DRUG HYPERSENSITIVITY: FROM MECHANISMS TO IMPROVED DIAGNOSIS AND STANDARDS OF CARE

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DRUG HYPERSENSITIVITY: FROM MECHANISMS TO IMPROVED DIAGNOSIS AND STANDARDS OF CARE

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EDITORIAL
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Editorial: Drug Hypersensitivity: From Mechanisms to Improved Diagnosis and Standards of Care

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Editorial on the Research Topic

Drug Hypersensitivity: From Mechanisms to Improved Diagnosis and Standards of Care

Adverse drugs reactions occur in 10–15% of hospitalized patients and cause 3–6% of hospital admissions, constituting adn important public health issue (Doña et al., 2012). Some of them are unpredictable and occur after exposure to a drug at doses normally tolerated. These reactions, called drug hypersensitivity reactions (DHRs), can be immunologically mediated (allergic reactions) or non-immunologically mediated (non-allergic hypersensitivity reactions), which comprise most reactions induced by nonsteroidal anti-inflammatory drugs (NSAIDs) (Pichler, 2019).

Their diagnosis and management is complex due to the lack of knowledge about underlying mechanisms, immunochemistry of the drugs that identify the epitope finally recognizes by the immunological system, variety of clinical symptoms, and heterogeneity among diagnostic protocols used in different centers (Torres et al., 2019). Therefore, an update on drug pharmacology, DHR classifications and mechanisms, and development of new tools and protocols to improve diagnosis and management is essential.

Regarding epidemiology, in a prospective study of 1,553 Kuwaiti patients reporting DHRs, performed by Al-Ahmad et al., NSAIDs and betalactams (BLs) were confirmed as the most commonly implicated drugs. In particular, reactions to BLs were mainly immediate (i.e., occurring within 1 h after drug administration) and the most common symptoms were urticaria, angioedema, and respiratory ones.

In patients with DHRs, diagnostic procedures must be performed by trained personnel in specialized facilities. These procedures include detailed clinical history, *in vivo* tests, mainly skin (STs) and drug provocation tests (DPTs), and *in vitro* assays (Romano et al., 2020). However, the allergy work-up of DHRs inevitably involves costs (Sobrino et al., 2020). In this regard, Sobrino-García et al. analyzed 20 studies regarding the costs of drug hypersensitivity assessment, especially those associated with mislabeling in NSAID or BL hypersensitivity. Their analysis revealed that the diagnosis of DHRs is not expensive, particularly considering the economic and clinical consequences of labeling patients with DHRs. Indeed, proper diagnostic work-ups of DHRs can save money to the health systems.

Nevertheless, the current diagnostic tests are not 100% sensitive (Mayorga et al., 2016). Regarding BLs, the chemical stability of benzylpenicillin reagents used for STs, the best validated *in vivo* method for immediate reactions to BLs, is essential to improve sensitivity. Mayorga et al. noted that butylamine-benzylpenicilloyl conjugates, present in commercial kits for STs, can

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suffer from an epimerization process, affecting their recognition by patients' IgE. This phenomenon may have important implications for the reproducibility and sensitivity of both *in vivo* and *in vitro* diagnostic tests.

Traditional diagnostic procedures include many steps, among which the last is DPT, although it is considered the gold standard. In fact, DPT can re-induce the index reaction and, for this reason, it is contraindicated in severe reactions (Romano et al., 2020). Felix and Kuschnir analyzed studies that used a different diagnostic approach, which consisted on performing DPTs with the suspected BLs without previous STs and which demonstrated that this strategy is safe and effective in children with mild non-immediate cutaneous reactions. However, they concluded that further studies involving various populations and age groups are needed to recommend this diagnostic approach, which may still prove to be a feasible and cost-effective strategy in the coming years.

DPT has been recommended not only to identify the culprit drug but also to identify safe alternatives in iodinated contrast media (ICM) allergy (Rosado Ingelmo et al., 2016). Doña et al. prospectively evaluated 101 patients reporting HSRs to ICMs by performing STs with a wide panel of ICMs. In negative STs, a single-blind placebo controlled DPT was carried out. If STs or DPTs were positive, tolerance was assessed with an alternative ICM negative in STs. Among the 101 subjects, 36 (35.6%) were allergic to more than one ICM. The percentage of patients reporting anaphylaxis was higher in patients allergic to multiple ICMs compared with patients allergic to a single ICM (50% vs. 25%).

Regarding *in vitro* tests, the lack of knowledge about the exact epitope, formed by part of the drug and part of the protein to which it is conjugated, able to induce the reaction, can be one of the causes for their non-optimal sensitivity (Ariza et al., 2015). This is specially challenging for clavulanic acid (CLV). Interestingly, Martín-Serrano et al. assessed the suitability of biotinylated analogues of CLV as probes to study protein haptenation by this BL. They demonstrated that these analogues could be valuable to identify protein targets and to get insights into the activation of the immune system by CLV and mechanisms involved in BL allergy. It is also challenging in drugs that are metabolized, like lapatinib, an anticancer drug generally used for breast and lung cancer. Andreu et al. showed that the parent drug and their metabolites have a high affinity to human proteins, especially to human serum albumin (HSA).

All these diagnostic approaches are aimed at identifying the responsible drug, which is essential for a correct patient management. Copaescu et al. provided an updated review of diagnostic methods in delayed T-cell-mediated DHRs, which include *in vivo* and *in vitro* tests. Regarding the latter methods, those most used are lymphocyte transformation test (LTT) and enzyme-linked ImmunoSpot (ELISpot) assay of cytokine release, typically of IFN-γ, after the patient's peripheral blood mononuclear cells are stimulated with the suspected drug. These tests and HLA-typing have shown to be really useful helping clinicians to prescribe safe and optimal treatments.

Regarding delayed DHRs mainly affecting the skin, it is crucial to consider the importance of a false allergy label due to viral-induced skin lesions leading to the unnecessary avoidance of drugs (Torres et al., 2003). Anci et al. reviewed current knowledge on the different aspects and potential roles of viruses in DHRs. They believed that further studies are needed to understand better the link between viruses and DHRs to improve management of patients presenting skin eruptions during treatments and, above all, to avoid useless drug avoidance, which is related to increased morbidity and mortality.

Once the culprit drug has been identified, the most common treatment is drug avoidance. However, sometimes this is not possible because the drug is the best or the only treatment for a certain pathology. In these situations, desensitization must be the preferred option (Broyles et al., 2020). This process is associated with inhibition of mast cell degranulation and cytokine production. Vultaggio et al. described the involvement of an antigen specific regulation of adaptive response, with an increase in regulatory cytokines, mainly represented by IL-10, and the appearance of IL-35 producing T regulatory cells during desensitization procedure.

Regarding NSAIDs hypersensitivity, the existence of a phenotypic heterogeneity of the NSAID-Exacerbated Respiratory Disease (NERD) has led to establishment of precision medicine strategies tailored/adapted to individual phenotypes/endotypes (Kowalski, 2019). Seong-Dae Woo et al. reviewed the current knowledge on pathophysiologic mechanisms and diagnosis/management approaches of this pathology. Therapeutical options may involve the avoidance of the drug, desensitization to acetylsalicylic acid (ASA), treatment with biologicals, or even dietary interventions.

There is still a lack of information on the complete molecular picture of the pathogenic mechanisms of NERD, although the involvement of platelet-adherent leukocytes and integrins has been described (Laidlaw et al., 2012). Jurado-Escobar et al. investigated such involvement in NSAID-induced urticaria/angioedema (NIUA), the most frequent clinical phenotype. Their results supported the participation of platelet-adherent leukocytes and integrins in this pathology and provided a link between these cells and arachidonic acid metabolism, although, unlike NERD, in NIUA they did not find a systemic imbalance in the frequency of $CD61^+$ cells/integrin expression or levels of LTE4.

Finally, Ghada et al. reviewed the current knowledge on hypersensitivity to rituximab, a chimeric monoclonal antibody used to treat various lymphoid malignancies, lymphoproliferative diseases, and rheumatologic disorders. Increased use of rituximab has been associated with an increase in several types of hypersensitivity reactions. The authors made a great effort to review several aspects of hypersensitivity reactions to this drug, in particular the clinical presentations, pathogenic mechanisms, and management of the reactions, including rapid desensitization.

In summary, in this Research Topic it has been revised the current knowledge on all aspects related to DHRs, especially epidemiology, pathogenic mechanisms, immunochemistry of the drugs, diagnosis, and management, including the latest developments.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

Ariza, A., Mayorga, C., Fernandez, T. D., Barbero, N., Martín-Serrano, A., Pérez-Sala, D., et al. (2015). Hypersensitivity Reactions to β-lactams: Relevance of Hapten-Protein Conjugates. *J. Investig. Allergol. Clin. Immunol.* 25 (1), 12–25.

Broyles, A. D., Banerji, A., and Castells, M. (2020). Practical Guidance for the Evaluation and Management of Drug Hypersensitivity: General Concepts. *J. Allergy Clin. Immunol. Pract.* 8 (9S), S3–S15. doi:10.1016/j.jaip.2020.08.002

Doña, I., Blanca-López, N., Torres, M. J., García-Campos, J., and García-Núñez, I., (2012). Drug Hypersensitivity Reactions: Response Patterns, Drug Involved, and Temporal Variations in a Large Series of Patients. *J. Investig. Allergol. Clin. Immunol.* 22 (5), 363–371.

Kowalski, M. L. (2019). Heterogeneity of NSAID-Exacerbated Respiratory Disease. *Curr. Opin. Pulm. Med.* 25 (1), 64–70. doi:10.1097/mcp.0000000000000530

Laidlaw, T. M., Kidder, M. S., Bhattacharyya, N., Xing, W., Shen, S., Milne, G. L., et al. (2012). Cysteinyl Leukotriene Overproduction in Aspirin-Exacerbated Respiratory Disease Is Driven by Platelet-Adherent Leukocytes. *Blood* 119 (16), 3790–3798. doi:10.1182/blood-2011-10-384826

Mayorga, C., Celik, G., Rouzaire, P., Whitaker, P., Bonadonna, P., Rodrigues-Cernadas, J., et al. (2016). In Vitrotests for Drug Hypersensitivity Reactions: an ENDA/EAACI Drug Allergy Interest Group Position Paper. *Allergy* 71 (8), 1103–1134. doi:10.1111/all.12886

Pichler, W. J. (2019). *Immune Pathomechanism and Classification of Drug Hypersensitivity*. Allergy. doi:10.1111/all.13765

Romano, A., Atanaskovic-Markovic, M., Barbaud, A., Bircher, A. J., Brockow, K., Caubet, J. C., et al. (2020). Towards a More Precise Diagnosis of Hypersensitivity to Beta-lactams - an EAACI Position Paper. *Allergy* 75 (6), 1300–1315. doi:10.1111/all.14122

Rosado Ingelmo, A., Doña Diaz, I., Cabañas Moreno, R., Moya Quesada, M., García-Avilés, C., García Nuñez, I., et al. (2016). Clinical Practice Guidelines for Diagnosis and Management of Hypersensitivity Reactions to Contrast Media. *J. Investig. Allergol. Clin. Immunol.* 26 (3), 144–155. doi:10.18176/jiaci.0058

Sobrino, M., Muñoz-Bellido, F. J., Macías, E., Lázaro-Sastre, M., de Arriba-Méndez, S., and Dávila, I. (2020). A Prospective Study of Costs Associated with the Evaluation of β-Lactam Allergy in Children. *J. Pediatr.* 223, 108–113. doi:10.1016/j.jpeds.2020.04.018

Torres, M. J., Celik, G. E., Whitaker, P., Atanaskovic-Markovic, M., Barbaud, A., and Bircher, A. (2019). *A EAACI Drug Allergy Interest Group Survey on How European Allergy Specialists deal with Beta-Lactam Allergy*. Allergy.

Torres, M. J., Corzo, J. L., Leyva, L., Mayorga, C., Garcia-Martin, F. J., Antunez, C., et al. (2003). Differences in the Immunological Responses in Drug- and Virus-Induced Cutaneous Reactions in Children. *Blood Cell Mol. Dis.* 30 (1), 124–131. doi:10.1016/s1079-9796(03)00004-4

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REVIEW
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NSAID-Exacerbated Respiratory Disease (NERD): From Pathogenesis to Improved Care

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Nonsteroidal antiinflammatory drug (NSAID)-exacerbated respiratory disease (NERD) is characterized by moderate-to-severe asthma and a higher prevalence of chronic rhinosinusitis/nasal polyps, but is a highly heterogeneous disorder with various clinical manifestations. Two major pathogenic mechanisms are: (1) overproduction of cysteinyl leukotrienes with dysregulation of arachidonic acid metabolism and (2) increased type 2 eosinophilic inflammation affected by genetic mechanisms. Aspirin challenge is the gold standard to diagnose NERD, whereas reliable *in vitro* biomarkers have yet not been identified. Therapeutic approaches have been done on the basis of disease severity with the avoidance of culprit and cross-reacting NSAIDs, and when indicated, aspirin desensitization is an effective treatment option. Biologic approaches targeting Type 2 cytokines are emerging as potential therapeutic options. Here, we summarize the up-to-date evidence of pathophysiologic mechanisms and diagnosis/management approaches to the patients with NERD with its phenotypic classification.

Keywords: nonsteroidal antiinflammatory drugs, hypersensitivity, asthma, rhinitis, eosinophil, leukotrienes, diagnosis, treatment

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INTRODUCTION

Aspirin (acetylsalicylic acid, ASA) and nonsteroidal antiinflammatory drugs (NSAIDs) are the most commonly prescribed drugs in the world (Doña et al., 2012); however, they are considered the most common causes of hypersensitivity reactions to drugs (Blanca-Lopez et al., 2018). Hypersensitivity reactions to NSAIDs have recently been classified by the European Academy of Allergy and Clinical Immunology (EAACI) and European Network of Drug Allergy (ENDA): 1) pharmacologic reactions (mediated by cyclooxygenase [COX]-1 inhibitions) include NSAID-exacerbated respiratory disease (NERD), NSAID-exacerbated cutaneous disease (NECD) and NSAID-induced urticarial/angioedema (NIUA), and present cross-intolerance to various COX-1 inhibitors; 2) selective responses (mediated by immunologic mechanisms) include single NSAIDs-induced urticaria, angioedema and/or anaphylaxis (SNIUAA) and single NSAIDs-induced delayed hypersensitivity reactions (SNIDHR) (Kowalski and Stevenson, 2013). NERD is a major phenotype among cross-intolerant categories of NSAID hypersensitivity and had been called ASA-induced asthma, ASA-intolerant asthma, ASA-sensitive asthma; however, NERD and ASA-exacerbated respiratory disease (AERD) are commonly used (Sánchez-Borges, 2019). The prevalence of NERD is reported to be 5.5% to 12.4% in the general population (Lee et al., 2018a;

Chu et al., 2019; Taniguchi et al., 2019), 7.1% among adult asthmatics and 14.9% among severe asthmatics (Rajan et al., 2015), while it rarely occurs in children (Taniguchi et al., 2019). No relationships were found with family history or NSAID administration history (Kowalski et al., 2011; Taniguchi et al., 2019).

NERD is characterized by moderate-to-severe asthma and a higher prevalence of chronic rhinosinusitis (CRS) nasal polyps (NPs) with persistent eosinophilic inflammation in the upper and lower airways (Taniguchi et al., 2019) as well as NSAID hypersensitivity where cysteinyl leukotrienes (CysLTs) over-production and chronic type 2 airway inflammation are key findings (Taniguchi et al., 2019). The diagnosis of NERD is confirmed by ASA challenge (*via* orally, bronchially or nasally route) and supported by potential biomarkers (Pham et al., 2017; Cingi and Bayar Muluk, 2020). In addition, *in vitro* cell activation tests and radiological imaging with nasal endoscopy can aid in NERD diagnosis (Taniguchi et al., 2019). This review updates the current knowledge on pathophysiologic mechanisms including molecular genetic mechanisms as well as the diagnosis and treatment of NERD.

CLINICAL FEATURES

NERD is characterized by chronic type 2 inflammation in the upper and lower airways; therefore, patients suffer from chronic persistent asthmatic symptoms and CRS with/without NPs, which are exacerbated by ASA/NSAID exposure and refractory to conventional medical or surgical treatment. Some patients are accompanied by cutaneous symptoms such as urticaria, angioedema, flushing or gastrointestinal symptoms (Buchheit and Laidlaw, 2016). Previous studies suggested that NERD is more common in females (middle-age onset) and non-atopics (Choi et al., 2015; Trinh et al., 2018). It was reported that rhinitis symptoms appear and then evolve into CRS which worsens asthmatic symptoms, subsequently followed by ASA intolerance (Szczeklik et al., 2000). However, their clinical presentations and courses have been found to be heterogeneous. It has been increasingly required to classify the subphenotypes of NERD according to its clinical features. One study demonstrated 4 subphenotypes by applying a latent class analysis in a Polish cohort: class 1 patients showing moderate asthma with upper airway symptoms and blood eosinophilia; class 2 patients showing mild asthma with low healthcare use; class 3 patients showing severe asthma with severe exacerbation and airway obstruction; and class 4 patients showing poorly controlled asthma with frequent and severe exacerbation (Bochenek et al., 2014). Another study showed 4 subtypes presenting distinct clinical/biochemical findings in a Korean cohort using a 2-step cluster analysis based on 3 clinical phenotypes (urticaria, CRS and atopy status): subtype 1 (NERD with CRS/atopy and no urticaria), subtype 2 (NERD with CRS and no urticaria/atopy), subtype 3 (NERD without CRS/urticaria), and subtype 4 (NERD with acute/chronic urticaria exacerbated by NSAID exposure) (Lee et al., 2017). Each subtype had distinct features in the aspect of female proportion, the degree of eosinophilia, leukotriene (LT) E_4 metabolite levels, the frequency of asthma exacerbation, medication requirements (high-dose ICS-LABA or systemic corticosteroids) and asthma severity, suggesting that stratified strategies according to subtype classification may help achieve better clinical outcomes in the management of NERD.

PATHOPHYSIOLOGY

The major upper and lower airway symptoms of NERD are mediated by increased levels of CysLTs with dysregulation of arachidonic acid (AA) metabolism and intense type 2/ eosinophilic inflammation (Cingi and Bayar Muluk, 2020).

CysLTs Overproduction

In the COX and LOX pathways, AA is metabolized to CysLTs (mostly LTE_4, *via* 5-lipoxygenase [5-LO] and LTC_4 synthase [LTC4S]), prostaglandin (PG) pathway (PGE_2, PGF_2, PGI_2 and PGD_2) and thromboxanes (TBX) A_2 by PG synthase and TBX synthase (Szczeklik, 1990), where enhanced synthesis of CysLTs synthesis with reduced level of PGE_2 is a major finding in NERD (Pham et al., 2016; Pham et al., 2017; Lee et al., 2018a; Yin et al., 2020). NERD patients have higher levels of CysLTs (especially LTE_4) mainly derived from various inflammatory cells, including neutrophils, monocytes, and basophils, eosinophils and mast cells, which further increases after ASA/NSAID exposure compared to asthmatic patients with ASA/NSAID tolerance (ATA). Moreover, the increased expression of 5-LO and LTC4S was noted in NERD patients with overproduction of CysLTs; increased CysLTs bind to CysLT receptor 1/2, subsequently inducing bronchoconstriction and amplifying inflammatory signal pathways (Jonsson, 1998; Yonetomi et al., 2015; Steinke and Wilson, 2016; Sekioka et al., 2017). Among PGs, PGE_2/PGD_2 play a major role in the pathogenesis of NERD. Increased PGD_2 (released from mast cells and eosinophils) binds to prostanoid receptors to induce bronchoconstriction (Säfholm et al., 2015), and also binds to chemoattractant receptor-homologous molecule expressed on TH2 cells (CRTH2) to induce chemotaxis and activate eosinophils/basophils/Th2 cells/innate lymphoid cells (ILC2) (Hirai et al., 2001; Woessner, 2017), accelerating type 2 airway inflammation (Chang et al., 2014). The down-regulation of PGE_2 biosynthesis, especially in peripheral blood leukocytes, nasal epithelial cells and nasal fibroblasts, was noted in patients with NERD (Laidlaw and Boyce, 2013; Cahill et al., 2016; Pham et al., 2017). PGE_2 has protective effects against bronchoconstriction, recruitment of eosinophils and degranulation of mast cells after binding to E prostanoid 2 (EP_2) receptors (Feng et al., 2006; Sturm et al., 2008); therefore, reduced levels of PGE_2 in NERD cannot suppress the signal of 5-LO pathways through IL-10-dependent mechanisms (Harizi et al., 2003). Furthermore, the lower expression of EP_2 receptors is closely associated with abnormal regulation of the autocrine loop involved in COX pathways (IL-1R1, COX-2, mPGES) in NERD patients (Cahill et al., 2015; Machado-Carvalho et al., 2016). This can be explained that COX-2 could not sufficiently produce PGH_2

(the first unstable precursors of PG products from AA metabolism) without COX-1 (Uematsu et al., 2002). Therefore, reduction in PGE_2 and its receptor levels could contribute to CysLTs overproduction in NERD patients. Lipoxin (LX) A4 and its epimer (15-epi-LXA4) are also called as the ASA-triggered lipoxins, and have antiinflammatory effects in airway inflammation (Pham et al., 2017; Sokolowska et al., 2020). Their receptor termed formyl peptide receptor 2 (FPR_2) is expressed on human neutrophils, eosinophils, macrophages, T cells, ILCs (ILC2 and NK cells) and epithelial cells of the respiratory tract. After binding their receptors, it leads to the restoration of epithelial barrier function and resolution of allergic inflammation through down-regulation of chemotaxis and cell activation (Barnig et al., 2013; Sokolowska et al., 2020). In the context of NERD, the concentration of LXA4 in the whole blood, sputum and bronchoalveolar lavage fluid, and 15-epi-LXA4 in the urine from NERD patients were lower than those in ATA patients. Additionally, their level has a negative correlation with worsening of airflow obstruction in patients with severe asthma (Christie et al., 1992; Sanak et al., 2000; Kupczyk et al., 2009; Yamaguchi et al., 2011). There was a significant increase in the FPR_2 expression of NK cells and ILC2s from patients with severe asthma compared with those with milder asthma (Barnig et al., 2013). All of the studies suggested that LXA4 and its epimer can be considered the potential therapeutics in the treatment of NERD (**Figure 1**). NSAID-induced inhibition of the COX pathway leads to shunting of AA metabolism down the 5-LO arm (Palikhe et al., 2009; Dominas et al., 2020). This is indirectly evidenced through the decreased level of antiinflammatory PG/LX (LXA_4, 15-epi-LXA_4, PGE_2) and increased levels of the pro-inflammatory CysLTs (Christie et al., 1992; Sanak et al., 2000; Harizi et al., 2003; Kupczyk et al., 2009; Yamaguchi et al., 2011).

Enhanced Type 2 Airway Inflammation

NERD is characterized by persistent eosinophil activation (presenting severe asthma, CRS and NPs) and CysLTs overproduction in which increased CysLTs contributes to driving type 2 inflammatory responses (Lee et al., 2018a; Rusznak and Peebles, 2019; Taniguchi et al., 2019). The key inflammatory cells in NERD are eosinophils and mast cells, which are closely interacting with other inflammatory and structural cells including basophils, platelets, neutrophils and epithelial cells. Regarding the activation mechanisms of eosinophils, both Th2 *cells* and ILC2 could activate eosinophils *via* release of IL-4, IL-5 and IL-13; moreover, activated eosinophils release the eosinophil extracellular traps (EETs), enhancing type 2 inflammation *via* interacting with epithelial cells and autocrine functions of eosinophils in the asthmatic airway (Pham et al., 2017; Choi et al., 2019b; Yin et al., 2020). There have been some data demonstrating epithelial dysfunction related to type 2 inflammation in NERD: 1) lower levels of SPD (protective function against eosinophilia) (Choi et al., 2019a), 2) increased epithelial folliculin and periostin levels (Kim M. A. et al., 2014; Trinh et al., 2018; Choi et al., 2019b), 3) increased CysLT-induced signaling (binding to CysLT2R or CysLT3R) in airway epithelial cells to induce the release of pro-inflammatory

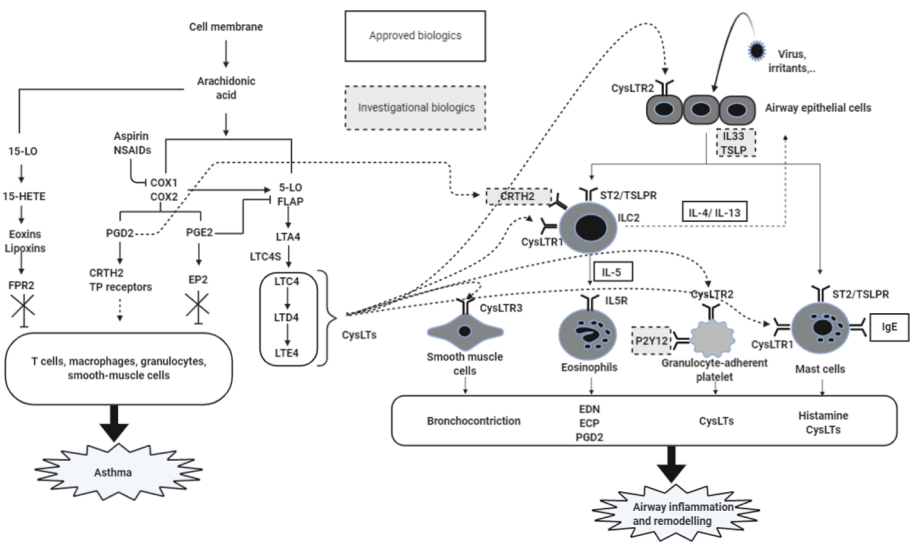

FIGURE 1 | Mechanisms of airway inflammation in NERD. Increased levels of CysLTs and PGD_2 as well as a decrease in the PGE_2 level caused by the AA metabolism dysregulation are the main mechanism for promoting the severity of NERD. Released CysLTs, PGD_2, and PGE_2 regulate inflammatory cells *via* receptors expressed on individual cells (eosinophils, ILC2, mast cells, smooth muscle cells, granulocyte-adherent platelet, and neutrophils). These activated cells release cytokines, histamine, CysLTs, and PGD_2, contributing to airway inflammation and remodeling in airway mucosa of NERD patients. 5-LO, 5-lipoxygenase; COX, cyclooxygenase; CysLTs, cysteinyl leukotrienes; PGs, prostaglandins; TBX, thromboxane; LT, leukotrienes; 15-HETE, 15-hydroeicosatetraenoic acid; FPR2, formyl peptide receptor 2; CysLTR, cysteinyl leukotrienes receptors; LTC4S, LTC4 synthase; EP2, E prostanoid 2; CRTH2, chemoattractant receptor-homologous molecule expressed on TH2 cells; TP receptors, T prostanoid receptors; IL, interleukin; TSLP, thymic stromal lymphopoietin; TSLPR, TSLP receptor; ILC2, innate lymphoid type 2 cells; Th2: T helper 2; ECP, eosinophil cationic protein; EDN: eosinophil-derived neurotoxin; IL5R, interleukin 5 receptor.

cytokines including IL-33, TSLP and IL-25 (Corrigan et al., 2005), leading to type 2/eosinophilic inflammation and remodeling in NERD (Ulambayar et al., 2019).

Recent studies suggested that the activation of neutrophils may be related to the severity of airway inflammation in NERD (Kim et al., 2019), although the exact mechanism is still not fully elucidated. Increased LTB4 levels (mostly formed from neutrophils) and reactive oxygen species release after N-formyl-methionyl-leucyl-phenylalanine stimulation were noted in patients with NERD compared to ATA patients (Mita et al., 2004; Kim et al., 2019). In addition, platelets are activated by CysLTR2 on their surfaces to release IL33 and to interact with leukocytes through binding P-selectin (CD62P)–P-selectin glycoprotein ligand 1, GPIIb/IIIa-Mac-1 and CD40 ligand (CD40L)–CD40 (Laidlaw et al., 2012; Mitsui et al., 2016; Liu et al., 2019; Taniguchi et al., 2019). The activation of platelets and adherent leukocytes with platelets leads to the transmigration of leukocytes into inflammatory airway tissue with increased CysLTs, suggesting that platelet-aggregated granulocytes promote severe and persistent airway inflammation in NERD patients (Laidlaw and Boyce, 2013; Laidlaw et al., 2014; Mitsui et al., 2016).

Genetic Mechanisms

Many genetic studies have focused on CysLTs-related and eosinophil activating genes (major pathogenic mechanisms) according to single nucleotide polymorphisms (SNPs) and genome-wide association studies (GWASs) (Pavón-Romero et al., 2017). (**Table 1**) *HLA* DPB1*0301 has been regarded as a strong genetic marker and replicated in the 2 ethnic groups Polish and Korean populations (Dekker et al., 1997; Choi et al., 2004a). Patients suffering from this allele manifested the typical clinical characteristics of NERD, and had lower FEV1 levels and a higher prevalence of CRS and/or NPs (Choi et al., 2004a). The GWAS demonstrated several significant SNPs (*HLA-DPB1*, rs3128965, *DPP10* rs17048175 in a Korean population, *TSLP* rs1837253 in a Japanese population, etc.) which were associated with the phenotypes of NERD (Park et al., 2013; Kim S. H. et al., 2014; Kim et al., 2015). The genetic polymorphism studies identifying the SNPs related to CysLTs synthesis demonstrated several significant SNPs: the promoter polymorphisms at the *LTC4S* -444 A>C in a Polish population (Sanak et al., 1997), although it was not replicated in the other populations as the US, Japanese and Korean (Van Sambeek et al., 2000; Kawagishi et al., 2002; Choi et al., 2004b). The SNPs of G-coupled receptors (*CysLTR1* -634C>T, -475 A>C, -336 A>G, *CysLTR2* -819 T>G, 2078 C>T, 2534 A>G) lead to amplify the biological activity of CysLTs, the SNPs of prostanoid receptor genes (*PTGER2* -616 C>G, -166 G>A, *PTGER3* -1709 T>A, *PTGER4* -1254 A>G, *PTGIR* 1915 T>A, *TBXA2R* -4684 C>T, 795 T>C) were associated with the development of NERD (Park et al., 2005; Kim et al., 2006; Kim et al., 2007). Regarding the SNPs related to eosinophil activation, including those of the chemokine CC motif receptor (*CCR3* −520 T>C), chemoattractant receptor molecular expressed in Th2 cells (*CRTH2* −466 T>C) and *IL5R* (-5993 G>A), were reported (Kim et al., 2008; Palikhe et al., 2010; Losol et al., 2013). Epigenetic factors, including exposure to NSAIDs and other stimuli, be also revealed to contribute to the development of NERD (Pham et al., 2017; Yin et al., 2020); DNA methylation associated with some SNPs (PGE synthesis, PGS, ALOX4AP, LTC4S, etc.) may contribute to presenting more severe phenotypes of NERD (Lee et al., 2019). Further replication studies in diverse ethnic groups are needed to clarify their functional roles in parallel with other omics markers with subphenotype classification.

DIAGNOSIS

A diagnosis of NERD is fundamentally based on the patient's history. NERD is suspected in patients having a history of upper/lower respiratory reactions after ingestion of ASA/NSAIDs or suffering from asthma along with CRS and NPs, (Choi et al., 2015). Some patients have a definitive history of adverse reactions to ASA/NSAIDs: however, many patients have not experienced hypersensitivity reactions (Palikhe et al., 2009). One study showed that 14% of patients who thought they had NERD based on symptoms were negative for oral aspirin challenge (Dursun et al., 2008). Thus, ASA challenge, as the gold standard for diagnosing NERD, is required to confirm or exclude hypersensitivity in patients with unclear history of adverse reactions.

There are 3 types of the ASA challenge test *via* the oral, bronchial and nasal routes. The oral challenge test is a more commonly used and convenient approach compared to other challenge tests in that it mimics natural exposure (Adkinson et al., 2013). It may be more suitable for investigating systemic adverse reactions to NSAIDs. Bronchial challenge with lysine-aspirin is safer and quicker, but shows lower sensitivity than the oral test. Nasal challenge is recommended for patients with predominant nasal symptoms, but the sensitivity is lower (Lee et al., 2018a; Kowalski et al., 2019). The EAACI recommended the oral challenge protocol with starting 20-40 mg of aspirin and gradually increasing the dose at 2 hour intervals. When no reactions occur within 3 hours after 325 mg of aspirin, the challenge is considered to be negative (Kowalski et al., 2019). Patients with lower FEV1 (<70% of the predicted value) or unstable asthma status are not recommended, and the test should be performed in a hospital with resuscitative equipment under the supervision of special training physicians (Adkinson et al., 2013). These tests may be influenced by bronchial hypersensitivity, ASA dosage, and the concurrent use of leukotriene modifier drugs and antihistamines (White et al., 2005; White et al., 2006). When patients are false-negative for ASA challenge, subsequent confirmatory challenges are recommended for holding leukotriene modifier drugs, antihistamines and oral corticosteroids for at least 1 week and employing high-dose ASA challenges (White et al., 2013).

There is no *in vitro* test available for the diagnosis of NERD. LTE_4 (especially in urine) is suggested to be the most reliable biomarker for the diagnosis of NERD. Several studies demonstrated that patients with NERD had higher baseline concentrations of urinary LTE_4 as well as greater increase after aspirin/NSAID exposure than in patients with ATA, suggesting that urine LTE_4 level could be used as a clinical diagnostic test

TABLE 1 | Genetic polymorphisms associated with NERD.

	Gene	SNP	Analysis methods	Ethnic group	Patients	OR (95% CI)	*P*-value (compared with ATA)	Reference
CysLTs overexpression	*LTC4S*	–444 A>C	Amplified-fragment single-strand conformation polymorphism	Polish	NERD: 47, ATA: 64, NC: 42	3.89 (1.57–8.98)	<0.001	(Sanak et al., 1997)
	CysLTR1	–634 C>T, –475 A<C, –336 A<G	Direct sequencing method	Korean	NERD: 105, ATA: 110, NC: 125	2.71 (1.10–6.68) 2.89 (1.14–7.28)	0.020	(Kim et al., 2006)
	CysLTR2	–819 T>G 2,078 C>T 2,534 A>G	ABI PRISM 3700 DNA analyzer	Korean	NERD: 134, ATA: 66, NC: 152	2.04 (1.06–3.85) 2.28 (1.19–4.40) 2.02 (1.07–3.84)	0.031 0.013 0.031	(Park et al., 2005)
	PTGER2	–616 C>G –166 G>A	Direct sequencing	Korean	NERD: 108, ATA: 93, NC: 140	0.64 (0.42–0.98) 2.60 (1.14–5.92)	0.038 0.023	(Kim et al., 2007)
	PTGER3	–1,709 T>A				3.02 (1.04–8.80)	0.043	
	PTGER4	–1,254 A>G				1.77 (1.08–2.90)	0.024	
	PTGIR	1,915 T>A				0.41 (0.20–0.86)	0.018	
	TBXA2R	–4,684 C>T 795 T>C				0.42 (0.19–0.91) 0.67 (0.45–1.00) 2.57 (1.09–6.09)	0.032 0.049 0.032	
Enhancement of type 2 inflammation	*CCR3*	–520 T>C	MDR method	Korean	NERD: 94, ATA: 152	ND	ND	(Kim et al., 2008)
	CRTH2	–466 T>C	Primer extension methods	Korean	NERD: 107, ATA: 115, NC: 133	ND	0.044 (TT) 0.037 (CC)	(Palikhe et al., 2010)
	IL5R	–5,993 G>A	Primer extension method	Korean	NERD: 139, ATA: 171, NC: 160	ND	0.685 (GG) 0.495 (AG) 0.408 (AA)	(Losol et al., 2013)
Others	*HLA*	DPB1*0301	DNA methods	Polish	NERD: 59, ATA: 57, NC: 48	5.3 (1.90–14.40)	<0.001	(Dekker et al., 1997)
			ABI 3100 Genetic analyzer	Korean	NERD: 76, ATA: 73, NC: 91	5.2 (1.80–14.70)	0.004	(Choi et al., 2004a)
	HLA-DPB1	rs3128965	Affymetrix Genome-Wide Human SNP array	Korean	NERD: 264, ATA: 387, NC: 238	1.8 (1.22–2.68) 3.1 (094–10.70)	0.098 (AG) 0.001 (AA)	(Kim S. H. et al., 2014)
	HLA-DPB1	rs104215	GoldenGate assay with the VeraCode microbead	Korean	NERD: 117, ATA: 685	2.40 (1.68–3.42)	<0.001 (fine-mapping study)	(Park et al., 2013)
	DPP10	rs17048175	Affymetrix Genome-Wide Human SNP array	Korean	NERD: 139, ATA: 171, NC: 160	ND	0.083 (TT) 0.072 (CT) 0.022 (CC)	(Kim et al., 2015)

NERD, NSAID-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; CysLTR, cysteinyl leukotriene receptor; LT, leukotriene; PG, prostaglandin; TX, thromboxane; CRTH2, chemoattractant receptor homolog expressed by type 2 helper T cells; CCR, chemokine receptor; HLA, human leukocyte antigen; DPP, dipeptidyl peptidase; IL, interleukin; ND, no data.

(Hagan et al., 2017; Bochenek et al., 2018). Recent studies demonstrated higher levels of serum periostin, and folliculin as potential biomarkers of NERD, however, further validation studies are needed in other cohorts (Kim M. A. et al., 2014; Trinh et al., 2018). The Polish group proposed the Aspirin-Sensitive Patients Identification Test (ASPI Test), however, it was not replicated in other centers (Kowalski et al., 2005). Despite the basophil activation test (BAT) has been investigated for *in vitro* diagnosis of NERD, variable values of sensitivity and specificity were reported depending on the protocols used, remaining limitations of the clinical use (Schafer and Maune, 2012). More efforts are needed to establish *in vitro* diagnostic tests for reducing the risks of challenge tests with identifying reliable biomarkers for the diagnosis of NERD and the classification of its subphenotypes.

MANAGEMENT

The standard management of NERD involves the guidelines established for the management of asthma and CRS with ASA/NSAID avoidance. The complete avoidance of culprit agents and cross-reacting NSAIDs with use of alternative agents (highly selective COX-2 inhibitors such as celecoxib, and partial inhibitors such as acetaminophen, meloxicam or nimesulide) is essential. ASA desensitization can be beneficial for NERD patients when indicated.

Pharmacologic Treatment

Treatment strategies for asthma should follow stepwise management guidelines with maintaining inhaled corticosteroids with or without long-acting beta 2 agonists, leukotriene modifier drugs and/or biologic agents on the basis of disease severity and rescue medications (GINA-guideline, 2020). Because the overproduction of CysLTs is a key feature in the pathogenic mechanisms, targeting the leukotriene pathway with CysLT1 receptor antagonists (montelukast, zafirlukast and pranlukast) and 5-LO inhibitors (zileuton) should be considered to improve upper and lower airway symptoms. Several studies have shown that these leukotriene modifiers lead to improvement in asthma symptoms, pulmonary function, quality of life, nasal function and lower use of bronchodilators (Rodriguez-Jimenez et al., 2018).

Initial treatment for CRS includes intranasal corticosteroids with intranasal saline irrigation. Intranasal corticosteroids have shown to be highly effective in reducing nasal inflammation and in shrinking NPs, which are recommended as a first-line treatment in patients with CRSwNP (Choi et al., 2015; Simon et al., 2015; Rodriguez-Jimenez et al., 2018). Because rinsing the nasal cavities with saline is helpful in removing secretions and washing away allergens and irritants, nasal irrigation prior to administration of topical medications can improve the response to the medications (Simon et al., 2015; Rodriguez-Jimenez et al., 2018). Systemic corticosteroids and broad-spectrum antibiotics can be additionally required according to the severity of nasal symptoms. Adding antihistamines or oral/nasal decongestants may provide symptom relief (Adkinson et al., 2013).

Despite the heterogeneity of NERD, therapeutic approaches have been proposed according to symptom severity. However, these different phenotypes contribute to the variability in response to treatment. A recent study found that clinical severity and courses differ among the 4 subtypes of NERD, which affect antiasthmatic medications required (Lee et al., 2017). Subtype 1/2 patients had severe clinical courses, requiring higher-dose of antiasthmatic medications including higher dose of ICS and systemic corticosteroids, while subtype 3 patients required low doses of these drugs with less frequent asthma exacerbation. These results suggest that a personalized approach according to subtype classification is needed to achieve better outcomes in the management of NERD.

ASA Desensitization

ASA desensitization is an effective treatment option when standard medical treatments are not effective or daily ASA/NSAIDs therapy is required for other medical conditions, such as coronary artery disease or chronic inflammatory disease (Stevenson and Simon, 2006). Multiple studies have demonstrated the effectiveness of ASA desensitization in reducing NP size and the need for sinus surgery as well as in improving nasal and bronchial symptoms with decrease in the doses of topical and oral corticosteroids (Swierczynska-Krepa et al., 2014; Waldram et al., 2018). A recent study showed the long-term safety and efficacy of ASA desensitization in patients who underwent continuous daily ASA therapy for more than 10 years (Walters et al., 2018). ASA desensitization is a provocative procedure by starting at low doses of ASA and gradually increasing to the dose of 650 to 1300 mg over a period of 1 to 3 days, which can induce hypersensitivity reactions (White and Stevenson, 2018). Thus, as safety is an important issue, ASA desensitization should be carried out in a well-equipped hospital under the supervision of special training physicians. The protocol with gradually increasing the dose over 2 days was suggested by the EAACI to secure safety and efficacy of aspirin desensitization (Kowalski et al., 2019).

Biologics

The emergence of biologics in the management of asthma and CRSwNP has represented potential and promising therapy for NERD. New biologics targeting type 2 cytokines, such as IL-4, IL-5 and IL-13 as well as IgE, have been reported in clinical trials, which could reduce asthma exacerbation and oral corticosteroid use, and improve lung function (Kim and Jee, 2018; McGregor et al., 2019). In addition, they have been shown to improve nasal symptom severity and reduce NP size in patients with CRSwNP, leading to a significant increase in quality of life (Bachert et al., 2020). Because NERD is strongly associated with mast cell activation and eosinophilic airway inflammation, the efficacy of biologics may be different from those usually observed in severe asthma (Hayashi et al., 2016). Here, we summarized the available studies for these biologics in patients with NERD (**Table 2**).

Omalizumab, a humanized recombinant monoclonal anti-IgE antibody, prevents IgE from binding to its high-affinity receptor and reduces Fc receptor expression on mast cells and basophils, subsequently suppressing their activation (Chang et al., 2015). Several studies have suggested the efficacy of omalizumab in the management of NERD, demonstrating a reduction in asthma exacerbation and the need for systemic steroids and short acting beta-2 agonist (SABA) as well as an improvement in upper and lower airway symptoms (Hayashi et al., 2016; (Lee et al., 2018b; Jean et al., 2019). Furthermore, there are some studies suggesting that omalizumab treatment can be beneficial for reducing respiratory symptoms during ASA desensitization and even can restore ASA tolerance without the need for ASA desensitization (Phillips-Angles et al., 2017; Lang et al., 2018; Hayashi et al., 2020). Omalizumab could improve upper and lower airway symptoms with suppression in urinary markers of mast cell activation, LTE_4 and PGD_2 metabolites, in patients with NERD and lead to the development of ASA tolerance with a reduction in urinary LTE_4 concentrations during oral ASA challenge (Hayashi et al., 2016;

Hayashi et al., 2020), suggesting that omalizumab has inhibitory effects on mast cell activation in NERD.

Dupilumab is a human monoclonal antibody that targets the IL-4α receptor and inhibits signaling of both IL-4 and IL-13. Although the study was conducted in a small number of patients with NERD, dupilumab could improve nasal and asthma-related symptom scores and lung functions (Laidlaw et al., 2019), although studies with a larger sample size are needed to confirm its effectiveness. Mepolizumab and reslizumab are both monoclonal antibodies that prevent IL-5 from binding to its receptor on eosinophils, and benralizumab is a monoclonal antibody that targets the alpha subunit of the IL-5 receptor. The respiratory tract of NERD patients is characterized by intense eosinophilic inflammation, with higher levels of eosinophils in NPs and bronchial mucosa biopsies than in ATA patients (Tuttle et al., 2018; Eid et al., 2020). These biologics inhibiting IL-5, eosinophilic maturation and differentiation factor could be effective in the management of patients with NERD (Choi et al., 2004b). In addition, based on recent study results on the pathogenic mechanisms, P2Y12 receptor antagonists, CRTH2 antagonists and anti-TSLP/IL-33 antibodies could be potential options in the management of NERD patients (Rodriguez-Jimenez et al., 2018).

Considering the heterogeneity of NERD phenotypes/endotypes, selecting right patients and right targets (biologics) are essential in the management of NERD. In phenotypic clusters of NERD, subtype 4 patients (NERD with urticaria) would need omalizumab as an effective option, which can inhibit activated basophils and mast cells, the key elements of NERD and urticaria (Lee et al., 2017); subtype 2 patients with severe eosinophilia may need anti-IL-5 as a first option. Despite the development of biologic therapies, unmet needs remain in NERD patients to be understood with regard to their comparative efficacy and long-term safety. Further studies are needed to answer questions on the selection of right patients and targets with right safety.

Dietary Interventions

Dietary intervention may be beneficial for controlling symptoms in patients with NERD. Some studies demonstrated that restricting dietary salicylates, including fruits, vegetables, berries, herbs, and spices, improves nasal and asthmatic symptoms, which can be explained by the known contribution of salicylates in the pathogenesis (Ta and White, 2015; Sommer et al., 2016). A previous study showed that alcohol ingestion can more commonly lead to upper and lower respiratory reactions in NERD patients, although the underlying mechanism is not clear (Cardet et al., 2014). Thus, restricting the diet, when experienced respiratory symptoms after the ingestion, can be additionally effective.

CONCLUSION

Patients with NERD present with a variety of clinical features affected by chronic type 2 eosinophilic inflammation with the overproduction of CysLTs in the upper and lower airways. Although NERD tend to be associated with severe asthma and CRSwNP, an improved understanding of clinical features and underlying pathogenesis of NERD will aid in diagnostic evaluations and new therapeutic strategies for improving clinical outcomes. With the increasing recognition of phenotypic heterogeneity of NERD, efforts are needed to

TABLE 2 | Biologics in NERD patients: Summary of available studies.

Biologics (Target)	Study design (Number of participants)	Route, Dose and Study period	Efficacy outcomes	Reference
Omalizumab (IgE)	Double-blind, randomized, placebo-controlled trial (16 Omalizumab vs. 16 Placebo)	Subcutaneous injection every 2 or 4 weeks based on total IgE level and body weight for 3 months	Improvement in ACT, ACQ-6, SNOT-22 and VAS scores in omalizumab group compared with placebo group after 3-month treatment. (All, *P*<.001) Improvement in FEV1 (%) in omalizumab group compared with placebo group after 3-month treatment (*P*=.003)	(Hayashi et al., 2020)
Omalizumab (IgE)	Retrospective analysis (29 Omalizumab)	Subcutaneous injection for 1 year	Reduction in use of OCS and SABA during 1 year on omalizumab treatment compared with 1 year before initiating omalizumab. (All, *P*=.001)	(Jean et al., 2019)
Dupilumab (IL-4Rα)	*Post hoc* analysis (8 Dupilumab vs. 11 Placebo)	Subcutaneous injection of 300 mg weekly for 16 weeks	Improvement in NPS, ACQ-5 and SNOT-22 total scores in dupilumab group compared with placebo group after 16-week treatment (All, *P*<.005) Changes in FEV1 (L) from baseline in dupilumab group compared with placebo group after 16-week treatment. (*P*<.05)	(Laidlaw et al., 2019)
Mepolizumab (IL-5)	Retrospective analysis (14 Mepolizumab)	Subcutaneous injection of 100 mg every 4 weeks for 3 months	Reduction in absolute eosinophil count from baseline after 3-month treatment. (*P*=.001) Improvement in SNOT-22 and ACT scores from baseline after 3-month treatment. (*P*=.005 and *P*=.002, respectively) No significant improvement in FEV1 (%) from baseline (*P*=.16)	(Tuttle et al., 2018)
Reslizumab (IL-5)	*Post hoc* analysis (28 Reslizumab vs. 28 Placebo)	Intravenous injection of 3 mg/kg every 4 weeks for 52 weeks	Difference in frequency of asthma exacerbation in reslizumab, 0.29 vs placebo, 1.95 (*P*=.001) during 52-week treatment. Changes in FEV1 (L) from baseline in reslizumab, 0.327L vs placebo, 0.002L (P<.001) after 52-week treatment.	(Weinstein et al., 2019)

IL, interleukin; IL-4Rα, interleukin-4 receptor alpha subunit; ACT, asthma control test; ACQ-6, 6-item asthma control questionnaire; SNOT-22, 22-item sino-nasal outcome Test; VAS, visual analog scale; OCS, oral corticosteroid; SABA, short-acting β2 agonist; NPS, nasal polyp score; ACQ-5, 5-item asthma control questionnaire.

establish precision medicine strategies tailored to individual phenotypes/endotypes with potential biomarkers.

