52 (28.5)
1–6 h	28 (15.4)
6–24 h	65 (35.7)
>24 h	11 (6.0)
Unknown	26 (14.3)
Day of reaction during index reaction	
Day 1	91 (50)
Day 2	28 (15.4)
Day 3 or more	35 (19.2)
Unknown	28 (15.4)
Semiology of the index reaction	
Anaphylaxis (w/o shock)	28 (15.3)
Anaphylactic shock	25 (13.7)
Urticaria/angioedema	78 (42.8)
MPE	43 (23.6)
Isolated respiratory symptoms	3 (1.6)
Unknown	5 (2.7)
Age at DPT	
Child (<18 years)	24 (13.2)
Adult (≥18 years)	158 (86.8)
DPT tested BL	
Culprit BL	160 (87.9)
Alternative BL	22 (12.1)
Semiology of DPT event	
Anaphylaxis (w/o shock)	21 (11.5)
Anaphylactic shock	4 (2.2)
Urticaria/angioedema	108 (59.3)
MPE‡	49 (26.9)

BL, β-lactam; DPT, drug provocation test.

*Sex was expressed according to the number of patients involved, and not DPT events.

†Immediate reactors.

‡Six cases were maculopapular exanthemas (MPEs) with systemic signs and symptoms.

The median (range) delay of DPT reaction after the last DPT dose was 1.5 h (0.5–7). In 96 DPT events (52.7%), the reaction occurred while the patient was either during DPT or during the 2-h surveillance period following DPT completion. In all the other cases, the reaction occurred after day hospital dismissal; they were handled properly with full recovery in all cases.

Eliciting dose thresholds of DPT

Reactive doses

Kaplan–Meyer curves in Fig. 2 show the reactive thresholds for the whole set of DPT events. The vertical and horizontal coloured lines give an example of how to generate the step doses to produce additional 10% of positive DPT cases at each step ('data-driven protocol'). The RD for 10% of cases (RD_{10}) was 0.6% of D_{ther}. Similarly, by deciles of reactive cases, RD_{20}, RD_{30} and RD_{40} were 3.2%, 20.2% and 39.7%, respectively. Interestingly, at RD = 71.8%, only 50% of cases had reacted, while another half (45.6%) reacted at (or after) RD = 100%. There were 29 cases (15.6%) who reacted with RD > 100% of the total DPT dose. Only three cases truly required higher D_{ther} for special clinical conditions (i.e. cystic fibrosis), while for the remaining 26 cases, an additional dose was needed to repeat a step during DPT, due to occurrence of subjective symptoms (23 with RD ≤ 112%, and three with RD = 118%, 124.4% and 130.3%, respectively).

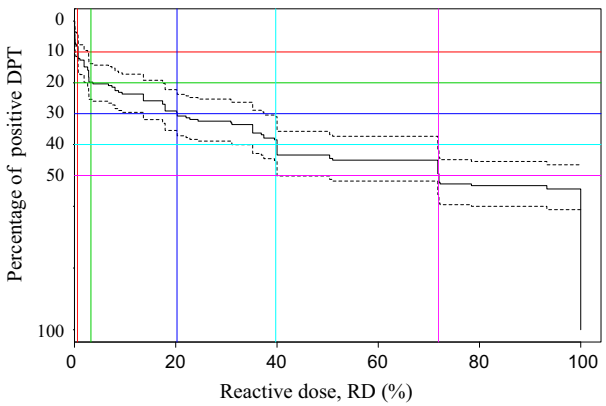

Figure 2 Kaplan–Meier curve (and 95% CI in dotted lines) for the whole set of drug provocation test (DPT) events, according to the data-driven protocol. The vertical and horizontal coloured lines identify the steps (RD) for deciles of positive DPT. These coloured lines generate an example of data-driven protocol if each dose was designed to induce positive DPT of 10% of cases. The RD for 10% of cases (RD_{10}) was 0.6%. Similarly, by deciles of reactive cases, RD_{20}, RD_{30}, RD_{40} and RD_{50} were 3.2%, 20.2%, 39.7% and 71.8%, respectively. RD, reactive dose.

Table 3 presents the details of the data-driven protocol. Each step dose was compared to the previous one, in terms of numerical (i.e. number of DPT events) and severity (i.e. number of anaphylactic events) added value.

