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Drug Allergy, Insect Sting Allergy, and Anaphylaxis

Safety of Drug Provocation Tests in Adults With and Without Clonal Mast Cell Disorders

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ABSTRACT

Background: Mastocytosis is a clonal mast cell disorder (CMD) characterized by the proliferation and accumulation of mast cells (MC) in different tissues. Anaphylaxis, resulting from massive MC activation and mediators' release, is reported in 22%–49% of mastocytosis cases, with drugs being potential triggers. Proper counseling on drug safety is crucial. We aim to demonstrate that, after a careful evaluation of clinical history and allergic work-up, drug provocation tests (DPT) are a safe and effective diagnostic tool in patients with CMD.

Methods: We enrolled 104 CMD patients with a suspicion of drug hypersensitivity reactions (DHR) or without known tolerated drugs and 100 control patients with DHR. The types of DHR and the results of DPT were compared between CMD and control groups.

Results: In both groups, previous DHR was mostly represented by skin reactions (46.4% in CMD and 82.9% in the control group); the most involved drugs were aminopenicillins and nonsteroidal anti-inflammatory drugs (NSAIDs). We performed 250 DPTs in the CMD group and 231 in the control group; challenges were well tolerated in both groups, except for 6 skin reactions: 1 in the CMD group (1.0%) and 5 in the control group (5%).

Conclusion: Drug challenge is a safe and effective diagnostic tool in patients with CMD. Moreover, patients that have never had adverse reactions to NSAIDs or antibiotics before the diagnosis of CMD do not need to undergo challenge tests. In contrast, patients with a history of reactions should avoid the culprit drugs and undergo DPT to identify a safe alternative drug.

Abbreviations: Adv-SM, advanced systemic mastocytosis; ASA, acetylsalicylic acid; BM, bone marrow; BMM, bone marrow mastocytosis; CM, cutaneous mastocytosis; CMD, clonal mast cell disorder; COX2, cyclooxygenase-2; DHR, drug hypersensitivity reactions; DPT, drug provocation test; GISM, Gruppo Italiano per lo Studio della Mastocitosi; ISM, indolent SM; LA, local anesthetic; MC, mast cells; MIS, mastocytosis in the skin; MMAS, monoclonal mast cell activation syndrome; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton pump inhibitor; RCM, radio contrast media; REMA, Spanish Network on Mastocytosis; s-BT, serum basal tryptase; SM, systemic mastocytosis; SSM, smoldering SM.

1 | Introduction

Mastocytosis is a clonal mast cell disorder (CMD) characterized by mast cell (MC) proliferation in skin and/or other organs. Cutaneous mastocytosis (CM) occurs mainly in children, while systemic mastocytosis (SM) mainly affects adults, often carrying the KIT D816V mutation [1].

According to the revised WHO classification [2], SM includes indolent SM (ISM), smoldering SM (SSM), aggressive SM (ASM), SM associated with another hematologic neoplasm (SM-AHN), and MC leukemia. Bone marrow mastocytosis (BMM) is a variant of ISM without skin involvement, low MC burden, absence of B findings (markers of a high mast cell burden without organ dysfunction, e.g., dense bone marrow [BM] infiltration with high serum tryptase, mild blood count changes, hepatomegaly, splenomegaly, or lymphadenopathy), and frequent association with anaphylaxis, mainly after hymenoptera sting [3]. Mastocytosis in the skin (MIS) refers to adults with CM not yet evaluated with BM biopsy [4]. Monoclonal MC activation syndrome (MMAS) describes patients without typical cutaneous lesions but with recurrent symptoms of MC activation and either the KIT D816V mutation or $CD25^+$ mast cells in their BM, without fulfilling sufficient SM diagnostic criteria [5].

The great majority of symptoms originate from MC activation and release of mediators; anaphylaxis is the most common expression of this situation and can be defined as an acute, suddenly occurring, severe hypersensitivity reaction involving at least two organ systems, including skin/mucosal tissue along with respiratory compromise, reduced blood pressure, severe gastrointestinal symptoms, or end-organ dysfunction. Alternatively, it can present as acute hypotension, bronchospasm, or laryngeal involvement after exposure to a known allergen, even without typical skin symptoms [6–8].

Mastocytosis in adults is associated with a history of anaphylaxis in 22%–49%, which represents a 100- to 1000-fold increased risk compared with the general population (0.05%–2%) [9, 10].

Drugs are known as triggers of anaphylaxis in patients with mastocytosis, although this association does not appear to be as strong as in the case of hymenoptera sting anaphylaxis, which represents the most common trigger in patients with SM or other CMD [11]. Nevertheless, patients with mastocytosis may experience fatal anaphylaxis after the administration of anesthetic drugs during the perioperative period, or following the intake of non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, and opioids [12]. Therefore, from a practical point of view, it is very important to know which drugs they can use and which ones they should avoid [13].

Several studies have analyzed the incidence of DHR in patients with mastocytosis, highlighting that NSAIDs, antibiotics, and perioperative agents are the most responsible for reactions [14–18]. The few guidelines available (ENDA/EAACI position paper [19], Carter et al. [20]) are based only on a few case reports and, therefore, lack studies on larger populations with CMD.

Drug provocation testing (DPT), also called drug challenge, is the gold standard for diagnosing drug hypersensitivity. In the general population, DPTs are considered safe when performed under appropriate medical supervision, with severe reactions occurring only rarely [21]. However, there are very few studies in the literature that evaluate the use of DPT in patients with mastocytosis. A study by the Italian group for the Study of Mastocytosis (GISM) demonstrated that the administration of local anesthesia in patients with CMD is to be considered a safe clinical practice [22]. Moreover, Hermans et al. [23] investigated the prevalence and severity of NSAID hypersensitivity reactions in patients with mastocytosis, concluding that it is safe to administer NSAIDs to most patients with mastocytosis if they do not have a history of prior NSAID hypersensitivity reactions. A recent study by Bent et al. [24] supported the safety of DPT in this population: in a large cohort study evaluating oral challenges with drugs and foods in patients with mastocytosis, anaphylaxis to drugs was reported in only 2.4% of cases (4 out of 170 challenges), with reactions occurring primarily to acetylsalicylic acid, tramadol, and flurbiprofen.