AUTHOR CONTRIBUTIONS

The clinical features, diagnosis and treatment of NERD were described by S-DW and the pathophysiologic mechanisms including molecular genetic mechanisms were described by QL. This article was written under supervision of H-SP. She, as corresponding author, performed the overall design and review of this article.

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REFERENCES

Adkinson, N.F. Jr, Bochner, B. S., Burks, A. W., Busse, W. W., Holgate, S. T., Lemanske, R. F., et al. (2013). *Middleton"s allergy E-Book: Principles and practice* (Elsevier Health Sciences).

Bachert, C., Zhang, N., Cavaliere, C., Weiping, W., Gevaert, E., and Krysko, O. (2020). Biologics for chronic rhinosinusitis with nasal polyps. *J. Allergy Clin. Immunol.* 145, 725–739. doi: 10.1016/j.jaci.2020.01.020

Barnig, C., Cernadas, M., Dutile, S., Liu, X., Perrella, M. A., Kazani, S., et al. (2013). Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci. Trans. Med.* 5 (174), 174ra26–174ra26. doi: 10.1126/scitranslmed.3004812

Blanca-Lopez, N., Somoza-Alvarez, M. L., Bellon, T., Amo, G., Canto, G., and Blanca, M. (2018). NSAIDs hypersensitivity: Questions not resolved. *Curr. Opin. Allergy Clin. Immunol.* 18, 291–301. doi: 10.1097/ACI.0000000000000454

Bochenek, G., Kuschill-Dziurda, J., Szafraniec, K., Plutecka, H., Szczeklik, A., and Nizankowska-Mogilnicka, E. (2014). Certain subphenotypes of aspirin-exacerbated respiratory disease distinguished by latent class analysis. *J. Allergy Clin. Immunol.* 133, 98–103.e101-106. doi: 10.1016/j.jaci.2013.07.004

Bochenek, G., Stachura, T., Szafraniec, K., Plutecka, H., Sanak, M., Nizankowska-Mogilnicka, E., et al. (2018). Diagnostic Accuracy of Urinary LTE4 Measurement to Predict Aspirin-Exacerbated Respiratory Disease in Patients with Asthma. *J. Allergy Clin. Immunol. Pract.* 6, 528–535. doi: 10.1016/j.jaip.2017.07.001

Buchheit, K. M., and Laidlaw, T. M. (2016). Update on the Management of Aspirin-Exacerbated Respiratory Disease. *Allergy Asthma Immunol. Res.* 8, 298–304. doi: 10.4168/aair.2016.8.4.298

Cahill, K. N., Bensko, J. C., Boyce, J. A., and Laidlaw, T. M. (2015). Prostaglandin D2: A dominant mediator of aspirin-exacerbated respiratory disease. *J. Allergy Clin. Immunol.* 135 (1), 245–252. doi: 10.1016/j.jaci.2014.07.031

Cahill, K. N., Raby, B. A., Zhou, X., Guo, F., Thibault, D., Baccarelli, A., et al. (2016). Impaired E prostanoid2 expression and resistance to prostaglandin E2 in nasal polyp Fibroblasts from subjects with aspirin-exacerbated respiratory disease. *Am. J. Respiratory Cell Mol. Biol.* 54 (1), 34–40. doi: 10.1165/rcmb.2014-0486OC

Cardet, J. C., White, A. A., Barrett, N. A., Feldweg, A. M., Wickner, P. G., Savage, J., et al. (2014). Alcohol-induced respiratory symptoms are common in patients with aspirin exacerbated respiratory disease. *J. Allergy Clin. Immunol. Pract.* 2, 208–213. doi: 10.1016/j.jaip.2013.12.003

Chang, J. E., Doherty, T. A., Baum, R., and Broide, D. (2014). Prostaglandin D2 regulates human type 2 innate lymphoid cell chemotaxis. *J. Allergy Clin. Immunol.* 133 (3), 899–901.e3. doi: 10.1016/j.jaci.2013.09.020

Chang, T. W., Chen, C., Lin, C. J., Metz, M., Church, M. K., and Maurer, M. (2015). The potential pharmacologic mechanisms of omalizumab in patients with chronic spontaneous urticaria. *J. Allergy Clin. Immunol.* 135, 337–342. doi: 10.1016/j.jaci.2014.04.036

Choi, J. H., Lee, K. W., Oh, H. B., Lee, K. J., Suh, Y. J., Park, C. S., et al. (2004a). HLA association in aspirin-intolerant asthma: DPB1*0301 as a strong marker in a Korean population. *J. Allergy Clin. Immunol.* 113, 562–564. doi: 10.1016/j.jaci.2003.12.012

Choi, J. H., Park, H. S., Oh, H. B., Lee, J. H., Suh, Y. J., Park, C. S., et al. (2004b). Leukotriene-related gene polymorphisms in ASA-intolerant asthma: An association with a haplotype of 5-lipoxygenase. *Hum. Genet.* 114 (4), 337–344. doi: 10.1007/s00439-004-1082-1

Choi, J. H., Kim, J. H., and Park, H. S. (2015). Upper airways in aspirin-exacerbated respiratory disease. *Curr. Opin. Allergy Clin. Immunol.* 15, 21–26. doi: 10.1097/aci.0000000000000122

Choi, Y., Lee, D. H., Trinh, H. K. T., Ban, G. Y., Park, H. K., Shin, Y. S., et al. (2019a). Surfactant protein D alleviates eosinophil-mediated airway inflammation and remodeling in patients with aspirin-exacerbated respiratory disease. *Allergy: Eur. J. Allergy Clin. Immunol.* 74 (1), 78–88. doi: 10.1111/all.13458

Choi, Y., Lee, Y., and Park, H. S. (2019b). Which factors associated with activated eosinophils contribute to the pathogenesis of aspirin-exacerbated respiratory disease? *Allergy Asthma Immunol. Res.* 11, 320–329. doi: 10.4168/aair.2019.11.3.320

Christie, P. E., Spur, B. W., and Lee, T. H. (1992). The effects of lipoxin A4 on airway responses in asthmatic subjects. *Am. Rev. Respiratory Dis.* 145 (6), 1281–1284. doi: 10.1164/ajrccm/145.6.1281

Chu, D. K., Lee, D. J., Lee, K. M., Schünemann, H. J., Szczeklik, W., and Lee, J. M. (2019). Benefits and harms of aspirin desensitization for aspirin-exacerbated respiratory disease: a systematic review and meta-analysis. *Int. Forum Allergy Rhinol.* 9, 1409–1419. doi: 10.1002/alr.22428

Cingi, C., and Bayar Muluk, N. (2020). *All Around the Nose E-Book Basic Science, Diseases and Surgical Management*. (Switzerland: Springer) doi: 10.1007/978-3-030-21217-9

Corrigan, C., Mallett, K., Ying, S., Roberts, D., Parikh, A., Scadding, G., et al. (2005). Expression of the cysteinyl leukotriene receptors cysLT1 and cysLT2 in aspirin-sensitive and aspirin-tolerant chronic rhinosinusitis. *J. Allergy Clin. Immunol.* 115 (2), 316–322. doi: 10.1016/j.jaci.2004.10.051

Dekker, J. W., Nizankowska, E., Schmitz-Schumann, M., Pile, K., Bochenek, G., Dyczek, A., et al. (1997). Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes. *Clin. Exp. Allergy* 27 (5), 574–577. doi: 10.1111/j.1365-2222.1997.tb00747.x

Dominas, C., Gadkaree, S., Maxfield, A. Z., Gray, S. T., and Bergmark, R. W. (2020). Aspirin-exacerbated respiratory disease: A review. *Laryngoscope Invest. Otolaryngol.* 5, 360–367. doi: 10.1002/lio2.387

Doña, I., Blanca-López, N., Torres, M. J., García-Campos, J., García-Núñez, I., Gómez, F., et al. (2012). Drug hypersensitivity reactions: Response patterns, drug involved, and temporal variations in a large series of patients. *J. Investigat. Allergol. Clin. Immunol.* 22 (5), 363–371.

Dursun, A. B., Woessner, K. A., Simon, R. A., Karasoy, D., and Stevenson, D. D. (2008). Predicting outcomes of oral aspirin challenges in patients with asthma, nasal polyps, and chronic sinusitis. *Ann. Allergy Asthma Immunol.* 100, 420–425. doi: 10.1016/s1081-1206(10)60465-6

Eid, R. C., Wudneh, E., Zahid, S., Cahill, K., and Jerschow, E. (2020). Poor control of asthma symptoms with interleukin-5 inhibitors in four patients with aspirin-exacerbated respiratory disease. *Ann. Allergy Asthma Immunol.* 124, 102–104. doi: 10.1016/j.anai.2019.09.023

Feng, C., Beller, E. M., Bagga, S., and Boyce, J. A. (2006). Human mast cells express multiple EP receptors for prostaglandin E 2 that differentially modulate activation responses. *Blood* 107 (8), 3243–3250. doi: 10.1182/blood-2005-07-2772

GINA-guideline (2020). *Global Initiative for Asthma. Global strategy for asthma management and prevention 2020* (Global Initiative for Asthma.; 2020). [cited 2020 Apr]. Available from: http://www.ginasthma.org

Hagan, J. B., Laidlaw, T. M., Divekar, R., O'Brien, E. K., Kita, H., Volcheck, G. W., et al. (2017). Urinary Leukotriene E4 to Determine Aspirin Intolerance in Asthma: A Systematic Review and Meta-Analysis. *J. Allergy Clin. Immunol. Pract.* 5, 990–997.e991. doi: 10.1016/j.jaip.2016.11.004

Harizi, H., Juzan, M., Moreau, J.-F., and Gualde, N. (2003). Prostaglandins Inhibit 5-Lipoxygenase-Activating Protein Expression and Leukotriene B 4 Production from Dendritic Cells Via an IL-10-Dependent Mechanism. *J. Immunol.* 170 (1), 139–146. doi: 10.4049/jimmunol.170.1.139

Hayashi, H., Mitsui, C., Nakatani, E., Fukutomi, Y., Kajiwara, K., Watai, K., et al. (2016). Omalizumab reduces cysteinyl leukotriene and 9alpha,11beta-prostaglandin F2 overproduction in aspirin-exacerbated respiratory disease. *J. Allergy Clin. Immunol.* 137, 1585–1587.e1584. doi: 10.1016/j.jaci.2015.09.034

Hayashi, H., Fukutomi, Y., Mitsui, C., Kajiwara, K., Watai, K., Kamide, Y., et al. (2020). Omalizumab for Aspirin-Hypersensitivity and Leukotriene Overproduction in Aspirin-Exacerbated Respiratory Disease: A Randomized Trial. *Am. J. Respiratory Crit. Care Med.* 137 (5), 1585–1587. doi: 10.1164/rccm.201906-1215OC

Hirai, H., Tanaka, K., Yoshie, O., Ogawa, K., Kenmotsu, K., Takamori, Y., et al. (2001). Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* 193 (2), 255–262. doi: 10.1084/jem.193.2.255

Jean, T., Eng, V., Sheikh, J., Kaplan, M. S., Goldberg, B., Jau Yang, S., et al. (2019). Effect of omalizumab on outcomes in patients with aspirin-exacerbated respiratory disease. *Allergy Asthma Proc.* 40, 316–320. doi: 10.2500/aap.2019.40.4241

Jonsson, E. W. (1998). Functional characterisation of receptors for cysteinyl leukotrienes in smooth muscle. *Acta Physiol. Scand. Suppl.* 641, 1–55. doi: 10.1006/pupt.1997.0075

Kawagishi, Y., Mita, H., Taniguchi, M., Maruyama, M., Oosaki, R., Higashi, N., et al. (2002). Leukotriene C4 synthase promoter polymorphism in Japanese patients with aspirin-induced asthma. *J. Allergy Clin. Immunol.* 109 (6), 936–942. doi: 10.1067/mai.2002.124466

Kim, D. H., and Jee, Y. K. (2018). Is Omalizumab a Problem-Solving Remedy in Severe Asthma? *Allergy Asthma Immunol. Res.* 10, 95–96. doi: 10.4168/aair.2018.10.2.95

Kim, S.-H., Oh, J.-M., Kim, Y.-S., Palmer, L. J., Suh, C.-H., Nahm, D.-H., et al. (2006). Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. *Clin. Exp. Allergy* 36, 433–439. doi: 10.1111/j.1365-2222.2006.02457.x

Kim, S.-H., Kim, Y.-K., Park, H.-W., Jee, Y.-K., Kim, S.-H., Bahn, J.-W., et al. (2007). Association between polymorphisms in prostanoid receptor genes and aspirin-intolerant asthma. *Pharmacogenet. Genomics* 17, 295–304. doi: 10.1097/01.fpc.0000239977.61841.fe

Kim, S.-H., Jeong, H.-H., Cho, B.-Y., Kim, M., Lee, H.-Y., Lee, J., et al. (2008). Association of Four-locus Gene Interaction with Aspirin-intolerant Asthma in Korean Asthmatics. *J. Clin. Immunol.* 28, 336–342. doi: 10.1007/s10875-008-9190-7

Kim, M. A., Izuhara, K., Ohta, S., Ono, J., Yoon, M. K., Ban, G. Y., et al. (2014). Association of serum periostin with aspirin-exacerbated respiratory disease. *Ann. Allergy Asthma Immunol.* 113 (3), 314–320. doi: 10.1016/j.anai.2014.06.014

Kim, S. H., Cho, B. Y., Choi, H., Shin, E. S., Ye, Y. M., Lee, J. E., et al. (2014). The SNP rs3128965 of HLA-DPB1 as a genetic marker of the AERD phenotype. *PLoS One.* 9 (12), e111220. doi: 10.1371/journal.pone.0111220

Kim, S. H., Choi, H., Yoon, M. G., Ye, Y. M., and Park, H. S. (2015). Dipeptidyl-peptidase 10 as a genetic biomarker for the aspirin-exacerbated respiratory disease phenotype. *Ann. Allergy Asthma Immunol.* 114 (3), 208–213. doi: 10.1016/j.anai.2014.12.003

Kim, S. H., Uuganbayar, U., Trinh, H. K. T., Le Pham, D., Kim, N., Kim, M., et al. (2019). Evaluation of neutrophil activation status according to the phenotypes of adult asthma. *Allergy Asthma Immunol. Res.* 11 (3), 381–393. doi: 10.4168/aair.2019.11.3.381

Kowalski, M. L., and Stevenson, D. D. (2013). Classification of Reactions to Nonsteroidal Antiinflammatory Drugs. *Immunol. Allergy Clin. North Am.* 33, 135–145. doi: 10.1016/j.iac.2012.10.008

Kowalski, M. L., Ptasinska, A., Jedrzejczak, M., Bienkiewicz, B., Cieslak, M., Grzegorczyk, J., et al. (2005). Aspirin-triggered 15-HETE generation in peripheral blood leukocytes is a specific and sensitive Aspirin-Sensitive Patients Identification Test (ASPITest). *Allergy* 60, 1139–1145. doi: 10.1111/j.1398-9995.2005.00836.x

Kowalski, M. L., Makowska, J. S., Blanca, M., Bavbek, S., Bochenek, G., Bousquet, J., et al. (2011). Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) - Classification, diagnosis and management: Review of the EAACI/ENDA and GA2LEN/HANNA. *Allergy* 66, 818–829. doi: 10.1111/j.1398-9995.2011.02557.x

Kowalski, M. L., Agache, I., Bavbek, S., Bakirtas, A., Blanca, M., Bochenek, G., et al. (2019). Diagnosis and management of NSAID-Exacerbated Respiratory Disease (N-ERD)-a EAACI position paper. *Allergy* 74, 28–39. doi: 10.1111/all.13599

Kupczyk, M., Antczak, A., Kuprys-Lipinska, I., and Kuna, P. (2009). Lipoxin A4 generation is decreased in aspirin-sensitive patients in lysine-aspirin nasal challenge in vivo model. *Allergy: Eur. J. Allergy Clin. Immunol.* 64 (12), 1746–175. doi: 10.1111/j.1398-9995.2009.02047.x

Laidlaw, T. M., and Boyce, J. A. (2013). Pathogenesis of Aspirin-Exacerbated Respiratory Disease and Reactions. *Immunol. Allergy Clin. North Am.* 33, 195–210. doi: 10.1016/j.iac.2012.11.006

Laidlaw, T. M., Kidder, M. S., Bhattacharyya, N., Xing, W., Shen, S., Milne, G. L., et al. (2012). Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. *Blood* 119 (16), 3790–3798. doi: 10.1182/blood-2011-10-384826

Laidlaw, T. M., Cutler, A. J., Kidder, M. S., Liu, T., Cardet, J. C., Chhay, H., et al. (2014). Prostaglandin E2 resistance in granulocytes from patients with aspirin-exacerbated respiratory disease. *J. Allergy Clin. Immunol.* 133 (6), 1692–1701.e3. doi: 10.1016/j.jaci.2013.12.1034

Laidlaw, T. M., Mullol, J., Fan, C., Zhang, D., Amin, N., Khan, A., et al. (2019). Dupilumab improves nasal polyp burden and asthma control in patients with CRSwNP and AERD. *J. Allergy Clin. Immunol. Pract.* 7, 2462–2465.e2461. doi: 10.1016/j.jaip.2019.03.044

Lang, D. M., Aronica, M. A., Maierson, E. S., Wang, X. F., Vasas, D. C., and Hazen, S. L. (2018). Omalizumab can inhibit respiratory reaction during aspirin desensitization. *Ann. Allergy Asthma Immunol.* 121, 98–104. doi: 10.1016/j.anai.2018.05.007

Lee, H. Y., Ye, Y. M., Kim, S. H., Ban, G. Y., Kim, S. C., Kim, J. H., et al. (2017). Identification of phenotypic clusters of nonsteroidal anti-inflammatory drugs exacerbated respiratory disease. *Allergy* 72, 616–626. doi: 10.1111/all.13075

Lee, J. H., Jung, C. G., and Park, H. S. (2018a). An update on the management of aspirin-exacerbated respiratory disease. *Expert Rev. Respiratory Med.* 12, 137–143. doi: 10.1080/17476348.2018.1417843

Lee, J. H., Lee, H. Y., Jung, C. G., Ban, G. Y., Shin, Y. S., Ye, Y. M., et al. (2018b). Therapeutic Effect of Omalizumab in Severe Asthma: A Real-World Study in Korea. *Allergy Asthma Immunol. Res.* 10, 121–130. doi: 10.4168/aair.2018.10.2.121

Lee, J. U., Park, J. S., Chang, H. S., and Park, C. S. (2019). Complementary participation of genetics and epigenetics in development of nsaid-exacerbated respiratory disease. *Allergy Asthma Immunol. Res.* 11, 779–794. doi: 10.4168/aair.2019.11.6.779

Liu, T., Barrett, N. A., Kanaoka, Y., Laidlaw, T. M., Yoshimoto, E., Garofalo, D., et al. (2019). Cysteinyl Leukotriene Receptor 2 Drives IL-33-Mediated Aspirin Sensitivity Through A Platelet Dependent Mechanism. *J. Allergy Clin. Immunol.* 143 (2), AB293. doi: 10.1016/j.jaci.2018.12.895

Losol, P., Kim, S.-H., Seob Shin, Y., Min Ye, Y., and Park, H.-S. (2013). A genetic effect of IL-5 receptor α polymorphism in patients with aspirin-exacerbated respiratory disease. *Exp. Mol. Med.* 45, e14–e14. doi: 10.1038/emm.2013.24

Machado-Carvalho, L., Martín, M., Torres, R., Gabasa, M., Alobid, I., Mullol, J., et al. (2016). Low E-prostanoid 2 receptor levels and deficient induction of the IL-1β/IL-1 type i receptor/COX-2 pathway: Vicious circle in patients with aspirin-exacerbated respiratory disease. *J. Allergy Clin. Immunol.* 137 (1), 99–107.e7. doi: 10.1016/j.jaci.2015.09.028

McGregor, M. C., Krings, J. G., Nair, P., and Castro, M. (2019). Role of Biologics in Asthma. *Am. J. Respir. Crit. Care Med.* 199, 433–445. doi: 10.1164/rccm.201810-1944CI

Mita, H., Higashi, N., Taniguchi, M., Higashi, A., and Akiyama, K. (2004). Increase in urinary leukotriene B4 glucuronide concentration in patients with aspirin-intolerant asthma after intravenous aspirin challenge. *Clin. Exp. Allergy* 34 (8), 1262–1269. doi: 10.1111/j.1365-2222.2004.02034.x

Mitsui, C., Kajiwara, K., Hayashi, H., Ito, J., Mita, H., Ono, E., et al. (2016). Platelet activation markers overexpressed specifically in patients with aspirin-

exacerbated respiratory disease. *J. Allergy Clin. Immunol.* 137 (2), 400–411. doi: 10.1016/j.jaci.2015.05.041

Palikhe, N. S., Kim, J. H., and Park, H. S. (2009). Update on recent advances in the management of aspirin exacerbated respiratory disease. *Yonsei Med. J.* 50, 744–750. doi: 10.3349/ymj.2009.50.6.744

Palikhe, N. S., Kim, S. H., Cho, B. Y., Ye, Y. M., Choi, G. S., and Park, H. S. (2010). Genetic variability in CRTH2 polymorphism increases eotaxin-2 levels in patients with aspirin exacerbated respiratory disease. *Allergy* 65, 338–346. doi: 10.1111/j.1398-9995.2009.02158.x

Park, J. S., Chang, H. S., Park, C.-S., Lee, J.-H., Lee, Y. M., Choi, J. H., et al. (2005). Association analysis of cysteinyl-leukotriene receptor 2 (CYSLTR2) polymorphisms with aspirin intolerance in asthmatics. *Pharmacogenet. Genomics* 15, 483–492. doi: 10.1097/01.fpc.0000166456.84905.a0

Park, B. L., Kim, T. H., Kim, J. H., Bae, J. S., Pasaje, C. F. A., Cheong, H. S., et al. (2013). Genome-wide association study of aspirin-exacerbated respiratory disease in a Korean population. *Hum. Genet.* 132 (3), 313–321. doi: 10.1007/s00439-012-1247-2

Pavón-Romero, G. F., Ramírez-Jiménez, F., Roldán-Alvarez, M. A., Terán, L. M., and Falfán-Valencia, R. (2017). Physiopathology and genetics in aspirin-exacerbated respiratory disease. *Allergol. Int.* 69, 138–140. doi: 10.1080/01902148.2017.1358776

Pham, D. L., Kim, J. H., Trinh, T. H. K., and Park, H. S. (2016). What we know about nonsteroidal anti-inflammatory drug hypersensitivity. *Korean J. Intern Med.* 31, 417–432. doi: 10.3904/kjim.2016.085

Pham, D. L., Lee, J. H., and Park, H. S. (2017). Aspirin-exacerbated respiratory disease: An update. *Curr. Opin. Pulmonary Med.* 23, 89–96. doi: 10.1097/MCP.0000000000000328

Phillips-Angles, E., Barranco, P., Lluch-Bernal, M., Dominguez-Ortega, J., Lopez-Carrasco, V., and Quirce, S. (2017). Aspirin tolerance in patients with nonsteroidal anti-inflammatory drug-exacerbated respiratory disease following treatment with omalizumab. *J. Allergy Clin. Immunol. Pract.* 5, 842–845. doi: 10.1016/j.jaip.2016.12.013

Rajan, J. P., Wineinger, N. E., Stevenson, D. D., and White, A. A. (2015). Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: A meta-analysis of the literature. *J. Allergy Clin. Immunol.* 135 (3), 676–681.e1. doi: 10.1016/j.jaci.2014.08.020

Rodriguez-Jimenez, J. C., Moreno-Paz, F. J., Teran, L. M., and Guani-Guerra, E. (2018). Aspirin exacerbated respiratory disease: Current topics and trends. *Respir. Med.* 135, 62–75. doi: 10.1016/j.rmed.2018.01.002

Rusznak, M., and Peebles, R. S. (2019). Prostaglandin E2 in NSAID-exacerbated respiratory disease: protection against cysteinyl leukotrienes and group 2 innate lymphoid cells. *Curr. Opin. Allergy Clin. Immunol.* 19, 38–45. doi: 10.1097/ACI.0000000000000498

Säfholm, J., Manson, M. L., Bood, J., Delin, I., Orre, A. C., Bergman, P., et al. (2015). Prostaglandin E2 inhibits mast cell–dependent bronchoconstriction in human small airways through the E prostanoid subtype 2 receptor. *J. Allergy Clin. Immunol.* 136 (5), 1232–1239.e1. doi: 10.1016/j.jaci.2015.04.002

Sanak, M., Simon, H. U., and Szczeklik, A. (1997). Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 350 (9091), 1599–1600. doi: 10.1016/S0140-6736(05)64015-9

Sanak, M., Levy, B. D., Clish, C. B., Chiang, N., Gronert, K., Mastalerz, L., et al. (2000). Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics. *Eur. Respiratory J.* 16 (1), 44–49. doi: 10.1034/j.1399-3003.2000.16a08.x

Sánchez-Borges, M. A. (2019). Aspirin or Nonsteroidal Drug-Exacerbated Respiratory Disease (AERD or NERD). *Allergy Asthma* 1–14. doi: 10.1007/978-3-319-58726-4_15-1

Schafer, D., and Maune, S. (2012). Pathogenic Mechanisms and In Vitro Diagnosis of AERD. *J. Allergy (Cairo)* 2012:789232. doi: 10.1155/2012/789232

Sekioka, T., Kadode, M., Yonetomi, Y., Kamiya, A., Fujita, M., Nabe, T., et al. (2017). CysLT2receptor activation is involved in LTC4-induced lung air-trapping in guinea pigs. *Eur. J. Pharmacol* 794, 147–153. doi: 10.1016/j.ejphar.2016.11.036

Simon, R. A., Dazy, K. M., and Waldram, J. D. (2015). Aspirin-exacerbated respiratory disease: characteristics and management strategies. *Expert Rev. Clin. Immunol.* 11, 805–817. doi: 10.1586/1744666x.2015.1039940

Sokolowska, M., Rovati, G. E., Diamant, Z., Untersmayr, E., Schwarze, J., Lukasik, Z., et al. (2020). Current perspective on eicosanoids in asthma and allergic diseases - EAACI Task Force consensus report, part I. *Allergy*. doi: 10.1111/all.14295

Sommer, D. D., Rotenberg, B. W., Sowerby, L. J., Lee, J. M., Janjua, A., Witterick, I. J., et al. (2016). A novel treatment adjunct for aspirin exacerbated respiratory disease: the low-salicylate diet: a multicenter randomized control crossover trial. *Int. Forum Allergy Rhinol.* 6, 385–391. doi: 10.1002/alr.21678

Steinke, J. W., and Wilson, J. M. (2016). Aspirin-exacerbated respiratory disease: Pathophysiological insights and clinical advances. *J. Asthma Allergy* 9, 37–43. doi: 10.2147/JAA.S88739

Stevenson, D. D., and Simon, R. A. (2006). Selection of patients for aspirin desensitization treatment. *J. Allergy Clin. Immunol.* 118, 801–804. doi: 10.1016/j.jaci.2006.06.019

Sturm, E. M., Schratl, P., Schuligoi, R., Konya, V., Sturm, G. J., Lippe, I. T., et al. (2008). Prostaglandin E 2 Inhibits Eosinophil Trafficking through E-Prostanoid 2 Receptors. *J. Immunol.* 181 (10), 7273–7283. doi: 10.4049/jimmunol.181.10.7273

Swierczynska-Krepa, M., Sanak, M., Bochenek, G., Strek, P., Cmiel, A., Gielicz, A., et al. (2014). Aspirin desensitization in patients with aspirin-induced and aspirin-tolerant asthma: a double-blind study. *J. Allergy Clin. Immunol.* 134, 883–890. doi: 10.1016/j.jaci.2014.02.041

Szczeklik, A., Nizankowska, E., and Duplaga, M. (2000). Natural history of aspirin-induced asthma. AIANE Investigators. European Network on Aspirin-Induced Asthma. *Eur. Respir. J.* 16, 432–436. doi: 10.1034/j.1399-3003.2000.016003432.x

Szczeklik, A. (1990). The cyclooxygenase theory of aspirin-induced asthma. *Eur. Respir. J.* 3, 588–593.

Ta, V., and White, A. A. (2015). Survey-Defined Patient Experiences With Aspirin-Exacerbated Respiratory Disease. *J. Allergy Clin. Immunol. Pract.* 3, 711–718. doi: 10.1016/j.jaip.2015.03.001

Taniguchi, M., Mitsui, C., Hayashi, H., Ono, E., Kajiwara, K., Mita, H., et al. (2019). Aspirin-exacerbated respiratory disease (AERD): Current understanding of AERD. *Allergol. Int.* 68, 289–295. doi: 10.1016/j.alit.2019.05.001

Trinh, H. K. T., Pham, D. L., Choi, Y., Kim, H. M., Kim, S. H., and Park, H. S. (2018). Epithelial folliculin enhances airway inflammation in aspirin-exacerbated respiratory disease. *Clin. Exp. Allergy* 48, 1464–1473. doi: 10.1111/cea.13253

Tuttle, K. L., Buchheit, K. M., Laidlaw, T. M., and Cahill, K. N. (2018). A retrospective analysis of mepolizumab in subjects with aspirin-exacerbated respiratory disease. *J. Allergy Clin. Immunol. Pract.* 6, 1045–1047. doi: 10.1016/j.jaip.2018.01.038

Uematsu, S., Matsumoto, M., Takeda, K., and Akira, S. (2002). Lipopolysaccharide-Dependent Prostaglandin E 2 Production Is Regulated by the Glutathione-Dependent Prostaglandin E 2 Synthase Gene Induced by the Toll-Like Receptor 4/MyD88/NF-IL6 Pathway. *J. Immunol.* 168 (11), 5811–5816. doi: 10.4049/jimmunol.168.11.5811

Ulambayar, B., Lee, S. H., Yang, E. M., Ye, Y. M., and Park, H. S. (2019). Association Between Epithelial Cytokines and Clinical Phenotypes of Elderly Asthma. *Allergy Asthma Immunol. Res.* 11, 79–89. doi: 10.4168/aair.2019.11.1.79

Van Sambeek, R., Stevenson, D. D., Baldasaro, M., Lam, B. K., Zhao, J., Yoshida, S., et al. (2000). 5' Flanking region polymorphism of the gene encoding leukotriene C4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J. Allergy Clin. Immunol.* 106 (1), 72–76. doi: 10.1067/mai.2000.107603

Waldram, J., Walters, K., Simon, R., Woessner, K., Waalen, J., and White, A. (2018). Safety and outcomes of aspirin desensitization for aspirin-exacerbated respiratory disease: A single-center study. *J. Allergy Clin. Immunol.* 141, 250–256. doi: 10.1016/j.jaci.2017.05.006

Walters, K. M., Waldram, J. D., Woessner, K. M., and White, A. A. (2018). Long-term Clinical Outcomes of Aspirin Desensitization With Continuous Daily Aspirin Therapy in Aspirin-exacerbated Respiratory Disease. *Am. J. Rhinol. Allergy* 32, 280–286. doi: 10.1177/1945892418770260

Weinstein, S. F., Katial, R. K., Bardin, P., Korn, S., McDonald, M., Garin, M., et al. (2019). Effects of Reslizumab on Asthma Outcomes in a Subgroup of Eosinophilic Asthma Patients with Self-Reported Chronic Rhinosinusitis with Nasal Polyps. *J. Allergy Clin. Immunol. Pract.* 7, 589–596.e583. doi: 10.1016/j.jaip.2018.08.021

White, A. A., and Stevenson, D. D. (2018). Aspirin-Exacerbated Respiratory Disease. *N. Engl. J. Med.* 379, 1060–1070. doi: 10.1056/NEJMra1712125

White, A. A., Stevenson, D. D., and Simon, R. A. (2005). The blocking effect of essential controller medications during aspirin challenges in patients with aspirin-exacerbated respiratory disease. *Ann. Allergy Asthma Immunol.* 95, 330–335. doi: 10.1016/s1081-1206(10)61150-7

White, A., Ludington, E., Mehra, P., Stevenson, D. D., and Simon, R. A. (2006). Effect of leukotriene modifier drugs on the safety of oral aspirin challenges. *Ann. Allergy Asthma Immunol.* 97, 688–693. doi: 10.1016/s1081-1206(10)61101-5

White, A. A., Bosso, J. V., and Stevenson, D. D. (2013). The clinical dilemma of "silent desensitization" in aspirin-exacerbated respiratory disease. *Allergy Asthma Proc.* 34, 378–382. doi: 10.2500/aap.2013.34.3670

Woessner, K. M. (2017). Update on Aspirin-Exacerbated Respiratory Disease. *Curr. Allergy Asthma Rep.* 17, 1–9. doi: 10.1007/s11882-017-0673-6

Yamaguchi, H., Higashi, N., Mita, H., Ono, E., Komase, Y., Nakagawa, T., et al. (2011). Urinary concentrations of 15-epimer of lipoxin A 4 are lower in patients with aspirin-intolerant compared with aspirin-tolerant asthma. *Clin. Exp. Allergy* 41 (12), 1711–1718. doi: 10.1111/j.1365-2222.2011.03839.x

Yin, W., Yeung, W., and Park, H. S. (2020). Update on the Management of Nonsteroidal Anti-Inflammatory Drug Hypersensitivity. *Yonsei Med. J.* 61, 4–14. doi: 10.3349/ymj.2020.61.1.4

Yonetomi, Y., Sekioka, T., Kadode, M., Kitamine, T., Kamiya, A., Matsumura, N., et al. (2015). Leukotriene C4 induces bronchoconstriction and airway vascular hyperpermeability via the cysteinyl leukotriene receptor 2 in S-hexyl glutathione-treated guinea pigs. *Eur. J. Pharmacol.* 754, 98–104. doi: 10.1016/j.ejphar.2015.02.014

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Direct Oral Provocation Test Is Safe and Effective in Diagnosing Beta-Lactam Allergy in Low-Risk Children With Mild Cutaneous Reactions

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INTRODUCTION

Beta-lactams (BLs) are frequent causes of drug allergies (Har and Solensky, 2017; Torres et al., 2019). Drug hypersensitivity reactions (DHRs) are defined by the World Allergy Organization (WAO) as "objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons. When immunologic mechanisms have been demonstrated, either antibody or cell-mediated, the reactions should be referred to as drug allergy" (Johansson et al., 2004).

DHRs are generally classified according to the onset time after drug exposure. Immediate reactions occur within the 1st h until 6 h following drug administration and are commonly mediated by IgE (Demoly et al., 2014). Examples include urticaria, angioedema, rhinitis, bronchospasm, or anaphylaxis (Demoly et al., 2014). Non-immediate reactions occur at least 1 h after drug exposure and are mediated by T cells (Demoly et al., 2014). They often appear as a maculopapular exanthema (MPE), but severe reactions may also emerge (Demoly et al., 2014).

About 10% of patients report allergy to penicillin, but 90% or more of these individuals may be able to tolerate penicillins (Har and Solensky, 2017). The misdiagnosis of penicillin allergy results from overestimated reports by health professionals and patients. Some symptoms (e.g., abdominal pain, nausea, headache) are usually side effects, but can be mistaken for allergic reactions (Har and Solensky, 2017). In addition, MPE may have been caused by an underlying infection or even by an interaction between a virus and the antibiotic (Har and Solensky, 2017). Another consideration is that there is a natural decrease in IgE antibodies against a penicillin over time (Har and Solensky, 2017). When assessing individuals with a clear history of immediate reaction and negative allergic tests, we must consider the time elapsed since the subject's last drug exposure.

The diagnosis of BL allergy begins with clinical history. Some tests, as *in vivo* (skin tests and drug provocation tests) and *in vitro* tests (specific IgE levels, basophil activation tests, lymphocytic transformation tests) may help elucidate the diagnosis (Demoly et al., 2014). A detailed history is crucial for evaluation of BL allergy (Macy, 2014). Nevertheless, it can be vague or imprecise, leading to an incorrect diagnosis (Har and Solensky, 2017). Skin tests (STs) are painful and have suboptimal sensitivity (Moral and Caubet, 2017). The oral provocation test (OPT) is considered the most

accurate test, with high positive and negative predictive values (PPV/NPV) (Moral and Caubet, 2017). Thereby, direct OPT without previous STs has been increasingly used in patients, especially children with a history of mild non-immediate reactions to BLs (Moral and Caubet, 2017; Graham et al., 2018; Torres et al., 2019).

Our aim was to review literature on diagnosis of allergy to BLs and to discuss the safety and efficacy of direct OPT in the diagnosis of BL allergy in children.

WHY IS IT IMPORTANT TO CORRECTLY DIAGNOSE BL ALLERGY?

The misdiagnosis of BL allergy may affect the health system in two ways: 1) false allergy label, with an unrealistic increase in incidence of BL allergy and impact on treatment options; and 2) false label of non-allergic, with important consequences in patient safety through prescription errors, leading to more severe reactions (Mayorga et al., 2019).

The patient with "penicillin allergy" is at increased risk of receiving broad-spectrum antibiotics, such as fluoroquinolones and clindamycin, which are associated with an increased prevalence of infections by multi-resistant bacteria (Lee et al., 2000; Macy and Contreras, 2014; van Dijk et al., 2016). In addition to antimicrobial resistance, studies have shown that patients "allergic to penicillin" have a higher frequency of postoperative complications, longer hospital stays, higher treatment costs and a higher rate of treatment failure (Solensky, 2014; Jefferson et al., 2018; Lucas et al., 2019).

WHO SHOULD BE EVALUATED FOR BL ALLERGY?

Due to the deleterious consequences of a false label of BL allergy, we should evaluate all individuals with a history suggestive of hypersensitivity to BLs (Torres et al., 2019). If the history is incompatible with an allergic reaction, for example, gastrointestinal symptoms, headache, dizziness, or other manifestations suggestive of side effects, the patient can receive treatment with BLs again (Har and Solensky, 2017). On the other hand, if the patient does not know details about his previous reaction, the best approach is to perform a complete investigation with *in vitro* tests, STs (immediate/delayed reading) and OPT. It is important to emphasize that patients who have a family history of allergy to BLs, with no personal history of previous reaction, do not need to be tested and can receive BLs safely (Har and Solensky, 2017).

HOW IS THE DIAGNOSIS OF BL HYPERSENSITIVITY PERFORMED TODAY?

Currently, the diagnosis of BL hypersensitivity is conducted by history, *in vitro* tests, STs and OPTs (Demoly et al., 2014; Har and Solensky, 2017; Torres et al., 2019). *In vitro* tests have low sensitivity, being performed in selected cases (Mayorga et al., 2016). If the patient had an immediate reaction, *in vitro* tests and STs (prick and intradermal tests) with immediate reading are performed. If they are negative, OPT is conducted (Blanca et al., 2009). In the case of mild non-immediate reactions in children, STs are less used and OPT is a safe procedure (Moral and Caubet, 2017; Graham et al., 2018). Mild non-immediate reactions include delayed-appearing urticaria and mild/moderate MPEs. OPT is formally contraindicated if there is history of severe cutaneous adverse reactions (SCARs) such as Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN) (Blanca et al., 2009; Macy, 2014).

The role of STs in mild non-immediate reactions to BLs has been questioned. The accuracy of STs suffers influence of various factors such as pre-test probability and type of reagents used. In Brazil, for example, the major and some of minor benzylpenicillin determinants are not commercially available, which reduces the sensitivity of STs. Recently, a systematic review and meta-analysis assessing the accuracy of STs and specific IgE in evaluation of patients who report allergy to BLs have been published (Sousa-Pinto et al., 2020). They included 105 primary studies (31,761 participants). STs had sensitivity of 30.7% and specificity of 96.8%. Specific IgE had sensitivity of 19.3% and specificity of 97.4% (Sousa-Pinto et al., 2020). Their results suggest that STs (at least in mild non-immediate reactions) and specific IgE have high specificity and NPV, but low sensitivity and PPV (Sousa-Pinto et al., 2020). In immediate reactions, it is difficult to define PPV in individuals with positive STs, as OPT is contraindicated in these patients. In non-immediate reactions, few studies have investigated PPV. Caubet et al. performed STs and OPTs in 88 children with non-immediate reactions to BLs and found a PPV of 36% (Caubet et al., 2011).

HOW CAN WE IMPROVE THE DIAGNOSIS OF PENICILLIN ALLERGY?

To simplify the algorithm and reduce the cost of diagnostic procedures, various investigators are performing direct OPTs without prior STs in low-risk patients. Whether there is agreement on the high-risk patient, there is no uniform definition of low-risk patient. The last EAACI position paper on diagnosis of BL hypersensitivity presented a risk stratification according to the index reaction (Romano et al., 2019). In summary, the high-risk group included those with history of severe immediate reactions (e. g., anaphylaxis, hypotension, laryngeal edema, bronchospasm, urticaria/angioedema) and severe non-immediate reactions (e. g., SJS, TEN, DRESS, serum-sickness-like disease, organ-specific manifestations) (Romano et al., 2019). Mild and moderate MPEs were considered of low risk, especially in children. Mild MPE was defined as "a more or less widespread rash, with less than a week of duration, without systemic involvement" and moderate MPE, those with more than a week of duration, without systemic symptoms (Romano et al., 2019).

First studies evaluating direct OPTs were done with children who presented non-immediate reactions, such as MPE. More recently, children with non-severe immediate reactions and adults were also investigated. Currently, there are already some studies suggesting that this strategy may be safe and effective for a selected group of "low-risk" patients (**Table 1**).

Vezir et al. evaluated 119 children with history of mild non-immediate cutaneous reactions induced by BL through direct OPTs (Vezir et al., 2016). Only four (3.4%) reacted with urticaria during OPTs, and there was no severe reaction (Vezir et al., 2016).

In Canada, Mill and colleagues investigated 818 children with suspect allergy (immediate and non-immediate) to amoxicillin (Mill et al., 2016). Exclusion criteria were SJS and TEN. There were no reactions in 770 (94.1%), mild immediate reactions in 17 (2.1%), and non-immediate reactions in 31 (3.8%) patients (Mill et al., 2016). Immediate reactions consisted of hives and non-immediate reactions varied from MPE to serum sickness-like reaction (Mill et al., 2016). They also conducted a follow-up of 250 participants. Of these, 55 received full treatment with amoxicillin, and 49 (89.1%) tolerated the treatment (Mill et al., 2016).

In United States (US), Iammatteo and colleagues investigated patients ≥ 7 years-old with history of mild penicillin allergy through direct OPT with no prior STs (Mill et al., 2016). Of 155 patients who completed OPTs, 120 (77.4%) had negative tests, 31 (20%) had non-allergic reactions and four participants (2.6%) experienced allergic reactions; all were mild (Mill et al., 2016).

In addition, also in the US, Mustafa et al. investigated children and adults with history of penicillin allergy and developed a randomized trial comparing two strategies: ST plus OPT versus direct OPT to amoxicillin in low-risk patients (Mustafa et al.,

TABLE 1 | Studies that evaluated efficacy and safety of direct beta-lactam provocation tests.

Study	Age	Country	Index reaction	Exclusion criteria	Diagnostic tests	Results	Safety
Vezir et al., 2016	Children and adolescents; 0-18 yo (n=119)	Turkey	Mild non-immediate reactions without systemic involvement (MPE or delayed-appearing urticaria/ angioedema)	Severe reactions (SJS, TEN, DRESS, AGEP, nephritis, pneumonitis, hepatitis, and vasculitis)	Direct OPT (5 doses were administered with 30-min intervals in increasing doses). OPT was continued for 5 days.	OPTs with the suspected drug were performed in 119 patients. Four patients (3.4%) had a reaction during OPT.	No severe reactions were recorded during OPT.
Mill et al., 2016	Children; median age 1.7 yo (n=818)	Canada	History of allergy to amoxicillin (immediate and non-immediate reactions)	SJS/TEN	Direct OPT (10% of the dose of amoxicillin, then 20 minutes later 90% of the dose).	Among all, 770 (94.1%) tolerated the OPT; 17 (2.1%) had immediate reactions and 31 (3.8%) non-immediate reactions.	No severe reactions were recorded during OPT.
Iammatteo et al., 2019	Children (≥ 7 yo) and adults (n=155)	US	History of non-life-threatening reactions to penicillin	Bronchospasm or laryngeal edema requiring intubation; anaphylactic shock; or severe non-IgE-mediated reactions (e. g. SJS, TEN, DRESS, nephritis, hepatitis, anemia, vasculitis, SSLR, pneumonitis). Pregnancy and antihistamine use.	Direct OPT (placebo followed by a 2-step OPT to amoxicillin).	Of 155 patients who completed OPT, 120 (77.4%) had negative tests, 31 (20%) had non-allergic reactions and four participants (2.6%) experienced mild allergic reactions.	No severe reactions were recorded during OPT.
Mustafa et al., 2019	Children and adults (n=363)	US	History of penicillin allergy. For the randomized study, patients needed to be aged 5 to 17 years with a history of cutaneous reaction (> 1 year ago) or aged 18 years and older with a history of cutaneous reaction (> 10 years ago).	Pregnancy. History of a severe cutaneous non IgE-mediated adverse drug reaction or SSLR.	Randomized trial comparing penicillin ST followed by OPT with amoxicillin versus direct OPT. Reagents used for STs were benzylpenicilloyl polylysine (Pre-Pen®) and penicillin G 10,000 U/mL.	13 children (< 5 yo) underwent direct OPT (all negative); 159 patients were randomized to direct OPT or ST. STs were negative in 70 of 80 (87.5%) patients. All 70 patients had negative OPTs. Direct OPT was negative in 76 of 79 (96.2%) patients.	No severe reactions were recorded during OPT.
Kuruvilla et al., 2019	Adults (n=50)	US	History of benign rash or benign somatic symptoms, or another unknown history associated with their last penicillin exposure if it occurred > 12 months ago.	Recent reaction (<12 months), a history of a penicillin-associated blistering rash, hemolytic anemia, or organ involvement. Antihistamines or steroids use.	OPT with a single dose of oral amoxicillin 500 mg was performed.	Four patients (8%) were de-labeled based on history. Twenty subjects (40%) were submitted to OPT, and none developed immediate, or delayed hypersensitivity reactions.	No severe reactions were recorded during OPT.

Acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS); MPE, maculopapular exanthema; OPT, oral provocation test; SJS, Stevens-Johnson syndrome; SSLR, serum sickness-like reaction; ST, skin test; TEN, toxic epidermal necrolysis; US, United States.

2019). Of the total, 13 children (<5 years) were submitted to direct OPTs (all were negative) and 159 patients (≥5 years old) with mild cutaneous reactions underwent ST or direct OPT. Eighty patients underwent STs and 70 (87.5%) had negative results. These 70 patients with negative STs underwent OPT (all were negative). Direct OPT was performed in 79 patients and, in 76 (96.2%) participants, they were negative. There was no severe reaction.

Lastly, Kuruvilla et al. evaluated direct OPT without prior STs in adults with a "low-risk" history of penicillin allergy (Kuruvilla et al., 2019). There were 50 patients with penicillin allergy label and 38 of them met their criteria for direct OPT. Four subjects were de-labeled based on history. Twenty participants were submitted to OPT, and none developed hypersensitivity reactions (Kuruvilla et al., 2019).