We observed sharp drops (Fig. 2) on the survival analysis curve corresponding to RD_{10} and RD_{20}, located within a tight dose range (0.6% and 3.2%, respectively). As shown in Table 3, up to 40% of DPT events in these two steps were anaphylactic events, which accounted for two-thirds of all anaphylactic DPT events. The following eliciting doses occurred on a rather smooth curve, but anaphylactic events could still occur, despite their low frequency (i.e. approximately 17.4%, 12.5% and 3.5% of DPT events were anaphylaxis at RD = 39.7%, 71.8% and 100%, respectively; Table 3).

When analysing positive DPT at very low doses (RD = 1%), 22 cases (12.1%) were identified, and half of them were anaphylactic DPT events. Indeed, 75% of anaphylactic shock and 62% of the anaphylaxis w/o shock during DPT occurred before or at RD = 1% (Table 3). By RD = 3.2%, 64% (16 cases) of all the anaphylactic DPT events had already occurred. As expected, in the subgroup of cases with index reaction of anaphylactic shock (25 cases; Fig. 3), the reactive thresholds were much lower. Index reaction/history of anaphylaxis was a strong risk factor of developing anaphylactic event at very low dose by RD = 5% [OR = 9.7, 95% CI = (1.5–62.7); $P = 0.01$]. Beyond RD = 5%, the data-driven eliciting doses corresponding to deciles of reactive patients showed a good safety profile (no anaphylactic events occurred between RD = 5% and RD_{30} = 20%, and the 4th and last anaphylactic shock occurred at RD = 50%) (Table 3). Therefore, imagining dose

Table 3 Details of the data-driven protocol. The steps are presented in terms of RD per deciles of positive DPT. The number of positive DPT since the previous step in the protocol (and more specifically, the number of anaphylactic shocks and anaphylaxis w/o shock) is noted for each RD. Of note, RD between (and excluding) RD = 3.2% and RD = 71.8% seem to provide an acceptable safety profile with respect to previous steps, as the number of added anaphylactic events is low. Therefore, dose steps corresponding to RD = 20%, followed directly by RD = 50% (instead of RD = 39.7%), are feasible

% positive DPT events	10%	20%	30%	40%	50%	100%
Data-driven steps (RD%)	0.6	3.2	20.2	39.7	71.8	100
New DPT events since previous step (cumulative cases)	19	18 (37)	19 (56)	23 (79)	16 (95)	87 (182)
New DPT events with shock since previous step (cumulative cases)	2	1* (3)	0 (3)	0 (3)	1† (4)	0 (4)
New DPT events with anaphylaxis since previous step (cumulative cases)	8	5 (13)	0 (13)	4 (17)	1 (18)	3 (21)

DPT, drug provocation test; RD, reactive dose.

*The anaphylactic shock is elicited at RD = 0.7%.

†The anaphylactic shock is elicited at RD = 50.4%.

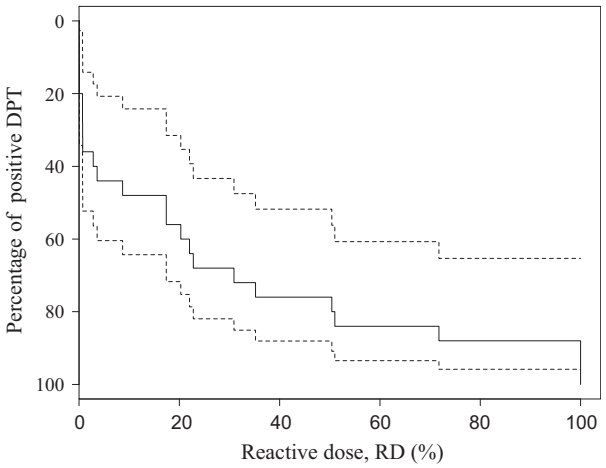

Figure 3 Kaplan–Meier curve (and 95% CI, in dotted lines) for the drug provocation test events with an index reaction of anaphylactic shock. Reactive dose (RD) RD_{20} was 0.1% and 60% of the cases had reacted by RD = 20%. Three cases (12%) reacted at RD = 100%.

steps corresponding to RD = 20%, followed directly by RD = 50% (instead of RD = 39.7%) could be acceptable.