The aim of this study is to evaluate the safety of DPTs in CMD patients and to retrospectively identify the drugs more frequently triggering reactions in this population. Additionally, we compare the outcomes of DPT in CMD patients with those observed in the general population of drug-allergic individuals to contextualize the risk. Ultimately, we aim to define the rationale and key principles for performing DPT in patients with CMD and to propose evidence-based recommendations for clinical practice.

2 | Methods

We analyzed the characteristics of previous DHR and the outcomes of DPTs in patients with mastocytosis and the general population, enrolling a total of 204 patients. Figure 1 illustrates the study design, showing patient classification, the number and type of DPTs performed, and their outcomes.

2.1 | Patients and Drug Reactions Characteristics

We included 104 adult patients with mastocytosis who were referred to our Interdisciplinary Study Group for Mastocytosis (GISM) of Verona and the Allergy and Asthma Unit of the Hospital of Verona from January 2006 to December 2021. The diagnosis of mastocytosis was established according to WHO criteria. All patients underwent bone marrow biopsy and met the required diagnostic standards, defined as either one major plus one minor criterion or at least three minor criteria [25]. These patients underwent DPTs either because they had a previous DHR or lacked a history of tolerated NSAIDs or antibiotics after the diagnosis of CMD.

Patients with a previous DHR were classified as "CMD group A"; in contrast, patients without a history of DHR but with no known tolerated drugs after the diagnosis were defined as "CMD group B."

In the control group, we collected data from 100 adult patients referred to our Allergy and Asthma Unit from July 2021 to December 2021 for a history of DHR, who showed no signs or symptoms suggestive of mastocytosis (absence of typical skin lesions and/or other MC activation symptoms and normal levels of s-BT), or who had a negative REMA (Spanish Network on Mastocytosis)

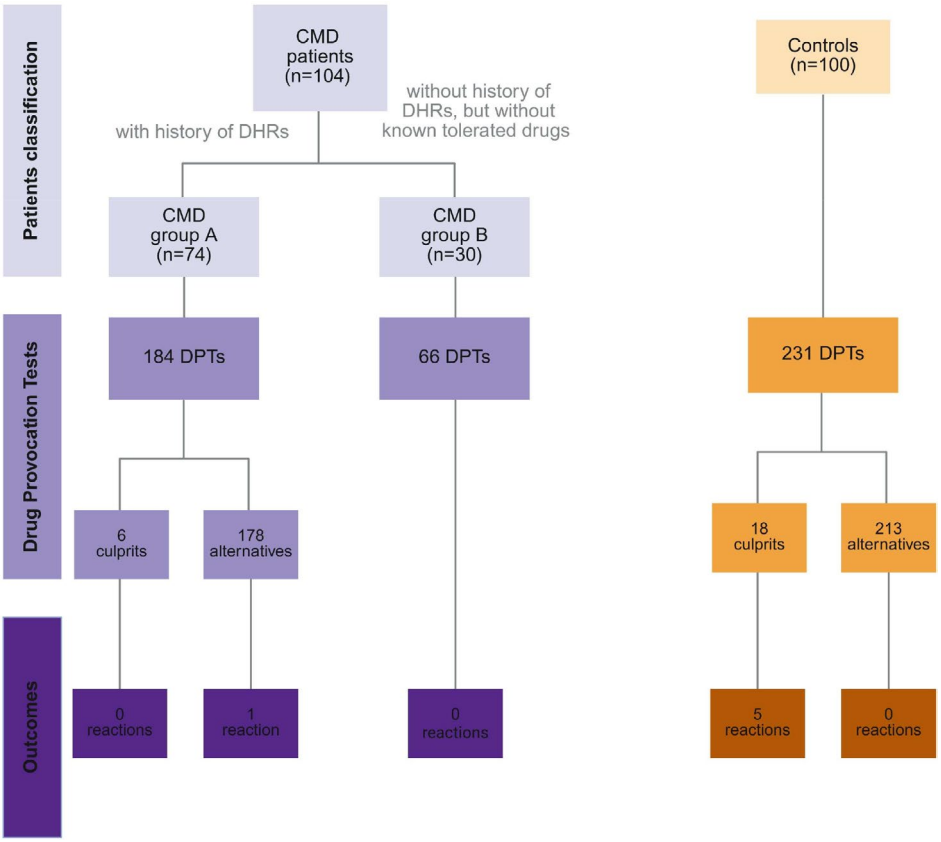

FIGURE 1 | Flowchart showing the classification of patients with clonal mast cell disorders (CMD) and controls, the number of drug provocation tests (DPTs) performed with culprit and alternative drugs, and the corresponding outcomes. CMD group A: Patients with a history of drug hypersensitivity reactions (DHRs); CMD group B: Patients without a history of DHRs but with no known tolerated drugs.

score [26]. For all patients, we collected demographic data, atopic disease, drug allergy history, severity of reactions, number of episodes, and, when available, allergy work-up results (skin prick test, intradermal test, specific IgE). Informed consent was obtained according to the guidelines of the Local Ethics Committee (protocol no. 1828, approved on May 12, 2010).

All reactions observed in our study were classified as immediate, typically occurring within 1 h, and in all cases within 6 h of drug administration. The severity of drug reactions was classified according to the Ring and Messmer Classification into four different grades [27]. For statistical analysis, reactions were pooled into two groups based on their severity: the "low-moderate" reaction group, which included grade I and grade II reactions, and the "severe systemic reaction" group, which included grade III and grade IV reactions.