DISCUSSION

Evidence is increasingly supporting direct OPT with no prior STs in mild non-immediate reactions to BLs, especially for the pediatric age group. Therefore, many guidelines are already suggesting this approach in the management of BL hypersensitivity in children (Mirakian et al., 2015; Gomes et al., 2016; Romano et al., 2019; Torres et al., 2019). There are some peculiarities in the management of DHRs in children (Gomes et al., 2016). Viral and bacterial infections are important differential diagnoses, as they may act as cofactors or triggers of exanthemas in young children (Gomes et al., 2016). In addition, diagnostic procedures such as intradermal STs are painful and less tolerated in this age group (Gomes et al., 2016). Another aspect is that lifelong avoidance of BLs in children is more difficult (Gomes et al., 2016). Thus, there are more studies investigating direct OPTs in children and adolescents with history of mild non-immediate reactions compared to adults, and their results suggest that this approach is safe and effective in this age group.

Optimization of diagnostic protocols is of outmost importance in penicillin allergy de-labeling programs. This optimization should balance accuracy, risks, costs, and labor-intensity. Direct OPT has many advantages such as being less time-consuming, less expensive and more accurate. To ensure safety, it is essential to perform risk stratification, as OPT can trigger a potentially serious hypersensitivity reaction (Romano et al., 2019). However, what constitutes a "low-risk" BL allergy history is highly variable. Recently, a multicenter Australian study investigated criteria to determine an optimal low-risk definition for penicillin testing (Stevenson et al., 2020). They evaluated 447 patients and found that 97.1% of 244 patients defined as low risk tolerated a direct OPT (Stevenson et al., 2020). They concluded that a history of penicillin-associated exanthema (without angioedema, mucosal ulceration, or systemic involvement), more than 1 year ago, was sufficient to select a patient for a direct OPT (Stevenson et al., 2020).

Several factors should be considered in risk stratification: type of manifestation (exanthema, urticaria, angioedema, and others), chronology (immediate vs. non-immediate), systemic involvement, comorbidities, pregnancy, and time elapsed since the last reaction. The "high risk" patient group generally includes recent reactions (< 1 year ago), patients with comorbidities, pregnant women, systemic involvement (encompassing all SCARs) and immediate reactions with angioedema or anaphylaxis. As highlighted in the previously reported studies, the evaluation of direct OPT was performed excluding high-risk patients (Mill et al., 2016; Vezir et al., 2016; Iammatteo et al., 2019; Kuruvilla et al., 2019; Mustafa et al., 2019).

In summary, BL allergy label is a major public health issue, as it leads to the use of non-BL antibiotics, which may be inappropriate and cause more side effects. On the other hand, traditional diagnostic procedures include many steps that can be an obstacle to the correct diagnosis. Thus, new strategies have been developed to improve the investigation of BL hypersensitivity. Direct OPTs without previous STs in low-risk patients can be a feasible and cost-effective approach in the coming years. This strategy seems to be safe and effective for children with mild non-immediate reactions. However, there are still controversies about which patients should undergo ST versus direct OPT. Further studies, including various populations and age groups, are needed to enable a stronger recommendation in this regard.

AUTHOR CONTRIBUTIONS

MF designed, drafted, reviewed and submitted the full document. FK designed and reviewed the full document.

REFERENCES

Blanca, M., Romano, A., Torres, M. J., Férnandez, J., Mayorga, C., Rodriguez, J., et al. (2009). Update on the evaluation of hypersensitivity reactions to betalactams. *Allergy* 64 (2), 183–193. doi: 10.1111/all.12886

Caubet, J. C., Kaiser, L., Lemaître, B., Fellay, B., Gervaix, A., and Eigenmann, P. A. (2011). The role of penicillin in benign skin rashes in childhood: a prospective study based on drug rechallenge. *J. Allergy Clin. Immunol.* 127 (1), 218–222. doi: 10.1016/j.jaci.2010.08.025

Demoly, P., Adkinson, N. F., Brockow, K., Castells, M., Chiriac, A. M., Greenberger, P. A., et al. (2014). International Consensus on drug allergy. *Allergy* 69 (4), 420–437. doi: 10.1111/all.12350

Gomes, E. R., Brockow, K., Kuyucu, S., Saretta, F., Mori, F., Blanca-Lopez, N., et al. (2016). Drug hypersensitivity in children: report from the pediatric task force of the EAACI Drug Allergy Interest Group. *Allergy* 71 (2), 149–161. doi: 10.1111/all.12774

Graham, F., Tsabouri, S., and Caubet, J. C. (2018). Hypersensitivity reactions to beta-lactams in children. *Curr. Opin. Allergy Clin. Immunol.* 18 (4), 284–290. doi: 10.1097/ACI.0000000000000453

Har, D., and Solensky, R. (2017). Penicillin and beta-lactam hypersensitivity. *Immunol. Allergy Clin. North Am.* 37 (4), 643–662. doi: 10.1016/j.iac.2017.07.001

Iammatteo, M., Alvarez Arango, S., Ferastraoaru, D., Akbar, N., Lee, A. Y., Cohen, H. W., et al. (2019). Safety and outcomes of oral graded challenges to

Amoxicillin without prior skin testing. *J. Allergy Clin. Immunol. Pract.* 7 (1), 236–243. doi: 10.1016/j.jaip.2018.05.008

Jefferson, A. A., Khan, D. A., and Shaker, M. S. (2018). The penicillin allergy label: expensive to maintain, inexpensive to remove. *J. Allergy Clin. Immunol. Pract.* 6 (3), 1028–1029. doi: 10.1016/j.jaip.2017.11.039

Johansson, S. G., Bieber, T., Dahl, R., Friedmann, P. S., Lanier, B. Q., Lockey, R. F., et al. (2004). Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J. Allergy Clin. Immunol.* 113 (5), 832–836. doi: 10.1016/j.jaci.2003.12.591

Kuruvilla, M., Shih, J., Patel, K., and Scanlon, N. (2019). Direct oral amoxicillin challenge without preliminary skin testing in adult patients with allergy and at low risk with reported penicillin allergy. *Allergy Asthma Proc.* 40 (1), 57–61. doi: 10.2500/aap.2019.40.4184

Lee, C. E., Zembower, T. R., Fotis, M. A., Postelnick, M. J., Greenberger, P. A., Peterson, L. R., et al. (2000). The incidence of antimicrobial allergies in hospitalized patients: implications regarding prescribing patterns and emerging bacterial resistance. *Arch. Intern. Med.* 160 (18), 2819–2822. doi: 10.1001/archinte.160.18.2819

Lucas, M., Arnold, A., Sommerfield, A., Trevenen, M., Braconnier, L., Schilling, A., et al. (2019). Antibiotic allergy labels in children are associated with adverse clinical outcomes. *J. Allergy Clin. Immunol. Pract.* 7 (3), 975–982. doi: 10.1016/j.jaip.2018.09.003

Macy, E., and Contreras, R. (2014). Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: a cohort study. *J. Allergy Clin. Immunol.* 133 (3), 790–796. doi: 10.1016/j.jaci.2013.09.021

Macy, E. (2014). Penicillin and beta-lactam allergy: epidemiology and diagnosis. *Curr. Allergy Asthma Rep.* 14 (11), 476. doi: 10.1007/s11882-014-0476-y

Mayorga, C., Celik, G., Rouzaire, P., Whitaker, P., Bonadonna, P., Rodrigues-Cernadas, J., et al. (2016). In vitro tests for drug hypersensitivity reactions: an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy* 71 (8), 1103–1134. doi: 10.1111/all.12886

Mayorga, C., Fernandez, T. D., Montañez, M. I., Moreno, E., and Torres, M. J. (2019). Recent developments and highlights in drug hypersensitivity. *Allergy* 74 (12), 2368–2381. doi: 10.1111/all.14061

Mill, C., Primeau, M. N., Medoff, E., Lejtenyi, C., O'Keefe, A., Netchiporouk, E., et al. (2016). Assessing the diagnostic properties of a graded oral provocation challenge for the diagnosis of immediate and nonimmediate reactions to Amoxicillin in children. *JAMA Pediatr.* 170 (6), e160033. doi: 10.1001/jamapediatrics.2016.0033

Mirakian, R., Leech, S. C., Krishna, M. T., Richter, A. G., Huber, P. A. J., Farooque, S., et al. (2015). Management of allergy to penicillins and other beta-lactams. *Clin. Exp. Allergy* 45 (2), 300–327. doi: 10.1111/cea.12468

Moral, L., and Caubet, J. C. (2017). Oral challenge without skin tests in children with non-severe beta-lactam hypersensitivity: Time to change the paradigm? *Pediatr. Allergy Immunol.* 28 (8), 724–727. doi: 10.1111/pai.12800

Mustafa, S. S., Conn, K., and Ramsey, A. (2019). Comparing direct challenge to Penicillin skin testing for the outpatient evaluation of Penicillin allergy: a randomized controlled trial. *J. Allergy Clin. Immunol. Pract.* 7 (7), 2163–2170. doi: 10.1016/j.jaip.2019.05.037

Romano, A., Atanaskovic-Markovic, M., Barbaud, A., Bircher, A., Brockow, K., Caubet, J., et al. (2019). Towards a more precise diagnosis of hypersensitivity to beta-lactams – an EAACI position paper. *Allergy* 75 (6), 1300–1315. doi: 10.1111/all.14122

Solensky, R. (2014). Penicillin allergy as a public health measure. *J. Allergy Clin. Immunol.* 133 (3), 797–798. doi: 10.1016/j.jaci.2013.10.032

Sousa-Pinto, B., Tarrio, I., Blumenthal, K. G., Araújo, L., Azevedo, L. F., Delgado, L., et al. (2020). Accuracy of Penicillin allergy diagnostic tests: a systematic review and meta-analysis [published online ahead of print, 2020 May 21]. *J. Allergy Clin. Immunol.* S0091-6749 (20), 30726–0. doi: 10.1016/j.jaci.2020.04.058

Stevenson, B., Trevenen, M., Klinken, E., Smith, W., Yuson, C., Katelaris, C., et al. (2020). Multicenter Australian study to determine criteria for low- and high-risk penicillin testing in outpatients. *J. Allergy Clin. Immunol. Pract.* 8 (2), 681–689.e3. doi: 10.1016/j.jaip.2019.09.025

Torres, M. J., Adkinson, N. F.Jr, Caubet, J. C., Khan, D. A., Kidon, M. I., Mendelson, L., et al. (2019). Controversies in drug allergy: Beta-Lactam hypersensitivity testing. *J. Allergy Clin. Immunol. Pract.* 7 (1), 40–45. doi: 10.1016/j.jaip.2018.07.051

van Dijk, S. M., Gardarsdottir, H., Wassenberg, M. W. M., Oosterheert, J. J., de Groot, M. C. H., and Rockmann, H. (2016). The high impact of penicillin allergy registration in hospitalized patients. *J. Allergy Clin. Immunol. Pract.* 4 (5), 926–931. doi: 10.1016/j.jaip.2016.03.009

Vezir, E., Dibek Misirlioglu, E., Civelek, E., Capanoglu, M., Guvenir, H., Ginis, T., et al. (2016). Direct oral provocation tests in non-immediate mild cutaneous reactions related to beta-lactam antibiotics. *Pediatr. Allergy Immunol.* 27 (1), 50–54. doi: 10.1111/pai.12493

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MINI REVIEW
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Rituximab Hypersensitivity: From Clinical Presentation to Management

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Rituximab is a chimeric monoclonal antibody (mAb) against CD20 molecule which is expressed on human B cells. It has been used for the treatment of various lymphoid malignancies, lymphoproliferative diseases, and rheumatologic disorders. Rituximab is generally well tolerated. However, increased use of rituximab has been associated with hypersensitivity reactions (HSRs), which can be classified as infusion-related, cytokine-release, type I (IgE/non-IgE), mixed, type III, and type IV reactions. Immediate infusion-related reactions to rituximab are quite common and decrease in frequency with subsequent infusions. However, in about 10% of patients, severe infusion-related reactions develop, which prevent its use. Some of the immediate infusion reactions are due to a cytokine-release but some reactions raise concerns for type I (IgE/non-IgE) hypersensitivity. Recent studies have shown the presence of serum anti-rituximab antibodies, either represented by the IgG or IgE isotype. In some cases, clinical manifestations of IgE-mediated reactions and cytokine-release reactions partially overlap, which is called a mixed reaction. Classified as Type III reaction, rituximab-induced serum sickness reactions have been reported in patients with autoimmune diseases and hematological malignancies. The classic serum sickness triad (fever, rash, and arthralgia) has been observed in patients mainly with an underlying rheumatologic condition. Severe delayed type IV hypersensitivity reactions including non-severe maculopapular rash to severe reactions such as Stevens–Johnson syndrome and toxic epidermal necrolysis have been rarely reported following rituximab injection. Comprehensive reviews focused on rituximab-induced HSRs are scarce. We aimed to review clinical presentations, underlying mechanisms of rituximab hypersensitivity, as well as management including rapid drug desensitization.

Keywords: biologic agents, rituximab, hypersensitivity, desensitization, drug allergy, serum sickness, monoclonal antibody, infusion reaction

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INTRODUCTION

Monoclonal antibodies (mAbs) have become mandatory for neoplastic targeted therapies as well as chronic inflammatory and autoimmune diseases (Beck et al., 2010; Li et al., 2013; Patel and Khan, 2017; Picard and Galvão, 2017; Özyiğit et al., 2020). Rituximab is a chimeric IgG mAb directed against CD20 antigen that is expressed on normal and malignant B cells (Plosker and Figgitt, 2003).

It was initially approved as an anti-neoplastic agent (Vikse et al., 2019). Later on, it became a bright treatment opportunity instead of the conventional treatment for chronic granulomatosis and inflammatory diseases (Wong and Long, 2017).

Rituximab treatment results in two main categories of adverse reactions, including immunodeficiency and hypersensitivity reactions (HSRs). The drug is considered to be of good and accepted tolerability, (Vidal et al., 2011; Makatsori et al., 2014); however, increased use of rituximab has been associated with HSRs (Brennan et al., 2009; Patel and Khan, 2017; Picard and Galvão, 2017; Isabwe et al., 2018). Comprehensive reviews focused on rituximab-induced HSRs are scarce. This article aimed to review and describe clinical presentations, underlying mechanisms of rituximab hypersensitivity as well as management including rapid drug desensitization (RDD).

HYPERSENSITIVITY REACTIONS TO RITUXIMAB

Classification and Prevalence

Adverse drug reactions are classified into two major types: type A reactions are common and may occur in any individual. Type B reaction, also called "Drug Hypersensitivity", is uncommon, unpredictable, and occurs only in susceptible individuals. Drug Hypersensitivity occurs *via* immune (allergic) or non-immune mechanisms. Allergic drug reactions can manifest with many different clinical presentations, and to better explain these variations in clinical presentations, traditional Gell and Coombs classification is used (Pichler, 2006).

Like other pharmaceutical agents, biological agents can cause adverse drug reactions. Because of the inherent differences between biologicals and pharmaceutical drugs, adverse reactions to biologicals cannot be classified according to the traditional classification. Therefore, alternative classification schemes have been suggested. One proposal by Pichler is based on the immunological activity of biologicals and to distinguish it from the classification of adverse drug reactions, the Greek alphabets, alfa, beta, gamma, delta, and epsilon have been used. This classification includes five groups: (Type α) high cytokine levels, (Type β) hypersensitivity reactions, IgE, IgG, and T cell mediate reactions, (Type δ) immune imbalance syndrome, (Type γ) cross-reactivity with native proteins, and (Type ε) non-immunologic adverse effects (Pichler, 2006; Patel and Khan, 2017; Wong and Long, 2017). Alfa reactions are associated with high cytokine levels, and in most cases, high cytokine levels occur due to endogenous cell activation, that is called a cytokine release reaction. Beta-type reactions are HSRs and are further defined as immediate and delayed and occur with IgE, IgG, and complement or T cell involvement. (Pichler, 2006; Corominas et al., 2014; Picard and Galvão, 2017).

However, recently a new classification was proposed considering phenotypes, endotypes, and biomarkers indicating underlying endotype. Phenotype and endotypes were based on the clinical presentation, the cells and mediators involved in the reaction respectively. The classification proposes four patterns of phenotypes: type I reactions (IgE/non-IgE), cytokine-release reactions, mixed reactions (type I/cytokine-release), and delayed-type IV reactions (Isabwe et al., 2018) (**Table 1**). This new classification encompasses the classic HSRs described by Gell and Coombs as well as reactions outside the classification, but it has no space for type II or III reactions since this classification focuses on the reactions that might benefit from desensitization.

Infusion-Related Reactions

Patients mostly suffer from common acute infusion reactions that occur in a short time after infusion. Although the pathogenesis of these reactions is not very clear, it's usually affected by the rate of infusion, pointing out to the possibility of a non-immunologic mechanism and the role of the inflammatory

TABLE 1 | Classifications of hypersensitivity reactions to biological agents including rituximab.

Type of reaction	Mechanism/Diagnostic criteria	References No≠
Infusion-related reaction	Non-immunologic, monocytes, macrophages, cytotoxic T cells, natural killer cells activation/clinical presentations and course, levels of IL-1, IL-6, and TNF-α	Pichler, 2006; Corominas et al., 2014; Galvão and Castells, 2015; Khan, 2016; Isabwe et al., 2018
Cytokine release reaction	Non-immunologic, monocytes, macrophages, cytotoxic T cells, natural killer cells activation/clinical presentations and course, levels of IL-1, IL-6. and TNF-α	Pichler, 2006; Corominas et al., 2014; Galvão and Castells, 2015; Khan, 2016; Isabwe et al., 2018
Type I reaction (IgE/non-IgE)	IgE or non- IgE dependent mast cell, basophil activation/clinical presentations, level of tryptase, skin tests, BAT	Pichler, 2006; Corominas et al., 2014; Galvão and Castells, 2015; Khan, 2016; Isabwe et al., 2018
Mixed reaction	IgE or non-IgE dependent mast cell, basophil activation and monocytes, macrophages, cytotoxic T cells, natural killer cells activation/clinical presentations, skin tests, BAT, levels of tryptase, IL-1, IL-6, and TNF-α	Pichler, 2006; Corominas et al., 2014; Galvão and Castells, 2015; Khan, 2016; Isabwe et al., 2018
Type III reaction	Not clear, may be related to C-fixing IgM and IgG antibodies and Fc-IgG receptor-mediated neutrophil activation clinical presentations, RF, immunoglobulins, and HACA levels	Karmacharya et al., 2015
Type IV reaction	T-cell mediated or other mechanisms/clinical presentations, immunohistological examination	Henning and Firoz, 2011; Macdonald et al., 2015; Fallon and Heck, 2015; Chen et al., 2018

C, complement; HACA, human anti-chimeric antibody; TNF-α, tumor necrosis factor-α.

cytokines such as IL-6 and tumor necrosis factor-α (Khan, 2016; Patel and Khan, 2017). Clinical presentations resemble type I or cytokine-release, but they are mild to moderate in severity and subside gradually with the following infusions (Plosker and Figgitt, 2003; Galvão and Castells, 2015).

Cytokine Release Reactions

The phenotype is defined as fever/chills, nausea, pain, headache, and rigors not responding to premedication/slower infusion rate during the first infusion. Clinical symptoms and signs are usually due to the cytokine release that is characterized by elevated serum TNF-α and IL-6 levels at the time of the reaction compared with their normal baseline (Isabwe et al., 2018).

Type I Reactions (IgE/Non-IgE)

The reaction is defined as flushing, pruritus, urticaria, shortness of breath, wheezing, hypotension, and life-threatening anaphylaxis. Reactions are associated with IgE or non-IgE mediated mast cell/basophil degranulation leading to massive histamine, leukotrienes and prostaglandins release. Skin test positivity and/or specific IgE to rituximab is indicative of both IgE-mediated and mixed reactions (Patel and Khan, 2017; Wong and Long, 2017; Isabwe et al., 2018).

Mixed Reactions

Mixed reactions are a combination of cytokine release and IgE-mediated reactions. Clinical presentations are characterized by wheezing, flushing, urticaria, pruritus, with fever/chills, nausea, pain, headache, and rigor. Skin test positivity and/or specific IgE to rituximab as well as increased levels of tryptase, IL-1, IL-6 and TNF-α can occur (Patel and Khan, 2017; Isabwe et al., 2018).

There are limited data on the frequency of HSRs and standard infusion reactions to rituximab. Additionally, the lack of consensus on the definition and classification of HSRs makes the data even confusing (**Table 2**). Among biological agents, rituximab has the highest reported infusion reactions, with up to 77% reported with the first infusion (van Vollenhoven et al., 2013). It also has a relatively high rate of HSRs, consistent with the IgE-mediated reactions, reported with 5 to 10% of infusions (Brennan et al., 2009; Galvão and Castells, 2015). In a study by Isabwe et al., prevalence of type I, cytokine-release, mixed type, and delayed-type IV reactions were reported as 63, 13, 21, and 3% respectively (Isabwe et al., 2018).

Brown classification is commonly used in the classification of severity of HSRs to mAbs. Grade 1 (mild) represents only skin/subcutaneous involvement, Grade 2 (moderate) presents gastrointestinal, cardiovascular or respiratory system affection, and Grade 3 (severe) consisted of failure of neurologic, respiratory, or cardiovascular systems (Brown, 2004).

Serum Sickness Reactions

Rituximab-induced serum sickness (RISS, type III) reactions have been observed less commonly. A systematic review reported 33 cases from 25 articles, the majority with underlying rheumatoid diseases. However, the systematic review has limitations such as the lack of confirmatory tests in all cases. Although the pathogenesis is not clear, it seems to be related to complement-fixing IgM and IgG antibodies targeted at an immunogenic part of the drug. The typical presentation has been found in 48.5% of cases. Symptoms are usually benign and self-limited in mild cases. Corticosteroid treatment may be beneficial, but premedication is not always effective. Correct diagnosis of RISS remains an unmet need (Karmacharya et al., 2015).

Type IV Reactions

Delayed type IV reactions are mostly presented with a maculopapular rash (Macdonald et al., 2015). Severe cutaneous reactions may occur, but they are probably rare. Two cases of Stevens–Johnson syndrome (SJS), one case of toxic epidermal necrolysis (TEN), and two cases of SJS-TEN caused by rituximab have been reported in a review (Chen et al., 2018). The U.S. FDA adverse events report seven cases of rituximab induced TEN (Fallon and Heck, 2015). There might be a false diagnosis of a case of SJS due to similarities in clinical findings, pathology, and prognosis resembling paraneoplastic pemphigus (PNP). To confirm cases of SJS/TEN in rituximab and differentiate them from PNP, direct and indirect immunofluorescence could be used (Joly et al., 2000; Henning and Firoz, 2011).

DIAGNOSIS

The detailed clinical history is crucial for determining the type and severity of the HSR (Picard and Galvão, 2017; Yang and Castells, 2019; Görgülü et al., 2019). Different hypersensitivity mechanisms such as type I IgE or non-IgE reactions could give rise to the same clinical picture (Isabwe et al., 2018). *In vivo* tests such as skin prick test (SPT) and intradermal testing (IDT), drug provocation test (DPT), and *in vitro* tests including specific IgE, basophil activation test (BAT), serum levels of tryptase, IL-1, IL-6, TNF-α, or lymphocyte transformation test (LTT) are used to define the phenotype of the HSR. Clinical history, *in vivo* and *in vitro* tests are all essential for personalized and precision medicine, but there is remarkable heterogeneity on diagnostic approaches (Santos and Galvao, 2017; Isabwe et al., 2018; Madrigal-Burgaleta et al., 2020).

Skin Testing as Diagnostic and Predictor for Breakthrough Reactions During Desensitization

Skin testing is the primary step for assessing HSRs to rituximab (Brennan et al., 2009). Skin test positivity demonstrated through SPT or IDT to rituximab suggests an IgE-mediated reaction. Despite insufficient evidence for optimal timing, skin testing with the culprit drug can be done within 2–4 weeks following the reaction to avoid false negative results (Alvarez-Cuesta et al., 2015; Santos and Galvao, 2017; Isabwe et al., 2018; Madrigal-Burgaleta et al., 2020). SPT is done using a drop of concentrated rituximab 10 mg/ml, and if it is negative, IDT is then performed with dilutions from 1:1,000 up to 1:1 (Wong and Long, 2017; Santos and Galvao, 2017; Isabwe et al., 2018). Positivity to rituximab is usually seen with IDT more than SPT. In a study,

TABLE 2 | Prevalence and severity of HSRs to rituximab.

References	Types, clinical features, and severity	
Brennan et al., 2009 n = 14 patients	**Clinical presentations** **Severity**	Approximately 57% cutaneous, 50% cardiovascular, 50% respiratory, 30% fever, 30% throat, 20% gastrointestinal, and 30% neurological reactions Grade I: 25%, Grade II: 50%, Grade III: 30%
van Vollenhoven et al., 2013 n = 3194 patients	-Acute infusion related reactions: 77% of patients	
Levin et al., 2017 n = 67 patients	**Incidence of reactions in relation to the number of infusions** **Clinical presentations** **Severity**	63% of reactions during 1st infusion, 9% during 2nd infusion, 15% during cycles 3–10, 7% during cycles 11–20, 6% during cycles 20–53 63% cutaneous reactions, 45% generalized pruritis, 21% flushing, 16% hives Grade 1A: 18%, Grade 1B: 9%, Grade 2: 61%, Grade 3: 10%, Grade 4: Less than 1%
Isabwe et al., 2018 n = 52 patients	**Reactions** **Severity**	Acute infusion reactions: 20-50% Type I reactions: 63% Cytokine-release reactions: 13% Mixed reactions: 21% Delayed reactions: 3% Grade I: 13%, Grade II: 60%, Grade III: 29%
Görgülü et al., 2019 n = 24 patients	**Reactions** (Based on Isabwe et al., 2018) **Clinical presentations** **Severity**	Type I (IgE/non-IgE): 46% Cytokine-release reaction: 12.5% Mixed reactions: 41.5% Cutaneous symptoms 92%, Respiratory symptoms 88%, Cardiovascular symptoms 67%, Gastrointestinal symptoms 55%, Neurologic/muscular symptoms 29%, Fever (≥38.3°C) 46% Grade I: 0%, Grade II: 63%, Grade III: 37%

30% of patients with HSRs to rituximab were positive in IDTs, and none of them were positive in SPT (Görgülü et al., 2019). Similarly, in an early study, IDT for rituximab was positive in six out of nine patients (Brennan et al., 2009). However, in another study, 20% of the patients were SPT positive, and 32% were IDT positive among 52% of skin test positive patients (Isabwe et al., 2018).

Skin test positivity was found to be correlated with the frequency of respiratory symptoms, but not to the severity of the initial reaction to rituximab (Görgülü et al., 2019). However, Isabwe et al. have found that positive rituximab skin testing was strongly associated with severe initial rituximab HSRs. The percentage of type I breakthrough reactions during desensitization was as high as 69% in patients with a positive skin test. Therefore, skin testing could be helpful in the prediction of the type of breakthrough reactions. On the other hand, breakthrough reactions for skin test negative patients were lower in severity during desensitization (Isabwe et al., 2018). Madrigal-Burgaleta et al. showed that patients with a positive SPT tend to encompass an important percentage of breakthrough reactions during desensitization (Madrigal-Burgaleta et al., 2019). Wong and Long have concluded that there is no significant difference in the risk of a breakthrough reaction if the patient is skin test positive or negative (Wong and Long, 2017). The differences may be due to patient selection, different concentrations and volume used for skin tests, the clinical symptoms of the patients, their severity, the time between the reaction and the study, such differences need to be clarified (Wong and Long, 2017).

Drug Provocation Test

Data on the use of DPT to biological agents are scarce and come from a few specialized centers. The Ramon y Cajal University Hospital (RCUH) group is the pioneer of DPT with the largest reported series with antineoplastics and biologicals. They positioned the use of DPT in patients with negative or equivocal skin test results provided the risk-assessment is favorable. There are no international guidelines for DPT with these drugs but the RCUH protocol is based on direct re-administration of the culprit drug under standard conditions (Alvarez-Cuesta et al., 2015; Madrigal-Burgaleta et al., 2019; Madrigal-Burgaleta et al., 2020; Martí-Garrido et al., 2020). If patients showed positive skin testing or positive DPT, then the HSR would be confirmed; conversely, a negative DPT would rule out an HSR. Thus, not doing DPTs routinely in the diagnosis of an HSR to biologics could bias the safety or the efficiency of RDD. However, this approach needs trained personnel and well-equipped center that limit its wide implementation (Madrigal-Burgaleta et al., 2019).

In Vitro Tests

In a patient with two immediate reactions to rituximab, non-isotype-specific and sIgE to rituximab were positive in the serum samples. More importantly, rituximab stimulated peripheral blood mononuclear cells displayed a response associated with a Th2 cytokine production profile (Vultaggio et al., 2012). The use of sIgE, BAT, serum levels of tryptase, IL-1, IL-6, TNF-α, and LTT as diagnostic tools to biologicals are restricted to selected patients in expert centers (Madrigal-Burgaleta et al., 2020).

MANAGEMENT

There's a general agreement about avoiding rituximab that has caused type IV HSR such as SJS, TEN, EM and DRESS as well as

RISS (Hong and Sloane, 2019; Yang and Castells, 2019). For mild to moderate common infusion reactions to rituximab, the manufacturer's instructions are to reduce the rate of infusion and premedicate the patients with an antihistamine, acetaminophen and methylprednisolone prior to dosing and liaise the allergist if required (https://www.ema.europa.eu/en/documents/product-information/mabthera-epar-product-information_en.pdf). In the event of an HSR to rituximab such as Type 1, cytokine release, mixed reaction and delayed maculopapular rash, RDD is a safe and valid alternative. The management by multidisciplinary teams led by expert allergists and access to adequate facilities for allergy procedures has shown to be the optimal approach, with the best efficacy and safety results (Isabwe et al., 2018; Görgülü et al., 2019; Hong and Sloane, 2019).

Patient Selection for Rapid Drug Desensitization

Patients with a clinical history of HSR to biologicals and who have a confirmation from their referring specialist to use the drug as their first line of choice with no better alternatives are possible candidates for RDD (Sloane et al., 2016; Patel and Khan, 2017; Picard and Galvão, 2017). Different groups use different criteria for patient selection for RDD. The Brigham and Women's Hospital (BWH), a very well-known center for RDD, recommends that RDD should always be performed on patients with positive *in vivo*/*in vitro* tests, regardless of the grade of the initial HSRs. If the test restults are negative and the initial HSR is Grade I (low risk), a challenge may be performed. If there is no reaction during the challenge, the patient can be sent back to regular infusion. However, if there is a reaction, RDD should be performed for the next drug exposure. If the test results are negative and the initial HSR is Grade II/III (moderate-high risk), RDD is indicated (Castells, 2009; Galvão and Castells, 2015; Isabwe et al., 2018). RCUH considers that DPT should always be performed systematically prior to RDD, but only if the risk-assessment for the patient is favorable. This risk-assessment strategy involves a number of factors (*e.g.* ST, serum biomarkers, patient comorbidities, and patient wishes) and is discussed in a collaborative decision-making process including the referring physician, the allergist, and the patient (who makes the final decision) (Alvarez-Cuesta et al., 2015; Madrigal-Burgaleta et al., 2019).

Premedication in Rapid Drug Desensitization

Premedication is a controversial issue and there is no categorical recommendation. BWH recommends routine pre-medications in all desensitization protocols such as cetirizine 10 mg, montelukast 10 mg, or zileuton to prevent bronchospasm, famotidine 20 mg for H1 and H2 blockage, and aspirin 81 to 325 mg to prevent flushing caused by prostaglandins. As well as ibuprofen 200 to 800 mg, meperidine 25 mg, or acetaminophen 650 mg for prevention of rigors, pain, and fever. 40 to 50 mg methylprednisolone or other steroids are needed in cases of more severe reactions. Benzodiazepines can be added to control anxiety (Brennan et al., 2009; Castells, 2009; Görgülü et al., 2019; Yang and Castells, 2019).

Contrary, RCUH Allergy Division Desensitization Program showed that in a limited number of patients with confirmed hypersensitivity to Paclitaxel, desensitization alone might be more than enough to control allergic reactions, and premedication with antihistamines and corticosteroids made no difference to the breakthrough reactions (Lopez-Gonzalez et al., 2018). The same group currently reported that they used only standard premedication for each drug (according to prescribing information by the manufacturer and institutional protocols) but for some cases additional premedication customized to their initial or breakthrough reactions (Madrigal-Burgaleta et al., 2019). Their results show no significant increase in the number or severity of breakthrough reactions when compared with results reported by other groups (Brennan et al., 2009; Sloane et al., 2016; Picard and Galvão, 2017; Isabwe et al., 2018).

Even if evidence is weak and the topic is controversial, whenever possible, β-blockers and ACE-Inhibitors should be avoided one day prior to desensitization as the former blocks the action of epinephrine and the latter may even aggravate immediate reaction (Aberer et al., 2003; Lebel et al., 2016; Görgülü et al., 2019; Madrigal-Burgaleta et al., 2019; Yang and Castells, 2019).

Available data suggest that systematic use of premedication may not play a significant role in improving the effectiveness and safety of RDD and should be carefully and individually discussed if their only purpose is to prevent breakthrough reactions. All groups seem to be recommended a personalized approach and further studies are needed for optimal premedication protocols (**Table 4**).

Rapid Desensitization Protocols

Several protocols have been proposed (Puchner et al., 2001; Jerath et al., 2009; Amorós-Reboredo et al., 2015; Madrigal-Burgaleta et al., 2019). However, the protocol developed by the BWH has gained wide acceptance. The 12-step protocol consists of three bags, where tolerance to the offending antigen dose is obtained by giving the patient 2 to 2.5 incremental doses of the drug, through increasing the rate of infusion and the concentration of the drug at fixed 15-min intervals. The remaining amount of the total dose is infused at a steady rate of infusion in the last step. A 4-bag, 16-step protocol can be initiated for more severe reactions (Castells, 2009). Few studies specifically focused on rituximab desensitization (**Table 3**) (Brennan et al., 2009; Amorós-Reboredo et al., 2015; Ataca et al., 2015; Tal et al., 2016; Wong and Long, 2017; Görgülü et al., 2019). A recent study with 141 RDD in reported that only 14 RDD were interrupted by breakthrough reactions leading to the incompletion of two desensitizations only due to development of anaphylaxis, with a success rate for RDD of 98.5%. Usually, breakthrough reactions are mild and develop in the last steps of the protocol (Görgülü et al., 2019). In another study, five patients have had 19 desensitizations to rituximab where all RDDs were successful. Only two patients have

TABLE 3 | Rapid desensitization protocols data from different studies.

Reference	Patients/Desensitization number	RDD protocol Success rate	BTRs	Skin test positivity
Brennan et al., 2009	14/55	12-step protocol 100% successful	40% of patients Grade 1 reaction	IDT: 6/9 patients
Amorós-Reboredo et al., 2015	5/19	12-step protocol 100% successful	2 patients had neuromuscular reactions	IDT: 2/5 patients
Tal et al., 2016	7/53	Modified 12-step protocol 100% successful	Grade 1 reaction in 3 RDDs	ND
Wong and Long, 2017	25/170	3 protocols: High-risk, intermediate and rapid protocols (3-8 steps) 100% successful	29% of patients (53% Grade 1 37% Grade 2 9% Grade 3)	IDT: 5/18 patients
Görgülü et al., 2019	24/141	12-step protocol 16-step in patients with severe reactions 98.5% successful	14 patients -Grade 1: 17% -Grade 2: 33% -Grade 3: 8% -2 RDDs couldn't be completed	IDT: 6/ 20patients
Madrigal-Burgaleta et al., 2019*	21/130	10-step protocol 100% successful*	Not reported	Not reported

BTRs, Break Through Reactions; NP, No data; IDT, Intradermal test.
**Data was not provided specifically on the rituximab-reactive patients but extracted from biological patients as a whole.*

TABLE 4 | Unmet needs and future research for HSRs to rituximab.

Classification	Clear and acceptable classification for the type of reaction Well defined criteria for this classification Clear clinical and laboratory criteria to differentiate the type of reactions Effective biomarkers for clear endotyping underlying reactions
Epidemiology	Lack of data about morbidity and mortality of each type of reactions
RISS*	Clinical trials in such patients with allergist involvement to obtain better evidence for the diagnostic criteria and management
SCARs**	Clearly understanding the mechanism and diagnostic methods The differential diagnosis in case of exposure to other concomitant medications that are known to cause SCARs
Skin tests	Non-irritant dose of rituximab The high cost of rituximab to use for skin tests Lack of skin test reagents containing all the immune epitopes Perfect timing for doing skin tests after an HSR Role of skin test in the prediction of breakthrough reactions The role of patch test in the diagnosis of delayed-type reaction
***In vitro* tests**	Their roles in diagnosis
Desensitization	Optimal premedication protocols Candidates for desensitization
Cross-reactivity	Between rituximab, obinutuzumab, ofatumumab, veltuzumab and ocrelizumab
OVERALL	A multidisciplinary team study including allergists, pharmacologists, nurses, oncologists, hematologists, and other specialties to improve the diagnostic approach and management of HSR s to mAbs and to overcome the unmet needs

**Rituximab induced serum sickness, **Severe cutaneous adverse reaction.*

developed breakthrough reactions in the form of neuromuscular reactions. Two out of five patients had positive IDT (Amorós-Reboredo et al., 2015). Wong and Long have demonstrated that out of 25 patients, 29% have experienced breakthrough reactions. IDTs only were positive in five of 18 patients. All 170 RDDs were conducted successfully using high-risk, intermediate or rapid protocols that consisted of 3–8 steps (Wong and Long, 2017). Brennan and colleagues have successfuly completed all 55 RDDs to rituximab for 14 patients with a 12-step protocol, where 40% of patients had symptoms of grade 1 breakthrough reactions. Only nine patients underwent IDT showing six positive results (Brennan et al., 2009). A different study has performed 53 RDDs on seven patients without skin testing. They used a modified 12-step protocol with 100% success. Grade 1 breakthrough reactions were reported in three RDDs (Tal et al., 2016).

Management of Breakthrough Reactions During Desensitization

The rapid desensitization protocol does not need to be suspended because of a breakthrough reaction. Once the reaction is controlled, the RDD protocol can be reinitiated and followed to completion.

Breakthrough reactions to rituximab were generally mild. However, moderate and severe reactions may appear, although less frequently than mild reactions, and the majority of desensitizations were completed (Lebel et al., 2016; Görgülü et al., 2019). Cutaneous involvement has been the main feature of breakthrough reactions (Brennan et al., 2009; Görgülü et al., 2019). If a breakthrough reaction occurs, the infusion must be immediately stopped, and specific medications should be given according to the symptoms experienced. For future RDD, protocols should be customized based on the severity,

symptoms and type of breakthrough reaction such as additional premedication and/or dilutions (Madrigal-Burgaleta et al., 2019; Yang and Castells, 2019).

CONCLUSION

Although rituximab is generally well tolerated, its widespread use has entailed an increase in the number of HSRs. There are different proposed classifications for HSRs to mAbs including rituximab with some degree of overlap. Each type of HSR has its features, course, and management. The new proposed classification seems to have clinical implication in terms of a personalized and precise approach. Skin tests, done 2–4 weeks after reaction, is the first step in the diagnostic algorithm and in case of negativity, should be followed by DPT in the availability of adequate settings. RDD is considered a cornerstone of treatment for patients with immediate-type HSRs to rituximab, whereas it seems to be performed in few centers. Therefore, desensitization approach needs more awareness and needs to gain more acceptance. Overall, institutional multidisciplinary teams promoted by allergists to manage HSRs to mAbs including rituximab is crucial.

PERSPECTIVES

New mAbs targeting CD20, obinutuzumab, ofatumumab, veltuzumab and ocrelizumab have been currently introduced to the market. Infusion reactions are the most common adverse event reported with these anti-CD20 mAbs, whether chimeric, humanized or human. However, the relative frequencies have not been studied in a head-to-head fashion. There is no data about cross-reactivity between these mAbs. This may be related to the exclusion of patients with a history of severe allergic reactions to these mAbs from such studies (Gelfand et al., 2017; Salles et al., 2017) (**Table 4**).

UNMET NEEDS AND FUTURE RESEARCH

There are several unmet needs (**Table 4**) that will lead to future research in this area.

AUTHOR CONTRIBUTIONS

SB directed the writing and was responsible for the overall guidance. GF and SB wrote and revised the manuscript.

REFERENCES

https://www.ema.europa.eu/en/documents/product-information/mabthera-epar-product- information_en.pdf.

Aberer, W., Bircher, A., Romano, A., Blanca, P., Campi, J., Fernandez, K., et al. (2003). Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. *Allergy* 58 (9), 854–863. doi: 10.1034/j.1398-9995.2003.00279.x

Alvarez-Cuesta, E., Madrigal-Burgaleta, R., Angel-Pereira, D., Ureña-Tavera, A., Zamora-Verduga, M., Lopez-Gonzalez, P., et al. (2015). Delving into cornerstones of hypersensitivity to antineoplastic and biological agents:value of diagnostic tools prior to desensitization. *Allergy* 70 (7), 784–794. doi: 10.1111/all.12620

Amorós-Reboredo, P., Sánchez-López, J., Bastida-Fernández, C., do Pazo-Oubiña, F., Borràs-Maixenchs, N., Giné, E., et al. (2015). Desensitization to rituximab in a multidisciplinary setting. *Int. J. Clin. Pharm.* 37 (5), 744–748. doi: 10.1007/s11096-015-0136-x

Ataca, P., Atilla, E., Kendir, R., Bavbek, S., and Ozcan, M. (2015). Successful Desensitization of a Patient with Rituximab Hypersensitivity. *Case Rep. Immunol.* 2015, 1–4. doi: 10.1155/2015/524507

Beck, A., Wurch, T., Bailly, C., and Corvaia, N. (2010). Strategies and challenges for the next generation of therapeutic antibodies. *Nat. Rev. Immunol.* 10 (5), 345–352. doi: 10.1038/nri2747

Brennan, P. J., Bouza, T. R., Hsu, F. I., Sloane, D. E., and Castells, M. C. (2009). Hypersensitivity reactions to mAbs: 105 desensitizations in 23 patients, from evaluation to treatment. *J. Allergy Clin. Immunol.* 124 (6), 1259–1266. doi: 10.1016/j.jaci.2009.09.009

Brown, S. G. A. (2004). Clinical features and severity grading of anaphylaxis. *J. Allergy Clin. Immunol.* Aug114 (2), 371–376. doi: 10.1016/j.jaci.2004.04.029

Castells, M. (2009). Rapid Desensitization for Hypersensitivity Reactions to Medications. *Immunol. Allergy Clin. North Am.* Aug29 (3), 585–606. doi: 10.1016/j.iac.2009.04.012

Chen, C.-B., Wu, M.-Y., Ng, C. Y., Lu, C.-W., Wu, J., Kao, P.-H., et al. (2018). Severe cutaneous adverse reactions induced by targeted anticancer therapies and immunotherapies. *Cancer Manag. Res.* 10, 1259–1273. doi: 10.2147/CMAR.S163391

Corominas, M., Gastaminza, G., and Lobera, T. (2014). Hypersensitivity Reactions to Biological Drugs. *J. Invest. Allergol. Clin. Immunol.* 24, 14.