Reactive time points

The reactive time points were divided into two chronologies: the delay of the index reaction after the first day of drug intake and the delay after the last administered dose. In our study population, 91 cases (50%) had reacted during Day 1 (Table 2), while only 52 cases (28.5%) were immediate reactors (i.e. within 1 h after the last administered dose). When reactive time points were set at 24, 48, and 72 h, the percentage of match with the chronology of the DPT was over 95% for all case groups (Fig. 4A). The chronology of DPT reactions also matched with the delay between the last intake and the reaction in the index reaction/history, even though the percentage of concordance was higher in immediate than nonimmediate reactors, 94.2% (49 of 52 cases) and 71.1% (74 of 104 cases), respectively. When the chronologies of these concordant nonimmediate reactors were studied in detail, it was noted that the patients reacted within the same delay frame as their index reaction (45.9%), more rapidly (39.2%) or later (14.8%). Overall, there was a discrepancy in chronology in 33 cases (18.1%), with three immediate reactors developing nonimmediate reactions, and conversely, 30 nonimmediate reactors developing reactions during DPT < 1 h after the last administered dose. Regarding the 26 DPT events with unknown chronology, most of them (16 cases, 61.5%) developed nonimmediate reactions during DPT.

DPT safety

Overall safety

The DPT resulted in benign cutaneous reactions in 82.9% of cases (108 with U/AO and 43 with MPE). There were

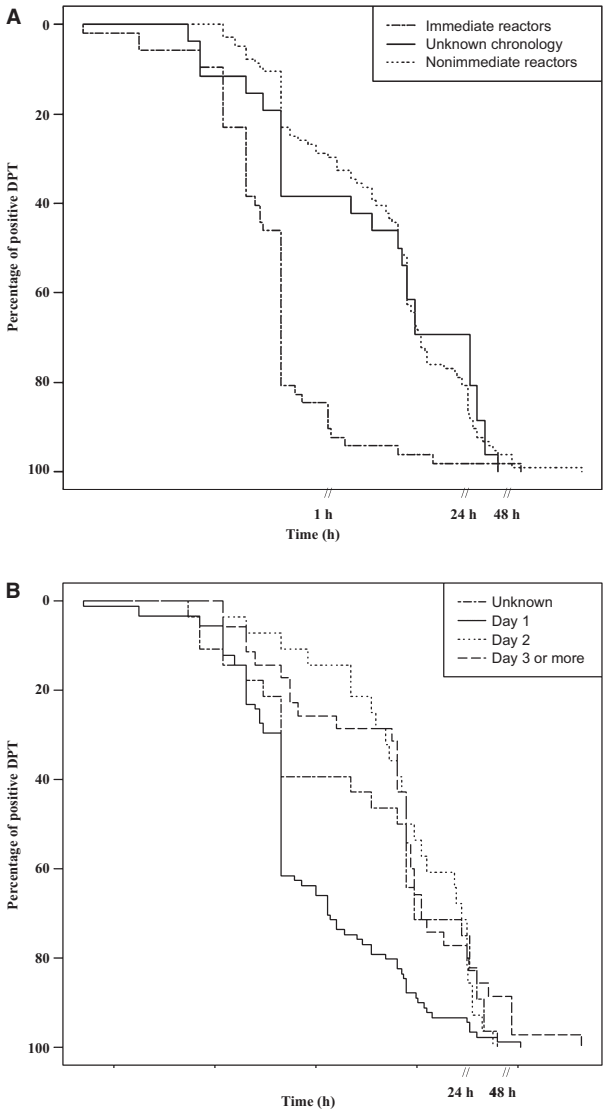

Figure 4 (A) Reactive time points for groups of patients according to their reactive day (after the first day of treatment) during the index reaction. Cases were divided into four groups, corresponding to index drug hypersensitivity reactions (DHR) on Day 1, Day 2 and Day 3 (or more) and of unknown chronology. The chronology of the index reaction was matched with the delay of reaction after the last dose administered during drug provocation test (DPT) (reaction within 24, 48 and 72 h). The percentage of match was 96.7% for Day 1 (88 of 91 cases reacting within 24 h), 100% for Day 2 (28 of 28 cases reacting within 48 h) and 97.1% for Day 3 (34 of 35 cases reacting within 48 h). D1 = Day 1; D2 = Day 2; D3 = Day 3. (B) Reactive time points for groups of patients according to the chronology after the last dose during the index reaction. Cases were divided into immediate reactors, nonimmediate reactors and of unknown chronology. The percentage of concordance for immediate and nonimmediate reactors was 94.2% and 72.5%, respectively. Most cases with unknown chronology (61.5%) turned out to be nonimmediate reactors.