2.2 | Drug Challenge

DPTs were performed according to the EAACI/ENDA position paper recommendations [21]. Indications included: exclusion of hypersensitivity when history was non-specific or low/intermediate risk, delabeling (especially penicillin allergy), identification of a safe alternative in cases where testing with the culprit was positive or not indicated, diagnosis when other tests were inconclusive/unavailable, and confirmation of tolerance to other drug classes. In most cases, the main indication was to identify a safe alternative to the culprit drug or to evaluate tolerance to drugs considered at lower risk (e.g., Cyclooxygenase-2 [COX-2] inhibitors among NSAIDs). DPT with the culprit drug was generally avoided in severe anaphylaxis (≥ Grade III) or when hypersensitivity was already proven. Alternative drugs were chosen with no structural similarity or known cross-reactivity, supported by available safety data, and, when possible, negative skin tests.

Skin prick tests, intradermal tests, and specific IgE were performed for those drug classes for which they were recommended. If negative, patients underwent DPT. Patients arrived at our Unit at 8:00 a.m. and remained under observation until 1:00 or 3:00 p.m., according to their allergic history and the challenge with culprit or alternative drugs. Vital signs (blood pressure, heart rate, peripheral oxygen saturation) were recorded before, during, and after the DPT. On the day of testing, patients were required to be in good health, without any signs of allergy or viral infection that could stimulate an immune response.

In patients with CMD, a venous access was placed, and saline was infused to keep it open. Any regular medication taken to maintain MC stability and limit the effect of MC mediators was continued during the test.

The oral route was favored when possible; typically, galenic formulations were used, but if unavailable, commercial preparations were administered. DPT involved administering the drug using

slow, incremental dose escalation at fixed time intervals and observed for the presence or absence of an objective reaction.

The challenges were single-blinded, and an inert compound always preceded the administration of the active drug to determine if psychological factors might influence patient reactivity through the nocebo effect [28]. All the tests were one-day DPT.

2.3 | Statistical Analysis

Comparisons among data were performed using the *T*-test to analyze mean values of continuous variables, and the Chi-square test to analyze categorical variables. All mean values are reported with their standard deviation, while median values are reported with the respective ranges. *p*-Values <0.05 were considered statistically significant.

3 | Results

3.1 | Patients and DHR Characteristics

The CMD group included 104 patients, of whom 46 were males (44.8%) and 58 (55.2%) were females. The median age was 58 years (range 26–87 years) (Table 1).

Sixty-four out of 104 patients (61.5%) had other atopic diseases (respiratory: $n=8$, 7.7%; food allergy: $n=20$, 19.2%; hymenoptera venom allergy: $n=36$, 34.6%). The median s-BT level was 25 ng/mL (range 4.2–505 ng/mL).

Anti-mediator therapies, such as antihistamines, histamine H2-receptor antagonists, and chromones, were ongoing for 41/104 patients (39.4%). No patients were receiving treatment with tyrosine kinase inhibitors or omalizumab.

Diagnoses were distributed as follows: CM ($n=3/104$; 2.9%), MIS ($n=2/104$; 1.9%), MMAS ($n=20/104$; 19.2%), and SM ($n=79/104$; 76%). In the SM group, BMM was the most frequent variant ($n=42/79$; 53.2%), followed by ISM ($n=29/79$; 36.7%), SM-AHN ($n=4/79$; 5.1%), smoldering SM (SSM) ($n=3/79$; 3.9%), and ASM ($n=1/79$; 1.3%).

Patients with SM were further divided into two groups according to the presence or absence of cutaneous involvement ($n=48/104$; 46.2% and $n=56/104$; 53.8%, respectively).

Among 104 patients with CMD, 74 (71.2%) were classified as CMD group A and 30 (28.8%) as CMD group B.

In CMD group A, no significant differences were found in the mean number of reactions between the 34 patients with

TABLE 1 | Comparison of patient features, previous DHR, and DPT outcomes between CMD (A and B) and control groups.

	CMD group A (74)	CMD group B (30)	Control group (100)	*p*-Value considering CMD A + B group and control group
Population characteristics				
Median age (years, range)	58 (26–87)	56 (32–82)	51 (7–80)	0.83
Female sex, N° (%)	30 (68.2%)	16 (53.3%)	71 (71%)	**0.02**
Atopic diseases, N° (%)	64 (61.5%)		41 (41%)	**0.003**
Respiratory	8 (7.7%)		35 (35%)	**<0.001**
Food allergy	20 (19.2%)		4 (4%)	**<0.001**
Hymenoptera venom allergy	36 (34.6%)		2 (2%)	**<0.001**
Drug hypersensitivity reactions (DHR) history				
Grade of more severe DHR				**0.004**
Low-moderate (I–II) n° pts. (%)	50 (67.6%)		86 (86.0%)	
Severe (III–IV) n° pts. (%)	24 (32.4%)		14 (14.0%)	
Drug Provocation Tests (DPT)				
N° of DPT	184	66	231	
Positive DPT, n° (%)	1 (0.4%)	0	5 (2.2%)	0.09
Reaction to placebo, n° (%)	8 (10%)	2 (6.6%)	6 (2.6%)	0.35

Note: Bold values indicate statistically significant differences ($p < 0.05$).

cutaneous involvement and the 40 patients without cutaneous involvement (1.71 ± 0.92 and 1.68 ± 0.94, *p*-value = 0.96).

Previous DHR were classified according to the Ring and Messmer classification based on severity. In CMD group A, out of 123 total reactions reported, 57 (46.3%) cases had a Grade I reaction, 27 (22.0%) had a Grade II reaction, 36 (29.3%) had a Grade III reaction, and 3 (2.4%) had a Grade IV reaction (Figure 2).