Fallon, M. J., and Heck, J. N. (2015). Fatal Stevens-Johnson syndrome/toxic epidermal necrolysis induced by allopurinol–rituximab–bendamustine therapy. *J. Oncol. Pharm. Pract.* 21 (5), 388–392. doi: 10.1177/1078155214533368

Galvão, V. R., and Castells, M. C. (2015). Hypersensitivity to Biological Agents—Updated Diagnosis, Management, and Treatment. *J. Allergy Clin. Immunol. Pract.* 3 (2), 175–185. doi: 10.1016/j.jaip.2014.12.006

Gelfand, J. M., Cree, B. A. C., and Hauser, S. L. (2017). Ocrelizumab and Other $CD20^+$ B-Cell-Depleting Therapies in Multiple Sclerosis. *Neurotherapeutics* 14 (4), 835–841. doi: 10.1007/s13311-017-0557-4

Görgülü, B., Seval, G., Kendirlinan, R., Toprak, S., Özcan, M., and Bavbek, S. (2019). Rapid Drug Desensitization With Rituximab in 24 Cases: A Single-Center Experience. *J. Invest. Allergol. Clin. Immunol.* 29 (6), 468–470. doi: 10.18176/jiaci.0445

Henning, J. S., and Firoz, B. F. (2011). Rituxan is not associated with Stevens-Johnson Syndrome. *Ann. Oncol.* 22 (6), 1463–1464. doi: 10.1093/annonc/mdr254

Hong, D., and Sloane, D. E. (2019). Hypersensitivity to monoclonal antibodies used for cancer and inflammatory or connective tissue diseases. *Ann. Allergy Asthma Immunol.* 123 (1), 35–41. doi: 10.1016/j.anai.2019.04.015

Isabwe, G. A. C., Garcia Neuer, M., de las Vecillas Sanchez, L., Lynch, D.-M., Marquis, K., and Castells, M. (2018). Hypersensitivity reactions to therapeutic monoclonal antibodies: Phenotypes and endotypes. *J. Allergy Clin. Immunol.* 142 (1), 159–170.e2. doi: 10.1016/j.jaci.2018.02.018

Jerath, M. R., Kwan, M., Kannarkat, M., Mirakhur, B., Carey, L., Valgus, J., et al. (2009). A desensitization protocol for the mAb cetuximab. *J. Allergy Clin. Immunol.* 123 (1), 260–262. doi: 10.1016/j.jaci.2008.09.046

Joly, P., Richard, C., Gilbert, D., Courville, P., Chosidow, O., Roujeau, J. C., et al. (2000). Sensitivity and specificity of clinical, histologic, and immunologic features in the diagnosis of paraneoplastic pemphigus. *J. Am. Acad. Dermatol.* 43 (4), 619–626. doi: 10.1067/mjd.2000.107488

Karmacharya, P., Poudel, D. R., Pathak, R., Donato, A. A., Ghimire, S., Giri, S., et al. (2015). Rituximab-induced serum sickness: A systematic review. *Semin. Arthritis Rheum.* 45 (3), 334–340. doi: 10.1016/j.semarthrit.2015.06.014

Khan, D. A. (2016). Hypersensitivity and immunologic reactions to biologics: opportunities for the allergist. *Ann. Allergy Asthma Immunol.* 117 (2), 115–120. doi: 10.1016/j.anai.2016.05.013

Lebel, E., Ben-Yehuda, D., Bohbot, E., Dranitzki, Z., Shalit, M., and Tal, Y. (2016). Hypersensitivity reactions to rituximab: 53 successful desensitizations in 7 patients with severe, near-fatal reactions. *J. Allergy Clin. Immunol. Pract.* 4 (5), 1000–1002. doi: 10.1016/j.jaip.2016.05.013

Levin, A., Otani, I., Lax, T., Hochberg, E., and Banerji, A. (2017). Reactions to Rituximab in an Outpatient Infusion Center: A 5-Year Review. *J. Allergy Clin. Immunol: Pract.* 5 (1), 107–113.e1. doi: 10.1016/j.jaip.2016.06.022

Li, G. N., Wang, S. P., Xue, X., Qu, X. J., and Liu, H. P. (2013). Monoclonal antibody-related drugs for cancer therapy. *Drug Discovery Ther.* 7 (5), 178–184. doi: 10.5582/ddt.2013.v7.5.178

Lopez-Gonzalez, P., Madrigal-Burgaleta, R., Carpio-Escalona, L. V., Bernal-Rubio, L., Guerra, E., Berges-Gimeno, M. P., et al. (2018). Assessment of Antihistamines and Corticosteroids as Premedication in Rapid Drug Desensitization to Paclitaxel: Outcomes in 155 Procedures. *J. Allergy Clin. Immunol. Pract.* 6 (4), 1356–1362. doi: 10.1016/j.jaip.2017.11.013

Macdonald, J. B., Macdonald, B., Golitz, L. E., LoRusso, P., and Sekulic, A. (2015). Cutaneous adverse effects of targeted therapies: Part I: Inhibitors of the cellular membrane. *J. Am. Acad. Dermatol.* 72 (2), 203–218. doi: 10.1016/j.jaad.2014.07.032

Madrigal-Burgaleta, R., Bernal-Rubio, L., Berges-Gimeno, M. P., Carpio-Escalona, L. V., Gehlhaar, P., and Alvarez-Cuesta, E. (2019). A Large Single-Hospital Experience Using Drug Provocation Testing and Rapid Drug Desensitization in Hypersensitivity to Antineoplastic and Biological Agents. *J. Allergy Clin. Immunol. Pract.* 7 (2), 618–632. doi: 10.1016/j.jaip.2018.07.031

Madrigal-Burgaleta, R., Vazquez-Revuelta, P., Marti-Garrido, J., Lleonart, R., Ali, F. R., and Alvarez-Cuesta, E. (2020). Importance of Diagnostics Prior to Desensitization in New Drug Hypersensitivity: Chemotherapeutics and Biologicals. *Curr. Treat Options Allergy* 7, 1–13. doi: 10.1007/s40521-020-00238-y

Makatsori, M., Kiani-Alikhan, S., Manson, A. L., Verma, N., Leandro, M., Gurugama, N. P., et al. (2014). Hypogammaglobulinaemia after rituximab treatment–incidence and outcomes. *QJM* 107 (10), 821–828. doi: 10.1093/qjmed/hcu094

Martí-Garrido, J., Vázquez-Revuelta, P., Lleonart-Bellfill, R., Molina-Mata, K., Muñoz-Sánchez, C., and Madrigal-Burgaleta, R. (2020). Pilot experience using drug provocation testing for the study of hypersensitivity to chemotherapy and biological agents [published online ahead of print, 2020 Jun 23]. *J. Invest. Allergol. Clin. Immunol.* 31 (1). doi: 10.18176/jiaci.0552

Özyiğit, L. P., Öztürk, A. B., and Bavbek, S. (2020). Anti-IL-5 Biologicals Targeting Severe Late Onset Eosinophilic Asthma. *Turk. Thorac. J.* 21 (1), 61–68. doi: 10.5152/TurkThoracJ.2019.180204

Patel, S. V., and Khan, D. A. (2017). Adverse Reactions to Biologic Therapy. *Immunol. Allergy Clin. North Am.* 37 (2), 397–412. doi: 10.1016/j.iac.2017.01.012

Picard, M., and Galvão, V. R. (2017). Current Knowledge and Management of Hypersensitivity Reactions to Monoclonal Antibodies. *J. Allergy Clin. Immunol. Pract.* 5 (3), 600–609. doi: 10.1016/j.jaip.2016.12.001

Pichler, W. J. (2006). Adverse side-effects to biological agents. *Allergy* 61 (8), 912–920. doi: 10.1111/j.1398-9995.2006.01058.x

Plosker, G. L., and Figgitt, D. P. (2003). Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs* 63 (8), 803–843. doi: 10.2165/00003495-200363080-00005

Puchner, T. C., Kugathasan, S., Kelly, K. J., and Binion, D. G. (2001). Successful desensitization and therapeutic use of infliximab in adult and pediatric Crohn's disease patients with prior anaphylactic reaction. *Inflammation Bowel Dis.* 7 (1), 34–37. doi: 10.1097/00054725-200102000-00005

Salles, G., Barrett, M., Foà, R., Maurer, J., O'Brien, S., Valente, N., et al. (2017). Rituximab in B-Cell Hematologic Malignancies: A Review of 20 Years of Clinical Experience. *Adv. Ther.* 34 (10), 2232–2273. doi: 10.1007/s12325-017-0612-x

Santos, B. R., and Galvao, V. R. (2017). Monoclonal antibodies hypersensitivity. Prevalence and management. *Immunol. Allergy Clin. N. Am.* 37, 695–711. doi: 10.1016/j.iac.2017.07.003

Sloane, D., Govindarajulu, U., Harrow-Mortelliti, J., Barry, W., Hsu, F. I., Hong, D., et al. (2016). Safety, Costs, and Efficacy of Rapid Drug Desensitizations to Chemotherapy and Monoclonal Antibodies. *J. Allergy Clin. Immunol. Pract.* 4 (3), 497–504. doi: 10.1016/j.jaip.2015.12.019

Tal, Y., Ben Yehuda, D., Shalit, M., and Lebel, E. (2016). Hypersensitivity Reactions to Rituximab: 53 Successful Desensitizations in 7 Patients with Severe, Near-Fatal Reactions. *J. Allergy Clin. Immunol.* 137 (2), AB39. doi: 10.1016/j.jaci.2015.12.131

van Vollenhoven, R. F., Emery, P., Bingham, C. O., Keystone, E. C., Fleischmann, R. M., Furst, D. E., et al. (2013). Long-term safety of rituximab in rheumatoid arthritis: 9.5-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients. *Ann. Rheum. Dis.* 72 (9), 1496–1502. doi: 10.1136/annrheumdis-2012-201956

Vidal, L., Gafter-Gvili, A., Salles, G., Dreyling, M. H., Ghielmini, M., Hsu Schmitz, S.-F., et al. (2011). Rituximab maintenance for the treatment of patients with follicular lymphoma: an updated systematic review and meta-analysis of randomized trials. *J. Natl. Cancer Inst.* 103 (23), 1799–1806. doi: 10.1093/jnci/djr418

Vikse, J., Jonsdottir, K., Kvaløy, J. T., Wildhagen, K., and Omdal, R. (2019). Tolerability and safety of long-term rituximab treatment in systemic inflammatory and autoimmune diseases. *Rheumatol. Int.* 39 (6), 1083–1090. doi: 10.1007/s00296-019-04272-1

Vultaggio, A., Matucci, A., Nencini, F., Pratesi, S., Petroni, G., Cammelli, D., et al. (2012). Drug-specific Th2 cells and IgE antibodies in a patient with anaphylaxis to rituximab. *Int. Arch. Allergy Immunol.* 159 (3), 321–326. doi: 10.1159/000336839

Wong, J. T., and Long, A. (2017). Rituximab Hypersensitivity: Evaluation, Desensitization, and Potential Mechanisms. *J. Allergy Clin. Immunol. Pract.* 5 (6), 1564–1571. doi: 10.1016/j.jaip.2017.08.004

Yang, B. C., and Castells, M. C. (2019). Rituximab hypersensitivity and desensitization. *Ann. Allergy Asthma Immunol.* 123 (1), 11–15. doi: 10.1016/j.anai.2019.03.008

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Hypersensitivity Reactions to Multiple Iodinated Contrast Media

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The incidence of hypersensitivity reactions (HSRs) to iodinated contrast media (ICM) has risen over last years, representing an important health problem. HSRs to ICMs are classified into immediate reactions (IRs) and non-immediate reactions (NIRs) according to if they occur within 1 h or longer after ICM administration. The diagnosis of HSRs to ICM is complex as skin test (ST) sensitivity ranges widely, and drug provocation test (DPT) protocols are heterogeneous. In this manuscript, we describe the clinical characteristics of a series of patients confirmed as HSR to ICM and the diagnosis procedure carried out, looking into those cases confirmed as HSRs to multiple ICMs. For this purpose, we prospectively evaluated patients suggestive of HSRs to ICMs and classified them as IRs or NIRs. STs were carried out using a wide panel of ICMs, and in those with a negative ST, a single-blind placebo controlled DPT was performed with the culprit. If ST or DPT were positive, then tolerance was assessed with an alternative negative ST ICM. We included 101 cases (12 IRs and 89 NIRs) confirmed as allergic. Among them, 36 (35.64%) cases were allergic to more than one ICM (8 IRs and 28 NIRs). The most common ICM involved were iomeprol and iodixanol. Although not statistically significant, the percentage of patients reporting anaphylaxis was higher in patients allergic to multiple ICMs compared with patients allergic to a single ICM (50 vs. 25%). Likewise, the percentage of positive results in STs was higher in patients allergic to multiple ICMs compared with those allergic to a single ICM (for IR 62.5 vs. 25%, $p > 0.05$; and for NIR, 85.71 vs. 24.59%, $p < 0.000$). In cases allergic to more than one ICM, DPT with negative-ST ICM was positive in more than 60% (24/36) of cases. Therefore, allergy to multiple ICMs is common, associated to severe reactions in IRs, and confirmed frequently by positive STs. The allergological work-up should include DPT not only to establish the diagnosis but also to identify safe alternative ICM, even if ICM is structurally unrelated and ST is negative. More studies are needed to clarify mechanisms underlying cross-reactivity among ICMs.

Keywords: anaphylaxis, drug allergy, drug provocation test, exanthema, hypersensitivity, iodinated contrast media, skin test, urticaria

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INTRODUCTION

Over the last decade, the incidence of hypersensitivity reactions (HSRs) to iodinated contrast media (ICM) has risen in parallel with their increased usage (Brockow et al., 2005; Brockow, 2020), being estimated to occur in about 0.5–2% of patients receiving ICMs (Brockow et al., 2005). HSRs to ICMs are classified into immediate (IRs) and non-immediate reactions (NIRs) according to if they occur within one hour or within hours or even days, respectively, after administration (Brockow et al., 2005; Brockow, 2020). Reactions may vary from mild to severe, being skin the organ most frequently involved (Brockow et al., 2005; Torres et al., 2012; Salas et al., 2013; Brockow, 2020). HSRs to ICMs have traditionally been considered as non-allergic, but growing evidence points to immune mechanisms. Positive results in skin tests (STs), basophil activation tests, and specific IgE detection in IRs suggests a likely IgE-mediated mechanism (Laroche et al., 1998; Mita et al., 1998; Laroche et al., 1999; Trcka et al., 2008; Brockow et al., 2009; Pinnobphun et al., 2011; Salas et al., 2013; Steiner et al., 2016); and the analysis of skin biopsies obtained from positive-ST and -drug provocation tests (DPTs) in NIR patients, the monitorization of the immune response during the acute and resolution phases, and the proliferative response in lymphocyte transformation test supports a T cell involvement (Romano et al., 2002; Kanny et al., 2005; Lerch et al., 2007; Torres et al., 2008; Antunez et al., 2011; Torres et al., 2012).

The diagnosis of HSRs to ICMs is complex. It is based on the clinical history, STs, and DPTs, although their role has not been fully established. The diagnostic sensitivity of STs has been reported to range from less than 5% to more than 90% (Vernassiere et al., 2004; Kvedariene et al., 2006; Trcka et al., 2008; Brockow et al., 2009; Dewachter et al., 2011; Goksel et al., 2011; Torres et al., 2012; Prieto-Garcia et al., 2013; Morales-Cabeza et al., 2017), being its routine use still matter of debate (Brockow et al., 2009; Caimmi et al., 2010; Goksel et al., 2011; Prieto-Garcia et al., 2013; Yoon et al., 2015; Soria et al., 2019). DPT is considered the gold standard for diagnosing HSRs to drugs (Aberer et al., 2003), and, in the case of HSRs to ICMs, it is recommended to be performed with the ICM giving negative results in STs for confirming diagnosis or looking for a safe alternative (Rosado Ingelmo et al., 2016; Brockow, 2020). However, its use is controversial as it is a not-risk free procedure (Aberer et al., 2003) and doses administered during the allergological work-up lack of consensus, varying from 10 to 120 cc and being injected on a single day or incrementally increased over several days (Vernassiere et al., 2004; Torres et al., 2012; Prieto-Garcia et al., 2013; Salas et al., 2013; Sese et al., 2016; Lerondeau et al., 2016; Morales-Cabeza et al., 2017; Gracia-Bara et al., 2019; Soria et al., 2019; Trautmann et al., 2019).

The management of patients diagnosed as having HSRs to ICMs involves prohibiting the use of the culprit ICM and identifying non–cross-reactive agents that can be safely used by the patient (Brockow, 2020). Currently, controversies exist regarding the pattern of cross-reactivity. Frequent cross-reactions between iodixanol, iopamidol, iomeprol, iohexol, ioversol, and ioxitalamate have been described. Cross-reactivity seems to be related to the chemical structure of ICMs, as the most frequent association has been observed between iodixanol and iohexol, being iohexol the monomer of iodixanol (Vernassiere et al., 2004; Hasdenteufel et al., 2011; Torres et al., 2012; Lerondeau et al., 2016). In fact, a classification of ICMs based on the cross-reactivity between the different molecules and related to chemical structure similarities has been proposed (Lerondeau et al., 2016). However, recommending a safe alternative in patients with HSRs to ICMs is in some cases difficult and exceptionally not possible due to the high degree of cross-reactivity. In clinical studies, reactions to several ICMs have been observed (Vernassiere et al., 2004; Torres et al., 2012; Morales-Cabeza et al., 2017; Schrijvers et al., 2018; Trautmann et al., 2019), ranging widely from 14.3% (Prieto-Garcia et al., 2013) to 88% (Brockow et al., 2009).

In this manuscript, we have analyzed a population of patients with a confirmed diagnosis of HSRs to ICMs focusing on those with HSRs to multiple ICMs.

METHODS

We prospectively evaluated patients with symptoms suggestive of HSRs to ICMs referred to the Allergy Unit of the Hospital Regional Universitario of Málaga for the period of October 2005–April 2020. Patients confirmed as allergic following a stardardized procedure including clinical history, STs, and DPTs were finally included (Rosado Ingelmo et al., 2016). In those with a confirmed diagnosis of allergy to ICM, cross-reactivity with a panel of ICMs was assessed.

Patients were classified as IRs or NIRs if reactions appeared within 1 h after ICM administration or after (Demoly et al., 2014). The clinical categories included urticaria, angioedema, and anaphylaxis for IRs, and maculopapular exanthema and delayed urticaria for NIRs (Brockow et al., 2019; Brockow, 2020). Patients with severe cutaneous reactions as Stevens-Johnson syndrome, toxic epidermal necrolysis, acute generalized pustulosis, or drug reaction with eosinophilia and systemic symptoms were excluded from the study. Severity was graded: mild when no treatment was required, moderate when the patient responded to treatment and did not require hospitalization, and severe when the patient required hospitalization (Brockow et al., 2009).

The study was conducted according to the principles of the Declaration of Helsinki. All the participants were orally informed about the study and signed the corresponding informed consent.

Skin Test

STs were carried out as described (Torres et al., 2012; Brockow et al., 2013; Salas et al., 2013; Brockow, 2020) using a battery that included the following ICMs: iomeprol (Iomeron, Rovi, Madrid, Spain), iodixanol (Visipaque, GE Healthcare Biosciences, Madrid, Spain), iobitridol (Xenetix, Guerbet, Madrid, Spain), iohexol (Omnipaque, GE Healthcare Biosciences, Madrid, Spain), iopromide (Clarograf, Bayer, Barcelona, Spain), ioversol (Optiray, Covidien, Barcelona, Spain), and ioxaglate (Hexabrix,

Guerbet, Madrid, Spain). For IRs, skin prick tests (SPTs) were performed using undiluted ICM and if negative, and intradermal tests (IDTs) were performed using 10-fold dilutions, being read 20 min after testing. For NIRs, IDTs were performed using 10-fold diluted, and if negative, undiluted ICM, being read at 20 min, 1, 2, and 3 days after testing. Positive responses were considered for SPTs if a wheal larger than 3 mm surrounded by erythema appeared with a negative response to the control saline; and for IDTs, if the size of the initial wheal increased 3 mm or more in diameter, surrounded by erythema (Brockow et al., 2002).

Drug Provocation Test

In case of negative STs, a single-blind placebo controlled DPT was performed with the ICM involved if known, as described (Aberer et al., 2003; Torres et al., 2012; Salas et al., 2013). Additionally, in patients in which the culprit ICM was unknown and in those with a positive ST or DPT, tolerance was assessed with an alternative negative-ST ICM. For IRs, ICM was administered intravenously in saline at 45-min intervals using 5, 15, 30, and 50 cc (cumulative dose 100 cc). For NIRs, this was performed in two runs sufficiently separated to detect reactions, according to the time interval between the ICM administration and the onset of the reaction reported in the clinical history. In the first run, 5, 10, and 15 cc of ICM at 1-h intervals were administered, and if no reaction occurred, in the second run, 20, 30, and 50 cc (cumulative dose of 100 cc). Concomitant medications were stopped before DPT as previously described (Aberer et al., 2003; Rosado Ingelmo et al., 2016). As prophylaxis against renal damage, DPT procedures were separated at least 1 week, renal function was checked before ICM injection, and hydration with intravenous saline solution (0.9%) was administered if needed (Rudnick et al., 2008).

DPT was considered positive if cutaneous and/or respiratory symptoms or alterations in vital signs appeared during the procedure, then it was stopped, and the symptoms were evaluated and treated. For IRs, positive response was considered if manifestations occur up to 1 h after the DPT, and for NIRs, if cutaneous eruptions with similar clinical characteristics to those with the initial reaction occurred up to 7 days after the DPT.

Statistical Analysis

Data analysis was performed using Chi-square analysis to test differences in nominal variables between groups, Fisher test was used when there were no criteria for using Chi-square test and Mann-Whitney test was used for quantitative variables. All reported p values represented two-tailed tests, with values <0.05 considered statistically significant.

RESULTS

A total of 321 subjects with a history of suggestive HSRs after at least one ICM were evaluated (106 reported IRs and 187 NIRs). From these, 220 were excluded from this study: 192 tolerated the culprit ICM (94 subjects reporting IRs and 98 NIRs) and in 28 the allergological work-up was not completed (17 IRs and 11 NIRs) due to comorbidities that contraindicated DPT (n = 15); the rejection by the patient (n = 12); and the severity of the reported reaction that contraindicated DPT (Stevens-Johnson syndrome) (n = 1). A total of 101 cases confirmed as allergic were included: 12 (11.3%) IRs and 89 (48.6%) NIRs (**Figure 1**). We included data from two previously published studies by our group that were performed in 2006–2011 (Torres et al., 2012; Salas et al., 2013).

Clinical Characteristics and Diagnosis Approach of the Patients Included

The median age of the subjects was 62 (interquartile range: 49–69) years, and 52 (51.48%) were women. The most common ICM involved in the reactions were iodixanol (31; 30.69%) and iomeprol (33; 32.67%), followed by iohexol (16; 15.84%), iobitridol (2; 1.98%), iopramide (2; 1.98%), and ioxaglate (1; 0.99%), and in 16 (15.84%) cases, the ICM was unknown. No differences regarding age, gender, and involved ICM were found comparing IRs and NIRs. A total of 87 cases reported a single episode after ICM administration, and 14 had two episodes (all of them NIRs). Regarding the time interval between ICM administration and development of symptoms, IR patients experienced the symptoms within 1 h after administration: 9 (75%) reacted within the 30 min after the ICM administration, and 3 (25%) after 30–59 min. NIR symptoms appeared 12 h (interquartile range: 12–21) after the administration: 35 (39.32%) after 13–24 h, 34 (38.2%) after 25–48 h, 11 (12.25%) after 7–12 h, 8 (8.98%) more than 48 h later, and 1 (1.12%) after 1–6 h. According to the information obtained from the clinical history, 7 out of 12 (58.33%) cases reporting IRs developed urticaria, and 5 (41.66%) symptoms compatible with anaphylaxis. According to the severity scale of Ring and Messmer (Ring and Messmer, 1977), seven cases had grade I reactions, two had grade II reactions, and three patients had grade III reactions. No patients had grade IV reactions. Regarding NIRs, 60 (67.41%) cases had maculopapular exanthema, and 29 (32.58%) had delayed-appearing urticarial. The median time interval between the last reaction and the study was 5 months (interquartile range: 3–10). No differences were found comparing IRs and NIRs.

Regarding the results of the diagnostic methods, 6 (50%) subjects reporting IRs were diagnosed by a positive ST: 3 by SPT (1 to iodixanol, 1 to iomeprol, and 1 to iohexol) and 3 by IDT (3 to iohexol, 2 to iodixanol, 2 to iomeprol, and 1 to iobitridol). In positive-ST patients, DPT was performed with an alternative ICM, being positive in 2: one to iobitridol and one to iodixanol. In cases with a negative ST to all ICM tested, DPT was carried out with the culprit ICM if known, being positive in six cases: 4 to iomeprol, 3 to iodixanol, 2 to iobitridol, and 1 to iohexol.

Regarding NIRs, 39 (43.82%) of the subjects had a positive IDT: 24 to iomeprol, 11 to iodixanol, 7 to iohexol, 5 to iobitridol, 4 to ioxaglate, and 1 to iopramide. In positive-ST patients, DPT was performed with an alternative ICM, being positive in 14 cases: 10 to iodixanol, 4 iohexol, 4 to iobitridol, 2 to iopramide, and 1 to iomeprol. In cases with a negative ST to all ICM tested, DPT was carried out with the culprit if known, being positive in 50 cases: 41 to iodixanol, 10 to iomeprol, 4 to iobitridol, and to 4 iohexol.

Patients with positive DPT experienced similar symptoms to those recorded in their clinical history; however, they were

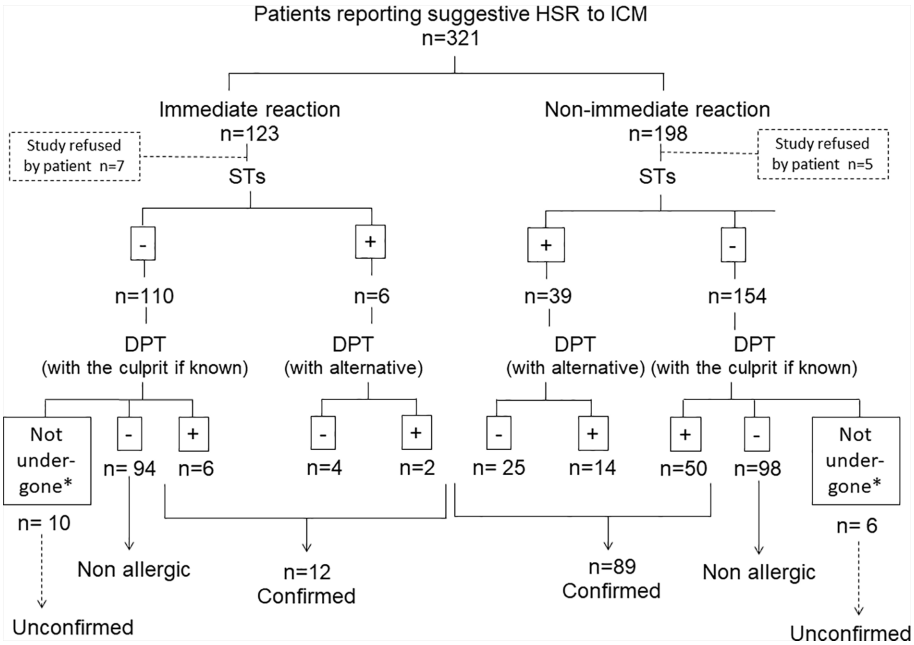

FIGURE 1 | Flow-chart for the patients included in the study. *Due to contraindications for DPT: 15 cases due to comorbidities and 1 case due to the severity of the reported reaction (Stevens-Johnson syndrome).

generally milder disappearing within 1–2 h after taking corticosteroid and antihistamine drugs. Only one patient reporting IR required a dose of 0.3 cc of adrenaline by intramuscular route to resolve their reaction within one hour.

Clinical Characteristics and Diagnosis approach of the Patients Allergic to Multiple ICMs

A total of 36 (35.64%) cases were allergic to more than one ICM, eight cases reporting IRs and 28 NIRs. This represents the 66.66% of all cases with a confirmed IR and the 31.46% of cases confirmed as NIR. The median age of the subjects was 64 (interquartile range: 49–69.5) years, and 20 (55.55%) were women. The ICMs involved in the reported reactions were iodixanol in 12 (33.33%), iomeprol in 10 (27.77%), iohexol in 7 (19.44%), ioxaglate in 1 (2.77%), and unknown in 6 (16.66%). No differences in age, gender and involved ICM were found comparing IRs and NIRs. Regarding the time interval between ICM administration and development of symptoms, IRs experienced the symptoms within 1 h after ICM administration: 7 (87.5%) cases within 30 min after the ICM administration and 1 (12.5%) with an interval if 31–59 min. NIRs appeared 10.3 (interquartile range: 6–12) h after ICM administration: 11 (39.28%) after 13–24 h, 10 (35.71%) after 25–48 h, four (14.28%) after 7–12 h, and three (10.71%) more than 48 h later. The clinical features of the reported reactions in cases allergic to multiple ICMs were urticaria in 12 (33.33%), anaphylaxis in 4 (11.11%), and MPE 20 (55.55%) (**Tables 1**, **2**).

The analysis of ST results in patients with allergy to multiple ICMs showed that 5 (62.5%) cases with IRs had a positive ST: 3 by SPT (1 to iodixanol, 1 to iomeprol, and one to iohexol) and two by IDT (2 to iohexol, 2 to iodixanol, 2 to iomeprol, and 1 to iobitridol). Regarding NIRs, 24 (85.71%) subjects had a positive IDT: 16 to iomeprol, eight to iodixanol, sto iohexol, 3 to iobitridol, 3 to ioxaglate, and 1 to iopramide (**Tables 1**, **2**). DPT was performed with negative-ST ICM, being positive in 5 cases with IRs: 3 to iomeprol, 3 to iodixanol, 2 to iobitridol, and 1 to iohexol. Six cases reporting IRs were confirmed as being allergic to 2 ICMs, 1 to 3 ICMs and 1 to 4 ICMs. DPT was positive in 19 cases reporting NIRs: 12 to iodixanol, 8 to iobitridol, 4 iohexol, 3 to iomeprol, and 2 to iopramide (**Tables 1**, **3**). A total of 18 subjects reporting NIRs were confirmed as being allergic to 2 ICMs, 8 to 3 ICMs, and 2 to 5 ICMs (**Table 1**). In 14 cases, no tolerated alternative was found: 12 cases refused to perform more DPTs with others negative-ST ICMs (patients 7, 9, 13, 21, 24, 25, 27, 29, 32, 33, and 35), and 2 cases (patients 28 and 31) were confirmed to be allergic to the 5 ICMs available in our hospital (**Table 1**). The most common associations detected were iodixanol and iomeprol in 17 cases (10 by ST plus DPT, 4 by STs, and 3 by DPT) and iodixanol and iohexol in 12 cases (7 by STs, 3 by DPT, and 2 by STs plus DPT) (**Table 1**).

Comparison of Clinical Characteristics and Diagnosis Approach in Both Patients Allergic to Multiple ICM and Those Allergic to a Single ICM

Comparing patients allergic to more than one ICM with those allergic to a single ICM, we found that the percentage of patients reporting anaphylaxis was higher in patients allergic to multiple ICM (50 vs. 25%; $p > 0.05$) (**Table 2**). The percentage of cases giving positive results in STs was higher in patients allergic to

TABLE 1 | Characteristics of patients allergic to multiple ICM.

Pat	Type of reaction	Symptoms	Culprit ICM	ST							DPT				
				IOME	IOHE	IODIX	IOBIT	IOPR	IOV	IOXGL	IOME	IOHE	IODIX	IOBIT	IOPR
1	IR	ANAPH	IODIX	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	ND	ND	ND	ND
2	IR	ANAPH	IODIX	Pos	Pos	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	Pos	Neg
3	IR	ANAPH	IOME	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	Neg	ND
4	IR	URT	IODIX	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	ND	ND	ND	ND
5	IR	URT	IODIX	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Pos	Neg	ND
6	IR	URT	IOME	Pos	Neg	Neg	Pos	Neg	Neg	Neg	ND	Neg	ND	ND	ND
7	IR	ANAPH	UK	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	ND	Pos	ND
8	IR	URT	IOHE	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	Pos	Neg	ND
9	NIR	MPE	IODIX	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	ND	ND
10	NIR	MPE	IOME	Pos	Neg	Neg	Neg	Pos	Neg	Neg	ND	Neg	ND	ND	ND
11	NIR	MPE	IODIX	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	ND	ND	ND	ND
12	NIR	URT	IOME	Pos	Neg	Neg	Neg	Neg	Neg	Pos	ND	Neg	ND	ND	ND
13	NIR	MPE	IOME	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	ND	ND
14	NIR	MPE	IOXGL	Pos	Neg	Neg	Neg	Neg	Neg	Pos	ND	ND	Pos	Neg	ND
15	NIR	URT	IOME	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	Pos	Pos	Neg	ND
16	NIR	URT	IOME	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Pos	ND	ND
17	NIR	MPE	IODIX	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	ND	ND	ND	ND
18	NIR	MPE	UK	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	Pos	Pos	Neg	ND
19	NIR	URT	IODIX	Pos	Pos	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	Neg	ND
20	NIR	MPE	UK	Pos	Neg	Neg	Pos	Neg	Neg	Neg	ND	ND	Neg	ND	ND
21	NIR	MPE	IOME	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	Pos	ND	ND	ND
22	NIR	URT	IOHE	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	ND
23	NIR	MPE	IOHE	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	ND
24	NIR	MPE	IOHE	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	ND	ND
25	NIR	MPE	IODIX	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	ND	ND
26	NIR	URT	IOHE	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Pos	ND	ND	ND
27	NIR	MPE	IODIX	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	ND	Pos	ND	ND
28	NIR	URT	IODIX	Pos	Pos	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	Pos	Pos
29	NIR	MPE	IODIX	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	Pos	ND
30	NIR	MPE	IOHE	Neg	Pos	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	Pos	ND
31	NIR	MPE	UK	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Pos	ND	ND	Pos	Pos
32	NIR	URT	UK	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	Pos	Pos	ND
33	NIR	MPE	UK	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	ND	Pos	ND
34	NIR	MPE	IOME	Pos	Pos	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	Neg	ND
35	NIR	MPE	IOME	Pos	Neg	Pos	Neg	Neg	Neg	Neg	ND	ND	ND	Pos	ND
36	NIR	MPE	IOHE	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Pos	ND

ANAPH, anaphylaxis; DPT, drug provocation test; IOBIT, iobitridol; IODIX, iodixanol; IOHE, iohexol; IOME; iomeprol; IOPR, iopramida; IOV, ioversol; IOXGL, ioxaglate; ND, not done; Neg, negative; Pat, patient; Pos, positive; UK, unknown; URT, urticaria. ST, Skin test.

multiple ICMs compared with those allergic to a single ICM in both IR and NIR groups (for IR, 62.5 vs. 25%, $p > 0.05$; and for NIR, 85.71 vs. 24.59%, $p < 0.000$), being iomeprol the most common ICM giving positive results, mainly in NIRs (**Table 2**). Iodixanol was the ICM giving most frequently positive results in DPT ($p = 0.002$) in both IRs and NIRs, whereas iomeprol was the most frequently tolerated ICM in DPT ($p > 0.05$) (**Table 3**). Although not statistically significant, patients allergic to multiple ICMs reacted in DPT to a lower dose than those cases allergic to a single ICM in both IRs [20 (20–50) vs. 35 cc [27.5–42.5), $p = 0.8079$) and NIRs [25 (20–82.5) vs. 50 cc (37.5–100), $p = 0.1207$)].

DISCUSSION

The incidence of HSRs to ICMs has increased over last decades (Brockow et al., 2005; Brockow, 2020), maybe due to the increase in the use of non-ionic ICMs, with approximately 75 million administrations conducted yearly worldwide (Sanchez-Borges et al., 2019). This increased incidence is a concern for doctors and patients as HSR diagnosis implies avoiding ICMs, which are required for radiological examination or treatment of different entities. The evaluation of HSRs to ICMs has been gaining attention over recent years (Brockow et al., 2005; Brockow et al., 2009; Hasdenteufel et al., 2011; Torres et al., 2012; Salas et al., 2013; Lerondeau et al., 2016; Sese et al., 2016; Soria et al., 2019; Trautmann et al., 2019; Brockow, 2020). The allergological work-up not only confirms or excludes the diagnosis but also finds safe alternative ICM. However, in some patients, finding a tolerated alternative may be difficult, as cross-reactivity among ICMs has been reported (Vernassiere et al., 2004; Kanny et al., 2005; Kvedariene et al., 2006; Brockow et al., 2009; Hasdenteufel et al., 2011; Torres et al., 2012; Salas et al., 2013; Lerondeau et al., 2016; Morales-Cabeza et al., 2017; Schrijvers et al., 2018). HSRs to multiple ICMs have been widely observed, ranging from 14% (Prieto-Garcia et al., 2013) to 88% (Brockow et al., 2009). This variability may be due to the different criteria used for patient inclusion and the different sample size in each study. In our population, 35.64% of patients were found to be allergic to two or more ICMs, being this percentage higher in IRs (66.66%) than in

TABLE 2 | Demograhic and clinical characteristics of patients allergic to multiple ICM and those allergic to a single ICM.

A. Immediate reactions.

		Allergic to multiple ICM n = 8	Allergic to a single ICMn = 4	p
Age; median (interquartile range) years		59 (49–65.5)	55 (38.25–62.25)	0.6278
Gender; n (%) female/n (%) male		5 (62.5)/3 (37.5)	2 (50)/2 (50)	1
Symptoms reported; n (%)	Anaphylaxis	4 (50)	1 (25)	0.5758
	Urticaria	4 (50)	3 (75)	
ICM involved	Iodixanol	4 (50)	–	NA
	Iomeprol	2 (25)	1 (25)	1
	Iohexol	1 (12.5)	–	NA
	Iopramide	–	1 (25)	NA
	Unkown	1 (12.5)	2 (50)	0.2364
Time interval between ICM administration and reaction onset; n (%)	≤30 min	7 (87.5)	2 (50)	0.2364
	31–59 min	1 (12.5)	2 50)	
N° of episodes	1 episode	8 (100)	4 (100)	1
	2 episodes	–	–	
Positive results in STs		5/8; 62.5% Iomeprol 3 Iohexol 3 Iodixanol 3 Iobitridol 1	1/4; 25 Iohexol 1	0.5455

B. Non-immediate reactions

		Allergic to multiple ICM n = 28	Allergic to a single ICMn = 61	p
Age; median (interquartile range) years		64.5 (49–69.25)	61 (52.35–63)	0.4356
Gender; n (%) female/n (%) male		15 (53.57)/13 (42.85)	30 (49.18)/31 (50.81)	0.7004
Symptoms reported; n (%)	Urticaria	8 (28.57)	21 (34.42)	0.5842
	MPE	20 (71.42)	40 (65.57)	
ICM involved	Iodixanol	8 (28.57)	19 (27.86)	0.8061
	Iomeprol	8 (28.57)	22 (32.78)	0.4874
	Iohexol	6 (21.42)	9 (13.11)	0.4348
	Iobitridol	–	2 (3.27)	NA
	Iopramida	–	1 (1.63)	NA
	Ioxaglate	1 (3.57)	–	NA
	Unkown	5 (17.85)	8 (13.11)	0.5564
Time interval between ICM administration and reaction onset; n (%)	1–6 h	–	1 (1.63)	NA
	7–12 h	4 (14.28)	–	NA
	13–24 h	11 (39.28)	28 (45.9)	0.8795
	25–48 h	10 (35.71)	27 (44.26)	0.7435
	>48 h	3 (10.71)	5 (8.19)	0.6998
No of episodes	1 episode	26 (92.85)	49 (80.32)	0.1317
	2 episodes	2 (7.14)	12 (19.67)	
Positive results in STs		24/28; 85.71% Iomeprol 16 Iohexol 7 Iodixanol 8 Iobitridol 3 Ioxaglate 3 Iopramida 1	15/61; 24.59% Iomeprol 8 Iodixanol 3 Iobitridol 2 Ioxaglate 1	0.00000006785

A.ICM, iodinated contrast media; MPE, maculopapular exanthema; NA, not applicable.

NIRs (31.46%). Indeed, 33.33% of our patients were allergic to three or more ICMs, and even in two patients, none of the available ICMs was tolerated. However, this percentage may be higher as in a percentage of the patients attending to our clinic because of a reaction after an ICM administration, the involved ICM was unknown, as in clinical practice, the exact name of the ICM is not always recorded in the radiologist clinical history. In these cases, as well as in those in which ICM was known but STs were negative, tolerance was assessed, and if no reaction occurs, no more ICMs are tested. This may also be the reason why the percentage of confirmed allergic patients in our population is low.

It has been considered that the diagnostic value of STs may be insufficient. A meta-analysis on STs in HSRs to ICM found an overall positive rate of STs of 17% in IRs and 26% in NIRs (Yoon et al., 2015). This may happen because the inclusion criteria are

TABLE 3 | Comparison of DPT results in patients allergic to multiple ICM.

	ICM used in DPT	DPT		p
		Positive (reacted)	Negative (tolerated)	
Total n = 36	Iomeprol	6 (40)	9 (60)	0.1243
	Iohexol	5 (50)	5 (50)	0.6187
	Iodixanol	15 (88.23)	2 (11.76)	0.002
	Iobitridol	10 (55.55)	8 (44.44)	0.8721
	Iopramida	–	3 (100)	NA
IR n = 8	Iomeprol	3 (50)	3 (50)	1
	Iohexol	1 (33.33)	2 (66.66)	1
	Iodixanol	3 (75)	1 (25)	0.3034
	Iobitridol	2 (40)	3 (60)	1
	Iopramida	–	1 (100)	NA
NIR n = 28	Iomeprol	3 (33.33)	6 (66.66)	0.05282
	Iohexol	4 (57.14)	3 (42.85)	1
	Iodixanol	12 (92.3)	1 (7.69)	0.006
	Iobitridol	8 (61.53)	5 (38.46)	0.9877
	Iopramida	2 (66.66)	1 (33.33)	1

DPT, drug provocation test; ICM, iodinated contrast media; NA, not applicable; IR, immediate reaction; NIR, non-immediate reaction.

based in many cases on the clinical history. In our study, we have only included patients with a confirmed diagnosis based on STs or DPTs and in this situation 50% of IRs and 43% of NIRs gave positive results in STs. Indeed, the percentage of positive results in STs was higher in cases allergic to more than one ICM (62.5% for IR and 85.71% for NIR). It is not known the reason for this observation. For IRs, it has been reported that positive STs are associated to severity reaction (Salas et al., 2013; Yoon et al., 2015; Trautmann et al., 2019). In our study, the percentage of patients reporting severe reactions (anaphylaxis) was higher in the group of patients allergic to multiple ICMs compared with those allergic to a single ICM, although this difference was not statistically significant, probably due to the small sample size. Moreover, the time interval between the reaction and the study may also influence in having positive results in STs (Salas et al., 2013; Yoon et al., 2015), however in our study no differences were found comparing patients allergic to multiple and to a single ICM. Another factor that must to be taken into account is the dilution used in STs. In a previous article by our group (Torres et al., 2012), we found a higher sensitivity for IDT using undiluted ICMs than 10-fold diluted ICM with 100% specificity. Moreover, no patient with negative IDT had a positive patch test. This is the reason why we did not include patch test in the allergological work-up for this study.

It has been proposed that STs should be performed with a wide panel of different ICMs in order to identify tolerated alternative ICM (Vernassiere et al., 2004; Kvedariene et al., 2006; Caimmi et al., 2010; Torres et al., 2012; Yoon et al., 2015; Gracia-Bara et al., 2019; Brockow, 2020), mainly when the culprit is unknown. However, choosing non–cross-reactive ICM basing only on a negative ST could not completely prevent the recurrence of HSR, as in our study, 55% of patients reacted in DPT despite being negative in STs, what it is in line with previous data (Vernassiere et al., 2004; Torres et al., 2012). Moreover, in the group of patients allergic to multiple ICMs with a positive ST, tolerance to a negative-ST ICM could not be guaranteed, as DPT was positive in almost 50% of cases. Therefore, DPT should be considered not only to establish the diagnosis but also to choose the alternative even if STs are negative. The underlying mechanism of HSRs to ICMs is not well known, mainly in those cases with negative STs and positive DPT, and there may be a non-immune mediated mechanism involved. However, previous evidence supports an underlying immune mechanism in these reactions. In this sense, positive results in basophil activation test in patients with IRs and negative STs and positive DPTs to ICMs (Salas et al., 2013), indicate that an IgE-mechanism may be involved in IRs to ICM. Regarding NIRs, it has been previously demonstrated similar results in skin biopsies obtained from positive IDTs and DPTs (Torres et al., 2012), supporting a T cell involvement.

The most frequent cross-reactivity associations detected in our study were iodixanol and iomeprol, and iodixanol and iohexol. This pattern agrees with other reports (Vernassiere et al., 2004; Brockow et al., 2009; Hasdenteufel et al., 2011; Torres et al., 2012; Gracia-Bara et al., 2019). Mechanisms underlying the cross-reactivities between ICMs are not fully understood and further studies are necessary. Cross-reactivity has been related to the chemical structure (Vernassiere et al., 2004; Hasdenteufel et al., 2011; Lerondeau et al., 2016). ICMs are monomeric or dimeric derivatives of triiodobenzoic acid, with different organic side chains attached to the central benzene ring shared by all ICMs (Lerondeau et al., 2016). According to their chemical structure, four groups have been described: ionic tri-iodized monomers, ionic hexa-iodized dimers, nonionic tri-iodized monomers, and nonionic hexaiodized dimers. It has been reported a higher cross-reactivity between ICMs from the same group and a lower one between ICMs from different groups (Hasdenteufel et al., 2011). Such a high cross-reactivity in NIRs has been proposed to be attributed to nonspecific stimulation or pharmacological interaction with immune receptors across ICM. The presence of T cell clones has been demonstrated in previous studies (Lerch et al., 2007) along with specific recognition of the ICM in T cell receptors (Keller et al., 2010). In fact, it has been reported that iobitridol shows low cross-reactivity, mainly in patients with NIRs. The results of an *in vitro* test of T cell clones have shown that iobitridol is the least stimulatory ICM (Lerch et al., 2007). In our study, the ICM that less frequently induced reactions in DPT were iobitridol and iohexol in IRs and iomeprol in NIRs. This difference compared with published data may be related to a bias in our study as we could not performed DPT with all ICMs in all patients. Nevertheless, our aim was to describe the clinical characteristics of a series of patients allergic to multiple ICMs and the role of the different methods used for their diagnosis in real allergological practice.

Summarizing, this study has investigated HSRs to multiple ICMs. It shows that allergy to multiple ICMs is common, associated to severe reactions in IRs and confirmed frequently by positive STs. However, even in the case of negative-ST results, tolerance to an alternative ICM (including structurally unrelated ones) must be proven by DPT, as ST sensitivity is not sufficient. Therefore, DPT is necessary not only to confirm the

diagnosis but also to identify safe alternative ICM before radiological examination.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Ética de la Investigación Provincial de Málaga. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ID, GB, MS, AT, and MT recruited patients and performed the clinical evaluations. ID, MT, JL, and EM contributed to study design. ID and MT wrote the first draft of the manuscript. ID, MT, JL, and EM corrected the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Aberer, W., Bircher, A., Romano, A., Blanca, M., Campi, P., Fernandez, J., et al. (2003). Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. *Allergy* 58 (9), 854–863. doi: 10.1034/j.1398-9995.2003.00279.x

Antunez, C., Barbaud, A., Gomez, E., Audonnet, S., Lopez, S., Guéant-Rodriguez, R. M., et al. (2011). Recognition of iodixanol by dendritic cells increases the cellular response in delayed allergic reactions to contrast media. *Clin. Exp. Allergy* 41 (5), 657–664. doi: 10.1111/j.1365-2222.2010.03693.x

Brockow, K., Romano, A., Blanca, M., Ring, J., Pichler, W., Demoly, P., et al. (2002). General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 57 (1), 45–51.

Brockow, K., Chistiansen, C., Kanny, G., Clément, O., Barbaud, A., Bircher, A., et al. (2005). Management of hypersensitivity reactions to iodinated contrast media. *Allergy* 60 (2), 150–158. doi: 10.1111/j.1398-9995.2005.00745.x

Brockow, K., Romano, A., Aberer, W., Bircher, A. J., Barbaud, A., Bonadonna, P., et al. (2009). Skin testing in patients with hypersensitivity reactions to iodinated contrast media - a European multicenter study. *Allergy* 64 (2), 234–241. doi: 10.1111/j.1398-9995.2008.01832.x

Brockow, K., Garvey, L. H., Aberer, W., Atanaskovic-Markovic, M., Barbaud, A., Bilo, M. B., et al. (2013). Skin test concentrations for systemically administered drugs – an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy* 68 (6), 702–712. doi: 10.1111/all.12142

Brockow, K., Ardern-Jones, M. R, Mockenhaupt, M., Werner, A., Barbaud, A., Caubet, J. C., et al. (2019). EAACI position paper on how to classify cutaneous manifestations of drug hypersensitivity. *Allergy* 74 (1), 14–27. doi: 10.1111/all.13562

Brockow, K. (2020). Medical algorithm: Diagnosis and treatment of radiocontrast media hypersensitivity. *Allergy* 75 (5), 1278–1280. doi: 10.1111/all.14147

Caimmi, S., Benyahia, B., Suau, D., Bousquet-Rouanet, L., Caimmi, D., Bousquet, P. J., et al. (2010). Clinical value of negative skin tests to iodinated contrast media. *Clin. Exp. Allergy* 40 (5), 805–810. doi: 10.1111/j.1365-2222.2010.03493.x

Demoly, P., Adkinson, N. F, Brockow, K., Castells, M., Chiriac, A. M., Greenberger, P. A., et al. (2014). International Consensus on drug allergy. *Allergy* 69 (4), 420–437. doi: 10.1111/all.12350

Dewachter, P., Laroche, D., Mouton-Faivre, C., Bloch-Morot, E., Cercueil, J. P., Metge, L., et al. (2011). Immediate reactions following iodinated contrast media injection: a study of 38 cases. *Eur. J. Radiol.* 77 (3), 495–501. doi: 10.1016/j.ejrad.2009.09.019

Goksel, O., Aydın, O., Atasoy, C., Akyar, S., Demirel, Y. S., Misirligil, Z., et al. (2011). Hypersensitivity reactions to contrast media: prevalence, risk factors and the role of skin tests in diagnosis–a cross-sectional survey. *Int. Arch. Allergy Immunol.* 155 (3), 297–305. doi: 10.1159/000320760

Gracia-Bara, M. T., Moreno, E., Laffond, E., Muñoz-Bellido, F., Lázaro, M., Macías, E., et al. (2019). Tolerability of iobitridol in patients with non-immediate hypersensitivity reactions to iodinated contrast media. *Allergy* 74 (1), 195–197. doi: 10.1111/all.13603

Hasdenteufel, F., Waton, J., Cordebar, V., Studer, M., Collignon, O., Luyasu, S., et al. (2011). Delayed hypersensitivity reactions caused by iodixanol: an assessment of cross-reactivity in 22 patients. *J. Allergy Clin. Immunol.* 128 (6), 1356–1357. doi: 10.1016/j.jaci.2011.05.034

Kanny, G., Pichler, W., Morisset, M., Franck, P., Marie, B., Kohler, C., et al. (2005). T cell-mediated reactions to iodinated contrast media: evaluation by skin and lymphocyte activation tests. *J. Allergy Clin. Immunol.* 115 (1), 179–185. doi: 10.1016/j.jaci.2004.09.012

Keller, M., Lerch, M., Britschgi, M., Tâche, V., Gerber, B. O., Lüthi, M., et al. (2010). Processing-dependent and -independent pathways for recognition of iodinated contrast media by specific human T cells. *Clin. Exp. Allergy* 40 (2), 257–268. doi: 10.1111/j.1365-2222.2009.03425.x

Kvedariene, V., Martins, P., Rouanet, L., and Demoly, P. (2006). Diagnosis of iodinated contrast media hypersensitivity: results of a 6-year period. *Clin. Exp. Allergy* 36 (8), 1072–1077. doi: 10.1111/j.1365-2222.2006.02532.x

Laroche, D., Aimone-Gastin, I., Dubois, F., Huet, H., Gérard, P., Vergnaud, M. C., et al. (1998). Mechanisms of severe, immediate reactions to iodinated contrast material. *Radiology* 209 (1), 183–190. doi: 10.1148/radiology.209.1.9769830

Laroche, D., Namour, F., Lefrançois, C., Aimone-Gastin, I., Romano, A., Sainte-Laudy, J., et al. (1999). Anaphylactoid and anaphylactic reactions to iodinated contrast material. *Allergy* 54 (Suppl 58), 13–16.