six cases of nonimmediate MPE elicited by DPT, which were accompanied by other signs/symptoms. Drug provocation test elicited anaphylaxis with or w/o shock in 4 (2.2%) and 21 cases (11.5%), respectively. Adrenaline was used in nine DPT events (4.9%). Overall, the reactions elicited by DPT were of similar semiology and severity to the index reaction in most cases (127, 69.8%). While the reaction was estimated to be more severe in 12 cases (6.6%; Table S1), it was less severe in 43 DPT events (23.6%).

*DPT events with more severe reactions than the index reaction (*N = *12 DPT events in 11 patients)*

The details are presented in Table S1. This group comprised five immediate reactors (two U/AO, two anaphylaxis, one MPE with hand oedema), five nonimmediate reactors (four delayed U/AO and one delayed anaphylaxis) and two cases of unknown semiology and chronology. Nonimmediate reactions elicited by DPT consisted mostly of MPE (six cases) that were considered more severe than the index reaction due to the association of general symptoms (fever, fatigue) or alteration of haematologic tests (i.e. eosinophilia). All the patients recovered from their reactions without sequelae. One patient (N#2928) described nonimmediate eyelid and possible laryngeal angioedema and dyspnoea, and consulted at the emergency department. He was treated with corticosteroids. Patient N#15547 clearly described immediate anaphylaxis as her index reaction (even after re-questioning), but instead she reacted with nonimmediate MPE. A similar discrepancy was noted for patient N#5946. Another patient (N#622) described what was likely to be immediate MPE and angioedema, but the reaction elicited during DPT was clearly of different semiology, although it occurred rapidly after the last intake (1 h).

When adjusted for all the studied variables, the only clinical risk factor for developing anaphylaxis (with or w/o shock) during DPT was an index reaction of anaphylaxis, with more than a 10-fold risk increase [OR = 13.7, 95% CI = (3.3–55.9); $P < 0.001$].

More details regarding anaphylactic DPT events w/o shock, anaphylactic shock DPT events, DPT events occurring after discharge from the hospital and DPT events in children can be found on the online repository.

Discussion

Studies aiming to define threshold dose distributions using interval-censoring survival analysis have already been undertaken in the area of food allergy (17), but not in drug allergy. In this study, we retrospectively analysed patients with positive DPT to BL, using the survival analysis method to identify RD and thus suggest optimal dose steps for future BL DPT protocols. To propose a standardization of DPT protocols, and encompass the multitude of patterns of possible reactions, a large number of positive DPT is required. We used our 20-year experience of prospectively explored patients with suspicious of DHR to BL (using the same protocol over the years), included in our historico-prospective cohort, the *Drug Allergy and Hypersensitivity Database* (DAHD®).

Drug provocation tests are routinely performed for diagnosis of BL hypersensitivity, when ST are negative, and in the absence of contraindications (15). Nevertheless, standardization of BL DPT is lacking and most protocols are based on hypothesis-driven (i.e. *a priori*) reasoning. However, these experience-tailored methods cannot optimally discriminate between and identify various groups of reactive patients. Learned societies create a frame for DPT protocol, advising to start 'low' and then reach a therapeutic daily dose, but no dose steps are mentioned between the first and the last dose, leaving the choice to the experience of each group (1, 2). Performing DPT with a reduced number of hypothesis-driven dose steps (e.g. 10–100%, or 1–100% (18) or 100% (8), 25–25–50% (5)) although safe and efficient, is bound to miss in-between steps of eliciting doses and presumably expose patients to unnecessary higher doses and risks. The DPT is an iatrogenic diagnostic test and not only the frequency, but also the intensity of DPT reactions should be kept to a minimum. On the other hand, long precautious protocols (like our initial hypothesis-driven protocol) are time-consuming (for the patient and for the medical staff), require important human resources and are uneasy for patients [being a reason of discontent related to DPT (19) and larger personal P. Demoly and A.-M. Chiriac unpublished data].