As regards the control group, there were 29 males and 71 females, with a median age of 51 years (range 7–80 years) (Table 1). Other atopic diseases were found in forty-one patients (41%): respiratory (35%), food allergy (4%), and hymenoptera venom allergy (2%).

Forty-six out of 100 control patients (46%) had DHR with one drug, whereas 54 (54%) had DHR with two or more drugs. We registered 186 DHRs with a mean number of 1.88 ± 1.03 events per patient, subdivided according to the Ring and Messmer classification; of these, 154 (82.9%) were classified as Grade I reactions, 15 (8%) as Grade II, and 17 (9.1%) as Grade III (Figure 2).

Among CMD group A patients, 42 (56.8%) reported reactions to a single drug (single reactors), whereas 32 patients (43.2%) reported reactions to several drugs (multiple reactors). The most frequently involved drugs were aminopenicillins (22; 17.7%) and NSAIDs (60; 48.4%). Other reactions were observed after the administration of radiocontrast media, proton pump inhibitors, local and general anesthesia, steroids, opioids, and antibiotics such as macrolides and quinolones. In the control group, aminopenicillins (35.9%) and anti-inflammatory drugs (35.3%) were the most frequently involved (Figure 3).

Comparing the CMD group A and the control group, statistically significant differences were observed in the frequency of reactions to ASA, which were more frequent in the CMD group (19 vs. 12, $p = 0.0136$). In contrast, reactions to paracetamol (3 vs. 14, $p = 0.0469$) and aminopenicillins (22 vs. 65, $p = 0.00056$) were more frequent in the control group. No significant differences were found for other drug classes, including other NSAIDs, cephalosporins, quinolones, local anesthetics, and steroids. Reactions to radiocontrast media and opioids were reported only in the CMD group, while a single reaction to pyrazolones occurred in the control group (Figure 3).

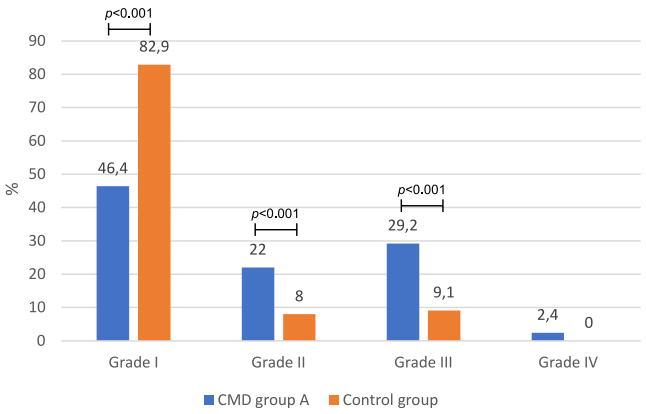

FIGURE 2 | Percentage distribution of previous drug hypersensitivity reactions classified by severity (Grade I–IV) according to the Ring and Messmer Classification among 74 patients in CMD group A (123 reactions) and 100 control patients (186 reactions).

Comparing the patients and DHR characteristics between the CMD group and the control group, statistically significant differences were found in terms of sex and severity of DHRs. In particular, the female sex was more prevalent in the control group ($p = 0.02$), and severe DHRs (grade III and IV) were more frequent in CMD group A compared to the controls ($p = 0.004$) (Table 1).

3.2 | Drug Provocation Tests

We performed a total of 250 DPTs in the CMD group (Figure 4). The drugs most frequently tested among antibiotics were macrolides and quinolones, while among anti-inflammatories were etoricoxib and nimesulide. Of note, we also performed 14 tests with ASA and 11 with ibuprofen.

In CMD group A, a total of 184 DPTs were performed. Of these, 6 were conducted with the culprit drug and were all well tolerated, while the remaining 178 involved alternative drugs. Among the latter, one patient developed a Grade I skin reaction after receiving a macrolide. This patient had a prior history of immediate skin rash (erythema) with aminopenicillins but tolerated DPTs with quinolones and cephalosporins (Table 2). In CMD group B, 66 drug provocation tests were performed to assess drug tolerance in the absence of prior reactions, and no adverse reactions occurred.

It is worth specifying that up to 9.4% of CMD patients presented the so-called "nocebo effect" after the administration of the inert compound, with reactions characterized by subjective symptoms such as itching, malaise, headache, dizziness, discomfort, shortness of breath, paresthesia, which were only objectively verified in two cases (tachycardia, flushing).

In the control group, we performed 231 DPTs (Figure 5). The most frequently tested drugs were cephalosporins (41; 17.7%), macrolides (29; 12.6%), and quinolones (25; 10.8%) among antibiotics, and etoricoxib (27; 11.7%) and nimesulide (19; 8.2%) among anti-inflammatories (Figure 5). Among the 231 DPTs performed, 18 involved the culprit drug, while the remaining 213 were conducted with alternative drugs (Table 2).

Only 5 DPTs (2.2%) resulted positive (2 with aminopenicillins, 1 with cephalosporins, 1 with ASA, 1 with paracetamol) and all of them presented with skin reactions (grade I). In particular:

- 3 patients with a suspected history of aminopenicillins allergy presented a positive DPT with beta-lactams (2 with aminopenicillins and 1 with cephalosporins), so they underwent DPT with alternative antibiotics without reactions;
- 1 patient with a suspected NSAID hypersensitivity reacted to paracetamol but tolerated ASA and ibuprofen;
- 1 patient with suspected NSAID hypersensitivity had a positive DPT with ASA but tolerated alternative NSAIDs (etoricoxib and nimesulide).