Lerch, M., Keller, M., Britschgi, M., Kanny, D., Tache, V., Schmid, D. A., et al. (2007). Cross-reactivity patterns of T cells specific for iodinated contrast media. *J. Allergy Clin. Immunol.* 119 (6), 1529–1536. doi: 10.1016/j.jaci.2007.02.007

Lerondeau, B., Trechot, P., Waton, J., Poreaux, C., Luc, A., Schmutz, J. L., et al. (2016). Analysis of cross-reactivity among radiocontrast media in 97 hypersensitivity reactions. *J. Allergy Clin. Immunol.* 137 (2), 633–635 e4. doi: 10.1016/j.jaci.2015.07.035

Mita, H., Tadokoro, K., and Akiyama, K. (1998). Detection of IgE antibody to a radiocontrast medium. *Allergy* 53 (12), 1133–1140. doi: 10.1111/j.1398-9995.1998.tb03832.x

Morales-Cabeza, C., Roa-Medellín, D., Torrado, I., De Barrio, M. I., Fernández-Álvarez, C., Montes-Aceñero, J. F., et al. (2017). Immediate reactions to iodinated contrast media. *Ann. Allergy Asthma Immunol.* 119 (6), 553–557. doi: 10.1016/j.anai.2017.08.014

Pinnobphun, P., Buranapraditkun, S., Kampitak, T., Hirankarn, N., and Klaewsongkram, J. (2011). The diagnostic value of basophil activation test in patients with an immediate hypersensitivity reaction to radiocontrast media. *Ann. Allergy Asthma Immunol.* 106 (5), 387–393. doi: 10.1016/j.anai.2010.12.020

Prieto-Garcia, A., Tomás, M., Pineda, R., Tornero, P., Herrero, T., Fuentes, V., et al. (2013). Skin test-positive immediate hypersensitivity reaction to iodinated contrast media: the role of controlled challenge testing. *J. Invest. Allergol. Clin. Immunol.* 23 (3), 183–189.

Ring, J., and Messmer, K. (1977). Incidence and severitiy of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1, 466–469. doi: 10.1016/S0140-6736(77)91953-5

Romano, A., Artesani, M. C., Andriolino, M., Viola, M., Pettinato, R., Vecchioli-Scaldazza, A., et al. (2002). Effective prophylactic protocol in delayed hypersensitivity to contrast media: report of a case involving lymphocyte transformation studies with different compounds. *Radiology* 225 (2), 466–470. doi: 10.1148/radiol.2251011654

Rosado Ingelmo, A., Doña Diaz, I., Cabañas Moreno, R., Moya Quesada, M. C., García-Avilés, C., García Nuñez, I., et al. (2016). Clinical Practice Guidelines for Diagnosis and Management of Hypersensitivity Reactions to Contrast Media. *J. Invest. Allergol. Clin. Immunol.* 26 (3), 144–151. doi: 10.18176/jiaci.0058

Rudnick, M. R., Goldfarb, S., and Tumlin, J. (2008). Contrast-induced nephropathy: is the picture any clearer? *Clin. J. Am. Soc. Nephrol.* 3 (1), 261–262. doi: 10.2215/CJN.04951107

Salas, M., Gomez, F., Fernandez, T. D., Doa, I., Aranda, A., Ariza, A., et al. (2013). Diagnosis of immediate hypersensitivity reactions to radiocontrast media. *Allergy* 68 (9), 1203–1206. doi: 10.1111/all.12214

Sanchez-Borges, M., Aberer, W., Brockow, K., Celik, G. E., Cernadas, J., Greenberger, P. A., et al. (2019). Controversies in Drug Allergy: Radiographic Contrast Media. *J. Allergy Clin. Immunol. Pract.* 7 (1), 61–65. doi: 10.1016/j.jaip.2018.06.030

Schrijvers, R., Breynaert, C., Ahmedali, Y., Bourrain, J. L, Demoly, P., Chiriac, A. M., et al. (2018). Skin Testing for Suspected Iodinated Contrast Media Hypersensitivity. *J. Allergy Clin. Immunol. Pract.* 6 (4), 1246–1254. doi: 10.1016/j.jaip.2017.10.040

Sese, L., Gaouar, H., Autegarden, J. E., Alari, A., Amsler, E., Vial-Dupuy, A., et al. (2016). Immediate hypersensitivity to iodinated contrast media: diagnostic accuracy of skin tests and intravenous provocation test with low dose. *Clin. Exp. Allergy* 46 (3), 472–478. doi: 10.1111/cea.12703

Soria, A., Masson, N., Vial-Dupuy, A., Gaouar, H., Amsler, E., Chollet-Martin, S., et al. (2019). Allergological workup with half-dose challenge in iodinated contrast media hypersensitivity. *Allergy* 74 (2), 414–417. doi: 10.1111/all.13638

Steiner, M., Harrer, A., and Himly, M. (2016). Basophil Reactivity as Biomarker in Immediate Drug Hypersensitivity Reactions-Potential and Limitations. *Front. Pharmacol.* 7, 171. doi: 10.3389/fphar.2016.00171

Torres, M. J., Mayorga, C., Cornejo-Garcia, J. A., Lopez, S., Chaves, P., Rondon, C., et al. (2008). Monitoring non-immediate allergic reactions to iodine contrast media. *Clin. Exp. Immunol.* 152 (2), 233–238. doi: 10.1111/j.1365-2249.2008.03627.x

Torres, M. J., Gomez, F., Doña, I., Rosado, A., Mayorga, C., Garcia, I., et al. (2012). Diagnostic evaluation of patients with nonimmediate cutaneous hypersensitivity reactions to iodinated contrast media. *Allergy* 67 (7), 929–935. doi: 10.1111/j.1398-9995.2012.02840.x

Trautmann, A., Brockow, K., Behle, V., and Stoevesandt, J. (2019). Radiocontrast Media Hypersensitivity: Skin Testing Differentiates Allergy From Nonallergic Reactions and Identifies a Safe Alternative as Proven by Intravenous Provocation. *J. Allergy Clin. Immunol. Pract.* 7 (7), 2218–2224. doi: 10.1016/j.jaip.2019.04.005

Trcka, J., Schmidt, C., Seitz, C. S., Bröcker, E. B., Gross, G. E., Trautmann, A., et al. (2008). Anaphylaxis to iodinated contrast material: nonallergic hypersensitivity or IgE-mediated allergy? *AJR Am. J. Roentgenol.* 190 (3), 666–670. doi: 10.2214/AJR.07.2872

Vernassiere, C., Trechot, P., Commun, N., Schmutz, J. L., and Barbaud, A. (2004). Low negative predictive value of skin tests in investigating delayed reactions to radio-contrast media. *Contact Dermatitis* 50 (6), 359–366. doi: 10.1111/j.0105-1873.2004.00367.x

Yoon, S. H., Lee, S. Y., Kang, H. R., Kim, J. Y., Hahn, S., Park, C. M., et al. (2015). Skin tests in patients with hypersensitivity reaction to iodinated contrast media: a meta-analysis. *Allergy* 70 (6), 625–637. doi: 10.1111/all.12589

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Protein Binding of Lapatinib and Its N- and O-Dealkylated Metabolites Interrogated by Fluorescence, Ultrafast Spectroscopy and Molecular Dynamics Simulations

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Lapatinib (LAP) is an anticancer drug generally used to treat breast and lung cancer. It exhibits hypersensitivity reactions in addition to dermatological adverse effects and photosensitivity. Moreover, LAP binds to serum proteins and is readily biotransformed in humans, giving rise to several metabolites, such as N- and O-dealkylated products (N-LAP and O-LAP, respectively). In this context, the aim of the present work is to obtain key information on drug@protein complexation, the first step involved in a number of hypersensitivity reactions, by a combination of fluorescence, femtosecond transient absorption spectroscopy and molecular dynamics (MD) simulations. Following this approach, the behavior of LAP and its metabolites has been investigated in the presence of serum proteins, such as albumins and α_1-acid glycoproteins (SAs and AGs, respectively) from human and bovine origin. Fluorescence results pointed to a higher affinity of LAP and its metabolites to human proteins; the highest one was found for LAP@HSA. This is associated to the coplanar orientation adopted by the furan and quinazoline rings of LAP, which favors emission from long-lived (up to the ns time-scale) locally-excited (LE) states, disfavoring population of intramolecular charge transfer (ICT) states. Moreover, the highly constrained environment provided by subdomain IB of HSA resulted in a frozen conformation of the ligand, contributing to fluorescence enhancement. Computational studies were clearly in line with the experimental observations, providing valuable insight into the nature of the binding sites and the conformational arrangement of the ligands inside the protein cavities. Besides, a good correlation was found between the calculated binding energies for each ligand@protein complex and the relative affinities observed in competition experiments.

Keywords: femtosecond transient absorption, fluorescence, hypersensitivity reactions, lapatinib, metabolites, molecular dynamics simulations, protein binding

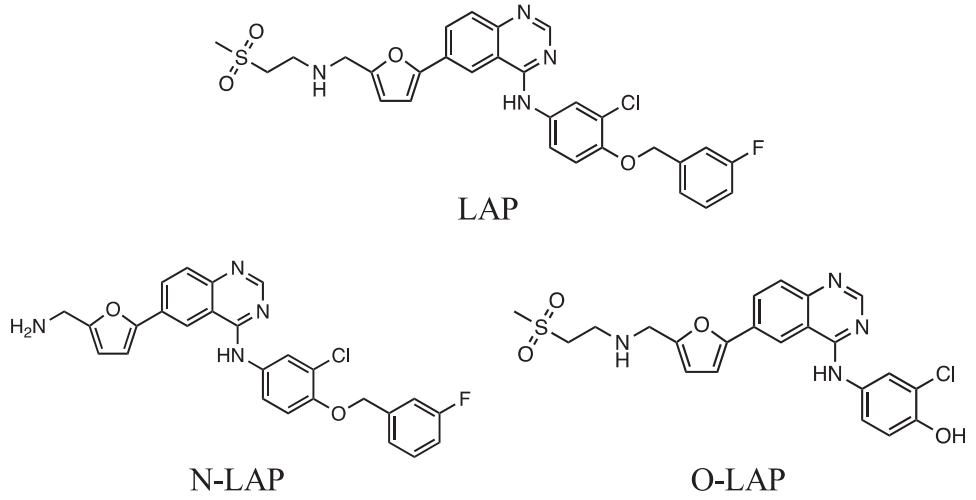

FIGURE 1 | Chemical structures of lapatinib (LAP) and its main metabolites N-LAP and O-LAP.

INTRODUCTION

The human epidermal growth factor receptor (HER) family is composed of four different members that have been thoroughly investigated due to their important role in cancer progression. HER receptors are transmembrane proteins that control a variety of cell functions such as cell differentiation, proliferation, apoptosis, migration and angiogenesis (Nicholson et al., 2001; Hynes and Lane, 2005; Thomas and Weihua, 2019). However, pathological alterations including overexpression or mutations in the tyrosine-kinase site to HER-1 and/or HER-2 are directly associated with the development of different types of human cancer (Hynes and Lane, 2005; González and Lage, 2007; Ross et al., 2016; Sigismund et al., 2018; Thomas and Weihua, 2019).

Lapatinib (LAP) is an orally administered drug that strongly inhibits HER-1 and HER-2. It is generally used to treat breast cancer, but due to its dual HER targeting it is expected to exhibit higher activity than monotargeted tyrosine-kinase inhibitors; in addition, its relatively small size allows LAP to cross the blood-brain barrier, evidencing antitumor activity against brain metastases (Lin et al., 2008; Lin et al., 2009; Johnston et al., 2017). The LAP mechanism of action involves reversible binding to the adenosine triphosphate site, stopping cellular growth and proliferation, which results in enhanced apoptosis (Spector et al., 2005; Schroeder et al., 2014). Furthermore, LAP undergoes extensive biotransformation in humans leading to a number of metabolites, including N- and O-dealkylated products (N-LAP and O-LAP, respectively; see **Figure 1**) (Medina and Goodin, 2008; Towles et al., 2016).

Lapatinib gives rise to T cell-mediated hypersensitivity reactions in the skin explant assay (Ahmed et al., 2019). Moreover, dermatological adverse events including papulopustular rash, pruritus, xerosis, and nail abnormalities have been reported with LAP, similar to those described for many other TKIs (Friedman et al., 2016). Generally, hypersensitivity reactions occur when a drug (acting as a hapten) has the ability to trigger an immune response. This can be accomplished by different pathways, including noncovalent interactions of drugs with immune receptors or covalent binding of drugs to a protein, producing hapten-protein conjugates, which can induce an immune response. It is noteworthy that sometimes the drug itself does not participate in the key event but instead a reactive metabolite or a photoactivated species becomes covalently bound to the protein and generates the allergic process (Nuin et al., 2016; Limones-Herrero et al., 2020). In this context, photoallergic reactions are related to immunological mechanisms, in which the photoactivated drug (photosensitizer) is the key chemical entity capable to interact with proteins (Andreu et al., 2010). Indeed, it has recently been demonstrated that LAP and N-LAP are able to induce phototoxicity and photogenotoxicity to cells, while O-LAP did not display any photosensitized damage. Besides, the parent drug showed higher activity in membrane phototoxicity and protein oxidation than N-LAP (García-Lainez et al., 2020).

Interestingly, LAP is known to bind to blood proteins (>99%), mainly serum albumins (SAs) and α_1-acid glycoproteins (AGs) (Medina and Goodin, 2008). The binding of drugs to transport proteins, such as SAs and AGs, attracts increasing attention because key properties such as drug function, pharmacokinetics, toxicity and transport to the target cells are strongly modulated through drug@protein complexation (Krasner, 1972; Peters, 1995). Moreover, exposure of these complexes to sunlight may result in photosensitivity disorders mainly associated with phototoxicity and photoallergy (Vargas et al., 1993; Miranda et al., 1994; Quintero and Miranda, 2000; Cosa, 2004; Montanaro et al., 2009; Nuin et al., 2016; Vayá et al., 2016; Blakely et al., 2019). In this context, it is known that drugs containing the quinazoline moiety (the core chromophore of LAP) can induce skin diseases such as allergic photodermatitis (Ishikawa et al., 1994; Selvam and Kumar, 2011).

Fluorescence and transient absorption spectroscopies are useful techniques to investigate drug/protein interactions. This is because excited state properties of drugs are very sensitive to the microenvironment; thus, the yield of transients formation, as well as their spectral profile and kinetic evolution, may be strongly affected by the surroundings of the investigated chromophore (Vayá et al., 2014). In particular, fluorescence and femtosecond transient absorption are highly sensitive techniques that may provide key information on the structural and dynamic features of drug@protein complexation, revealing the nature of the early primary processes occurring from the excited states in a time window from the fs to the ns range, such as intersystem crossing, energy/electron transfer or charge separation. In a parallel approach, molecular dynamics (MD) simulations can be used to study drug@biomolecule interactions (Spitaleri and Rocchia, 2019). Thus, properties such as the strength of interaction and the conformational orientation of a drug in the neighborhood of the amino acid residues of the protein binding sites can be investigated in detail (Pérez-Ruiz et al., 2017; Pinheiro and Curutchet, 2017; Molins-Molina et al., 2019; Spitaleri and Rocchia, 2019).

In this framework, preliminary findings on LAP@SA complexation point to a moderate binding of the drug in the so-called site III (subdomain IB) of the protein (Shen et al., 2015; Wilson et al., 2015; Kabir et al., 2016). In addition, a recent photophysical study on the interactions between LAP, N-LAP or O-LAP and HSA (Vayá et al., 2020) has shown that within the constrained environment provided by the HSA cavities emission occurs from long-lived locally excited (LE) states, whereas in the bulk solution shorter-lived (70–90 ps) intramolecular charge transfer (ICT) states predominate. This is related to the relative conformational orientation of the furan vs. the quinazoline ring in the different media.

With this background, the aim of the present work is to obtain relevant information about the complexation of LAP and its N- and O-dealkylated metabolites with serum proteins (SAs and AGs) from human and bovine origin, as a model for the first step involved in (photo)sensitivity reactions. To this end, a combination of photophysical techniques (fluorescence and femtosecond transient absorption spectroscopies) with MD simulation studies has been employed. The obtained results are relevant in connection with the capability of LAP to elicit hypersensitivity reactions with special emphasis on its photosensitizing potential.

EXPERIMENTAL SECTION

Chemicals and Reagents

Lapatinib (CAS 231277-92-2), serum albumins and α_1-acid glycoproteins, from human and bovine origin, were purchased from Sigma-Aldrich (Madrid, Spain). N-De[2-(methylsulfonyl) ethyl lapatinib (N-LAP, CAS 697299-82-4) and O-De(3-fluorobenzyl) lapatinib ditosylate salt (O-LAP; CAS 1268997-70-1) were provided by Santa Cruz Biotechnology (Dallas, United States) and Toronto Research Chemicals (North York, Canada), respectively. PBS buffer was prepared by dissolving phosphate-buffered saline tablets (Sigma-Aldrich) using ultrapure water from a Millipore (Milli-Q Synthesis) system.

Spectroscopic Measurements

Steady-state absorption spectra were recorded in a JASCO V-760 spectrophotometer. Steady-state fluorescence spectra were obtained using a JASCO-8500 spectrofluorometer system provided with a monochromator in the wavelength range 200–900 nm, with an excitation wavelength of 295 or 340 nm at 25°C. Measurements on drug or metabolite@protein complexes were performed in aerated PBS of 1:1 M ratio mixtures at 5 μM. Competing interactions were evaluated for solutions containing LAP (or its metabolites) within a mixture of proteins in a 1:1:1 M ratio (each component at 5 μM).

Time-resolved fluorescence measurements were done using an EasyLife X system containing a sample compartment composed of an automated Peltier cuvette holder to control the temperature, a pulsed LED excitation source and a lifetime detector. The employed LED excitation source was 340 nm, with emission filter of GG400.

The UV and fluorescence measurements were recorded using 10×10 mm^2 quartz cells at 25°C. The absorbance of the samples at the excitation wavelength was kept below 0.1.

Femtosecond transient absorption experiments were performed using a typical pump-probe system. The femtosecond pulses were generated with a mode-locked Ti-Sapphire laser of a compact Libra HE (4 W power at 4 kHz) regenerative amplifier delivering 100 fs pulses at 800 nm (1 mJ/pulse). The output of the laser was split into two parts to generate the pump and the probe beams. Thus, tunable femtosecond pump pulses were obtained by directing the 800 nm light into an optical parametric amplifier. In the present case, the pump was set at 330 nm and passed through a chopper prior to focus onto a rotating cell (1 mm optical path) containing the samples in organic or aqueous solution. The white light used as probe was produced after part of the 800 nm light from the amplifier traveled through a computer controlled 8 ns variable optical delay line and impinge on a CaF_2 rotating crystal. This white light was in turn split in two identical portions to generate reference and probe beams that then were focused on the rotating cell containing the sample. The pump and the probe were made to coincide to interrogate the sample. The power of the pump beam was set to 180 μW. A computer-controlled imaging spectrometer was placed after this path to measure the probe and the reference pulses to obtain the transient absorption decays/spectra. The experimental data were treated and compensated by the chirp using the ExciPro program.

Molecular Docking

These calculations were performed using GOLD 5.8.1 program (CCDC, 2020) and the protein coordinates were taken from: 1) the crystal structure of HSA in complex with hemin and myristic acid (PDB entry 1O9X) (Zunszain et al., 2003); 2) the crystal structure of BSA in complex with 3,5-diiodosalicylic acid (PDB entry 4JK4, chain A) (Sekula et al., 2013); 3) the crystal structure of HAG in the unbound form (PDB entry 3KQ0) (Schonfeld et al., 2008); and 4) our previously reported homology model of BAG

using the Phyre2 (Kelley et al., 2015) homology modeling web server (Limones-Herrero et al., 2017). The experimental procedure was similar to that described for: 1) 2-acetoxy-4-trifluoromethylbenzoic acid (triflusal) (Molins-Molina et al., 2019) and HSA protein with the exception that the position of hemin was used to define the binding pocket, and the radius was set to 10 Å; and 2) carprofen methyl ester and the homology model of BSA (Limones-Herrero et al., 2017). The protonated forms of the ligands (secondary and primary amines) were employed.

Molecular Dynamics Simulation Studies

The highest score solution obtained by docking was subjected to 100 ns of dynamic simulation. The experimental protocol involved: 1) the minimization of the ligands (LAP, N-LAP and O-LAP); 2) the generation and minimization of the binary LAP@protein, N-LAP@protein, and O-LAP@protein complexes (protein = HSA, BSA, HAG, and BAG) using the poses obtained by docking; and 3) MD simulations of the resulting minimized ligand@protein complexes. The protocol was performed as described for triflusal and carprofen methyl ester (Limones-Herrero et al., 2017; Molins-Molina et al., 2019). The analysis of the trajectories and the rmsd of the atomic positions of the protein and the ligands during the simulation were analyzed by using the cpptraj module in AMBER 16 (Case et al., 2016). The binding free energies of LAP in the LAP@HSA and LAP@BSA complexes were calculated using the MM/PBSA (Miller et al., 2012) approach in explicit water (generalized Born, GB) as implemented in Amber. The protein figures disclosed were created by using the molecular graphics program PyMOL (DeLano, 2020). For figures related to HSA, BSA and HAG proteins, the amino acid numbering described in PDB entries 1O9X, 4JK4, and 3KQ0, respectively, was employed. For figures related to BAG protein, the numbering of the protein sequence was used.

RESULTS AND DISCUSSION

The photobehavior of LAP and its metabolites was investigated in aqueous buffer solution and in the presence of an equimolar amount of protein. For this purpose, two types of transport proteins were selected: serum albumins and α_1-acid glycoproteins from human (HSA and HAG) and bovine (BSA and BAG) origin, respectively. As previously observed, LAP and N-LAP formed aggregates in PBS solution (Wilson et al., 2015; Vayá et al., 2020); however, upon interaction with proteins they were completely solubilized. The UV absorption spectra of LAP, N-LAP and O-LAP bound to HSA, BSA, HAG and BAG did not reveal significant differences (**Supplementary Figure S1**).

Emission of the protein-bound LAP (or metabolites) was first investigated at λ_{exc} = 295 nm, where both the protein and the drug absorb light. In PBS, the fluorescence of LAP was weak and unstructured (λ_{max} ~ 475 nm) due to aggregation but most importantly due to emission from intramolecular charge transfer (ICT) states, which are favored in a twisted orientation between the furan and quinazoline rings due to the freedom in the degrees of movement of the drug in solution (Vayá et al., 2020). However, in the presence of protein, which provides a more constrained environment, a clear enhancement of LAP fluorescence in addition to quenching of the protein emission (λ_{max} ~ 340 nm) was noticed (see **Supplementary Figure S2**). This effect was previously detected for the drug complexed to HSA, and was associated to singlet-singlet energy transfer (SSET) from HSA to LAP (Vayá et al., 2020). On the other hand, the spectra within each protein were unstructured and displayed their maxima at shorter wavelengths (*ca*. 450 nm) compared to the free drug in PBS. Quenching of the protein fluorescence (λ_{max} ~ 340 nm) upon interaction with LAP, N-LAP and O-LAP was different for each complex (**Supplementary Figures S2–S4**; **Supplementary Table S1**). In general, higher quenching due to SSET was detected for the human proteins; besides, SAs appeared to induce greater deactivation than AGs. This can be associated to the encapsulation of the drug (or metabolite) inside the protein, which was stronger for LAP@protein compared to N-LAP and O-LAP.

More relevant from the photobiological point of view was the emission behavior of LAP and its metabolites upon excitation with UVA light (λ_{exc} = 340 nm), as this type of radiation is selectively absorbed by the drug chromophore and does not interact directly with biomolecules. The obtained results for LAP, N-LAP and O-LAP, upon excitation at 340 nm are shown in **Figure 2**.

In contrast to the strong and structured fluorescence with maxima at *ca*. 425 nm detected for LAP and N-LAP within HSA (**Figures 2A,B**), the emissions of the other ligand@protein complexes were much weaker and unstructured, and the maxima were found at longer wavelengths (~450 nm). The behavior within HSA was associated to the more constrained environment provided by the protein but also to the frozen coplanar orientation adopted by the furan and quinazoline rings of LAP or N-LAP, which favors emission from LE (locally excited) states (Vayá et al., 2020). On the contrary, binding to BSA, HAG or BAG might disfavor emission from LE states due to a certain degree of freedom for twisting between both rings, resulting in a diminished and red-shifted emission of the encapsulated LAP or N-LAP. This would imply that the confinement and the conformational arrangement of the ligands within the protein cavities are key factors controlling the photobehavior. As regards the fluorescence lifetimes, decay kinetics were measured upon excitation at 340 nm (**Supplementary Figure S5**). In general, shorter lifetimes (<1 ns) were determined for LAP and N-LAP bound to AGs than those determined within SAs, which ranged from 1 to 1.5 ns (**Supplementary Table S2**). Besides, the decay traces were faster for N-LAP than for the parent drug, which is in line with the steady-state results. Again, little if any emission was detected for O-LAP in the presence of the different proteins, suggesting the feasibility of alternative deactivation pathways, for instance excited state deprotonation of the phenol moiety.

Furthermore, both SAs and AGs are transport proteins present in the plasma. Under normal conditions, the concentration of the former is higher; however, this situation may change significantly under a variety of conditions (for instance, during inflammatory processes), where AGs can play a significant role (Kremer et al.,

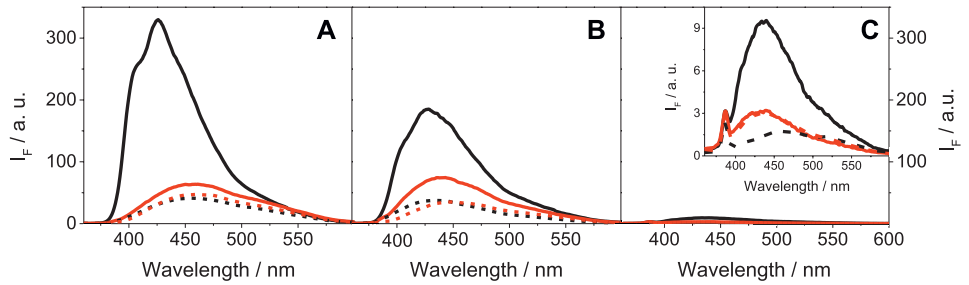

FIGURE 2 | Fluorescence spectra at λ_{exc} = 340 nm for **(A)** LAP@HSA (solid black), LAP@BSA (solid red), LAP@HAG (dashed black) and LAP@BAG (dashed red); **(B)** N-LAP@HSA (solid black), N-LAP@BSA (solid red), N-LAP@HAG (dashed black) and N-LAP@BAG (dashed red); **(C)** O-LAP@HSA (solid black), O-LAP@BSA (solid red), O-LAP@HAG (dashed black) and O-LAP@BAG (dashed red). All mixtures were at 1:1 M ratio (5 μM) in PBS under air, using isoabsorptive solutions at the excitation wavelength. The inset in **(C)** shows a zoom of the low emissive states for the O-LAP@protein complexes.

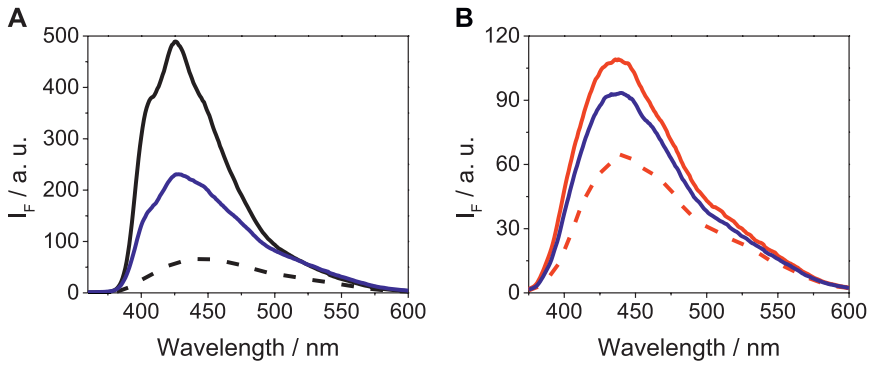

FIGURE 3 | Fluorescence spectra at λ_{exc} = 340 nm for **(A)** LAP@HSA (solid black), LAP@HAG (dashed black) and LAP@HSA+HAG (solid blue), and **(B)** LAP@BSA (solid red), LAP@BAG (dashed red) and LAP@BSA+BAG (solid blue). For LAP@protein, solutions of 1:1 M ratio (5 μM) were prepared, while for LAP@protein1+protein2, solutions of 1:1:1 M ratio (5 μM) were used.

1988; Bteich, 2019). Thus, it makes sense to investigate competing interactions of LAP or N-LAP with SAs and AGs in protein mixtures, upon selective excitation of the drug chromophore, to check the relative affinities to both types of proteins.

As it is shown in **Figure 3**, the emission spectra of LAP@HSA+HAG upon excitation at 340 nm was intermediate between the LAP@HSA and the LAP@HAG profiles; however, the shape and position of the band was more similar to that of LAP@HSA. A similar trend was also detected for LAP in a mixture of BSA and BAG, where LAP preferentially binds to BSA. Competing interactions of LAP in mixtures containing other protein combinations are shown in **Supplementary Figure S6**. Thus, it can be concluded that LAP interacts more strongly with the human proteins than with those of bovine origin; the weakest interaction was evidenced for BAG. A similar behavior was revealed for N-LAP in a mixture of proteins, as it can be deduced from the emission spectra shown in **Supplementary Figure S7**.

In order to get a deeper insight into the nature of LAP-protein binding, the photobehavior of the complexes was examined in the very early stages after exposure to UVA light, by means of femtosecond transient absorption measurements, upon excitation at 330 nm. The spectral shapes of the species detected for LAP in its complexes with BSA, HAG and BAG (**Supplementary Figure S8**) were in good accordance with that obtained previously for LAP@HSA (Vayá et al., 2020). Thus, a transient absorption band with relative maxima at *ca.* 425 and 530 nm was observed for LAP@BSA; similar profiles were noticed for LAP@AGs, with maxima around 425 and 550 nm. These transients were assigned to the LE singlet-singlet absorption species; their kinetic traces decayed double-exponentially (**Figure 4**). Thus, for SAs, short components with lifetimes of *ca.* 12 ps were evidenced, while the longer components were species-dependent and decayed faster for BSA than for HSA, reaching the ns time-scale. For the AGs, the short components decayed with lifetimes around 6 ps while the long ones survived until the ns time-scale. The two lifetime components could be associated to the LE states of LAP within the constrained environment provided by the protein; the short components would arise from the reorganization of the drug in the initial steps after excitation, whereas the long ones would correspond to the stabilized conformation within the protein cavities. Actually, the signature of ICT states was not evidenced in any of the LAP@protein complexes.

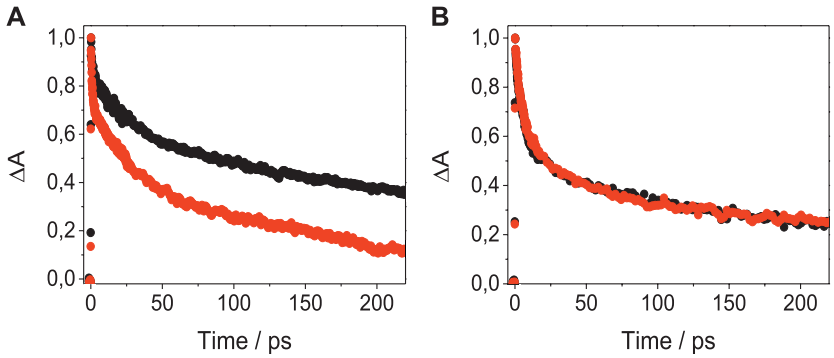

FIGURE 4 | Femtosecond transient absorption decays for **(A)** LAP@HSA (black) and LAP@BSA (red) monitored at 530 nm, and for **(B)** LAP@HAG (black) and LAP@BAG (red) monitored at 550 nm, in PBS after excitation at 330 nm.

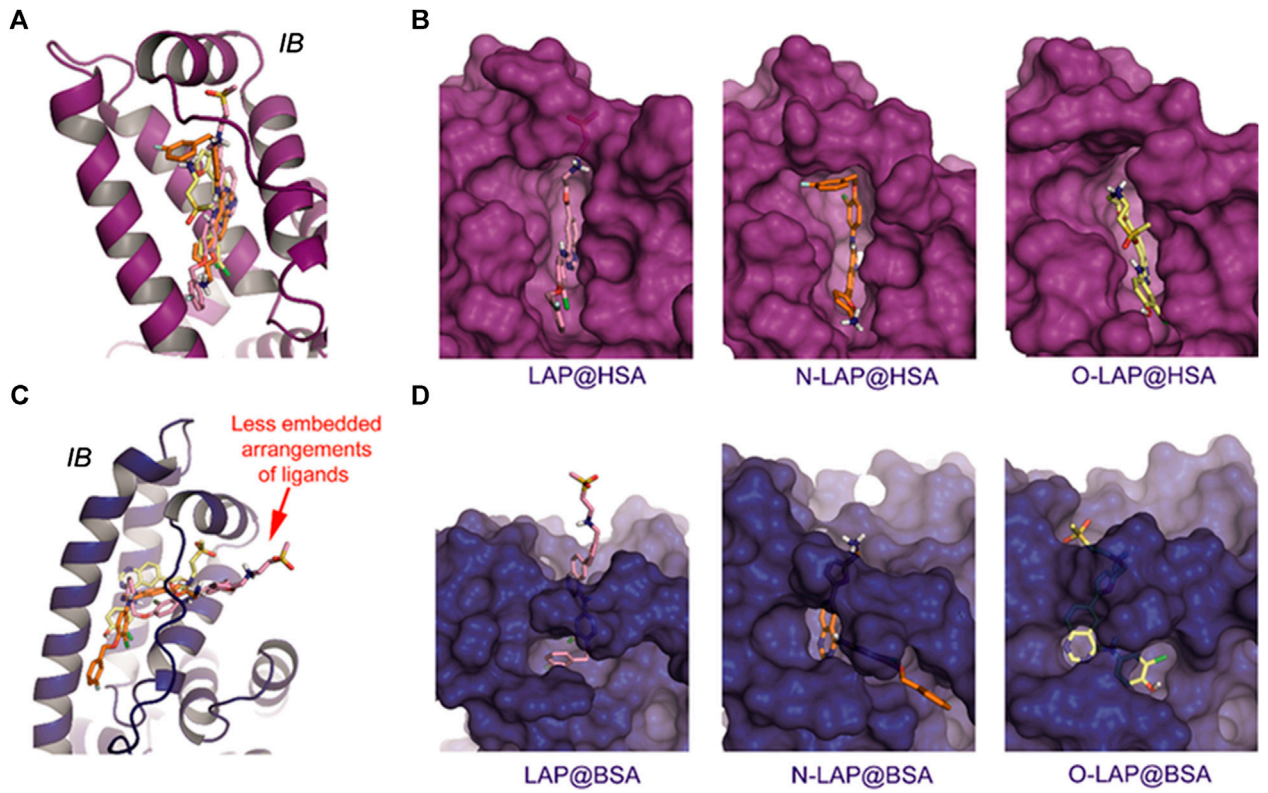

FIGURE 5 | Overall view of the proposed binding modes of LAP (pink), N-LAP (orange) and O-LAP (yellow) to HSA (magenta, **A,B**) and BSA (blue, **C,D**) as obtained by MD simulation studies. **(A,C)** Superposition of the binding modes of the three ligands to subdomain IB of HSA **(A)** and BSA **(C)**. **(B,D)** Detailed views of the proposed binary ligand@HSA **(B)**, and ligand@BSA **(D)** complexes.

In order to understand the marked experimental differences in the photobehavior of LAP and its metabolites interacting with SAs and AGs, the binding mode was investigated by computational studies. This was first analyzed by molecular docking using the program GOLD, version 5.8.1, and further validated by MD simulation studies.

For SAs, the ligands LAP, N-LAP and O-LAP were docked to subdomain IB. Experimental evidence to assign subdomain IB as the preferred binding site in HSA was previously obtained using selective site I and site II probes such as warfarin (WRF) and ibuprofen (IBP), respectively (Vayá et al., 2020). The resulting ligand@protein binary complexes were submerged in a truncated octahedron of water molecules and further analyzed by MD simulations (100 ns) using the molecular mechanics force field AMBER to achieve reliable models (Götz et al., 2012; Le Grand et al., 2013; Salomon-Ferrer et al., 2013). All ligand@protein

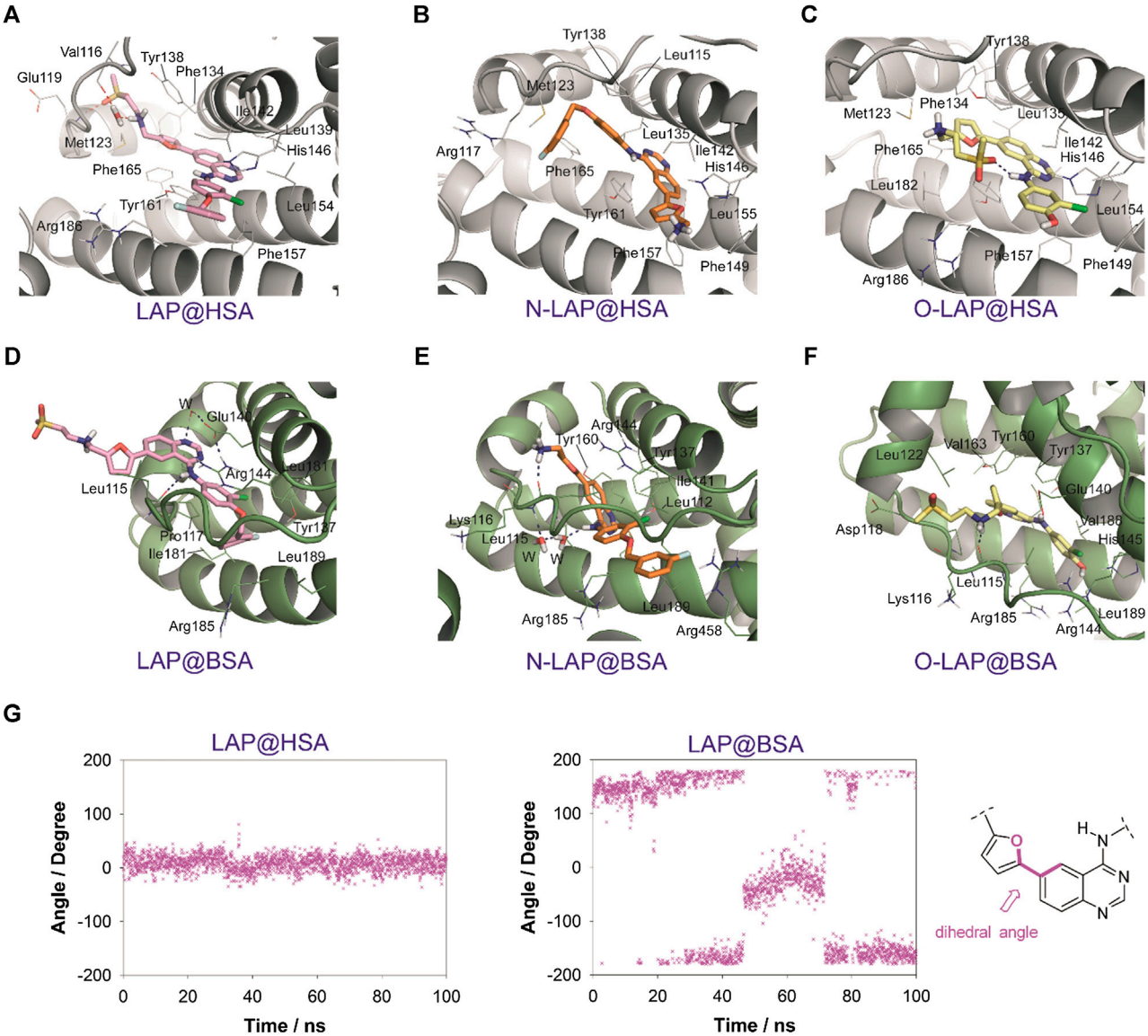

FIGURE 6 | Interactions of LAP (pink, **A,D**), N-LAP (orange, **B,E**), and O-LAP (yellow, **C,F**) to HSA (gray, **A–C**) and BSA (green, **D–F**) as obtained by MD simulation studies. Snapshots after 80 ns are shown. Relevant side chain residues are shown (lines) and labeled. Ligands are indicated as sticks. Hydrogen bonding interactions between the ligands and the protein are shown as blue dashed lines. **(G)** Variation of the dihedral angle between the furyl and quinazoline rings for LAP bound to HSA or BSA during the whole simulation. The bonds involved in the dihedral angle are highlighted in pink.

complexes proved to be very stable during simulation, as evidenced by the low values of the root-mean-square deviation (rmsd) for the whole protein backbone (C^{α}, C, N and O atoms) calculated for all complexes (average values range from 0.7 to 2.0 Å) (**Supplementary Figures S9–S12**).

The results of the MD simulation studies carried out for the ligand@SA complexes revealed that: 1) all ligands displayed a more deeply embedded arrangement in subdomain IB when bound to HSA than to BSA; 2) for the BSA complexes, a portion of the ligands was exposed to the solvent environment, particularly in the case of LAP. Specifically, the overall arrangement adopted by LAP or N-LAP was found to be clearly different in HSA compared to BSA (**Figure 5**). Thus, for LAP@BSA only part of the ligand interacted within the protein binding site, while N-LAP was rotated 180° within BSA to locate the ammonium moiety in close contact with the solvent. In any case, all ligands would be stabilized by numerous apolar interactions with the side chain residues within subdomain IB, as well as hydrogen bonding interactions through the ammonium group and the nitrogen atoms of the quinazoline ring. In addition, conformational analysis of the ligands within subdomain IB showed that: 1) only LAP and O-LAP would achieve an almost coplanar arrangement of the furyl and quinazoline rings upon binding to HSA, showing average dihedral angles

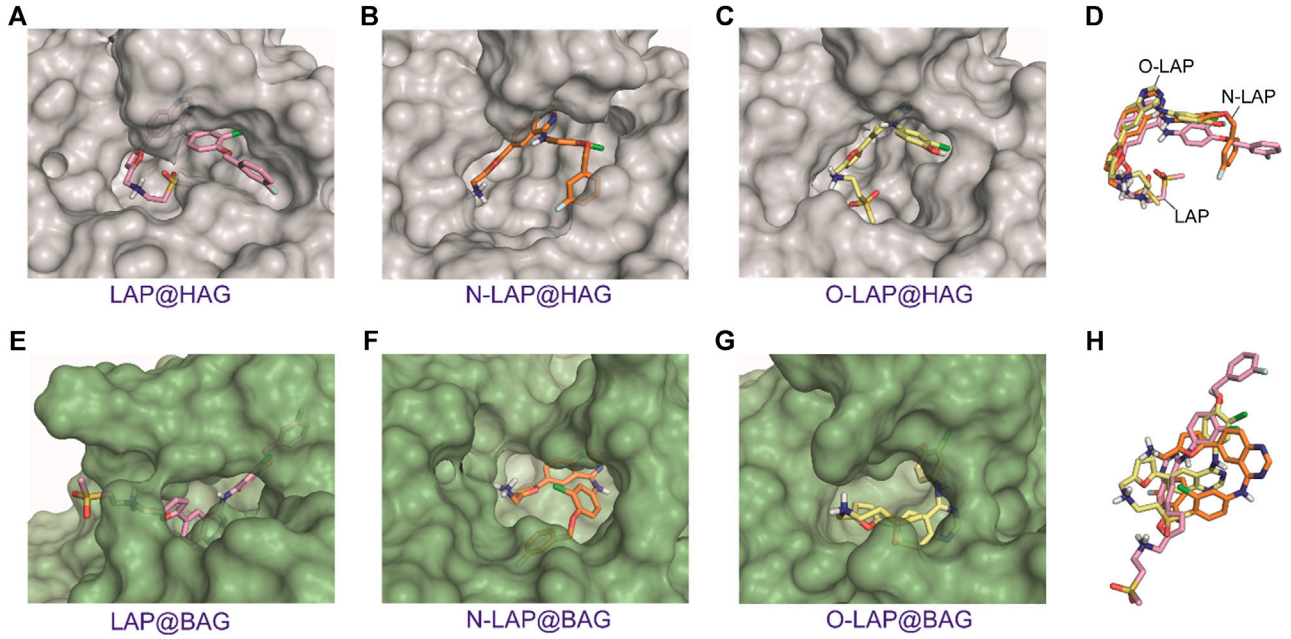

FIGURE 7 | Overall view of the proposed binding modes of LAP (pink, **A,E**), N-LAP (orange, **B,F**) and O-LAP (yellow, **C,G**) to HAG (gray) and BAG (green) as obtained by MD simulation studies. **(D,H)** Superposition of the binding modes of the tree ligands in the recognition pocket of HAG **(D)** and BAG **(H)**. Note how the furyl and quinazoline rings of the ligands for the HAG complexes would be almost coplanar and all ligands would show a similar arrangement.

of 8.3° and 7.3°, respectively, being their relative conformations frozen within the pocket; 2) on the contrary, free rotation around the linkage of the furyl and quinazoline rings was possible for LAP@BSA, O-LAP@BSA and the two N-LAP@SA complexes (**Figure 6**; **Supplementary Figure S13**).

Unlike SAs that can undergo large conformational changes upon binding a variety of ligands, specifically in domains I and III (Curry et al., 1998; Pérez-Ruiz et al., 2017), the plasticity of AGs is intrinsically more limited. Their central motif, which is composed by an eight-stranded β-barrel and is flanked by an α-helix, makes them constrained receptacles for ligand recognition. It is therefore not surprising that the MD simulation studies carried out for the ligand@AG complexes showed that the overall flexibility of LAP or its metabolites within the binding pockets would be restricted to some extent (**Figures 7A–C**; **Supplementary Figure S14**). This effect was found to be more pronounced for the ligand@HAG complexes, where the drug and its metabolites would be well surrounded by the protein. In addition, a similar arrangement of LAP, N-LAP and O-LAP within HAG was observed, with an almost coplanar arrangement of the furyl and quinazoline rings (**Figure 7D**; **Supplementary Figure S15**). However, although this effect would favor emission from LE states, the weaker intensity observed for HAG compared to HSA could be associated with the larger binding site of the former (**Supplementary Figure S16**), which may reduce the matrix effect provided by the protein cavities. On the contrary, for ligand@BAG it can be summarized that: 1) no common pattern of recognition was identified; 2) more solvent-exposed complexes were obtained; and 3) no coplanarity between the aromatic moieties was observed (**Figures 7E–H**; **Supplementary Figures S14 and S15**). In general, the MD simulation results are in line with the experimental data, as they justify the higher emission of LAP bound to HSA compared with the other proteins, in addition to the stronger affinity to the human proteins, as a result of the deeper embedded arrangement with higher restriction in the degrees of movement compared to the bovine proteins. Besides, the stronger emission of N-LAP within HSA and the weaker emission of the drug and its metabolites in BAG are also explained.

Finally, the binding free energies of LAP, N-LAP and O-LAP interacting with the investigated proteins (HSA, BSA, HAG and BAG) in the corresponding ligand@protein complex were calculated using the MM/PBSA approach (Miller et al., 2012)

TABLE 1 | Calculated binding free energies using MM/PBSA.[a]

Ligand	Protein	Complex	Energy
LAP	HSA	LAP@HSA	−56.7 ± 0.2[b]
—	BSA	LAP@BSA	−36.2 ± 0.3[b]
—	HAG	LAP@HAG	−54.3 ± 0.3[b]
—	BAG	LAP@BAG	−50.4 ± 0.4[b]
N-LAP	HSA	N-LAP@HSA	−43.5 ± 0.2[b]
—	BSA	N-LAP@BSA	−35.3 ± 0.2[b]
—	HAG	N-LAP@HAG	−39.1 ± 0.2[b]
—	BAG	N-LAP@BAG	−40.2 ± 0.3[b]
O-LAP	HSA	O-LAP@HSA	−37.2 ± 0.2[b]
—	BSA	O-LAP@BSA	−35.9 ± 0.2[b]
—	HAG	O-LAP@HAG	−41.3 ± 0.2[b]
—	BAG	O-LAP@BAG	−34.0 ± 0.2[b]

[a]*Energy units = kcal mol^{-1}.*
[b]*Standard error of mean.*

in explicit water (generalized Born, GB) as implemented in Amber (**Table 1**). These energies were estimated by subtracting the free energy of the corresponding unbound components, i.e. ligand and protein, to the free energy of the ligand@protein complex. The results revealed that the binding affinity of LAP to the human proteins is higher than to the bovine ones, especially in the case of SAs. Thus, LAP would have a 1.6-fold higher affinity for HSA than for BSA. This is mainly caused by the distinct amino acid sequence of the subdomain IB for both SAs (**Supplementary Figure S17**). These differences in the intraprotein microenvironments that surround the ligands are responsible for the type and strength of the stabilization interactions, as it can be observed from **Figures 6A–F**. This effect is even greater when comparing SAs and AGs in which the ligand-binding pockets are markedly different, either in amino acid sequence (**Supplementary Figure S18**) as well as in their tridimensional arrangement. In addition, the affinity of both metabolites to the investigated proteins was found to be weaker than that of the parent drug, following the order LAP >> N-LAP > O-LAP. Again, these results are qualitatively in line with those obtained from the fluorescence measurements, where higher affinity of either LAP or its metabolites was found for the human proteins, with the highest one observed for LAP@HSA.