In this study, due to the slow increase of dose steps, we were able to capture low RD at the very beginning of the DPT protocol. They were elicitors of anaphylactic events (64% of the total anaphylactic events occurred by RD = 3.2%), and therefore, these low doses should not be ignored. Additionally, we identified an index reaction of anaphylaxis as the risk factor to develop anaphylaxis during DPT, at RD ≤ 5%. This is not surprising, as a clinical history of anaphylaxis is also known as a risk factor for systemic reactions during BL ST, and the allergen exposure during ST is much lower than during DPT (20). The survival analysis method enabled us to identify RD and compare them in terms of numerical and severity added values. With all the above considerations in mind, and to meet practical purposes, we recommend the following dose increase steps for BL DPT: 5–15–30–50% of therapeutic daily dose (which would correspond to RD of 5%, 20%, 50% and 100% and actually matches the data-driven RD). We suggest keeping the additional steps of 0.01%, 0.1% and 1% in the DPT protocol for patients with an index reaction of anaphylaxis, as they will offer a greater level of protection to patients during DPT. In our experience, this theoretically long protocol would concern <1 in four tested patients (as of all our tested patients, about 25% have had a clinical history of anaphylaxis and ¾ of them would be positive in ST). In our case, the transition from a 7–8 step protocol to a shorter one will allow to gain time by reducing dose escalation duration (which would drop down to 1.5 h, compared to a median duration of 3 h in the present study, i.e. a 50% reduction) while still preserving patients' safety. This time gain can be easily translated into patient and hospital benefits.

All our calculations are on dose escalation. Regarding time steps, in our practice, we use the same protocols (30-min time intervals) for immediate and nonimmediate index reactions and we perform 1-day DPT till full therapeutic dose. Therefore, we cannot answer the question as to whether the reaction elicited by DPT is related to the time elapsed since previous dose steps, or to the RD itself. This theoretical question could be answered by sequential-dose DPT, performed on different days, with different doses in the same patients, but this is not a pragmatic solution.

There is indeed an ongoing debate in the drug allergy field, as to whether 1-day DPT protocol is sensitive enough (*vs* several-day DPT or full therapeutic course of treatment DPT), especially in patients with nonimmediate index reactions developing on Day 2 or further on during the initial course of treatment (2, 3, 10). There have not been any head-to-head comparisons between these different dosing approaches (as well as their cumulative doses) and the results reported by several studies are inconsistent. In the present study, we aimed to answer this question indirectly, and we analysed the chronology concordance of the DPT reaction and the index reaction (both in terms of delay after the last dose, and after the first day of treatment). We found a good match (excellent for immediate reactors 94.2% and 71.1% for nonimmediate reactors) between the chronology of the index reaction and the one elicited by DPT. All in all, only 7.7% of cases (16.5%, if cases with unknown delay are considered) reacted after a delay that was longer compared to the chronology of the index reaction. Moreover, data-driven analysis showed that irrespective of the chronology of the initial reaction after the first day of treatment, all but two patients (1.1%) reacted within 48 h after the last dose administered during DPT. This seems to strengthen the clinical empirical evidence that upon re-exposure, most DHR would occur faster or within the same lapse of time as the initial DHR. It also links up to the pharmacokinetics of BL antibiotics. In patients with normal renal function, the serum half-life ($t_{1/2}$) of most BL is 1–2 h, with a few exceptions (e.g. ceftazidime and ceftriaxone are more long-lasting, with a $t_{1/2}$ of 4–6 and 8–10 h, respectively) and it is generally considered that by $7t_{1/2}$, the drug has been eliminated (21). On the other hand, our analysis proves that 1-day DPT up to D_{ther} can elicit reactions, as far as 48 h after the last administered dose (in some singular cases, even further). Prolonged DPT do not take into account this window, because BL prolonged administration is immediately continued after Day 1 of DPT, for 3–7 days. It is therefore reasonable to assume that some of these positive DPT could have been elicited by the 1-day DPT only. Prolonged DPT seem to pick up as much as 11.5–15% more positives than 1-day DPT (10, 11). Nevertheless, the majority of papers reporting results of prolonged DPT report numbers (5, 12, 22) that are no different from those assimilated to the incidence of proven BL DHR in the general population, around 5–8% (23). Whether or not prolonged DPT can account for new sensitizations should be taken into consideration. The reactions elicited by prolonged DPT are classified as 'mild' cutaneous reactions. Therefore, the number needed to harm (NNH) is also an important issue. Drug provocation test is an intervention with not only potential allergic consequences. Prolonged DPT courses of antibiotics in subjects that are otherwise healthy have additional drawbacks (microbial resistance) and if the NNH is high, to pick up merely mild reactions, prolonged DPT might not be an efficient intervention. Specific memory T (and B) cells and effector T cells are by definition present in BL allergic patients, and there is no *in vivo* published evidence showing that a several-day DPT would perform better than a 1-day DPT to activate them. We believe that the adequate cumulative dose to trigger immune reaction would be more important than the timing of exposure alone as a 1-day DPT allows at least a 48 h stimulation of immune cells when one considers the above described $7t_{1/2}$ elimination time. Moreover, an *a posteriori* argument to validate the 1-day DPT comes from the evaluation of the NPV of DPT to BL, which has been shown to be high (94.1%) in a multicentre study using 1-day DPT and including more than 81% of patients with nonimmediate or unknown index reaction (13). An ongoing survey on a higher number of patients (multicentre study, from Montpellier and Rome) shows similar results (NPV 96%, unpublished data). Moreover, this high NPV has been confirmed in paediatric studies, using 1-day, but also prolonged DPT (24).