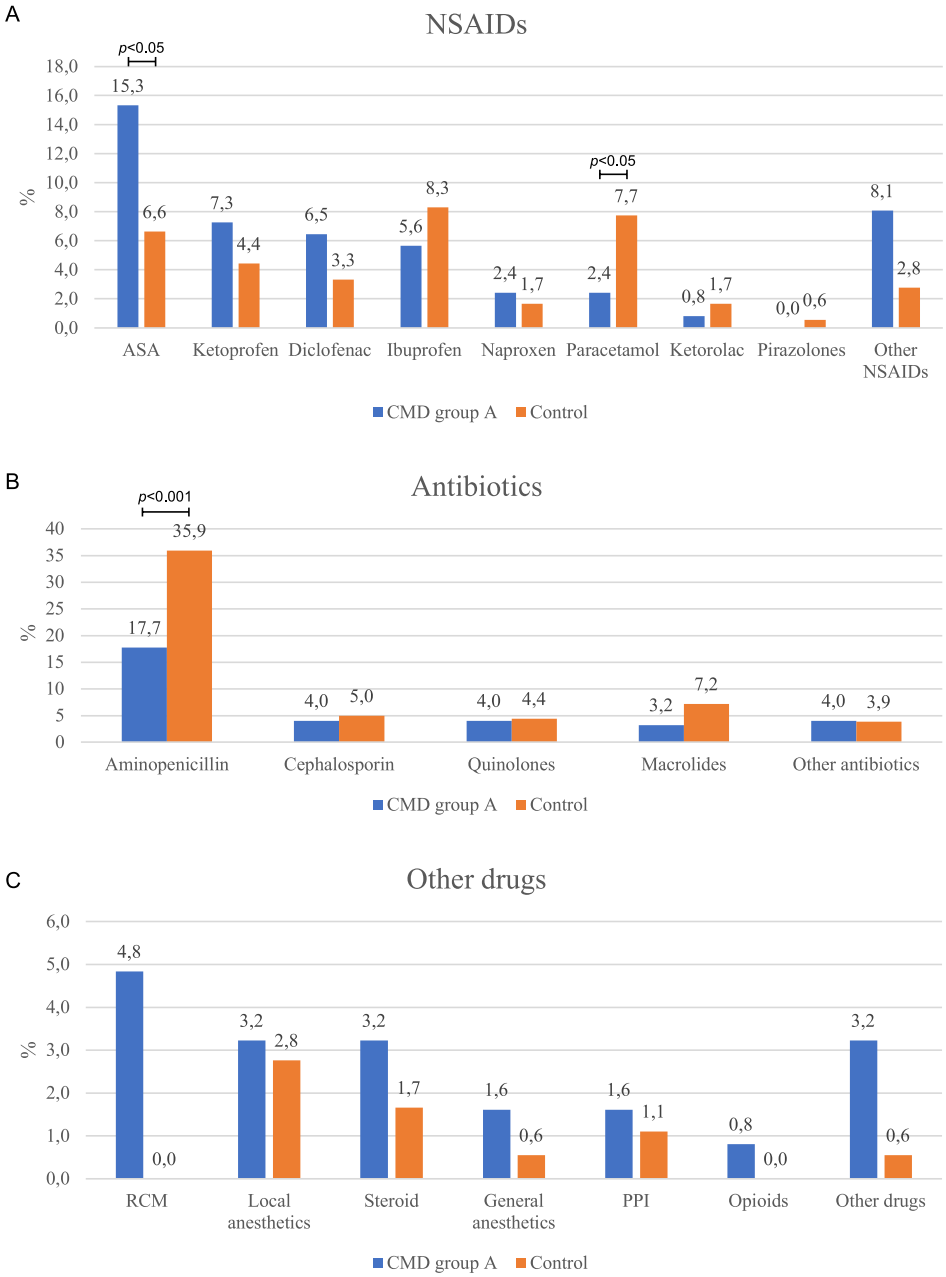

FIGURE 3 | (A) Percentage distribution of reported reactions to NSAIDs in CMD group A patients versus control subjects. (B) Percentage distribution of reported reactions to antibiotics in CMD group A patients versus control subjects. (C) Percentage distribution of reported reactions to other drugs in CMD group A patients versus control subjects. ASA, acetylsalicylic acid; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton pump inhibitors; RCM, radio contrast media.

In the control group we observed 6 reactions to placebo (6%), characterized by diarrhea, dizziness, itching, paresthesia, and shortness of breath. There were no significant differences between the CMD and control groups in the frequency of positive DPTs ($p = 0.09$). Similarly, no differences were observed between the two groups in the occurrence of nocebo effects ($p = 0.35$) (Table 1).

4 | Discussion

Drugs represent 5%–9% of the triggers of anaphylaxis in CMD patients, but data concerning the severity of hypersensitivity episodes, as well as the type of drug involved, are often missing [14–16]. Elicitors of these reactions frequently remain patient-reported, as it is difficult to verify them due to insufficient data, lack of reliable in vitro tests, and absence of DPTs. Recent literature has started to address the safety of DPTs in patients with CMD. A recent study demonstrated that anaphylaxis during drug and food challenges in mastocytosis patients is rare, challenging the perception that these tests pose an excessive risk in this population [24]. However, studies specifically evaluating the safety of DPTs in CMD patients remain scarce. Importantly, our study is the first to compare the outcomes of DPTs in CMD patients with those in a control group of drug-allergic individuals from the general population. This comparison provides a novel