CONCLUSION

The interaction of Lapatinib (LAP) and its N- and O-dealkylated metabolites (N-LAP and O-LAP) with model proteins (serum albumins and α_1-acid glycoproteins, SAs and AGs), of human and bovine origin, has been investigated by a combined photophysical and computational approach. In this context, the present work reveals key information on drug@protein complexation, the first step involved in a number of hypersensitivity reactions, including photosensitivity disorders. Photophysical results (fluorescence and ultrafast transient absorption) agree with strong and specific interactions of the drug and its metabolites with the selected proteins, pointing to a higher affinity to the human proteins, especially in the case of the LAP@HSA complex. The observed behavior can be rationalized by the coplanar orientation adopted by the furan and quinazoline rings within subdomain IB of HSA, in conjunction with the degree of confinement provided by this constrained intraprotein microenvironment. The experimental findings can be undoubtedly explained by the MD simulation studies. Thus, the weaker emission observed within BSA is explained by the higher exposure of part of the ligand to the bulk solution and also by the conformational arrangements and the degrees of freedom within the BSA binding site. Finally, the calculated binding energies for ligand@protein complexes are also in line with the relative affinities found in the competition experiments.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

All authors have realized substantial, direct, and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.576495/full#supplementary-material

REFERENCES

Ahmed, S. S., Whritenour, J., Ahmed, M. M., Bibby, L., Darby, L., Wang, X. N., et al. (2019). Evaluation of a human *in vitro* skin test for predicting drug hypersensitivity reactions. *Toxicol. Appl. Pharmacol.* 369, 39–48. doi:10.1016/j.taap.2019.02.005

Andreu, I., Mayorga, C., and Miranda, M. A. (2010). Generation of reactive intermediates in photoallergic dermatitis. *Curr. Opin. Allergy Clin. Immunol.* 10 (4), 303–308. doi:10.1097/ACI.0b013e32833bc68c

Blakely, K. M., Drucker, A. M., and Rosen, C. F. (2019). Drug-induced photosensitivity-an update: culprit drugs, prevention and management. *Drug Saf.* 42 (7), 827–847. doi:10.1007/s40264-019-00806-5

Bteich, M. (2019). An overview of albumin and alpha-1-acid glycoprotein main characteristics: highlighting the roles of amino acids in binding kinetics and molecular interactions. *Heliyon* 5 (11), e02879. doi:10.1016/j.heliyon.2019.e02879

Case, D. A., Betz, R. M., Cerutti, D. S., Cheatham, T. E., Darden, T. A., Duke, R. E., et al. (2016). *Amber*. (San Francisco, CA, USA: University of California).

CCDC *GOLD-Protein Ligand Docking Software*. Available at: http://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/ (Accessed January 22, 2020).

Cosa, G. (2004). Photodegradation and photosensitization in pharmaceutical products: assessing drug phototoxicity. *Pure Appl. Chem.* 76 (2), 263–275. doi:10.1351/pac200476020263

Curry, S., Mandelkow, H., Brick, P., and Franks, N. (1998). Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat. Struct. Biol.* 5 (9), 827–835. doi:10.1038/1869

Delano, W. L. (2020). *The PyMOL molecular graphics system*. Available at: http://www.pymol.org/ [Online].

Friedman, M. D., Lacouture, M., and Dang, C. (2016). Dermatologic adverse events associated with use of adjuvant lapatinib in combination with paclitaxel and trastuzumab for HER2-positive breast cancer: a case series analysis. *Clin. Breast Canc.* 16 (3), e69-74. doi:10.1016/j.clbc.2015.11.001

García-Lainez, G., Vayá, I., Marín, M. P., Miranda, M. A., and Andreu, I. (2020). In Vitro assessment of photo(geno)toxicity associated with lapatinib tyrosine kinase inhibitor. *Arch. Toxicol.* doi:10.1007/s00204-020-02880-6.

González, G., and Lage, A. (2007). Cancer vaccines for hormone/growth factor immune deprivation: a feasible approach for cancer treatment. *Curr. Cancer Drug Targets* 7 (3), 229–241. doi:10.2174/156800907780618310

Götz, A. W., Williamson, M. J., Xu, D., Poole, D., Le Grand, S., and Walker, R. C. (2012). Routine microsecond molecular dynamics simulations with AMBER on GPUs. 1. Generalized Born. *J. Chem. Theor. Comput.* 8 (5), 1542–1555. doi:10.1021/ct200909j

Hynes, N. E., and Lane, H. A. (2005). ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat. Rev. Canc.* 5 (5), 341–354. doi:10.1038/nrc1609

Ishikawa, T., Kamide, R., and Niimura, M. (1994). Photoleukomelanodermatitis (kobori) induced by afloqualone. *J. Dermatol.* 21 (6), 430–433. doi:10.1111/j.1346-8138.1994.tb01768.x

Johnston, S. R. D., Hegg, R., Im, S.-A., Park, I. H., Burdaeva, O., Kurteva, G., et al. (2017). Phase III, randomized study of dual human epidermal growth factor receptor 2 (HER2) blockade with lapatinib plus trastuzumab in combination with an aromatase inhibitor in postmenopausal women with HER2-positive, hormone receptor-positive metastatic breast cancer: alternative. *J. Clin. Oncol.* 36, 741–748. doi:10.1200/JCO.2017.74.7824

Kabir, M. Z., Mukarram, A. K., Mohamad, S. B., Alias, Z., and Tayyab, S. (2016). Characterization of the binding of an anticancer drug, lapatinib to human serum albumin. *J. Photochem. Photobiol., B* 160, 229–239. doi:10.1016/j.jphotobiol.2016.04.005

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* 10 (6), 845–858. doi:10.1038/nprot.2015.053

Krasner, J. (1972). Drug-protein interaction. *Pediatr. Clin.* 19 (1), 51–63. doi:10.1016/s0031-3955(16)32666-9

Kremer, J. M., Wilting, J., and Janssen, L. H. (1988). Drug binding to human alpha-1-acid glycoprotein in health and disease. *Pharmacol. Rev.* 40 (1), 1–47.

Le Grand, S., Götz, A. W., and Walker, R. C. (2013). SPFP: speed without compromise-A mixed precision model for GPU accelerated molecular dynamics simulations. *Comput. Phys. Commun.* 184 (2), 374–380. doi:10.1016/j.cpc.2012.09.022

Limones-Herrero, D., Palumbo, F., Vendrell-Criado, V., Andreu, I., Lence, E., Gonzalez-Bello, C., et al. (2020). Investigation of metabolite-protein interactions by transient absorption spectroscopy and in silico methods. *Spectrochim. Acta Mol. Biomol. Spectrosc.* 226, 117652. doi:10.1016/j.saa.2019.117652

Limones-Herrero, D., Pérez-Ruiz, R., Lence, E., González-Bello, C., Miranda, M. A., and Jiménez, M. C. (2017). Mapping a protein recognition centre with chiral photoactive ligands. An integrated approach combining photophysics, reactivity, proteomics and molecular dynamics simulation studies. *Chem. Sci.* 8, 2621–2628. doi:10.1039/c6sc04900a

Lin, N. U., Carey, L. A., Liu, M. C., Younger, J., Come, S. E., Ewend, M., et al. (2008). Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. *J. Clin. Oncol.* 26, 1993–1999. doi:10.1200/JCO.2007.12.3588

Lin, N. U., Dieras, V., Paul, D., Lossignol, D., Christodoulou, C., Stemmler, H. J., et al. (2009). Multicenter phase II study of lapatinib in patients with brain metastases from HER2-positive breast cancer. *Clin. Canc. Res.* 15, 1452–1459. doi:10.1158/1078-0432.CCR-08-1080

Medina, P. J., and Goodin, S. (2008). Lapatinib: a dual inhibitor of human epidermal growth factor receptor tyrosine kinases. *Clin. Therapeut.* 30, 1426–1447. doi:10.1016/j.clinthera.2008.08.008

Miller, B. R., 3rd, Mcgee, T. D., Jr., Swails, J. M., Homeyer, N., Gohlke, H., and Roitberg, A. E. (2012). MMPBSA.py: an efficient program for end-state free energy calculations. *J. Chem. Theor. Comput.* 8 (4), 3314–3321. doi:10.1021/ct300418h

Miranda, M. A., Bosca, F., Vargas, F., and Canudas, N. (1994). Photosensitization by fenofibrate. II. In vitro phototoxicity of the major metabolites. *Photochem. Photobiol.* 59 (2), 171–174. doi:10.1111/j.1751-1097.1994.tb05018.x

Molins-Molina, O., Perez-Ruiz, R., Lence, E., Gonzalez-Bello, C., Miranda, M. A., and Jimenez, M. C. (2019). Photobinding of triflusal to human serum albumin investigated by fluorescence, proteomic analysis, and computational studies. *Front. Pharmacol.* 10, 1028. doi:10.3389/fphar.2019.01028

Montanaro, S., Lhiaubet-Vallet, V., Iesce, M. I., Previtera, L., and Miranda, M. A. (2009). A mechanistic study on the phototoxicity of atorvastatin: singlet oxygen generation by a phenanthrene-like photoproduct. *Chem. Res. Toxicol.* 22 (1), 173–178. doi:10.1021/tx800294z

Nicholson, R. I., Gee, J. M. W., and Harper, M. E. (2001). EGFR and cancer prognosis. *Eur. J. Canc.* 37, S9–S15. doi:10.1016/s0959-8049(01)00231-3

Nuin, E., Perez-Sala, D., Lhiaubet-Vallet, V., Andreu, I., and Miranda, M. A. (2016). Photosensitivity to triflusal: formation of a photoadduct with ubiquitin demonstrated by photophysical and proteomic techniques. *Front. Pharmacol.* 7, 277. doi:10.3389/fphar.2016.00277

Pérez-Ruiz, R., Lence, E., Andreu, I., Limones-Herrero, D., González-Bello, C., Miranda, M. A., et al. (2017). A new pathway for protein haptenation by beta-lactams. *Chem. Eur J.* 23 (56), 13986–13994. doi:10.1002/chem.201702643

Peters, T. (1995). "Ligand binding by albumin," in *All about albumin - biochemistry, genetics, and medical applications*, 1st edn. (San Diego, CA, USA: Academic Press), 76–132.

Pinheiro, S., and Curutchet, C. (2017). Can forster theory describe stereoselective energy transfer dynamics in a protein-ligand complex? *J. Phys. Chem. B* 121 (10), 2265–2278. doi:10.1021/acs.jpcb.7b00217

Quintero, B., and Miranda, M. A. (2000). Mechanisms of photosensitization induced by drugs: a general survey. *Ars. Pharm* 41 (1), 27–46. doi:10.30827/ARS.V41I1.5732

Ross, J. S., Gay, L. M., Wang, K., Ali, S. M., Chumsri, S., Elvin, J. A., et al. (2016). Nonamplification ERBB2 genomic alterations in 5605 cases of recurrent and metastatic breast cancer: an emerging opportunity for anti-HER2 targeted therapies. *Cancer* 122 (17), 2654–2662. doi:10.1002/cncr.30102

Salomon-Ferrer, R., Götz, A. W., Poole, D., Le Grand, S., and Walker, R. C. (2013). Routine microsecond molecular dynamics simulations with AMBER on GPUs. 2. Explicit solvent particle mesh ewald. *J. Chem. Theor. Comput.* 9 (9), 3878–3888. doi:10.1021/ct400314y

Schonfeld, D. L., Ravelli, R. B., Mueller, U., and Skerra, A. (2008). The 1.8-A crystal structure of alpha1-acid glycoprotein (Orosomucoid) solved by UV RIP reveals the broad drug-binding activity of this human plasma lipocalin. *J. Mol. Biol.* 384 (2), 393–405. doi:10.1016/j.jmb.2008.09.020

Schroeder, R. L., Stevens, C. L., and Sridhar, J. (2014). Small molecule tyrosine kinase inhibitors of ErbB2/HER2/Neu in the treatment of aggressive breast cancer. *Molecules* 19 (9), 15196–15212. doi:10.3390/molecules190915196

Sekula, B., Zielinski, K., and Bujacz, A. (2013). Crystallographic studies of the complexes of bovine and equine serum albumin with 3,5-diiodosalicylic acid. *Int. J. Biol. Macromol.* 60, 316–324. doi:10.1016/j.ijbiomac.2013.06.004

Selvam, T. P., and Kumar, P. V. (2011). Quinazoline marketed drugs – a review. *Res. Pharm.* 1 (1), 1–21.

Shen, G. F., Liu, T. T., Wang, Q., Jiang, M., and Shi, J. H. (2015). Spectroscopic and molecular docking studies of binding interaction of gefitinib, lapatinib and sunitinib with bovine serum albumin (BSA). *J. Photochem. Photobiol., B* 153, 380–390. doi:10.1016/j.jphotobiol.2015.10.023

Sigismund, S., Avanzato, D., and Lanzetti, L. (2018). Emerging functions of the EGFR in cancer. *Mol. Oncol.* 12 (1), 3–20. doi:10.1002/1878-0261.12155

Spector, N. L., Xia, W., Burris, H., 3rd, Hurwitz, H., Dees, E. C., Dowlati, A., et al. (2005). Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J. Clin. Oncol.* 23 (11), 2502–2512. doi:10.1200/JCO.2005.12.157

Spitaleri, A., and Rocchia, W. (2019). "Molecular dynamics-based approaches describing protein binding," in *Biomolecular simulations in structure-based drug discovery*. Editors F. L. Gervasio and V. Spiwok (Weinheim, Germany: Wiley VCH), 29–39.

Thomas, R., and Weihua, Z. (2019). Rethink of EGFR in cancer with its kinase independent function on board. *Front. Oncol.* 9. doi:10.3389/fonc.2019.00800

Towles, J. K., Clark, R. N., Wahlin, M. D., Uttamsingh, V., Rettie, A. E., and Jackson, K. D. (2016). Cytochrome P450 3A4 and CYP3A5-catalyzed bioactivation of lapatinib. *Drug Metab. Dispos.* 44, 1584–1597. doi:10.1124/dmd.116.070839

Vargas, F., Canudas, N., Miranda, M. A., and Bosca, F. (1993). Photodegradation and in vitro phototoxicity of fenofibrate, a photosensitizing anti-hyperlipoproteinemic drug. *Photochem. Photobiol.* 58 (4), 471–476. doi:10.1111/j.1751-1097.1993.tb04917.x

Vayá, I., Andreu, I., Lence, E., González-Bello, C., Cuquerella, M. C., Navarrete-Miguel, M., et al. (2020). Characterization of the locally excited and charge-transfer states of the anticancer drug lapatinib by ultrafast spectroscopy and computational studies. *Chem. Eur J.* doi:10.1002/chem.202001336

Vayá, I., Andreu, I., Monje, V. T., Jimenez, M. C., and Miranda, M. A. (2016). Mechanistic studies on the photoallergy mediated by fenofibric acid: photoreactivity with serum albumins. *Chem. Res. Toxicol.* 29 (1), 40–46. doi:10.1021/acs.chemrestox.5b00357

Vayá, I., Lhiaubet-Vallet, V., Jimenez, M. C., and Miranda, M. A. (2014). Photoactive assemblies of organic compounds and biomolecules: drug-protein supramolecular systems. *Chem. Soc. Rev.* 43 (31), 4102–4122. doi:10.1039/c3cs60413f

Wilson, J. N., Liu, W., Brown, A. S., and Landgraf, R. (2015). Binding-induced, turn-on fluorescence of the EGFR/ERBB kinase inhibitor, Lapatinib. *Org. Biomol. Chem.* 13 (17), 5006–5011. doi:10.1039/c5ob00239g

Zunszain, P. A., Ghuman, J., Komatsu, T., Tsuchida, E., and Curry, S. (2003). Crystal structural analysis of human serum albumin complexed with hemin and fatty acid. *BMC Struct. Biol.* 3, 6. doi:10.1186/1472-6807-3-6

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Biotin-Labelled Clavulanic Acid to Identify Proteins Target for Haptenation in Serum: Implications in Allergy Studies

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Clavulanic acid (CLV) and amoxicillin, frequently administered in combination, can be independently involved in allergic reactions. Protein haptenation with β-lactams is considered necessary to activate the immune system. The aim of this study was to assess the suitability of biotinylated analogues of CLV as probes to study protein haptenation by this β-lactam. Two synthetic approaches afforded the labeling of CLV through esterification of its carboxylic group with a biotin moiety, via either direct binding (CLV-B) or tetraethylenglycol linker (CLV-TEG-B). The second analogue offered advantages as solubility in aqueous solution and potential lower steric hindrance for both intended interactions, with the protein and with avidin. NMR reactivity studies showed that both CLV and CLV-TEG-B reacts through β-lactam ring opening by aliphatic amino nitrogen, however with different stability of resulting conjugates. Unlike CLV conjugates, that promoted the decomposition of clavulanate fragment, the conjugates obtained with the CLV-TEG-B remained linked, as a whole structure including biotin, to nucleophile and showed a better stability. This was a desired key feature to allow CLV-TEG-B conjugated protein detection at great sensitivity. We have used biotin detection and mass spectrometry (MS) to detect the haptenation of human serum albumin (HSA) and human serum proteins. MS of conjugates showed that HSA could be modified by CLV-TEG-B. Remarkably, HSA preincubation with CLV excess only reduced moderately the incorporation of CLV-TEG-B, which could be attributed to different protein interferences. The CLV-TEG-B fragment with opened β-lactam was detected bound to the $^{404–430}$HSA peptide of the treated protein. Incubation of human serum with CLV-TEG-B resulted in the haptenation of several proteins that were identified by 2D-electrophoresis and peptide mass fingerprinting as HSA, haptoglobin, and heavy and light chains of immunoglobulins. Taken together, our results show that tagged-CLV keeps some of the CLV features. Moreover, although we observe a different behavior in the conjugate stability and in the site of protein modification, the similar reactivity indicates that

it could constitute a valuable tool to identify protein targets for haptenation by CLV with high sensitivity to get insights into the activation of the immune system by CLV and mechanisms involved in β-lactams allergy.

Keywords: betalactam, biotin tag, biotinylation, clavulanate, drug allergy, haptenation

INTRODUCTION

β-lactam antibiotics are the second most consumed drugs and the most frequent ones eliciting allergic reactions (Doña et al., 2012). This poses an important clinical problem since, in the most severe cases, allergic reactions may be life-threatening and reduce the therapeutic options against infections. The frequency of allergic reactions associated with each drug varies over time according to consumption patterns (Martin-Serrano, 2018). Hence, currently, amoxicillin (AX) is the antibiotic most frequently eliciting immediate (IgE-mediated) allergic reactions (Fernandez et al., 2017). In addition, AX is nowadays frequently administered in combination with clavulanic acid (CLV), a β-lactam compound which inhibits β-lactamases activity (Torres et al., 2016). As a consequence of the increase in the frequency of AX-CLV administration, selective reactions to CLV are on the rise, reaching 30% of the allergic reactions induced by this combination (Torres et al., 2010; Blanca-Lopez et al., 2015; Fernandez et al., 2017; Salas et al., 2018).

Diagnosis of a suspected reaction after AX-CLV intake is a challenge, as it involves determining which of the two drugs is the responsible one (Torres et al., 2016). Skin test to CLV is only available in some countries and its sensitivity is not optimal. Therefore, drug provocation tests must be performed indirectly using both AX and AX-CLV to establish a diagnosis of CLV allergy (Doña et al., 2019). However, these *in vivo* tests are contraindicated in severe life threatening reactions. The ideal alternative is the performance of risk-free *in vitro* tests (Mayorga et al., 2016), although immunoassays for quantifying IgE specific to CLV are not available and have never been reported. Only *in vitro* tests such as basophil activation tests or histamine release tests have been used for the evaluation of patients (Torres et al., 2010; Pineda et al., 2015; Salas et al., 2018; Barbero et al., 2019). These functional assays use the CLV molecule to evaluate whether the drug induces cellular activation, but they show suboptimal sensitivity (Ariza et al., 2016b; Doña et al., 2017).

In the context of IgE-mediated reactions to β-lactams, drugs behave as haptens as they are assumed to covalently bind to carrier proteins to induce an immunological response (Ariza et al., 2011; Gonzalez-Morena et al., 2016). Both, the resulting structure of the conjugated drug (antigenic determinant) and part of the protein to which it is attached may be involved in the IgE recognition process (Ariza et al., 2015; Ariza et al., 2016a; Martín-Serrano et al., 2016). Developing new approaches for diagnosing CLV allergy and improving the existing ones requires the inclusion of CLV derivative structures recognized by the immune system, whose identification is much more complex compared with other β-lactam drugs. Complex reactivity of CLV and instability after protein conjugation have delayed the isolation and characterisation of the main CLV antigenic determinants (Barbero et al., 2019), and the lack of monoclonal antibodies against CLV has impeded the identification of proteins involved. Therefore, elucidating the mechanisms and structures involved in the immune system activation by CLV is required to advance in diagnosis.

Protein haptenation by CLV is assumed to occur similarly to other β-lactams (Edwards et al., 1988; Barbero et al., 2019), i.e., benzylpenicillin and AX, by nucleophilic opening of the β-lactam ring by protein amino groups from lysine residues (Batchelor et al., 1965; Yvon et al., 1990; Garzon et al., 2014). However, unlike penicillins, which render stable penicilloyl determinants, the resulting acylated structure of CLV is unstable and degrades, leading to small and heterogeneous epitopes with a very low density in the carrier (Edwards et al., 1988; Torres et al., 2016). We have recently reported the identification of a CLV determinant: N-protein, 3-oxopropanamide, which was addressed through a synthetic approach of its analogues and their ability to activate basophils in a higher proportion of patients compared with the native CLV (Barbero et al., 2019). Moreover, the same CLV fragments bound to protein were identified by proteomic approaches (Barbero et al., 2019). Based on its extraordinary ligand-binding capacity, human serum albumin (HSA) has been traditionally considered the main target protein in the haptenation process for β-lactams, and most studies have focused on characterisation of the penicilloyl-HSA conjugates (Jenkins et al., 2009; Martin et al., 2010; Whitaker et al., 2011; Garzon et al., 2014; Meng et al., 2016; Azoury et al., 2018). Regarding CLV, only a couple of recent studies have reported HSA haptenation, identifying stable N-protein, 3-oxopropanamide determinant on lysine residues in *in vitro* conjugation at physiological pH (Barbero et al., 2019) and in patients treated with the drug (Meng et al., 2016), whereas direct binding of CLV to lysine residues, subsequent degradation products, pyrazine conjugates and cross-linking conjugates were identified at high concentrations *in vitro* (Meng et al., 2016).

Besides HSA, other proteins can be target of haptenation with β-lactams. Transferrin (Magi et al., 1995; Ariza et al., 2012; Garzon et al., 2014) and immunoglobulins (light and heavy chains) (Ariza et al., 2012; Garzon et al., 2014) have been reported to be target serum proteins to be modified *in vitro* by ampicillin and/or AX. Besides serum proteins, surface and intracellular proteins have been reported to form antigenic determinants with β-lactams (Binderup and Arrigoni-Martelli, 1979; Watanabe et al., 1986; Watanabe et al., 1987; O´Donnell et al., 1991; Gonzalez-Morena et al., 2016; Sánchez-Gómez et al., 2017) and the intracellular haptenation process

has been suggested to be cell type-dependent (Ariza et al., 2014). The identification of novel drugs or reactive metabolite conjugates with proteins is extremely challenging and relevant due to the role of these structures in the activation of the immune system (Labenski et al., 2009). The gold standard to detect and identify drug-protein conjugates is high resolution mass spectrometry (Garzon et al., 2014); nevertheless this approach requires sophisticated equipment that is not available to every laboratory. Alternatively, specific antibodies can be used through immunoassays (ELISA, immunoblotting) for the detection of these conjugates (Martin-Serrano, 2018); however, the lack of specific antibodies for some epitopes and the lack of sensitivity for the detection of low concentrations of conjugates or low degree of protein modification is the major drawback of these detection techniques (Tailor et al., 2016). The use of labeled drugs or reactive metabolites to enrich the drug modified fraction in complex samples and to improve the detection is an option to be considered (Koizumi et al., 2011; Ariza et al., 2014; Gonzalez-Morena et al., 2016). The avidin-biotin interaction provides great affinity and sensitivity, as well as the possibility of coupling modification of proteins by biotinylated compounds with several methods for detection, purification, and imaging (Ariza et al., 2014; Martin-Serrano, 2018). Previous studies have combined biotin labeling with proteomic techniques for the identification of potential protein targets for haptenation (Garzón et al., 2010; Ariza et al., 2014; Sánchez-Gómez et al., 2017; Shan et al., 2017) or modified protein residues (Havelund et al., 2017). The use of biotinylated AX (AX-B) has been shown to increase the detection sensitivity of AX modified serum proteins by immunological methods (Ariza et al., 2014) and has allowed the detection of novel cellular targets (Sánchez-Gómez et al., 2017).

Unlike AX, no antibody specifically targeting CLV is available, which has hampered the immunological detection of protein-CLV conjugates. To overcome this issue, in this work we envisaged an alternative strategy consisting in the design of appropriate biotinylated derivatives of CLV as highly sensitive and straightforward tools to study haptenation and developing methods to identify CLV target proteins. Two different structures, CLV-Biotin (CLV-B) and CLV-tetraethylenglycol-Biotin (CLV-TEG-B), were synthesized as probes for detecting haptenated serum proteins through streptavidin-based amplification technique. Both compounds bear biotin moiety linked through CLV carboxylic group without altering β-lactam group, which is reactive against proteins. The reactivity of biotinylated analogs of CLV and their ability to form conjugates was studied with a simple nitrogen nucleophile, as well as with HSA as protein model for haptenation. In addition, conjugation to a simple model peptide was analyzed, choosing $^{182-195}$HSA peptide due to its previous identification as target of AX (Garzon et al., 2014) and CLV (Barbero et al., 2019). The biotinylated derivative was demonstrated to be a straightforward tool to identify serum proteins target of modification.

MATERIALS AND METHODS

Chemical Synthesis

Synthesis of Clavulanate-Biotin

Methyl Biotinate

The reported procedure (Martin-Serrano, 2018) was followed and adapted (Soares da Costa et al., 2012). Thionyl chloride (45 μL) was added slowly to a suspension of biotin (50 mg, 0.204 mmol) in methanol (1.2 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* to give quantitatively the methyl ester as a white solid (52 mg). Spectral data are in agreement with those reported in the literature (Tao et al., 2007).

Biotinol

The reported procedure(Martin-Serrano, 2018) was followed and adapted (Soares da Costa et al., 2012). To a suspension of methyl biotinate (52 mg, 0.204 mmol) in dry THF (2 mL) was added carefully $LiAlH_4$ (31 mg, 0.816 mmol) and stirred at room temperature overnight. The reaction mixture was quenched with methanol (1 mL) and water (1 mL). $MgSO_4$ was added to the mixture and it was stirred for additional 20 min. Then, the reaction mixture was concentrated *in vacuo*, filtered and washed with 1:4 MeOH/CH_2Cl_2 (10 mL). The filtrate was concentrated *in vacuo* to give quantitatively the target product as a white solid (47 mg). Spectral data are in agreement with those reported in the literature (Corona et al., 2006).

Biotin Tosylate

The reported procedure (Martin-Serrano, 2018) was followed and adapted (Soares da Costa et al., 2012). Tosyl chloride (47 mg, 0.245 mmol) was added to a suspension of biotinol (47 mg, 0.204 mmol) in dry pyridine (1.0 mL) in an ice bath. The reaction mixture was stirred at zero degrees for 1 h and at room temperature overnight. Then, it was diluted with CH_2Cl_2 (5 mL) and washed with aqueous HCl 1M (5 mL), aqueous saturated $NaHCO_3$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, concentrated *in vacuo* and purified by flash chromatography eluting with 5% methanol in CH_2Cl_2 to give a white solid (29%). Spectral data are in agreement with those reported in the literature (DeLaLuz et al., 1995).

Biotin Iodide

The reported procedure (Martin-Serrano, 2018) was followed (Iglesias-Sánchez et al., 2010). Biotin tosylate (75 mg, 0.196 mmol) and NaI (60 mg, 0.391 mmol) were stirred at reflux in acetone (10 mL) for 24 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 and the organic layer was successively washed with aqueous saturated sodium thiosulfate (10 mL) and water, dried over anhydrous Mg_2SO_4, and concentrated *in vacuo*. Purification of the crude material by flash chromatography eluting with 5% methanol in CH_2Cl_2 gave the target compound as a white solid (52 mg, 78%). Spectral data are in agreement with those reported in the literature (Iglesias-Sánchez et al., 2010).

2-Biotin Clavulanate (CLV-B)

The reported procedure (Martin-Serrano, 2018) was followed and adapted (Brown et al., 1984). Commercially available potassium clavulanate (44 mg, 0.173 mmol) and previously synthesized biotin iodide (49 mg, 0.144 mmol) under nitrogen atmosphere were stirred in dry DMF (2 mL) at room temperature overnight. The solvent was removed *in vacuo* and the crude was purified by flash chromatography eluting with 5% methanol in CH_2Cl_2 to give the target compound as a white solid (35 mg, 60%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.43 (1H, s, NH), 6.36 (1H, s, NH), 5.69 (1H, d, J = 2.7 Hz, H_5), 5.18 (1H, s, H_2), 4.73 (1H, t, J = 6.8 Hz, H_1''), 4.30 (1H, t, J = 7.3 Hz, H_{12}'), 4.14–3.94 (5H, m, H_8' + H_2' + H_2''), 3.62 (1H, dd, J = 16.8, 2.7 Hz, H_6, diastereotopic protons), 3.13–3.09 (2H, m, H_6 + H_7'), 2.82 (1H, dd, J = 12.5, 5.1 Hz, H_{13}', diastereotopic protons), 2.58 (1H, d, J = 12.5 Hz, H_{13}'), 1.60–1.23 (8H, m, H_6' + H_5' + H_4' + H_3'); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 175.5 (C7), 167.2 (C1′), 162.7 (C10′), 150.4 (C3), 101.2 (C1′), 87.5 (C5), 65.5 (C2′), 61.0 (C8′), 69.9 (C2), 59.2 (C12′), 55.5 (C2″), 55.4 (C7'), 46.0 (C6), 28.14, 28.11, 27.7 (C5′, C4′, C3′), 25.2 (C6′). C13′ signal overlaps with DMSO-d6 solvent signal (see HSQC). HRMS ($C_{18}H_{25}N_3O_6S$-H, 410.1385), found: 410.1385.

Synthesis of Clavulanate-Tetraethylenglycol-Biotin

2-(2-(2-(2-Iodoethoxy)ethoxy)ethoxy)ethyl Biotinate

The reported procedure (Martin-Serrano, 2018) was followed and adapted (Li et al., 2008). DMSO (5 mL) was added into a 25-mL schlenk containing biotin (244 mg, 1.0 mmol) under nitrogen atmosphere at room temperature. Then, NaH (44 mg, 1.1 mmol, 60% dispersion in mineral oil) was added under N_2 atmosphere and the reaction mixture was allowed to stir for 10 min. Then, previously prepared 1-iodo-2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy) ethane (I-TEG-I) (555 mg, 1.4 mmol) in DMSO (2 mL) was added to the reaction and the mixture was stirred at room temperature overnight. Then, saturated aqueous NH_4Cl was added. Subsequently, it was extracted with EtOAc (3 × 10 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography eluting with 5% methanol in CH_2Cl_2 to give the target compound as a white solid (184 mg, 35%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.29 (1H, s, NH), 5.83 (1H, s, NH), 4.38–4.35 (1H, m, H_8), 4.18–4.15 (1H, m, H_4), 4.10–4.07 (2H, m, H_6'), 3.62 (2H, t, J = 6.7 Hz, H_{12}'), 3.57 (2H, t, J = 4.9 Hz, H_7'), 3.53 (s, 8H, H_8', H_9', H_{10}', H_{11}', H_{12}'), 3.13 (2H, t, J = 7.0 Hz, H_{13}'), 3.04–2.99 (1H, m, H_5), 2.76 (1H, dd, J = 12.8, 5.0 Hz, H_7, diastereotopic protons), 2.61 (1H, d, J = 12.7 Hz, H_7), 2.24 (2H, t, J = 7.6 Hz, H_4'), 1.64–1.48 (4H, m, H_1', H_3'), 1.36–1.27 (2H, m, H_2'). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 173.6 (C5′), 164.0 (C2), 71.8, 70.5, 70.46, 70.40, 70.07, 69.04 (C7′, C8′, C9′, C10′, C11′, C12′), 63.3 (C6′), 61.8 (C8), 60.0 (C4), 55.5 (C5), 40.4 (C7),33.7 (C4′), 28.26, 28.11 (C2′, C3′), 24.6 (C1′), 3.1 (C13′). HRMS ($C_{18}H_{31}IN_2O_6S$ + H, 531.1020), found: 531.1015.

2-Tetraoxadodecane-Biotin Clavulanate (CLV-TEG-B)

The reported procedure (Martin-Serrano, 2018) was followed and adapted as follows (Brown et al., 1984). Commercially available potassium clavulanate (53 mg, 0.223 mmol) and previously synthesized 2-(2-(2-(2-Iodoethoxy)ethoxy)ethoxy)ethyl biotinate (130 mg, 0.246 mmol) under nitrogen atmosphere were stirred in dry DMF (2 mL) at room temperature overnight. The solvent was removed under vacuum and the crude was purified by flash chromatography eluting with 5% methanol in CH_2Cl_2 to give the target compound as colourless oil (20 mg, 15%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.90 (1H, s, NH), 5.68 (1H, d, J = 2.3 Hz, H_5), 5.35 (1H, s, NH), 5.05 (1H, d, J = 1.1 Hz, H_2), 4.97 (1H, dt, J = 6.9, 1.3 Hz, H_1''), 4.51–4.47 (1H, m, H_{16}'), 4.31–4.19 (7H, m, H_{20}', H_2', H_2'', H_9'), 3.71–3.58 (12H, m, H_3', H_4', H_5', H_6', H_7', H_8'), 3.48 (1H, dd, J = 16.7, 2.8 Hz, H_6, diastereotopic protons), 3.16–3.11 (1H, m, H_{15}'), 3.08 (1H, d, J = 16.7 Hz, H_6), 2.89 (1H, dd, J = 12.8, 5.0 Hz, H_{21}', diastereotopic protons), 2.74 (1H, d, J = 12.8 Hz, H_{21}'), 2.36 (2H, t, J = 7.3 Hz, H_{11}'), 1.74–1.63 (4H, m, H_{12}', H_{14}'), 1.48–1.39 (2H, m, H_{13}'). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 174.8, 173.8, 167.3, 163.7 (C=O), 152.0 (C3), 101.0 (C1′″), 87.9 (C5), 70.8, 70.6, 70.4, 69.3, 69.8 (oxygenated chain), 63.51, 63.49 (C2′, C9′), 62.0 (C20′), 61.7 (oxygenated chain), 60.5, 60.2 (C2, C16′), 57.2 (C2″), 55.6 (C15′), 46.5 (C6), 40.6 (C21′), 33.9 (C11′), 28.4, 28.3 (C12′, C13′), 24.8 (C14′). HRMS ($C_{26}H_{40}N_3O_{11}S$ + H, 602.2378), found: 602.2378.

Stability Studies

Freshly prepared solutions of CLV or CLV-TEG-B 1:1, at 10 mM concentration, in deuterated PBS were placed in an NMR tube and incubated at 37°C. The reactions were monitored by ^{1}H-NMR registration after 1, 16, and 40 h (Martin-Serrano, 2018).

Evaluation of CLV and CLV-TEG-B Reactivity Toward Amino Nucleophiles

Mixture solutions of CLV or CLV-TEG-B 1:1 with butylamine, at 10 mM concentration for each species were prepared in deuterated PBS and incubated at 37°C. The reactions were monitored by ^{1}H-NMR registration after 15 min, 1, 16 and 40 h (Martin-Serrano, 2018).

In vitro Modification of HSA or Serum Proteins by CLV–B or CLV-TEG-B

HSA-CLV-TEG-B conjugates were prepared by incubation of HSA at 10 mg/mL (0.15 mM) in PBS for 16 h at 37°C with decreasing concentrations of CLV-B and CLV-TEG-B (15 mM, 1.5 and 0.15 mM) in PBS, at 1:100, 1:10 and 1:1 protein/drug molar ratio. They were purified by dialysis filtration using Amicon filters (Merck- Millipore) and analyzed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS).

In addition, each biotinylated derivative, CLV-B and CLV-TEG-B, were freshly dissolved in DMSO and PBS (pH 7.4), respectively. For modification by biotinylated CLV derivatives, HSA at 10 mg/mL (0.15 mM) in PBS was incubated for 16 h at 37°C with decreasing concentrations of CLV-B or CLV-TEG-B (90 mM–0.05 μM). Then, conjugates were purified by dialysis

filtration using Amicon filters (Merck-Millipore). For modification of serum proteins, human serum from healthy donors was incubated with CLV-TEG-B freshly prepared in PBS (pH 7.4) at 0.03 mM for 16 h at 37°C. All conjugates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by modification detection with streptavidin-horseradish peroxidase (HRP) and ECL (Martin-Serrano, 2018), as described below.

In Vitro Modification of HSA Peptide by CLV-TEG-B

CLV-TEG-B was freshly prepared in PBS (pH 7.4) at 0.15 mM (Ariza et al., 2012) and incubated with 13.5 mM $^{182-195}$HSA peptide (1,518.68 Da) in PBS (pH 7.4) for 24 h at 37°C. Resulting conjugates were purified using PD G-10 Desalting Columns (GE Healthcare) and then analyzed by mass spectrometry (Martin-Serrano, 2018), as described below.

Competition Between CLV and CLV-TEG-B for HSA Modification

HSA (10 mg/mL, 0.15 mM) was preincubated with CLV (80–96,000 μM) in PBS (pH 7.4) for 16 h at 37°C. After preincubation, CLV-B was added to each sample to a final concentration of 80 μM and then incubated for 2 h at 37°C. Resulting conjugates were than analyzed by SDS-PAGE and drug modification was detected by transfer to membrane and biotin detection with avidin-HRP (Martin-Serrano, 2018).

CLV- Enrichment of Protein-Biotinylated Drug Fraction by Avidin Affinity Chromatography

Resulting samples of the incubation of proteins with biotinylated compounds were filtered and then purified using agarose beads coated with Neutravidin (Thermo Fisher Scientific). Samples were incubated with the beads for 2 h at room temperature and then resin was washed with 50 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM EGTA, 1% NP-40 and 5% SDS buffer to eliminate unspecifically bound proteins (Garzón et al., 2010; Martin-Serrano, 2018). Finally, biotinylated fraction was released from the resin using a buffer containing SDS and β-mercaptoethanol. In parallel, control HSA was subjected to affinity purification to assay the possibility of unspecific retention with the resin (Garzón et al., 2010; Martin-Serrano, 2018).

Mass Spectrometry Analysis of CLV-TEG-B- Modified HSA

The MALDI-TOF mass spectra of HSA protein and peptide modification with CLV-TEG-B were acquired at Central Service for Research Support (SCAI, University of Malaga), Proteomic Unit, using a MALDI TOF TOF Bruker UltraFlextreme (Martin-Serrano, 2018). Experiments were recorded by dissolving conjugates in milliQ water containing 0.1% trifluoroacetic acid (TFA) and using sinapinic acid (SPA) or α-cyano-4-hydroxycinamic acid (CHCA) as the matrix for proteins or peptides, respectively.

In vitro HSA modification with CLV-TEG-B (enriched biotinylated fraction prepared at 1:10 protein/drug molar ratio) was studied by liquid chromatography-mass spectrometric (LC-MS/MS) analysis, acquired at Proteomic Laboratory at National Center of Biotecnology (CNB,CSIC, Madrid). After sample digestion with trypsin, chymotrypsin and LysC, collision-induced dissociation (CID) fragmentation was performed using a TripleTOF 5600 Q-TOF mass spectrometer (SCIEX) for peptide sequencing and protein matching (MASCOT). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD021727.

SDS-PAGE Electrophoresis and Biotinylation Detection by Blot Followed by Biotin Detection

Samples of HSA and human serum conjugates with CLV-B or CLV-TEG-B containing 2–4 μg of protein were separated in 12% SDS-PAGE. Then, proteins were transferred to a PVDF membrane (Trans-Blot Turbo Mini PVDF Transfer Packs, Bio-Rad) using Trans-Blot Turbo Transfer System from Bio-Rad following manufacturer's indications. For biotin detection, blots were incubated with HRP-streptavidin (Amersham, GE Biosciences) at 1/1,000 dilution and ECL detection (Clarity Western ECL Substrate, Bio-Rad). Estimation of the biotinylation degree was made by comparison with a biotinylated BSA standard (Pierce) (Gharbi et al., 2007). To check HSA load, blots were previously incubated with anti-HSA primary antibody (Santa Cruz Biotechnology) at 1 μg/mL and polyclonal rabbit HRP-anti-mouse IgG (DAKO) at 1/2,000 dilution and then stripped with HCl guanidine 8 M before biotinylation detection. Chemiluminiscence was used for detection (Clarity Western ECL Substrate, Bio-Rad) and images were analyzed using ImageQuant LAS4000 (GE Healthcare) (Martin-Serrano, 2018). We analyzed images obtained with ImageJ software (National Institutes of Health) for three replicates and expressed the results as pmol biotin/pmol HSA.

Two-Dimensional Electrophoresis and Protein Identification

For two-dimensional electrophoresis, samples were processed by a procedure similar to that previously described (Ariza et al., 2012) (Martin-Serrano, 2018). Aliquots of control and CLV-TEG-B-treated human serum (protein:drug ratio 1:0.192) containing 20 μg of protein were precipitated with cold acetone stirring for 16 h at 4°C. Then, acetone was decanted and the pellet completely dried using a SpeedVac system. The dried pellet was resuspended in 278.4 μL of IEF simple buffer (4% CHAPS, 2 M thiourea, 7 M urea, 100 mM DTT, and 0.4% Bio-lyte ampholytes). Sample was then divided in two aliquots and loaded on two ReadyStrip IPG Strips (pH 3–10 lineal, 7 cm, Bio-Rad) for isoelectric focusing on a Protean IEF cell (Bio-Rad), following the instructions of the manufacturer. Before the second dimension, strips were equilibrated in 6 M urea, 2% SDS, 0.375 M Tris-HCl

pH 8.8, 20% glycerol and bromophenol blue containing 130 mM DTT for the first equilibration step and 135 M iodoacetamide for second step. Strips were then placed on top of duplicated 10% polyacrilamide SDS gels. One of the gels was subsequently transferred to a PVDF membrane and used for localization of proteins modified by CLV-TEG-B by biotin detection.

The duplicate gel was stained for total protein with Comassie staining and it was used for spot excising and identification at the CAI Técnicas Biológicas, Unidad de Proteómica, Facultad de Farmacia (Universidad Complutense de Madrid, Madrid, Spain). The spots of interest were then manually excised from gels. Proteins selected for analysis were in-gel reduced, alkylated and digested with trypsin according to previous literature (Sechi and Chait, 1998; Martin-Serrano, 2018). Briefly, the samples were reduced with 10 mM DTT in 25 mM ammonium bicarbonate for 30 min at 56°C and subsequently alkylated with 25 mM iodoacetamide in 25 mM ammonium bicarbonate for 15 min in the dark. Finally, samples were digested with 12.5 ng/μL sequencing grade trypsin (Roche Molecular Biochemicals) in 25 mM ammonium bicarbonate (pH 8.5) overnight at 37°C. After digestion, the supernatant was collected and 1 μL was spotted onto a MALDI target plate and allowed to air-dry at room temperature. Then, 0.6 μL of a 3 mg/mL of α-cyano-4-hydroxy-cinnamic acid matrix (Sigma) in 50% acetonitrile were added to the dried peptide digest spots and allowed again to air-dry at room temperature. MALDI-TOF MS analyses were performed in a 4800 Plus Proteomics Analyzer MALDI-TOF/TOF mass spectrometer (Applied Biosystems, MDS Sciex, Toronto, Canada). The MALDI-TOF/TOF operated in positive reflector mode with an accelerating voltage of 20,000 V. All mass spectra were calibrated internally using peptides from the auto digestion of trypsin. For protein identification SwissProt 20170116 (553222 sequences; 198133818 residues) with taxonomy restriction to human was searched using MASCOT 2.3 (www.matrixscience.com) through the software Global Protein Server v 3.6 (ABSciex). Search parameters were set as follows: enzyme, trypsin; fixed modifications, carbamidomethyl (C); variable modification, oxidized methionine; one missed cleavage allowed; peptide tolerance, 50 ppm. Probability scores greater than the score fixed by MASCOT were considered significant if $p < 0.05$.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD021675.

RESULTS

Synthesis and Characterisation of Biotinylated Clavulanic Acid (CLV-B and CLV-TEG-B)

Two different biotin derivatives of CLV were designed as probes for detecting haptenated serum proteins. Both approaches consisted in the successful labeling of CLV through its carboxylic group with a biotin moiety, via either direct binding or hydrophilic spacer linker, without affecting the CLV β-lactam ring.

The first approach consisted in the straight labeling CLV resulting in CLV-B (**Figure 1A**). The synthetic sequence involved biotin esterification to form the methyl ester, further reduction to alcohol and subsequent tosylation that eventually allowed substitution by an iodide group. The resulting biotin iodide was used for esterification of CLV allowing biotinylation of the drug. The product was isolated with 73% average yield. The chemical structure of CLV-B and the synthetic intermediates were confirmed by conventional techniques of NMR and MS. The resulting compound was not completely soluble in water and DMSO was used to make it soluble in aqueous media for NMR characterisation (**Supplementary Figures S1, S2**) as well as for further protein incubation experiments.

The second approach employed an extending tetraethylenglycol (TEG) linker between CLV and the biotin moiety by means of a two-step synthetic process (**Figure 1B**). First, hydroxyl groups from TEG chain were tosylated and further substituted by iodide. Esterification reaction of biotin with TEG di-iodide (I-TEG-I) afforded biotin-TEG-iodide (I-TEG-B), which was used for the esterification of CLV leading to the target molecule CLV-TEG-B, isolated with 62% average yield. The hydrophilic TEG linker provides solubility to the compound in aqueous media. Reactions were monitored using ^{1}H-NMR (**Supplementary Figure S3**) to confirm the completion of reactions. Final and intermediate products were appropriately purified and chemical structures were confirmed by conventional NMR (**Supplementary Figures S4, S5**) and MS techniques. This second derivative was completely soluble in water. Since aqueous solubility was a valuable property to perform following protein modification experiments, we mainly focused on CLV-TEG-B further on.

Stability and Reactivity of CLV and CLV-TEG-B

Due to their similar structures it is expected that both biotinylated compounds, i.e., CLV-B and CLV-TEG-B, present similar stability and reactivity. The main difference between both molecules is the presence of the PEG spacer that confers water solubility to CLV-TEG-B, which are the optimal conditions to evaluate stability and reactivity for further protein experiments.

We compared both the stability of CLV and CLV-TEG-B at neutral pH and physiological salt concentration and their acylation reactivity toward nitrogen nucleophile at NMR scale. Proton chemical shifts for H5 and H6 in the β-lactam ring were used to monitor these processes.