Finally, whether or not slow increase of doses, as in our former hypothesis-driven protocol, can induce desensitization is another controversial issue. However, in most cases, desensitization protocols start lower (1 : 10 000 to 1 : 1 000 000 of D_{ther}) and use smoother progression (25). Additionally, the evidence of high NPV while using this 30-min interval protocol is a counter argument to desensitization.

When analysing the clinical characteristics of the DPT events, we confirm that DPT needs an adequate environment and trained staff, as anaphylaxis may be elicited even in patients with nonanaphylactic index reaction. Discordant cases may occur, such as immediate reactors presenting with nonimmediate DPT DHR, and vice versa. Regarding nonimmediate reactions, clinicians should be aware of the risk of inducing more severe reactions than an index clinical history of mild cutaneous reaction, and therefore, contraindications to DPT are to be strictly observed. We did not find any difference between children and adults, which links up to the idea that DHR, although less frequent in children (26), have no age limitation in their clinical expression.

Our study has a few limitations. The choice of using a 'standardized' measure, that is RD, to describe thresholds for different BL and different protocols might raise issues regarding the heterogeneity of data. It is self-evident that BL differ in terms of D_{ther}. Moreover, over the past two decades, D_{ther} has been in continuous progression for certain BL, such as amoxicillin, due to antibiotic resistance. Nowadays, higher doses are used, compared to when we started performing DPT. To integrate this change and provide a common standard, we reported all RD as a percentage of the total dose reached by DPT at present. This allowed us to identify and communicate correct and practical data. Another limitation arises from having analysed single-centre data. This limitation will be overcome in a prospective phase (registration on

clinicaltrials.gov; No. NCT02839551), involving three centres in southern France, that will now evaluate the data-driven protocol.

In conclusion, we retrospectively analysed our experience with positive DPT to BL. Using the survival analysis method, we identified eliciting thresholds and we suggest the following optimal steps for future DPT: 5–15–30–50% of daily therapeutic dose (N.B.: with the above mentioned additional steps for index reactions of anaphylaxis). We confirm a 1-day protocol for immediate and mild nonimmediate reactors (while observing contraindications for DPT), for both children and adults, with a surveillance period of 2 h after the last administered dose, and a prolonged surveillance after discharge of 48 h.

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Author contributions

TR, AMC and PD initiated the study. TR, AMC, PJB and PD contributed to data collection. AMC, PB and NM performed the statistical analysis. AMC and TR wrote the initial manuscript. PD, PJB and NM revised the manuscript. All authors participated in the design of the study and interpretation of the results and approved the final version.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of patients who developed a more severe reaction during drug provocation tests, compared to their index reaction.

Table S2. Subgroup of peculiar patients who presented anaphylaxis w/o shock during or following drug provocation tests.

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