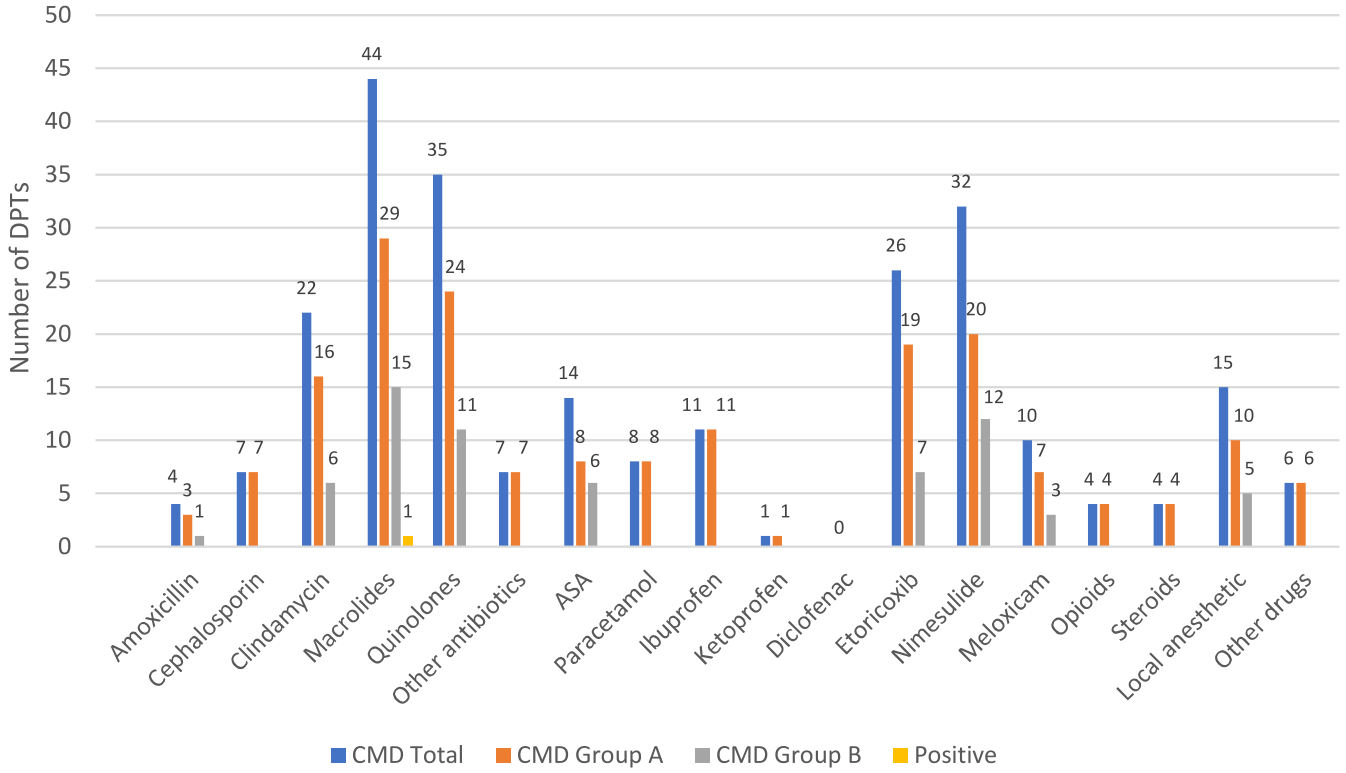

FIGURE 4 | Number of DPTs performed, divided by type of drug, in 104 patients with CMD (CMD total), CMD group A, CMD group B, and positive challenges (positive).

perspective, reinforcing that DPTs can be safely performed in CMD patients when conducted under appropriate medical supervision, with the aim of identifying safe pharmacological alternatives.

This study has some limitations that should be acknowledged. In most CMD patients, DPTs were performed with alternative drugs that have no known cross-reactivity with the culprit drug. This reflects real-life clinical practice, where both physicians and patients are often reluctant to re-expose themselves to the culprit drug in mastocytosis, even under controlled conditions. Conversely, in the control group, DPTs involved the culprit drug more often (18 patients) compared with the CMD group (6 patients). Despite this difference between groups, our findings still provide clinically relevant information: they support the safety of DPTs with appropriately selected alternative drugs in patients with CMD when performed under specialist supervision. This is of particular importance given the widespread hesitation to prescribe or administer untested drugs to patients with CMD—a practice that can unnecessarily limit therapeutic options even in the absence of prior hypersensitivity reactions.

Here we report data on 104 patients with mastocytosis and 100 patients without findings or symptoms suspected of mastocytosis who underwent DPT for previous DHR (control group). In CMD patients, we tested both antibiotics and NSAIDs in the majority of cases in order to provide patients with safe options for their use, as they would otherwise be reluctant to use such drugs.

As reported in the literature [12, 15, 20], drug reactions in CMD patients were primarily attributed to aminopenicillins (17.7%) and NSAIDs (48.4%), while in the control group, aminopenicillins accounted for 35.9% and anti-inflammatory drugs for 35.3%. Moreover, our findings revealed that patients who suffered from DHRs showed a female predominance, which is consistent with previous data indicating a higher risk for DHRs among females. The female prevalence was significantly higher in the control group (p-value = 0.02); this is probably due to the high number of BMM patients in the CMD group, who are typically males.

In our cohort, patients with a CMD were at a higher risk of developing severe systemic DHRs compared to patients in the control group (p-value 0.004). Cutaneous involvement does not appear to predispose to a greater risk of DHR.

Overall, we performed 250 drug challenges in the mastocytosis group and 231 in the control group. In both groups, macrolides and quinolones were the most frequently tested antibiotics, whereas etoricoxib and nimesulide were the more frequently tested anti-inflammatories.

Notably, only 6 out of 204 patients developed skin reactions after DPT, which were consequently considered positive: one (0.5%) among drug challenges performed in the CMD group (with macrolides) and 5 (2.5%) among those performed in the control group (2 with aminopenicillins, 1 with cephalosporins, 1 with paracetamol and 1 with ASA). There was no significant difference in the frequency of positive DPTs between the CMD and control groups, supporting the safety of DPTs even in patients with CMD.