We performed stability studies of CLV-TEG-B dissolved in deuterated PBS (**Supplementary Figure S6**). Spectra revealed that after 1 h of incubation 20% of the β-lactam ring was opened and kept on opening over time, with 50% of opened β-lactam at 16 h and 58% at 40 h. In addition, after 1 h, the peak corresponding to the oxazolidine ring (signal 1′) seems to be affected, which could indicate some kind of degradation (as oxazolidine ring opening or double bond isomerisation). Spectra were analyzed to compare the stability between CLV

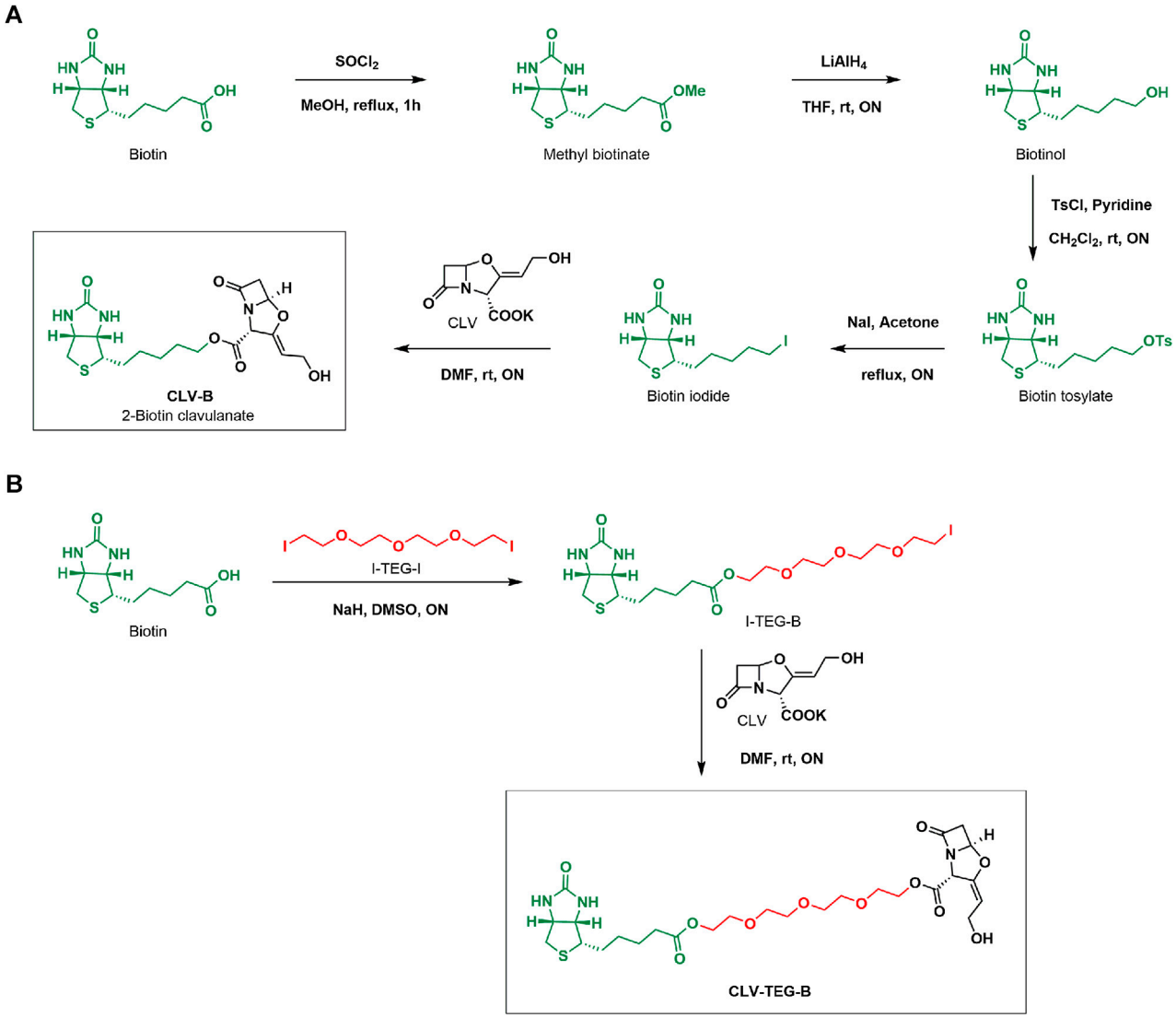

FIGURE 1 | Synthesis of biotinylated derivatives of clavulanic acid (CLV). **(A)** CLV-B and **(B)** CLV-TEG-B. CH_2Cl_2, dichloromethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; $LiAlH_4$, lithium aluminum hydride; MeOH, methanol; NaI, sodium hydride; ON, overnight; rt, room temperature; $SOCl_2$, thionyl chloride; THF, tetrahydrofurane; TsCl, tosyl chloride.

and CLV-TEG-B in deuterated PBS, at neutral pH and physiological salt concentration, showing that CLV is more stable than CLV-TEG-B since β-lactam opening did not take place for the former (**Supplementary Figure S7**).

The acylation abilities of CLV-TEG-B and CLV were studied toward butylamine, a simple nitrogen nucleophile molecule that mimics lateral chain of lysine residues, and therefore can conjugate to β-lactam (**Figure 2**). Results of CLV-TEG-B reactivity studies showed that after 15 min incubation with 1 equivalent of butylamine there was no remaining signal corresponding to methylene protons next to the amine in butylamine at 2.6 ppm and the appearance of a triplet at 3.2 ppm, both consistent with amide bond formation (**Supplementary Figure S8**). A shift in signals corresponding to β-lactam protons (H5 and H6) was observed after 15 min of incubation and the shift was complete after 16 h (see changes in signals H5 and H6), which could evidence β-lactam ring opening and/or other kind of ring modification. Spectra of CLV incubated with butylamine for 15 min showed that 60% of butylamine was forming amide and 40% remained as butylamine, as deduced by integration of signals corresponding to methylene protons closest to the butylamine nitrogen (**Supplementary Figure S9**). Moreover, the percentage of CLV conjugated to butylamine seems to degrade into other compounds whose structures cannot be elucidated with obtained data. Only a decreased integration of original shifts of CLV can be observed, consistent with the percentage of amide formed, however

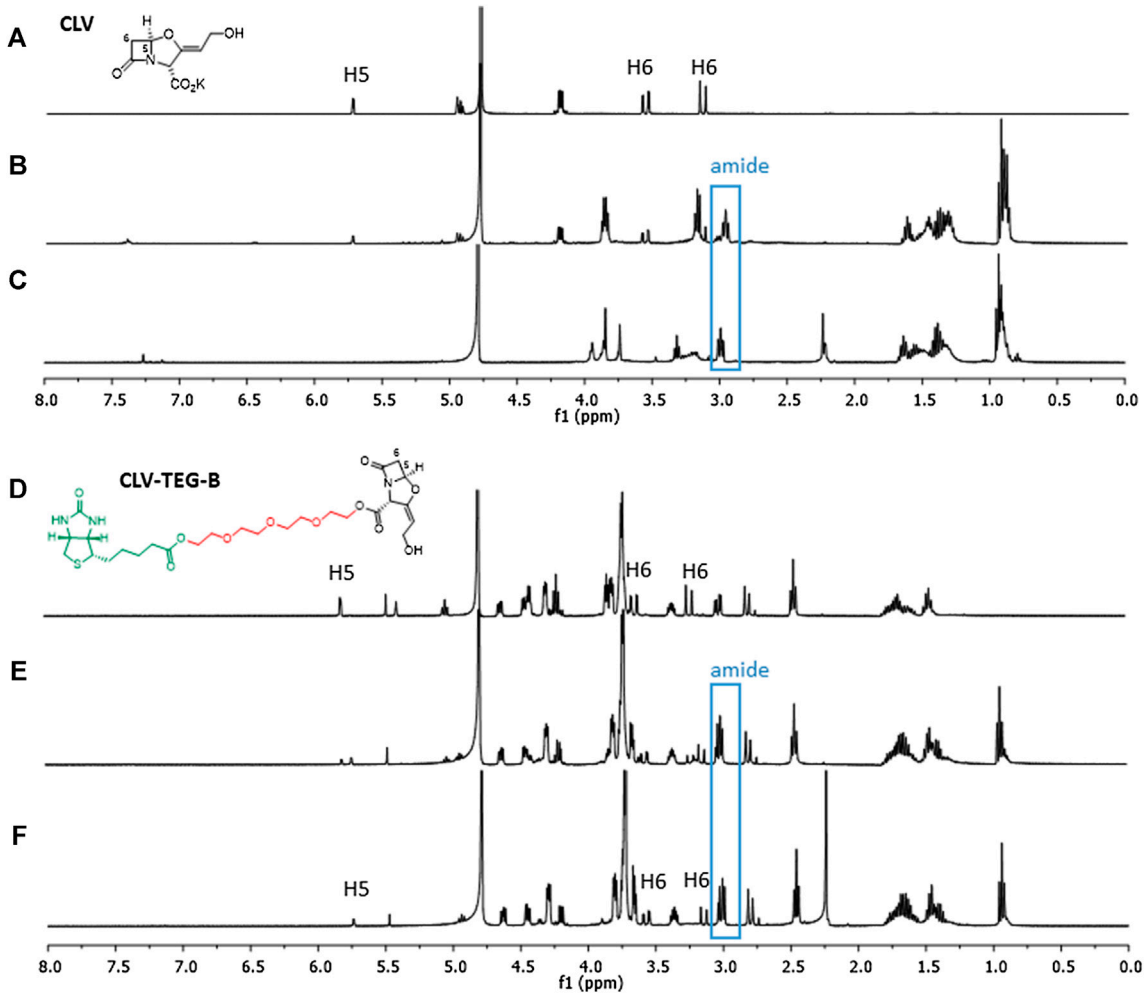

FIGURE 2 | Reactivity of clavulanic acid (CLV) and CLV-tetraethylenglycol-Biotin (CLV-TEG-B). ^{1}H NMR of each compound at similar conditions: **(A)** CLV control and **(D)** CLV-TEG-B control, after 15 min incubation in deuterated PBS. **(B)** CLV and **(E)** CLV-TEG-B, incubated with 1 equivalent of butylamine for 15 min **(C)** CLV and **(F)** CLV-TEG-B, incubated with 1 equivalent of butylamine for 16 h min. After addition of butylamine is observed amide formation for both compounds. Subsequently, degradation occurs in the case of CLV and shifted signals for protons corresponding to β-lactam (H5 and H6) are observed in the case of CLV-TEG-B.

discerning where signals shift is not clear, unlike in the case of CLV-TEG-B.

Characterisation of HSA-CLV-TEG-B Conjugates by MALDI-TOF and LC-MS

To confirm that conjugation takes place with proteins similarly with CLV and CLV-TEG-B, we used CLV- and CLV-TEG-B treated-HSA as model and analyzed the conjugates using proteomics. First MALDI-TOF MS techniques showed that incubation of HSA in the presence of CLV-TEG-B at 1:100, 1:10 and 1:1 M ratio caused an increase in the mass of the protein, which indicates incorporation of the drug derivative. (**Supplementary Figures S10–S13**). MS spectra of the dialyzed conjugates shows a peak at 66,467, 66,596 and 68,091 Da, shifted by 35, 164 and 1,659 Da with regard to the HSA control sample, for 1:100, 1:10 and 1:1 respectively, indicating a modification dependent of the biotinylated drug concentration, as previously reported with CLV treated-HSA (Barbero et al., 2019). The shift of the peak correspond to an average of different species as no homogeneous haptenation of the HSA may happen, and therefore we could not confirm neither the number of CLV derivatives bound to the protein nor the molecular weight of the added fragments.

To study in depth HSA residues modified by CLV-TEG-B and compared with previous results obtained with CLV (Barbero et al., 2019), HSA-CLV-TEG-B 1:10 conjugate was analyzed on a SCIEX 5600 TripleTOF spectrometer with CID fragmentation. The attempts to analyze the dialyzed conjugate were unsuccessful, however we observed that for the enriched

biotinylated conjugate digested with chymotrypsin, a peptide modified with a mass increment of 600.2 Da was found (**Supplementary Figures S14, S15**): $^{404-430}$HSA (QNALLVRYTKKVPQVSTPTLVEVSRNL). Both MS1, showing the m/z value corresponding to the parental ion (**Supplementary Figure S14**) and MS2 spectra (**Supplementary Figure S15**), are fully compatible with this modified $^{404-430}$HSA peptide. The fragmentation spectrum (**Supplementary Figure S15**) shows interesting information corresponding to the y2, y3, y4, y5, y6, y7, y8, y9, y10 and y11 ion fragments, as well as b1 and b6 ions, in addition to some secondary fragments. The involved residue cannot be identified based on these ion series, although they suggest the discarding the TPTLVEVSRNL and QNALLV fragments, and therefore the addition could be in any nucleophilic aminoacid of the RYTKKVPQVS fragment.

The mass increment (600.2 Da) is consistent with the *in vitro* CLV-TEG-B covalent binding to HSA, by the β-lactam opening. In order to characterize the modification in a simpler model, an HSA peptide containing several lysine residues, $^{182-195}$HSA peptide (LDELRDEGKASSAK), previously identified as target of AX (Garzon et al., 2014) and CLV (Barbero et al., 2019), was incubated with a 90 M excess of CLV-TEG-B and analyzed by MALDI-TOF MS (**Supplementary Figure S16**). Spectra show that only a low proportion of peptide was modified, but the mass increment (600.2 Da) is in agreement with the previous approach, indicating the coupling of one moiety of CLV-TEG-B.

Detection of HSA-Biotinylated CLV Conjugates by Blot and Biotin Detection

We analyzed the ability of biotinylated derivatives of CLV to bind covalently to proteins and their usefulness for the detection of CLV-protein conjugates by blot followed by biotin detection, since no anti-CLV antibodies are available. We incubated HSA with increasing concentrations of biotinylated derivatives of CLV in PBS (pH 7.4) (protein/biotinylated CLV ratios from 1:3.07 × 10^{-4} to 1:600) Results showed that both biotinylated derivatives of CLV bind covalently to HSA and that protein modification was dose-dependent. Signal detected by blot and detection with avidin-HRP was specific for biotinylated derivatives bound on HSA since no signal was detected for control HSA, and it was detected even at very low protein/biotinylated ratio, which confirms the high sensitivity of the method. A similar haptenation pattern was obtained for both biotinylated derivatives, and CLV-TEG-B was selected for performing further studies due its better water solubility properties compared with CLV-B (**Supplementary Figure S17**). Then, a more detailed dose-response analysis was performed with HSA-CLV-TEG-B conjugates, confirming their usefulness in the detection of HSA conjugates with high sensitivity (**Figure 3A**). The degree of incorporation of CLV-TEG-B was estimated by comparison with a biotinylated BSA standard and results representative of three assays showed values of 0.0935; 0.013; 0.00055 pmol biotin/pmol HSA for molar ratios of HSA:CLV-TEG-B 1:100; 1:10 and 1:1, respectively (**Figure 3B**). These results suggest that the extent of protein modification is not complete. Since the presence of non-conjugated protein could interfere in further detection analysis of the drug-protein conjugate, enrichment of the fraction of modified proteins would improve the characterisation and identification of conjugates. Protein fractions purified on neutravidin-agarose were analyzed by SDS-PAGE, and as it is shown in **Supplementary Figure S18**, the enrichment of HSA-CLV-TEG-B fraction increase the signal detection.

CLV and CLV-TEG-B Competition for Protein Binding Sites

In order to compare the binding capacity of both structures, competition assays were performed. HSA was incubated for 2 h with 80 μM CLV-TEG-B, after preincubation (16 h) with increasing concentrations of CLV. Results showed that the preincubation of HSA in the presence of an excess of CLV reduced the formation of conjugates containing CLV-TEG-B (**Figure 4**), which may indicate that both compounds may bind on HSA common binding sites. Incubations using the highest concentration of CLV resulted in protein aggregation, which could be explained by the formation of cross-linking conjugates that has been reported at high CLV concentration *in vitro* (Meng et al., 2016). This is similar to the observed behavior in HSA incubations with high concentrations of AX (Ariza et al., 2012).

Detection of CLV-TEG-B Candidate Target Proteins in Human Serum

In addition to HSA, other plasma proteins may be covalently modified by CLV-TEG-B and, to analyze that possibility, we incubated human serum with CLV-TEG-B and modified proteins were detected by transfer to membrane and biotin detection with avidin-HRP. Monodimensional SDS-PAGE allowed the observation of multiple positive bands, even with the lowest concentration of CLV-TEG-B used (**Figure 5A**), confirming that besides HSA, other serum proteins were target of haptenation. In order to identify the target proteins, samples were analyzed by two-dimensional electrophoresis followed by peptide fingerprint analysis by tryptic digestion and MALDI-TOF MS (**Figure 5B**; **Table 1**). Identified proteins included HSA, heavy and light immunoglobulin chains and haptoglobin. Although not included in this analysis, the haptenation of transferrin could be proposed on the bases of its molecular weight and isoelectric point in 2D gels.

DISCUSSION

CLV is a potent inhibitor of β-lactamase enzyme of increasing interest. Due to the increasingly worrying problem of antibiotic resistance, its consumption is on the rise and, as a consequence, the number of reported selective allergic responses induced by CLV after AX-CLV intake has increased significantly (Fernandez et al., 2017; Montañez et al., 2017). Unfortunately, the diagnostic

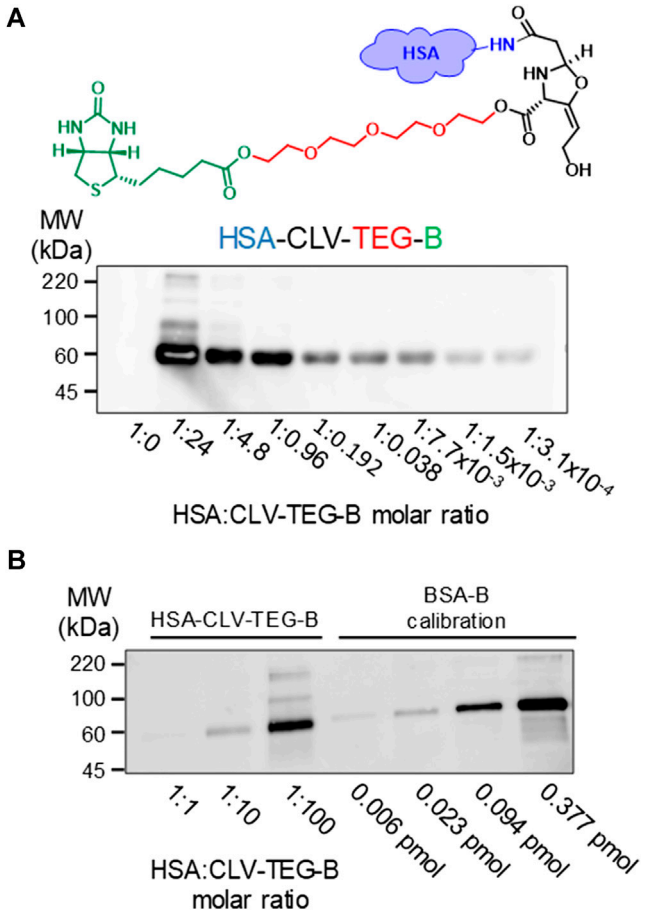

FIGURE 3 | Detection of modification of HSA by CLV-tetraethylenglycol-Biotin (CLV-TEG-B). Human serum albumin (HSA) was incubated in the presence of CLV-TEG-B for 16 h at 37°C. HSA:CLV-TEG-B molar ratios are shown in figure **(A)** Aliquots of the haptenation reactions containing 2 µg of protein were analyzed by SDS-PAGE and CLV-TEG-B modification was detected by blot with streptavidin-HRP and ECL detection **(B)** 0.15 µg aliquots of HSA-CLV-TEG-B **(left)** and 0.006–0.377 pmol aliquots of BSA-B standards **(right)** were analyzed by SDS-PAGE followed by blot with streptavidin-HRP and ECL detection.

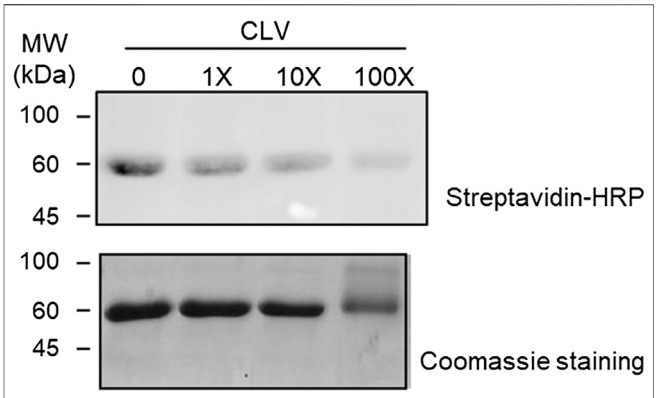

FIGURE 4 | Competition assay between clavulanic acid (CLV) and CLV-tetraethylenglycol-Biotin (CLV-TEG-B). Human serum albumin (HSA) was preincubated with increasing concentrations of CLV (80, 800 and 8,000 µM) for 16 h at 37°C and then with a fixed concentration of CLV-TEG-B (80 µM) for 2 h at 37°C. Resulting adducts were analyzed by SDS-PAGE and CLV-TEG-B modification was detected by blot with streptavidin-HRP and ECL detection **(top panel)**. Total protein was detected by Coomassie staining **(bottom panel)**.

work-up aiming to identify which drug of the AX-CLV combination is responsible for a reaction is not trivial. Risky *in vivo* tests show suboptimal sensitivity for CLV due to the fact that testing must be performed with AX-CLV combination, in which CLV is always in a lower ratio. This impedes both discriminating the drug eliciting the reaction and reaching CLV concentrations high enough to trigger reactions (Fernandez et al., 2017). On the other hand, *in vitro* tests to diagnose IgE-mediated reactions to CLV are limited to the basophil activation test using native CLV, with 40–50% of sensitivity (Mayorga et al., 2019). However, a recent study has reported that the inclusion of synthetic determinants of CLV increased the sensitivity of this assay up to 69%. Interestingly, only determinants with a N-protein, 3-oxopropanamide structure and ability for protein conjugation (spontaneous reactivity against amino groups) were found to induce basophil activation (Barbero et al., 2019). This finding indicates that drug-protein conjugates play a crucial role in the induction of allergies. Therefore, after getting insight into potential determinants responsible for allergies to CLV, herein we investigate potential proteins targets of CLV haptenation. A convenient strategy would be to follow a procedure similar to that reported for immunological detection of AX–protein conjugates with antibodies recognizing the lateral chain of the AX molecule, which successfully allowed the identification of serum proteins coupled to AX (Ariza et al., 2012). However, the lack of a suitable antibody against CLV has prevented the use of immunological detection approaches for our objective. Therefore, we considered label-drug techniques. Despite the fact that label-dependent techniques cannot be used to study conjugate formation in patients and usually do not provide information on the site of modification and/or structure of the conjugates, they are quite useful to visualize conjugates and to identify the modified proteins (Gonzalez-Morena et al., 2016). This is a big step to gain insight into conjugates formation in cases in which there are no specific antibodies against the drug or the conjugation takes place in such a low extension that it hampers detection using label-free approaches.

With the aim of detecting serum proteins that conjugate to CLV, biotin was chosen as a tag due to its extremely high affinity for streptavidin and the lack of impact on the properties of its substrate in most cases (Diamandis and Christopoulos, 1991). The strength of the biotin-streptavidin interaction makes this approach very sensitive. However, in order to retain their proper biological activity, different factors should be taken into account to design tagged molecules, such as the introduction of a biotin moiety into the parent molecule that could result in steric hindrances of its interaction with certain targets (site of protein recognition), the preferential solubility in aqueous media, the presence of intact functional groups that are

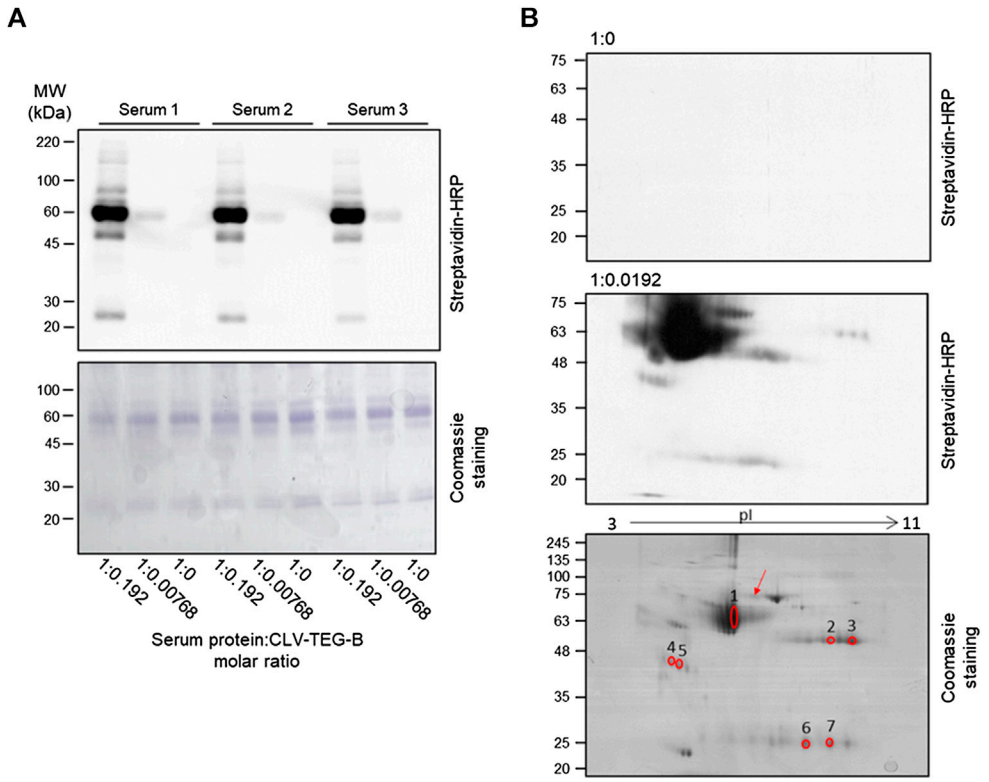

FIGURE 5 | Detection of modification of serum proteins by clavulanic acid (CLV)-tetraethylenglycol-Biotin (CLV-TEG-B) and identification of targets. Human sera were incubated in the presence of CLV-TEG-B for 16 h at 37°C. **(A)** 4 µg aliquots of resulting adducts were analyzed by SDS-PAGE and CLV-TEG-B modification was detected by blot with streptavidin-HRP and ECL detection. Lower panel shows the Coomassie staining for total proteins visualization. **(B)** 50 µg aliquots of resulting adducts were subjected to 2D-eletrophoresis on duplicate gels, after which, one of the gels was used for detection of modified proteins by transfer to membrane and biotin detection with avidin-HRP and the other one was used for protein staining with Coomassie. Matched spots were excised from the gel and used for tryptic digestion and peptide fingerprint analysis. The blot in the middle panel is deliberately overexposed in order to show the signals corresponding to modified immunoglobulin chains. The red arrow point towards a spot that could be proposed as transferrin, on the bases of its molecular weight and isoelectric point.

TABLE 1 | Identification of human serum proteins as targets for haptenation by CLV-tetraethylenglycol-Biotin by mass spectrometry. Data under superscripts c through h are from MASCOT.

Spot number[a]	Accession code[b]	Protein name	Total score[c]	Limit score	MW (Da)[d]	pI[e]	Matched peptides[f]	Coverage (%)[g]
1	P02768	Human serum albumin	470	56	71,317	5.92	43	69
2	P01857	Ig gamma-1 chain C region human	119	56	36,596	8.46	13	52
3	P01857	Ig gamma-1 chain C region human	197	56	36,596	8.46	18	66
4	P00738	Haptoglobin human	128	56	45,861	6.13	18	35
5	P00738	Haptoglobin human	102	56	45,861	6.13	16	33
6	P01834	Ig kappa chain C region human	101	56	11,773	5.58	8	76
7	P01834	Ig kappa chain C region human	81	56	11,773	5.58	7	76

[a]*Spot numbering as shown in 2-DE Coomassie gel in* ***Figure 5****.*
[b]*Protein accession code from NCBI database.*
[c]*Mascot total score.*
[d]*Theoretical molecular weight (Da).*
[e]*Theoretical pI.*
[f]*Number of matched peptides.*
[g]*Protein sequence coverage for the most probable candidate as provided by Mascot.*

involved in the protein conjugation, and the stability of resulting biotinylated drug coupled to protein after conjugation. In search of all these aspects is a challenge in the case of CLV, especially due to its complex reactivity and instability after β-lactam opening (Finn et al., 1984; Martin et al., 1989; Baggaley et al., 1997; Brethauer et al., 2008). This probably has hampered any success in the detection of specific IgE antibodies through immunoassays as well as the production of antibodies against CLV.

CLV was successfully labeled with a biotin moiety in the carboxylic group at C3, through two different approaches that keep the β-lactam ring intact. Direct coupling rendered CLV-B, which was not soluble in water, needing proportions of DMSO to solubilize in aqueous media for performing protein incubation experiments. An improved design consisted in CLV-TEG-B, which included an extending TEG linker between CLV and the biotin moiety. Such hydrophilic linker increases the hydrophilicity of the compound, which provides solubility in aqueous media, besides the flexibility and length of the spacer would allow enough distance between CLV molecule and the biotin tag to potentially result in less steric hindrance interactions, both in the protein-CLV conjugation process, and making biotin moiety more available to interaction with streptavidin, leading to higher detection efficiency. Due to these advantages we focused mainly on CLV-TEG-B conjugation studies.

Since CLV degrades at basic pH (Martin et al., 1989), physiological pH conditions were used for the biotinylated derivative conjugation as previously optimized for CLV (Barbero et al., 2019). This differs from optimized AX or other penicillins conjugations which have been reported to be performed in basic pH and which ensure lysine amino group deprotonation and favor nucleophilic attack to β-lactam carbonyl (Ariza et al., 2012; Pajares et al., 2020). Here we observed that CLV is very stable at physiological pH whereas 50% of CLV-TEG-B suffers hydrolysis in its β-lactam at 16 h, which would still permit 50% of CLV-TEG-B to conjugate during incubation time. We also have observed that both CLV and CLV-TEG-B display reactivity toward simple amines, through β-lactam ring opening. In spite of their similar reactivity in presence of nitrogen nucleophiles, they differ in terms of stability subsequent to conjugation. After amide formation the resulting conjugate for CLV breaks down into multiple compounds, whereas the conjugate formed with CLV-TEG-B does not suffer degradation in its biotinylated drug linked (**Figure 6**). Similar reactivity behavior was desired for both, parent and biotinylated drug, in order the tagged drug can emulate conjugation process occurring with the native drug (Ariza et al., 2014; Gonzalez-Morena et al., 2016). Nevertheless, the stability of the conjugated biotinylated analogue was a critical aspect for keeping the biotinylated moiety attached to the drug, and therefore their usefulness for detecting haptenated proteins.

Because published data identifies HSA as the main target for haptenation with drugs, we used this protein as a model for our study (Ariza et al., 2014). We observed that addition of CLV-TEG-B to HSA takes place *in vitro*, as deduced by MALDI-TOF MS, and that this modification is concentration-dependent, consistent with recent studies involving CLV (Meng et al., 2016; Barbero et al., 2019). These results are in agreement with the SDS-PAGE analysis of these conjugates, that showed that the extent of biotinylation was concentration-dependent of CLV-TEG-B and CLV-B used during incubation. MALDI-TOF MS analysis provided not a single mass but a molecular weight distribution containing protein species modified to a different extent, including unmodified protein, thus yielding only an estimate of the mass increment that may be related to the degree of haptenation. Moreover, MS comparison between conjugates with CLV-TEG-B and CLV (Barbero et al., 2019), and taken into account that the molecular weight of identified determinants of native CLV is 70 Da, indicates that haptenation taking place with CLV-TEG-B occurs in a lower extent than with CLV, which could be explained by the steric hindrances of the biotinylated moiety. The identification of HSA sites modified by CLV-TEG-B was only carried out for the conjugate formed using 1:10 protein:drug ratio, as performed previously with the native CLV (Barbero et al., 2019), although this concentration is higher than that of the drug *in vivo* under therapeutic conditions (Nagarajan et al., 2013). In the case of the biotinylated CLV, to get an exact mass increment of the fragment that links to the protein, enrichment of the biotinylated fraction was required to get any result by LC-MS/MS. We searched a 601.2 Da mass increase, related to the incorporation of a 602.2 Da with loss of the hydrogen atom of the HSA lysine fragment, compatible with the haptenation by one CLV-TEG-B thorough β-lactam ring opening without any other fragmentation. However, only a mass increment of 600.2 was found in the $^{404–430}$HSA peptide. An identical mass increment was found in a CLV-TEG-B treated-HSA peptide, used in a controlled experiment as a simpler model, which confirms that CLV-TEG-B adds to the protein with a 600.2 Da mass addition, although a low level of haptenation was obtained and the attached structure could not be elucidated. This $^{182–195}$HSA peptide has been previously reported to be modified *in vitro* by CLV-derived structures, consisting of *N*-protein, 3-oxopropanamide identified determinant of CLV (Barbero et al., 2019) or others bearing higher molecular weight (Meng et al., 2016), and by a series of penicillins, such as benzylpenicillin (Meng et al., 2011), AX (Garzon et al., 2014), flucloxacillin (Jenkins et al., 2009), and piperacillin (Whitaker et al., 2011).

Besides lysine residues, the modified $^{404–430}$HSA peptide contains other amino acid nucleophiles as arginine, which could be modified by acylation of the β-lactam. For instance, HSA haptenation with native CLV has been reported to occur also through histidine residues that are modified with the open β-lactam of the complete CLV molecule or with its pyrazine metabolite (Meng et al., 2016). However, modification of residues different from lysine was not observed in our previous study with native CLV (Barbero et al., 2019). In the present study, the modified $^{404–430}$HSA peptide seems to be adducted on the $^{410–419}$HSA sequence (RYTKKVPQVS). From these residues, we assume lysine as the residue with the highest reactivity toward this compound, due to its lower pka and previous literature regarding CLV protein binding (Meng et al., 2016; Barbero et al., 2019); however, at this point, there is no experimental evidence about which aminoacid forms the adduct with CLV-TEG-B. In any case, the modified peptide

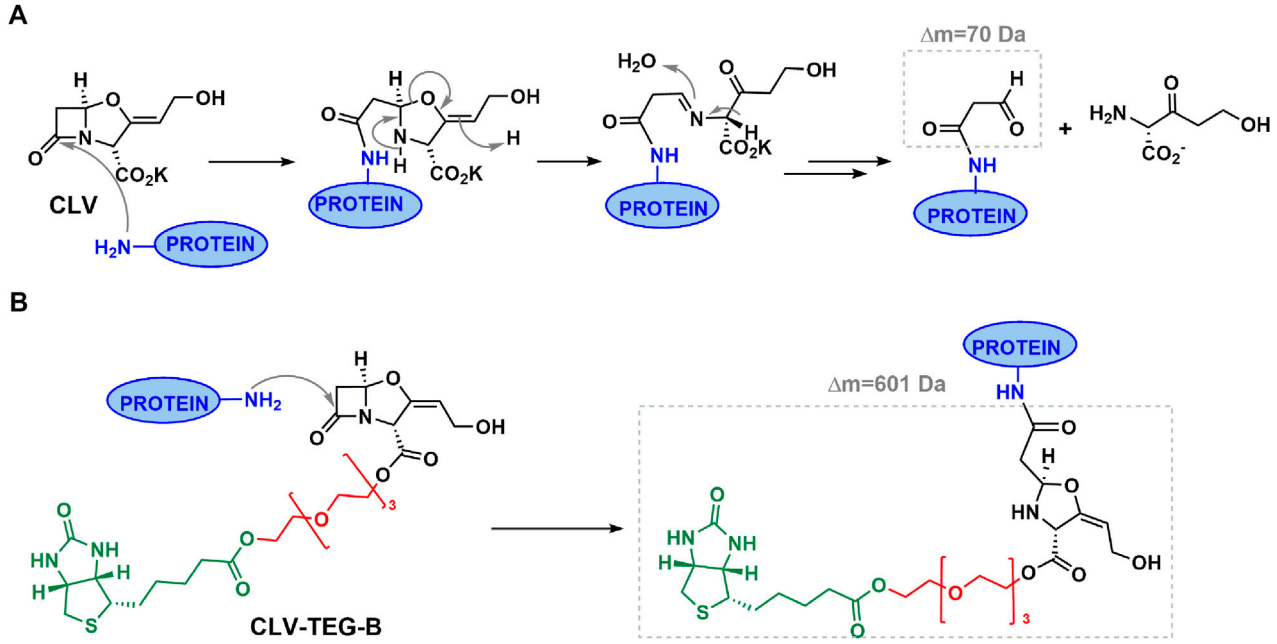

FIGURE 6 | Hypothesized mechanisms for protein conjugation with clavulanic acid (CLV) derivatives. **(A)** Proposed reaction mechanism for covalent binding of CLV to proteins leading to an unstable structure that can degrade through different pathways resulting in different determinants. Represented only the structure of identified potential antigenic determinant (N -protein, 3-oxopropanamide). **(B)** Proposed reaction mechanism for binding of CLV-tetraethylenglycol-Biotin to proteins leading to a stable conjugate structure. Theorycal increment of mass added to protein is indicated in each case (see text for details).

with CLV-TEG-B differ from those modified by original CLV, attached as 70 Da antigenic determinant, observed at two different residues, Lys 195 and Lys 475 (Barbero et al., 2019). This was somewhat expected, and could be explained by the presence of the biotin moiety that may impose steric impediments for binding to some targets or it may shield part of the molecule (Ariza et al., 2014). In spite of this, both compounds seem to compete for binding to proteins. This is in agreement with a previous study suggesting that AX and its biotinylated derivative could bind to protein common sites (Ariza et al., 2014). Besides competition for common site binding, other interferences in the protein could explain this behavior such as a change in the conformation structure of the protein and a binding/addition to closed sites that involves changes in steric and electronic effects of target lysine residues.

The chance of using biotinylated derivatives of CLV for the identification of modified serum proteins was studied by SDS-PAGE and blot followed by biotin detection since biotinylated CLV could be detected with a high sensibility using streptavidin conjugated with peroxidase. The analysis of HSA modification by biotinylated-CLV showed that protein extent modification was drug concentration-dependent. Importantly, the biotin moiety remained linked to the protein after conjugation to provide detection, and the method showed a great sensitivity for this application. Moreover, a semiquantitative estimation of biotinylation indicated that at 1:100 protein/drug molar ratio, only a 9% of the protein would form conjugate, if only one site were modified. This confirm the remarkable sensitivity of the method and may suggest a low protein modification extension, in agreement with the required enrichment of biotinylated fractions for high resolution MS techniques.

Serum proteins identified as candidate targets of CLV-TEG-B by 2D-electrophoresis and mass spectrometry were HSA, haptoglobin, and immunoglobulin heavy and light chains. In addition, the haptenation of transferrin could be proposed on the bases of its molecular weight and isoelectric point in 2D gels. This finding represents a great progress in our understanding of the mechanisms driving CLV allergy. Furthermore, these CLV derivatives could be useful for complex systems study, in which modified proteins could be purified with streptavidin columns. Previous studies of protein haptenation by AX have allowed to confirm the results obtained with biotinylated drug with those obtained with the native drug. By assaying serum protein conjugates with AX or biotinylated AX by 1D and 2D-electrophoresis and following Western blot using anti-AX antibodies for the AX detection or streptavidin for the biotin detection, it was observed that both compounds bind to the same serum targets (HSA, transferrin and IgE light and heavy chains), with the only difference of a weak binding to haptoglobin, which is undetectable using immunological AX detection, but is detectable with biotinylated AX due to the higher sensitivity of this method (Ariza et al., 2014; Martin-Serrano, 2018). The lack of antibodies against CLV impedes the immunological evaluation of protein-CLV conjugates and, therefore, we could

not assess if the proteins haptenated by CLV are the same as the modified by its biotinylated derivative. However, this comparison study in the case of AX (Ariza et al., 2012; Ariza et al., 2014) could be somewhat extrapolated to the case of CLV.

CONCLUSION

We have set up a model that may shed light into the process of protein haptenation by CLV through the use of highly sensitive approaches, such as labeling with biotinylated analogues, which allow the detection of its target serum proteins. These results strongly suggest that both, CLV and biotinylated CLV, are able to bind proteins through nucleophilic attack of the β-lactam carbonyl group by the protein amino nitrogen, process leading to the opening of the β-lactam ring. Unlike CLV protein conjugation, that promotes the decomposition of clavulanate fragment, the protein conjugates obtained with the CLV-TEG-B are stable enough to allow detection at great sensitivity. The results herein reported are of great interest since, for the first time, serum proteins that may act as carriers in allergic reactions to CLV are identified. Other alternative approaches for these studies are hampered by the complex reactivity of CLV and instability after conjugation. Further structural information on the binding sites on various targets would provide potential antigenic determinants to be used in diagnostic procedures and in studies on the mechanisms of CLV induced allergy.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Ética de la Investigación Provincial. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DP-S, EP-I, MJT and MIM conceived and designed the study. NB optimized synthetic approach and performed characterization of compounds. AM-S prepared compound in higher scale and performed reactivity studies. AS, JMM and FJSG performed proteomic experiments. AM-S, AA, DP-S and MIM analyzed all data, prepared figures, and wrote the manuscript, with input from MJT and EP-I.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.594755/full#supplementary-material

REFERENCES

Ariza, A., Collado, D., Vida, Y., Montañez, M. I., Pérez-Inestrosa, E., Blanca, M., et al. (2014). Study of protein haptenation by amoxicillin through the use of a biotinylated antibiotic. *PLoS One* 9 (3), e90891. doi:10.1371/journal.pone.0090891

Ariza, A., Fernandez, T. D., Mayorga, C., Barbero, N., Martin-Serrano, A., Perez-Sala, D., et al. (2015). Hypersensitivity reactions to betalactams: relevance of the hapten-protein conjugates. *J. Investig. Allergol. Clin. Immunol.* 25 (1), 12–25.

Ariza, A., Garzon, D., Abánades, D. R., de los Ríos, V., Vistoli, G., Torres, M. J., et al. (2012). Protein haptenation by amoxicillin: high resolution mass spectrometry

analysis and identification of target proteins in serum. *J. Proteo.* 77, 504–520. doi:10.1016/j.jprot.2012.09.030.

Ariza, A., Mayorga, C., Salas, M., Doña, I., Martín-Serrano, Á., Pérez-Inestrosa, E., et al. (2016a). The influence of the carrier molecule on amoxicillin recognition by specific IgE in patients with immediate hypersensitivity reactions to betalactams. *Sci. Rep.* 6, 35113. doi:10.1038/srep35113

Ariza, A., Montanez, M. I., Fernandez, T. D., Perkins, J. R., and Mayorga, C. (2016b). Cellular tests for the evaluation of drug hypersensitivity. *Curr. Pharmaceut. Des.* 22 (45), 6773–6783. doi:10.2174/1381612822666161107165917

Ariza, A., Montanez, M. I., and Perez-Sala, D. (2011). Proteomics in immunological reactions to drugs. *Curr. Opin. Allergy Clin. Immunol.* 11 (4), 305–312. doi:10.1097/ACI.0b013e3283489ae5

Azoury, M. E., Fili, L., Bechara, R., Scornet, N., de Chaisemartin, L., Weaver, R. J., et al. (2018). Identification of T-cell epitopes from benzylpenicillin conjugated to human serum albumin and implication in penicillin allergy. *Allergy* 73, 1662–1672. doi:10.1111/all.13418.

Baggaley, K. H., Brown, A. G., and Schofield, C. J. (1997). Chemistry and biosynthesis of clavulanic acid and other clavams. *Nat. Prod. Rep.* 14 (4), 309–333. doi:10.1039/np9971400309

Barbero, N., Fernández-Santamaría, R., Mayorga, C., Martin-Serrano, Á., Salas, M., Bogas, G., et al. (2019). Identification of an antigenic determinant of clavulanic acid responsible for IgE-mediated reactions. *Allergy* 74 (8), 1490–1501. doi:10.1111/all.13761

Batchelor, F., Dewdney, J., and Gazzard, D. (1965). Penicillin allergy: the formation of the penicilloyl determinant. *Nature* 206 (982), 362–364. doi:10.1038/206362a0

Binderup, L., and Arrigoni-Martelli, E. (1979). [14C]-D-Penicillamine: uptake and distribution in rat lymphocytes and macrophages. *Biochem. Pharmacol.* 28 (2), 189–192. doi:10.1016/0006-2952(79)90501-x

Blanca-Lopez, N., Perez-Alzate, D., Ruano, F., Garcimartin, M., de la Torre, V., Mayorga, C., et al. (2015). Selective immediate responders to amoxicillin and clavulanic acid tolerate penicillin derivative administration after confirming the diagnosis. *Allergy* 70, 1013–1019. doi:10.1111/all.12636

Brethauer, S., Held, M., and Panke, S. (2008). clavulanic acid decomposition is catalyzed by the compound itself and by its decomposition products. *J. Pharmaceut. Sci.* 97 (8), 3451–3455. doi:10.1002/jps.21225

Brown, A. G., Corbett, D. F., Goodacre, J., Harbridge, J. B., Howarth, T. T., Ponsford, R. J., et al. (1984). Clavulanic acid and its derivatives. structure elucidation of clavulanic acid and the preparation of dihydroclavulanic acid, isoclavulanic acid, esters and related oxidation products. *J. Chem. Soc., Perkin Transact.* 1, 635–650. doi:10.1039/P19840000635

Corona, C., Bryant, B. K., and Arterburn, J. B. (2006). Synthesis of a biotin-derived alkyne for PD-catalyzed coupling reactions. *Org. Lett.* 8 (9), 1883–1886. doi:10.1021/ol060458r

DeLaLuz, P. J., Golinski, M., Watt, D. S., and Vanaman, T. C. (1995). Synthesis and use of a biotinylated 3-azidophenothiazine to photolabel both amino- and carboxyl-terminal sites in calmodulin. *Bioconjug. Chem.* 6 (5), 558–566. doi:10.1021/bc00035a009

Diamandis, E. P., and Christopoulos, T. K. (1991). The biotin-(strept)avidin system: principles and applications in biotechnology. *Clin. Chem.* 37 (5), 625–636. doi:10.1093/clinchem/37.5.625

Doña, I., Blanca-Lopez, N., Torres, M. J., Garcia-Campos, J., Garcia-Nunez, I., Gomez, F., et al. (2012). Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J. Investig. Allergol. Clin. Immunol.* 22 (5), 363–371.