It is interesting to observe that up to 35 patients tolerated quinolones, a class of antibiotics well known for its ability

TABLE 2 | Outcomes of DPTs with culprit and alternative drugs in CMD Group A and control group.

	Reaction culprit	DPT	Outcome
CMD group A (74 patients, 184 DPTs)			
DPT with culprit or same-class drug ($n = 6$)	Ibuprofen	Ibuprofen	Tolerated
	Ibuprofen	Ibuprofen	Tolerated
	Hydrocortisone	Betamethasone	Tolerated
	Methylprednisolone	Methylprednisolone	Tolerated
	Betamethasone	Betamethasone	Tolerated
	Mepivacaine	Mepivacaine	Tolerated
DPT with alternatives ($n = 178$) resulted positive ($n = 1$)	Aminopenicillins	Macrolides *Quinolones* *Cephalosporins*	**Reacted (grade I)** *Tolerated* *Tolerated*
Control group (100 patients, 231 DPTs)			
DPT with culprit or same-class drug ($n = 18$)	Naproxen	Acetylsalicylic acid	Tolerated
	Ibuprofen, Ketoprofen	Acetylsalicylic acid	Tolerated
	Paracetamol	Acetylsalicylic acid	Tolerated
	Nimesulide	Nimesulide	Tolerated
	Ketoprofen	Ketoprofen	Tolerated
	Ibuprofen	Ibuprofen	Tolerated
	Paracetamol	Paracetamol *Acetylsalicylic acid* *Ibuprofen*	**Reacted (grade I)** *Tolerated* *Tolerated*
	Ketoprofen, Ibuprofen	Acetylsalicylic acid *Etoricoxib* *Nimesulide*	**Reacted (grade I)** *Tolerated* *Tolerated*
	Betamethasone	Betamethasone	Tolerated
	Betamethasone	Methylprednisolone	Tolerated
	Mepivacaine	Mepivacaine	Tolerated
	Lidocaine	Lidocaine	Tolerated
	Lansoprazole	Lansoprazole	Tolerated
	Amoxicillin (Aminopenicillins)	Amoxicillin	**Reacted (grade I)**
	Amoxicillin (Aminopenicillins)	Amoxicillin	**Reacted (grade I)**
	Aminopenicillins	Cephalosporins	**Reacted (grade I)**
	Cephalosporins	Cephalosporins	Tolerated
	Ciprofloxacin	Ciprofloxacin	Tolerated
DPT with alternatives ($n = 213$) resulted positive ($n = 0$)			All tolerated

Note: Bold text indicates positive DPT outcomes and the corresponding reaction grade according to the Ring and Messmer classification.

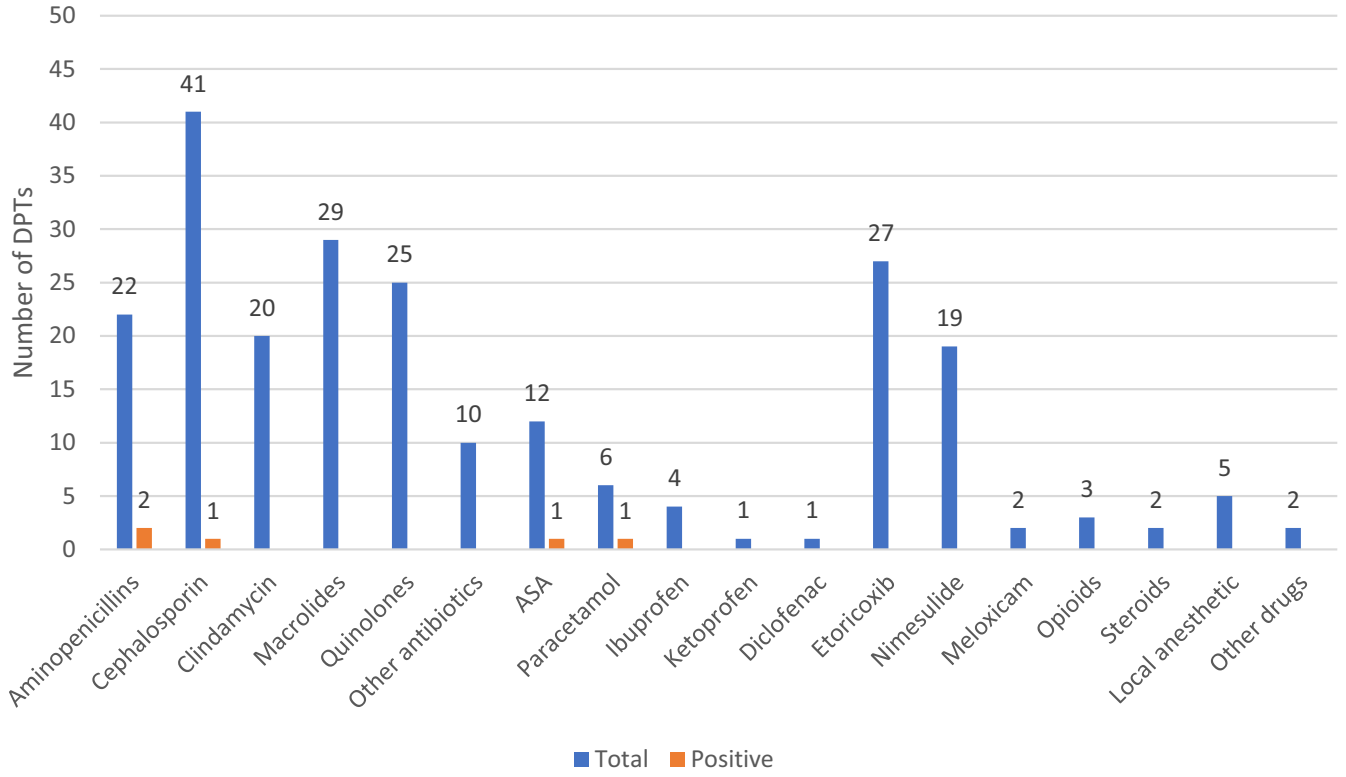

FIGURE 5 | Numbers and outcomes of DPT performed in our Unit, divided by type of drug in 100 control group patients.

to activate the Mas-related G protein-coupled receptor X2 (MRGPRX2), triggering direct mast cell degranulation in CMD contexts [29].

Drug challenges with ASA were safe in all 14 CMD patients tested. Despite concerns about prescribing NSAIDs to SM patients due to reported adverse reactions, the actual risk of anaphylaxis remains unclear. In a retrospective study of 470 mastocytosis patients, NSAIDs were the main trigger for drug-induced anaphylaxis (56%) [18], but another study of 388 adults found a lower frequency of NSAID hypersensitivity (11.3%), with severe reactions being rare (2.8%) [30]. Anxiety about NSAID-related hypersensitivity reactions leads to confusion among both physicians and patients, often resulting in NSAID avoidance. However, mastocytosis patients may require ASA for several reasons. Firstly, these patients are at an increased risk of cardiovascular morbidity, often necessitating ASA for secondary prevention. Secondly, aspirin is a well-known treatment for flushing in mastocytosis. Lastly, patients with mastocytosis frequently experience various types of pain for which effective analgesics might be necessary. Currently, they are often advised to only take acetaminophen, which is not always sufficient [23].

Hermans et al. [23] performed a double-blind placebo-controlled ASA challenge up to a cumulative dose of 520 mg among 50 adult patients with mastocytosis, finding ASA hypersensitivity in only 2%. In addition, a retrospective search of the entire outpatient cohort was conducted to obtain "real-life" data on NSAID hypersensitivity, finding a prevalence of 4.1%. They concluded that it is safe to administer NSAIDs to most patients with mastocytosis if they do not have a history of prior NSAID hypersensitivity reactions, and they can safely start at home. Extra caution should be taken in patients with a history of hypersensitivity reactions to other drugs or traditional risk factors for NSAID hypersensitivity.

In a study by Matito et al. [31], 418 adult and 223 pediatric mastocytosis patients were retrospectively reviewed. Overall, 87% of adults and 91% of pediatric patients tolerated NSAIDs. Their goal was to design an algorithm that could contribute to the early identification of mastocytosis patients who are at higher risk of being multiple reactors.

In our study, 14 patients with CMD underwent DPT with ASA, either for anti-inflammatory or cardiovascular reasons, and 11 patients with ibuprofen. Most patients did not have a history of DHR with NSAIDs, but after the diagnosis of CMD, they avoided using any anti-inflammatory or antiaggregant drugs, or they reported reactions with other classes of drugs. All patients had a negative DPT, and NSAIDs were well tolerated without any signs of cutaneous, respiratory, or cardiovascular symptoms.

Based on this finding and the fact that the severity of hypersensitivity reactions was not increased in patients with increased s-BT values, avoiding NSAIDs in CMD might be unwarranted and should be approached on a case-by-case basis [20]. Therefore, patients with a known tolerance to NSAIDs can continue the treatment, whereas those with a prior reaction to NSAIDs should undergo an allergy workup [19]. A retrospective study in 388 mastocytosis patients across Sweden and Italy confirmed that patients with a history of NSAID tolerance can safely continue their use [30]. Up to 9.4% of the CMD group and 6% of the

control group experienced a nocebo effect, developing symptoms after receiving an inert substance before the active drug. These were mostly subjective (nausea, headache, itching, temperature sensations), with tachycardia and flushing documented in only two cases. The nocebo effect is influenced by expectations, past experiences, setting, and drug appearance. An Italian study on 600 patients with DHR found nocebo reactions in 27% of cases across three centers (Verona, Naples, Genoa) [28].

The nocebo effect is frequently observed in patients with previous DHR, and it is mandatory to recognize false positive responses of oral challenges. In our study, the nocebo effect was observed in both groups without any significant difference (p-value 0.35).

In conclusion, MC proliferation in mastocytosis, as well as the increased releasability of MCs in CMD, may increase the risk of drug anaphylaxis; nevertheless, data concerning the frequency and severity of DHR in mastocytosis patients are lacking.

The diagnosis of DHR is often challenging and requires the same careful approach, regardless of the class of drug involved. Moreover, despite the potential use of certain allergy tests for specific drug classes, the diagnosis primarily relies on clinical findings.

This is one of the few studies in literature analyzing DTPs in patients with CMD. Our findings suggest that, after a careful evaluation of clinical history and an allergic work-up, drug challenges could represent a safe and effective diagnostic tool in patients with CMD as well as in the general population. Based on our findings, we propose a new algorithm: in patients without a positive history of adverse reactions to drugs, medications tolerated before the diagnosis of CMD are allowed, and DPT could be avoided. In contrast, in patients with a history of drug reactions, the culprit drugs should be avoided, and an alternative drug should be tested with a DPT.

Author Contributions

Patrizia Bonadonna and Francesca Nalin conceived the idea for the study and wrote the protocol. Patrizia Bonadonna, Francesca Nalin, Bianca Olivieri, Elisa Olivieri, Roberta Zanotti, Ilaria Tanasi, Andrea Bernardelli, Massimiliano Bonifacio, Francesca Norelli, Giorgia Marta, Valentina Gueli, and Giovanna Sfriso were responsible for the clinical follow-up and management of the patients. Francesca Nalin, Bianca Olivieri, and Francesco Olivieri wrote the manuscript. Francesco Olivieri performed the statistical analysis. Patrizia Bonadonna and Bianca Olivieri critically revised the manuscript. The final version was revised and approved by all authors.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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