Doña, I., Romano, A., and Torres, M. J. (2019). Algorithm for betalactam allergy diagnosis. *Allergy* 74 (9), 1817–1819. doi:10.1111/all.13844

Doña, I., Torres, M. J., Montañez, M. I., and Fernández, T. D. (2017). *In Vitro* diagnostic testing for antibiotic allergy. *Allergy Asthma Immunol. Res.* 9 (4), 288–298. doi:10.4168/aair.2017.9.4.288

Edwards, R. G., Dewdney, J. M., Dobrzanski, R. J., and Lee, D. (1988). Immunogenicity and allergenicity studies on two beta-lactam structures: a clavam, clavulanic acid, and a carbapenem: structure-activity relationships. *Int. Arch. Allergy Appl. Immunol.* 85 (2), 184–189. doi:10.1159/000234500

Fernandez, T. D., Mayorga, C., Salas, M., Barrionuevo, E., Posadas, T., Ariza, A., et al. (2017). Evolution of diagnostic approaches in betalactam hypersensitivity. *Expet Rev. Clin. Pharmacol.* 10 (6), 671–683. doi:10.1080/17512433.2017.1313110

Finn, M. J., Harris, M. A., Hunt, E., and Zomaya, I. I. (1984). Studies on the hydrolysis of clavulanic acid. *J. Chem. Soc., Perkin Transact.* 1, 1345–1349. doi:10.1039/P19840001345

Garzón, B., Gayarre, J., Gharbi, S., Díez-Dacal, B., Sánchez-Gómez, F. J., Timms, J. F., et al. (2010). A biotinylated analog of the anti-proliferative prostaglandin A1 allows assessment of PPAR-independent effects and identification of novel cellular targets for covalent modification. *Chem. Biol. Interact.* 183 (1), 212–221. doi:10.1016/j.cbi.2009.09.019

Garzon, D., Ariza, A., Regazzoni, L., Clerici, R., Altomare, A., Sirtori, F. R., et al. (2014). Mass spectrometric strategies for the identification and characterization of human serum albumin covalently adducted by amoxicillin: *ex vivo* studies. *Chem. Res. Toxicol.* 27 (9), 1566–1574. doi:10.1021/tx500210e

Gharbi, S., Garzón, B., Gayarre, J., Timms, J., and Pérez-Sala, D. (2007). Study of protein targets for covalent modification by the antitumoral and anti-inflammatory prostaglandin PGA1: focus on vimentin. *J. Mass Spectrom.* 42 (11), 1474–1484. doi:10.1002/jms.1291

Gonzalez-Morena, J. M., Montanez, M. I., Aldini, G., Sanchez-Gomez, F. J., and Perez-Sala, D. (2016). Adduct formation and context factors in drug hypersensitivity: insight from proteomic studies. *Curr. Pharmaceut. Des.* 22 (45), 6748–6758. doi:10.2174/1381612822666160927113748

Havelund, J. F., Wojdyla, K., Davies, M. J., Jensen, O. N., Moller, I. M., and Rogowska-Wrzesinska, A. (2017). A biotin enrichment strategy identifies novel carbonylated amino acids in proteins from human plasma. *J. Proteo.* 156, 40–51. doi:10.1016/j.jprot.2016.12.019

Iglesias-Sánchez, J. C., María, D. S., Claramunt, R. M., and Elguero, J. (2010). Molecular recognition studies on naphthyridine derivatives. *Molecules* 15 (3), 1213. doi:10.3390/molecules15031213

Jenkins, R. E., Meng, X., Elliott, V. L., Kitteringham, N. R., Pirmohamed, M., and Park, B. K. (2009). Characterisation of flucloxacillin and 5-hydroxymethyl flucloxacillin haptenated HSA *in vitro* and *in vivo*. *Proteono. Clin. Appl.* 3 (6), 720–729. doi:10.1002/prca.200800222

Koizumi, A., Yamano, K., Schweizer, F., Takeda, T., Kiuchi, F., and Hada, N. (2011). Synthesis of the carbohydrate moiety from the parasite *Echinococcus multilocularis* and their antigenicity against human sera. *Eur. J. Med. Chem.* 46 (5), 1768–1778. doi:10.1016/j.ejmech.2011.02.030

Labenski, M. T., Fisher, A. A., Lo, H.-H., Monks, T. J., and Lau, S. S. (2009). Protein electrophile-binding motifs: lysine-rich proteins are preferential targets of quinones. *Drug Metabol. Dispos.* 37 (6), 1211–1218. doi:10.1124/dmd.108.026211

Li, Y., Chase, A. R., Slivka, P. F., Baggett, C. T., Zhao, T. X., and Yin, H. (2008). Design, synthesis, and evaluation of biotinylated opioid derivatives as novel probes to study opioid pharmacology. *Bioconjugate Chem.* 19 (12), 2585–2589. doi:10.1021/bc8003815

Magi, B., Marzocchi, B., Bini, L., Cellesi, C., Rossolini, A., and Pallini, V. (1995). Two-dimensional electrophoresis of human serum proteins modified by ampicillin during therapeutic treatment. *Electrophoresis* 16 (7), 1190–1192. doi:10.1002/elps.11501601198

Martin, J., Mendez, R., and Alemany, T. (1989). Studies on clavulanic acid. Part 1. Stability of clavulanic acid in aqueous solutions of amines containing hydroxy groups. *J. Chem. Soc., Perkin Transact.* 2 (3), 223–226. doi:10.1039/p29890000223

Martin, S. F., Esser, P. R., Schmucker, S., Dietz, L., Naisbitt, D. J., Park, B. K., et al. (2010). T-cell recognition of chemicals, protein allergens and drugs: towards the development of *in vitro* assays. *Cell. Mol. Life Sci.* 67 (24), 4171–4184. doi:10.1007/s00018-010-0495-3

Martin-Serrano, A. (2018). Allergic reactions to β-lactam antibiotics: chemical approaches for improving in vitro diagnosis. Dissertation. University of Malaga. [RIUMA, UMA Editorial].

Martín-Serrano, A., Barbero, N., Agundez, J. A., Vida, Y., Pérez-Inestrosa, E., and Montañez, M. I. (2016). New advances in the study of IgE drug recognition. *Curr. Pharmaceut. Des.* 22, 1–14. doi:10.2174/1381612822666160921142231

Mayorga, C., Celik, G., Rouzaire, P., Whitaker, P., Bonadonna, P., Cernadas, J. R., et al. (2016). *In vitro* tests for drug hypersensitivity reactions. An ENDA/EAACI drug allergy interest group position paper. *Allergy* 71 (8), 1103–1134. doi:10.1111/all.12886

Mayorga, C., Fernandez, T. D., Montañez, M. I., Moreno, E., and Torres, M. J. (2019). Recent developments and highlights in drug hypersensitivity. *Allergy* 74 (12), 2368–2381. doi:10.1111/all.14061

Meng, X., Earnshaw, C. J., Tailor, A., Jenkins, R. E., Waddington, J. C., Whitaker, P., et al. (2016). Amoxicillin and clavulanate form chemically and immunologically distinct multiple haptenic structures in patients. *Chem. Res. Toxicol.* 29 (10), 1762–1772. doi:10.1021/acs.chemrestox.6b00253

Meng, X., Jenkins, R. E., Berry, N. G., Maggs, J. L., Farrell, J., Lane, C. S., et al. (2011). Direct evidence for the formation of diastereoisomeric benzylpenicilloyl haptens from benzylpenicillin and benzylpenicillenic acid in patients. *J. Pharmacol. Exp. Therapeut.* 338 (3), 841–849. doi:10.1124/jpet.111.183871

Montañez, M. I., Mayorga, C., Bogas, G., Barrionuevo, E., Fernandez-Santamaria, R., Martin-Serrano, A., et al. (2017). Epidemiology, mechanisms, and diagnosis of drug-induced anaphylaxis. *Front. Immunol.* 8, 614. doi:10.3389/fimmu.2017.00614

Nagarajan, J. S. K., Vimal, C. S., George, R., and Dubala, A. (2013). Simultaneous pharmacokinetic assessment of cefadroxil and clavulanic acid in human plasma by LC–MS and its application to bioequivalence studies. *J. Pharm. Anal.* 3 (4), 285–291. doi:10.1016/j.jpha.2013.02.003

O´Donnell, C. A., Foster, A. L., and Coleman, J. W. (1991). Penicillamine and penicillin can generate antigenic determinants on rat peritoneal cells *in vitro*. *Immunology* 72, 571–576.

Pajares, M. A., Zimmerman, T., Sánchez-Gómez, F. J., Ariza, A., Torres, M. J., Blanca, M., et al. (2020). Amoxicillin inactivation by thiol-catalyzed cyclization reduces protein haptenation and antibacterial potency. *Front. Pharmacol.* 11 (189). doi:10.3389/fphar.2020.00189

Perez-Riverol, Y., Csordas, A., Bai, J., Bernal-Llinares, M., Hewapathirana, S., Kundu, D. J., et al. (2019). The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res.* 47 (D1), D442–D450. doi:10.1093/nar/gky1106

Pineda, F., Ariza, A., Mayorga, C., Arribas, F., González-Mendiola, R., Blanca-López, N., et al. (2015). Role of histamine release test for the evaluation of patients with immediate hypersensitivity reactions to clavulanic acid. *Int. Arch. Allergy Immunol.* 168 (4), 233–240. doi:10.1159/000443274

Sánchez-Gómez, F. J., González-Morena, J. M., Vida, Y., Pérez-Inestrosa, E., Blanca, M., Torres, M. J., et al. (2017). Amoxicillin haptenates intracellular proteins that can be transported in exosomes to target cells. *Allergy* 72, 385–396. doi:10.1111/all.12958

Salas, M., Fernandez-Santamaria, R., Mayorga, C., Barrionuevo, E., Ariza, A., Posadas, T., et al. (2018). Use of the basophil activation test may reduce the need for drug provocation in amoxicillin-clavulanic allergy. *J. Allergy Clin. Immunol. Pract.* 6 (3), 1010–1018 e1012. doi:10.1016/j.jaip.2017.08.009

Sechi, S., and Chait, B. T. (1998). Modification of cysteine residues by alkylation. A tool in peptide mapping and protein identification. *Anal. Chem.* 70 (24), 5150–5158. doi:10.1021/ac9806005

Shan, K., Zhenhua, S., Jiawei, L., Wuguo, L., Qiaoling, S., Qing, Z., et al. (2017). Synthesis of biotinylated 2-methoxystrypandrone and identification of JAK2 and IKK as its targets. *Anti Canc. Agents Med. Chem.* 17, 1–6. doi:10.2174/1871520617666171106123226

Soares da Costa, T. P., Tieu, W., Yap, M. Y., Pendini, N. R., Polyak, S. W., Sejer Pedersen, D., et al. (2012). Selective inhibition of biotin protein ligase from *Staphylococcus aureus*. *J. Biol. Chem.* 287 (21), 17823–17832. doi:10.1074/jbc.M112.356576

Tailor, W. J., Meng, X., and Park, B. K. (2016). Mass spectrometric and functional aspects of drug–protein conjugation. *Chem. Res. Toxicol.* 12, 1912–1935. doi:10.1021/acs.chemrestox.6b00147.

Tao, L., Geng, J., Chen, G., Xu, Y., Ladmiral, V., Mantovani, G., et al. (2007). Bioconjugation of biotinylated PAMAM dendrons to avidin. *Chem. Commun.* (33), 3441–3443. doi:10.1039/B709080C

Torres, M. J., Ariza, A., Mayorga, C., Doña, I., Blanca-Lopez, N., Rondon, C., et al. (2010). Clavulanic acid can be the component in amoxicillin-clavulanic acid responsible for immediate hypersensitivity reactions. *J. Allergy Clin. Immunol.* 125 (2), 502–505. doi:10.1016/j.jaci.2009.11.032

Torres, M. J., Montañez, M. I., Ariza, A., Salas, M., Fernandez, T. D., Barbero, N., et al. (2016). The role of IgE recognition in allergic reactions to amoxicillin and clavulanic acid. *Clin. Exp. Allergy* 46 (2), 264–274. doi:10.1111/cea.12689

Watanabe, H., Grimsley, G., Major, G. A., and Dawkins, R. L. (1986). Increased binding of D-penicillamine to monocytes in rheumatoid arthritis. *Clin. Immunol. Immunopathol.* 39 (1), 173–178. doi:10.1016/0090-1229(86)90217-5

Watanabe, H., Kelly, H., and Dawkins, R. L. (1987). Association of HLA DR1 with high D-penicillamine binding to monocytes in females. *Microbiol. Immunol.* 31 (1), 83–88. doi:10.1111/j.1348-0421.1987.tb03070.x

Whitaker, P., Meng, X., Lavergne, S. N., El-Ghaiesh, S., Monshi, M., Earnshaw, C., et al. (2011). Mass spectrometric characterization of circulating and functional antigens derived from piperacillin in patients with cystic fibrosis. *J. Immunol.* 187 (1), 200–211. doi:10.4049/jimmunol.1100647

Yvon, M., Anglade, P., and Wal, J.-M. (1990). Identification of the binding sites of benzyl penicilloyl, the allergenic metabolite of penicillin, on the serum albumin molecule. *FEBS Lett.* 263 (2), 237–240. doi:10.1016/0014-5793(90)81382-x

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Platelet-Adherent Leukocytes Associated With Cutaneous Cross-Reactive Hypersensitivity to Nonsteroidal Anti-Inflammatory Drugs

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most highly consumed drugs worldwide and the main triggers of drug hypersensitivity reactions. The most frequent reaction, named cross-reactive NSAID-hypersensitivity, is due to the pharmacological activity of these drugs by blocking the cyclooxygenase-1 enzyme. Such inhibition leads to cysteinyl-leukotriene synthesis, mainly LTE4, which are responsible for the reaction. Although the complete molecular picture of the underlying mechanisms remains elusive, the participation of platelet-adherent leukocytes ($CD61^+$) and integrins have been described for NSAID-exacerbated respiratory disease (NERD). However, there is a lack of information concerning NSAID-induced urticaria/angioedema (NIUA), by far the most frequent clinical phenotype. Here we have evaluated the potential role of $CD61^+$ leukocytes and integrins (CD18, CD11a, CD11b, and CD11c) in patients with NIUA, and included the other two phenotypes with cutaneous involvement, NSAID-exacerbated cutaneous disease (NECD) and blended reactions (simultaneous skin and airways involvement). A group NSAID-tolerant individuals was also included. During the acute phase of the reaction, the three clinical phenotypes showed increased frequencies of $CD61^+$ neutrophils, eosinophils, and monocytes compared to controls, which correlated with urinary LTE4 levels. However, no correlation was found between these variables at basal state. Furthermore, increased expressions of CD18 and CD11a were found in the three $CD61^+$ leukocytes subsets in NIUA, NECD and blended reactions during the acute phase when compared with $CD61^-$leukocyte subpopulations. During the acute phase, $CD61^+$ neutrophils, eosinophils and monocytes showed increased CD18 and CD11a expression when compared with $CD61^+$ leukocytes at basal state. No differences were found when comparing controls and $CD61^+$ leukocytes at basal state. Our results support the participation of platelet-adherent leukocytes and integrins in cutaneous cross-hypersensitivity to NSAIDs and provide a link between these cells and arachidonic acid metabolism. Our findings also suggest that these reactions do not involve a systemic

imbalance in the frequency of $CD61^+$ cells/integrin expression or levels of LTE4, which represents a substantial difference to NERD. Although further studies are needed, our results shed light on the molecular basis of cutaneous cross-reactive NSAID-hypersensitivity, providing potential targets for therapy through the inhibition of platelet-leukocyte interactions.

Keywords: nonsteroidal anti-inflammatory drugs-hypersensitivity, cysteinyl-leukotrienes, transcellular metabolism, platelet-adherent leukocytes, integrins

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most highly consumed drugs worldwide because of their adequacy for treating pain and inflammatory processes (Fosbol et al., 2008; Conaghan, 2012; Duong et al., 2014). However, they are also responsible for 21–25% of adverse drug reactions, including drug hypersensitivity (Kowalski et al., 2011). The most frequent NSAID-hypersensitivity type belongs to the cross-reactive category, with patients reacting to NSAIDs from different chemical groups in the absence of specific immunological recognition (Dona et al., 2012; Dona et al., 2014; Dona et al., 2020).

Three cross-reactive clinical phenotypes have been recognized in the latest classification of NSAID-hypersensitivity by the European Academy of Allergy and Clinical Immunology; NSAID-exacerbated respiratory disease (NERD), in patients with rhinitis and/or asthma with or without nasal polyposis; NSAID-exacerbated cutaneous disease (NECD), in patients with underlying chronic spontaneous urticaria; and NSAID-induced urticaria/angioedema (NIUA), in otherwise healthy individuals (Kowalski et al., 2013). The latter is the most frequent clinical entity induced by drug hypersensitivity (Dona et al., 2014). Our group has recently described a frequent phenotype, blended reactions, with patients suffering from simultaneous cutaneous and respiratory involvement (Dona et al., 2018).

Concerning the underlying mechanisms, the precipitation of asthma attacks after acetylsalicylic acid (ASA) intake in NSAID-hypersensitive asthmatics was linked to cyclooxygenase (COX)-1 inhibition, and subsequent prostaglandin synthesis blockage. Such inhibition shunts the arachidonic acid (AA) metabolism toward pro-inflammatory cysteinyl-leukotrienes (CysLTs; LTC4, LTD4, and LTE4) biosynthesis, responsible for triggering a reaction in susceptible individuals (Szczeklik et al., 1975; Stevenson et al., 2001; Kowalski et al., 2019).

AA released from cellular membranes by cytosolic phospholipase A2 (cPLA2) is oxidized by 5-lipooxygenase (5-LO) to leukotriene (LT) A4 in inflammatory leukocytes (Reid et al., 1990). In monocytes, mast cells, eosinophils, and basophils LTA4 is conjugated to reduced glutathione by LTC4 synthase (LTC4S) to form LTC4. This is exported by the cell and enzymatically converted into LTD4, and then into the stable metabolite LTE4. In neutrophils, which lack LTC4S activity, LTA4 is hydrolyzed by LTA4 hydrolase (LTA4H) to form LTB4 (Lam et al., 1994).

This pathogenic model was initially proposed for NERD (Szczeklik et al., 1975), and supported by the presence of increased levels of CysLTs after ASA challenge (Szczeklik et al., 1996; Antczak et al., 2002; Swierczynska et al., 2003; Sanak et al., 2004; Gaber et al., 2008), and further extended to NECD (Mastalerz et al., 2004; Setkowicz et al., 2009). Lower baseline levels of PGE2 and increased values of CysLTs have been found in induced sputum from NERD when compared with ASA-tolerant asthmatics and chronic rhinosinusitis with nasal polyposis patients (Mastalerz et al., 2019). Additionally, PGE2 decreased and CysLTs increased after ASA challenge in NERD (Mastalerz et al., 2019), with significant differences compared with their basal values and with ASA-tolerant asthmatics. In both NIUA and NECD, we have recently reported increased LTE4 and 9a,11b-PGF2 levels after ASA challenge, which decreased at the basal state to values similar to those found in controls (Dona et al., 2019).

It is known that eosinophils, basophils, mast cells, and macrophages synthesize LTC4 but not how LTA4 is provided at sufficient quantities to produce the high basal levels of CysLTs described in NERD (Oosaki et al., 1997; Mita et al., 2001). Neutrophils have the highest 5-LO activity and their production of LTA4 exceeds their capacity to form LTB4 via LTA4H. The lack of LTC4S activity in neutrophils seems to be balanced by platelets, which possess abundant LTC4S activity in the absence of 5-LO (Penrose et al., 1995; Sala et al., 1999). In fact, *ex vivo* studies have shown that platelets can convert LTA4 from neutrophils or monocytes into LTC4 by a transcellular pathway that requires P-selectin-dependent interactions between platelets and leukocytes (Bigby and Meslier, 1989; Maclouf et al., 1994; Maugeri et al., 1994). Moreover, a key role of P-selectin-dependent platelets-leukocytes adherence have been described in an asthma mouse model of allergen-induced pulmonary eosinophilia and airway remodeling, which includes a subsequent augmentation of leukocyte integrin function (Pitchford et al., 2005). The underlying platelet-dependent pathway in this model of asthma requires the binding of platelet-associated P-selectin to leukocyte associated PSGL-1 (Pitchford et al., 2005). Such interaction primes leukocytes for adhesion to endothelial cells by up-regulating the expression and avidity of integrins, as it has been demonstrated in eosinophils, neutrophils, and monocytes (da Costa Martins et al., 2006; Xu et al., 2007; Johansson and Mosher, 2011). Concerning NSAID-hypersensitivity, a key role of platelet-adherent leukocytes and integrins (CD18, CD11a, CD11b, and CD11c) have been proposed for NERD (Laidlaw et al., 2012).

As platelet adherence to leukocytes permit the adhesion of both platelets and leukocytes to the endothelium, potentially increasing transcellular metabolism, alterations in platelet-

leukocytes interactions may influence CysLTs production and trigger a cutaneous hypersensitivity reaction to NSAIDs, as reported for NERD (Laidlaw et al., 2012). However, despite its frequency, there is a lack of information concerning the role of platelet-leukocytes interactions in NIUA.

The aim of this work was to evaluate the potential participation of platelet-adherent leukocytes in NIUA, the most common phenotype in drug hypersensitivity. In addition, we have included a group of patients suffering from NECD and other with blended reactions, the other two phenotypes displaying cutaneous involvement.

METHODS

Subjects

We included patients aged 18–60 years with a confirmed diagnosis of NSAID cross-reactive hypersensitivity who attended the Allergy Unit of the Malaga Regional University Hospital (Malaga, Spain) between March 2017 and February 2020.

Only patients reporting at least three episodes of acute urticaria, i.e., NIUA, exacerbation of their underlying chronic spontaneous urticaria, i.e., NECD, or blended reactions (skin and airways involvement) to NSAIDs were considered. Cross-reactive hypersensitivity was confirmed by a drug provocation test (DPT) with ASA.

We also included a control group of age and sex-matched individuals who reported regularly taking NSAIDs, including strong COX-1 inhibitors such as ASA and indomethacin, without developing a clinical reaction, and had no history of chronic spontaneous urticaria, drug hypersensitivity, rhinitis and/or asthma or nasal polyposis. A subset of these controls was also administered ASA.

All participants gave informed consent. The study was approved by the Ethics Committee of Malaga Regional University Hospital and conducted according to the principles of the Declaration of Helsinki.

Oral Drug Provocation Test

ASA DPT was performed in a single-blind manner as reported previously (Dona et al., 2018), giving placebo capsules at different times on the first day. ASA and placebo were given in opaque capsules prepared by the hospital pharmacy service. Other medications were withheld before testing, in accordance with international guidelines (Dona et al., 2018).

For DPT to ASA, two doses were administered orally with an interval of 3 h (50 and 100 mg) on the second day. If negative, two larger doses of ASA (250 and 500 mg) were administered on the third day, with a 3 h interval. The procedure was stopped if cutaneous and/or respiratory symptoms or changes in vital signs (cardiac rhythm alterations, decrease in peak expiratory flow or hypotension) appeared, and symptoms were evaluated and treated (Dona et al., 2018). If no symptoms appeared during these periods, this was followed by a 2 days/8 h course of the therapeutic dose (500 mg) after a gap of 24 h (Dona et al., 2018).

Flow Cytometry Analysis

Peripheral blood was collected in heparinised tubes from both patients and controls, and immediately assayed. For patients, a blood sample was obtained in the absence of clinical symptoms (basal state) and another one during the first half an hour after a positive DPT result (acute phase). For flow cytometry studies, in a subset of controls taking ASA, a blood sample was obtained before ASA intake and another during an hour after intake, whereas for the rest of controls blood samples were obtained at the moment of their enrollment in the study.

One hundred microliters of whole blood were directly incubated with specific antibodies for CD45, CD16, CCR3 (CD193), CD61, CD11a, CD11b, CD11c, P-selectin glycoprotein ligand 1 (PSLG-1; CD162), and/or CD18, or adequate isotype controls (BioLegend) for 20 min. After erythrocyte lysis and washing, at least 20,000 $CD45^+$ cells were obtained in a FACSCanto cytometer (BD Biosciences), and analyzed with the FlowJo software Version 10.6 (TreeStar). According to their side scatter characteristics, $CD45^+$ leukocytes were classified as granulocytes, monocytes, or lymphocytes. In addition to their side scatter properties, neutrophils and eosinophils were further defined from the granulocyte population by the expression of CD16 or CCR3 (**Supplementary Figure S1**). All these populations were assessed for the presence of adherent platelets by the expression of CD61. Finally, in both platelet-adherent and platelet-nonadherent subsets, adhesion markers were determined through their mean fluorescence intensity (MFI).

LTE4 Determination

Patient urine samples were collected at basal state, that is, before challenge, and within the first 3 h after a positive challenge as described (Dona et al., 2019). One urine sample was also obtained from controls regularly taking NSAIDs. LTE4 was determined by high-perfomance liquid chromatography-tandem mass spectrometry, and results were expressed in pg/mg of creatinine (Dona et al., 2019).

Statistical Analysis

Descriptive statistics (frequency, mean, and SD) were used to summarize data. Comparison between groups were performed using the Kruskal-Wallis test followed by Mann-Whitney when necessary, and related samples were evaluated with the Wilcoxon test. Correlation between variables was estimated with the Pearson correlation coefficient. All analyses were performed with GraphPad version 7.04 for Windows (GrapPad Software, La Jolla, CA, United States). All p-values ≤ 0.05 were considered statistically significant.

RESULTS

Demographic and Clinical Data

We finally included a total of 59 patients and 19 controls. Patients were classified as having NIUA (n = 35), NECD (n = 14) or blended reactions (n = 10). A subset of controls was administered ASA (n = 10). The distribution of individuals between groups is

TABLE 1 | Demographic and clinical data for patients and controls.

	Controls (n = 19)	NIUA (n = 35)	NECD (n = 14)	Blended (n = 10)	p-value
Sex (female/male)	10/9	20/15	9/5	6/5	0.926
Age, median (range)	37 (33.2–45)	40.5 (31.5–48)	41 (30.2–50)	53.5 (43.7–60)	0.082
Acetylsalicylic acid cumulative dose	NA	355 ± 312.9	352.9 ± 265.7	232 ± 186.8	0.710
Time interval	NA	54 ± 34.3	75.7 ± 43.9	28 ± 19.2	0.082

NA, not applicable; NIUA, NSAID-induced acute urticaria/angioedema; NECD, NSAID-exacerbated cutaneous disease.

shown in **Table 1**. None of the patients in the blended reactions group suffered from nasal polyposis. No significant differences in sex were found between patients and controls (p = 0.926). In addition, although patients with blended reactions showed a higher median age, no statistically significant differences were found between the groups (p = 0.082) (**Table 1**).

Concerning the cumulative ASA dose that elicited a reaction during this procedure, no significant differences were found between the three groups of patients (p = 0.710), although the lowest dose was found for those with blended reactions (**Table 1**). Finally, no significant differences were found between the three NSAID-hypersensitive groups of patients regarding the time interval elapsed between the last dose administered via DPT and the appearance of clinical symptoms (p = 0.082), although the lowest interval corresponded to blended reactions (**Table 1**).

Platelet-Adherent Leukocytes

We evaluated the presence of platelet-adherent leukocytes in whole blood by flow cytometry using the protein tyrosine phosphatase CD45, which is a pan-leukocyte antigen. Positive CD45 cells were further grouped into different categories based only on their specific light side scatter characteristics (monocytes

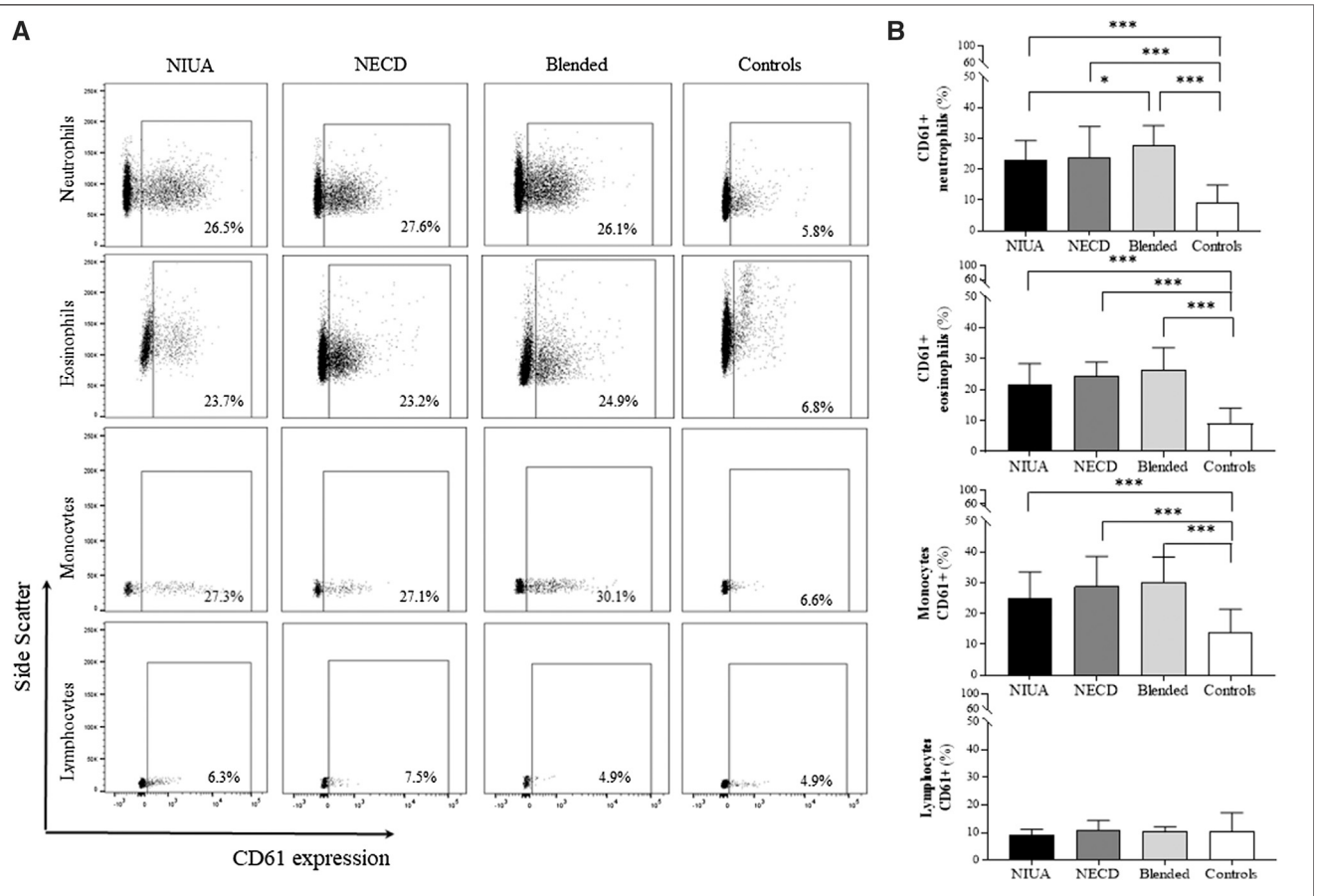

FIGURE 1 | Platelet-adherent leukocytes in peripheral blood during the acute phase in NIUA, NECD or blended reactions, and from controls. **(A)** Representative histograms of platelet-adherent neutrophils [identified as $CD45^+CD16^+$ cells in the granulocyte side scatter (SSC) gate], eosinophils (CD45 + CCR3+ in the granulocyte SSC gate), monocytes ($CD45^+$ in the monocyte SSC gate), and lymphocytes ($CD45^+$ in the lymphocyte SSC gate). The percentage of each cell type with adherent platelets is presented. **(B)** Percentages of platelet-adherent leukocytes ($CD61^+$) in patients during the acute phase and controls. Data are expressed as mean ± SD (*$p \leq 0.05$; **$p \leq 0.001$; ***$p < 0.0001$).

TABLE 2 | Platelet-adherent leukocytes in the different phenotypes of cross-hypersensitivity to NSAIDs during the acute phase and the basal state.

		% CD61$^+$ (mean ± SD)			
		Neutrophils	**Eosinophils**	**Monocytes**	**Lymphocytes**
NIUA	Acute	22.9 ± 6.6	21.8 ± 6.8	24.3 ± 8.3	8.8 ± 2.7
	Basal	8.9 ± 4.3	14.8 ± 8	11.9 ± 6.4	9.7 ± 5.2
	p-value	<0.001	0.001	<0.001	0.489
NECD	Acute	23 ± 9.1	24.2 ± 4.4	27.4 ± 11.1	10.8 ± 2.7
	Basal	9.4 ± 4.2	11.3 ± 5.4	12.3 ± 4.2	10.3 ± 4.1
	p-value	0.003	0.003	0.003	0.807
Blended	Acute	28.4 ± 6.5	27.1 ± 7.3	30.8 ± 8.6	10.3 ± 1.8
	Basal	10.2 ± 5.4	12.2 ± 6.2	12.6 ± 8.2	9.1 ± 6.4
	p-value	0.008	0.011	0.008	0.859

NIUA, NSAID-induced acute urticaria/angioedema; NECD, NSAID-exacerbated cutaneous disease.

and lymphocytes) or also considering the expression of CD16 or CCR3 (neutrophils and eosinophils, respectively) (**Supplementary Figure S1**). Platelet-adhesion was determined through the CD61 antigen, which is an integrin expressed in platelets (Pitchford et al., 2005). Our preliminary results did not find any differences between in the ASA-controls group before and after ASA intake for any of the variables analyzed (data not shown), therefore ASA administration in controls was not considered necessary for subsequent comparisons.

We detected the presence of platelet-adherent leukocytes in both patients and controls (**Figure 1A**). During the acute phase, i.e., after a positive challenge result, CD61$^+$ neutrophils were more frequent in NIUA, NECD, and blended reactions than in controls ($p < 0.0001$ for all comparisons) (**Figure 1B**). Similar results were also found when evaluated CD61$^+$ eosinophils and CD61$^+$ monocytes in all groups of patients respect to control individuals ($p < 0.0001$ for all comparisons) (**Figure 1B**). In addition, CD61$^+$ neutrophils were also increased in blended reactions when compared with NIUA ($p = 0.034$). No statistically significant differences were found between any of the groups regarding CD61$^+$ lymphocytes (**Figure 1B**).

We further analyzed if there were differences in the percentage of platelet-adherent leukocytes between the acute phase and the basal state (**Table 2**). Such percentage significantly decreased when compared these two time points in the three groups of patients in neutrophils (22.9 ± 6.6 vs. 8.9 ± 4.3 in NIUA, $p < 0.001$; 23 ± 9.1 vs. 9.4 ± 4.2 in NECD, $p = 0.003$; and 28.4 ± 6.5 vs. 10.2 ± 5.4 in blended reactions, $p = 0.008$); eosinophils (21.8 ± 6.8 vs. 14.8 ± 8 in NIUA, $p = 0.001$; 24.2 ± 4.4 vs. 11.3 ± 5.4 in NECD, $p = 0.003$; and 27.1 ± 7.3 vs. 12.2 ± 6.2 in blended reactions, $p = 0.011$), and monocytes (24.3 ± 8.3 vs. 11.9 ± 6.4 in NIUA, $p < 0.001$; 27.4 ± 11.1 vs. 12.3 ± 4.2 in NECD, $p = 0.003$; and 30.8 ± 8.6 vs. 12.6 ± 8.2 in blended reactions, $p = 0.008$) (**Table 2**). No statistically significant differences were found between these two states in lymphocytes for any of the groups considered (**Supplementary Figure S2**).

Expression of Integrins

MFI of CD18, CD11a, CD11b, and CD11b for both platelet-adherent and platelet-nonadherent leukocytes (CD61$^+$ and CD61$^-$, respectively) during the acute phase are shown in **Figure 2**. We found a statistically significant increased expression in CD18 and CD11a in neutrophils, eosinophils and monocytes in the CD61$^+$ subset in the three groups of cross-hypersensitive patients during the acute phase. Such increase was also detected in the control group (**Figure 2**). Concerning CD11b, we only found a statistically significant increase in platelet-adherent monocytes. Regarding CD11c, no differences were found for any of these three leukocyte populations in patients and controls when compared the CD61$^+$ and CD61$^-$populations. Finally, there were no differences between the platelet-adherent and platelet-nonadherent lymphocytes for any of the integrins evaluated in patients and controls (**Supplementary Figure S2**).

We also explored potential changes in integrin expression in the CD61$^+$ subset between the acute phase and the basal state in the four cell types considered. MFI of CD18 and CD11a were significantly increased during the acute phase in neutrophils, eosinophils and monocytes for NIUA, NECD and blended reactions, with no differences found in lymphocytes. CD11b and CD11c did not show expression changes when compared the acute phase and the basal state for any of the group of patients included (**Table 3**).

Finally, NIUA, NECD, and blended reactions showed a similar pattern in the expression levels of PSLG-1 in all leukocytes subsets, with no differences between the acute phase and the basal state (**Supplementary Figure S3**).

LTE4 Levels and Platelet-Adherent Leukocytes

We determined urinary LTE4 levels during the acute phase and in the basal state in all patients with NECD or blended reactions as well as in a subset of NIUA patients ($n = 24$) and controls ($n = 17$). Urinary LTE4 levels during the acute phase were increased when compared with controls in the three clinical phenotypes: NIUA ($p = 0.01$), NECD ($p < 0.0001$) and blended reactions ($p = 0.0002$) (**Figure 3A**, top). In addition, these levels were also increased when compared the acute phase with the basal state ($p = 0.045$ for NIUA, $p = 0.0006$ for NECD, and $p = 0.001$ for blended reactions) (**Figure 3A**, bottom). We did not find differences between urinary LTE levels when compared the basal state of the three groups of patients with those from the control group (data not shown).

We also evaluated the potential correlation between urinary LTE levels and platelet-adherent leukocytes during the acute phase. We found a positive correlation between this variable and platelet adherent-neutrophils in NIUA ($r = 0.68$, $p < 0.0001$), NECD ($r = 0.71$, $p = 0.0001$), and blended reactions ($r = 0.65$, $p = 0.005$). Concerning platelet-adherent eosinophils, statistically significant correlation was found only for blended reactions ($r = 0.68$, $p = 0.003$). Moderate correlations were also found with platelet-adherent monocytes in NIUA ($r = 0.52$, $p < 0.001$), NECD ($r = 0.53$, $p = 0.003$), and blended reactions ($r = 0.6$, $p = 0.008$). No correlations were detected regarding urinary LTE levels and platelet-adherent lymphocytes. Besides, no correlations were found between these levels and platelet-adherent leukocytes during the basal state (data not shown).

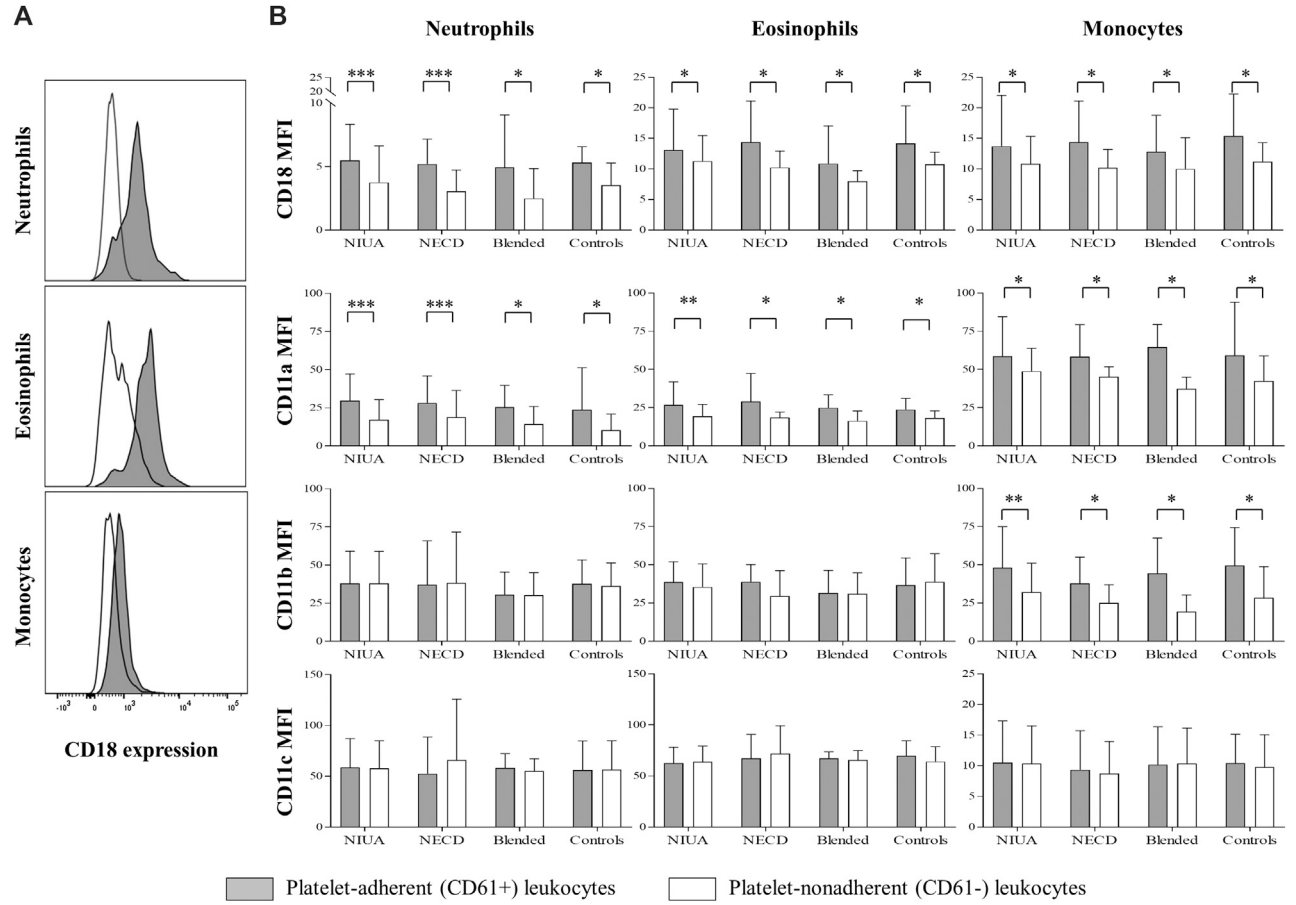

FIGURE 2 | A) Representative histograms of relative CD18 expression by platelet-adherent ($CD61^+$; solid gray) and platelet-nonadherent ($CD61^-$; white line) leukocytes. **(B)** Relative expression of CD18, CD11a, CD11b, and CD11c integrins by platelet-adherent ($CD61^+$) and platelet-nonadherent ($CD61^-$) leukocytes subpopulations during the acute phase in NSAID-induced urticaria/angioedema (NIUA), NSAID-exacerbated cutaneous disease (NECD) or blended reactions, and in controls. Data are expressed as mean ± SD (*$p \leq 0.05$; **$p \leq 0.001$; ***$p < 0.0001$).

TABLE 3 | $CD61^+$ leukocyte expression of the integrins CD18, CD11a, CD11b, and CD11c in the different phenotypes of cross-hypersensitivity to NSAIDs during the acute phase and the basal state.

		NSAID-induced urticaria/angioedema			NSAID-exacerbated cutaneous disease			Blended		
Integrins	**Leukocytes**	**Acute**	**Basal**	***p*-value**	**Acute**	**Basal**	***p*-value**	**Acute**	**Basal**	***p*-value**
CD18	Neutrophils	5.3 ± 2.7	3.7 ± 1.2	0.006	5.3 ± 2.3	3.5 ± 1.1	0.011	4.8 ± 4.1	2.2 ± 0.4	0.021
	Eosinophils	12.8 ± 6.5	9.6 ± 3.6	0.023	12.5 ± 6.8	5.6 ± 2.1	0.003	10.1 ± 4.6	3.5 ± 1.8	0.015
	Monocytes	13.4 ± 8.3	9.5 ± 3.4	0.005	14.7 ± 7.1	3.8 ± 1.2	0.001	12.4 ± 5.2	4.9 ± 1.3	0.018
	Lymphocytes	10.6 ± 4.4	9.8 ± 3.3	0.427	10.3 ± 3.6	8.6 ± 2.9	0.249	11.8 ± 4.2	10.22 ± 4.2	0.310
CD11a	Neutrophils	30 ± 17.7	20.5 ± 3.8	0.018	28.2 ± 17.8	17.9 ± 3.3	0.016	22.1 ± 11.1	14.1 ± 3.9	0.038
	Eosinophils	25.8 ± 14.3	18.4 ± 4.9	0.036	32.3 ± 19.5	16.4 ± 5	0.013	22.7 ± 8.3	11.8 ± 2.9	0.008
	Monocytes	57.7 ± 25.7	42.4 ± 11.4	0.011	60.3 ± 22.5	17.3 ± 5.1	0.001	63.4 ± 14.2	21.2 ± 4.3	0.018
	Lymphocytes	43 ± 20.1	42.5 ± 16.3	0.993	49.6 ± 27.5	35.8 ± 9.6	0.279	45.7 ± 11.8	45.9 ± 9.6	0.176
CD11b	Neutrophils	37.8 ± 21.3	43.4 ± 49.7	0.533	39.5 ± 28	27.1 ± 37.2	0.101	27.7 ± 15.2	31.6 ± 44.1	0.374
	Eosinophils	39.3 ± 12.5	37.5 ± 10.7	0.317	38.1 ± 12.3	29.4 ± 33.4	0.133	33.4 ± 13.3	33.9 ± 39.9	0.441
	Monocytes	49.2 ± 26.4	42.2 ± 14.2	0.235	37.6 ± 17.6	26.5 ± 28.8	0.101	36.8 ± 16.9	34.8 ± 32	0.441
	Lymphocytes	10.7 ± 6.8	11.5 ± 6.1	0.412	9.2 ± 6.5	11.6 ± 8.7	0.463	9.9 ± 6.3	9.5 ± 11.1	0.374
CD11c	Neutrophils	59.7 ± 28.3	49.1 ± 25.6	0.104	52.1 ± 36.4	49.1 ± 16.1	0.861	56.3 ± 16.7	48.8 ± 21.9	0.265
	Eosinophils	62.6 ± 15.9	62.1 ± 14.8	0.837	67.3 ± 23.4	65.1 ± 14.1	0.917	66.2 ± 7.2	63.3 ± 12.8	0.515
	Monocytes	37.7 ± 22.6	31.7 ± 21.4	0.238	41.9 ± 30.6	25.9 ± 13.8	0.196	29.8 ± 10.8	28.9 ± 4.6	0.859
	Lymphocytes	7.9 ± 6	9.9 ± 12.5	0.688	7.3 ± 8.4	5 ± 3.4	0.701	9.6 ± 4.6	9.9 ± 12.6	0.261

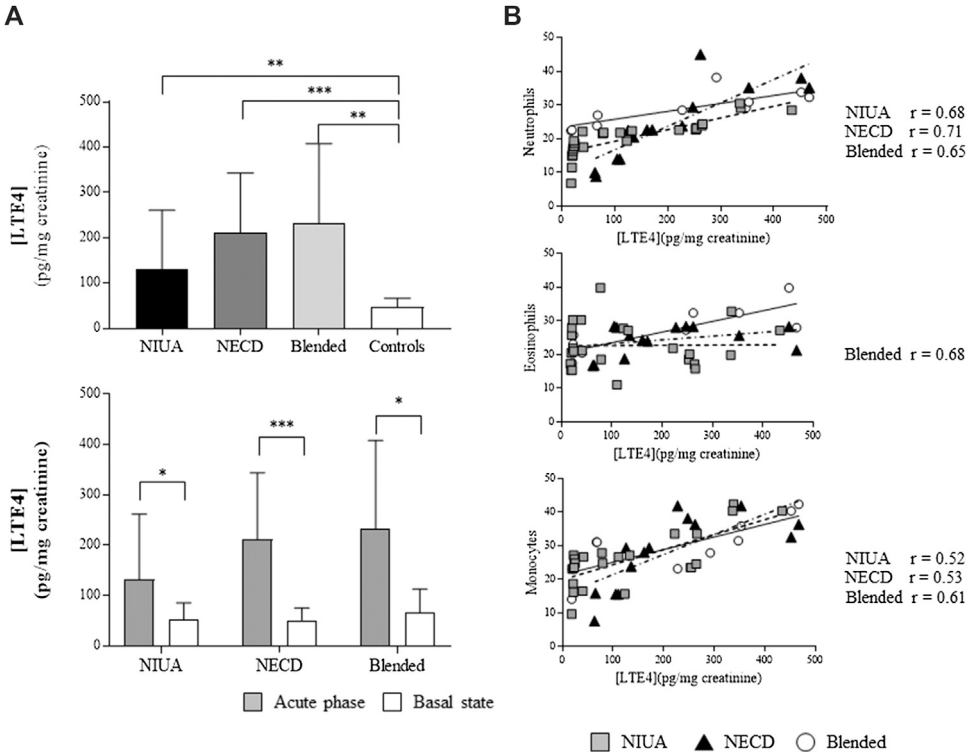

FIGURE 3 | Platelet-adherent leukocytes and urinary LTE4. **(A)** LTE4 levels in patients with NSAID-induced urticaria/angioedema (NIUA), NSAID-exacerbated cutaneous disease (NECD), or blended reactions during the acute phase, and controls (**top**), and comparisons between the acute phase and the basal state in patients (**bottom**). **(B)** LTE4 in the three clinical entities induced by NSAID-hypersensitivity vs. the percentage of platelet-adherent neutrophils (**top**), eosinophils (**middle**), and monocytes (**bottom**) in peripheral blood. Data are expressed as mean ± SD (*$p \leq 0.05$; **$p \leq 0.001$; ***$p < 0.0001$). The value of r represents the effect size, determined by the Pearson correlation coefficient. Gray squares, NIUA; black triangles, NECD; white circles, blended reactions.

DISCUSSION

NSAIDs are widely accepted to be the main cause of drug hypersensitivity reactions, and NIUA the most frequent phenotype. In addition to NIUA, two other clinical entities induced by cross-reactive hypersensitivity to NSAIDs show cutaneous symptoms, i.e., NECD and blended reactions. The underlying mechanism in cross-reactive hypersensitivity, initially proposed for NERD, involves the pharmacological inhibition of COX-1 by NSAIDs, blocking prostaglandins synthesis and shunting the AA metabolism toward CysLTs production (Szczeklik et al., 1975; Szczeklik et al., 1996). This mechanism has been supported by multiple studies (Antczak et al., 2002; Swierczynska et al., 2003; Sanak et al., 2004; Gaber et al., 2008), and further extended to NECD and NIUA (Dona et al., 2020).

Although in more than 70% of patients with blended reactions diagnosis can be achieved by nasal provocation test with Lys-ASA (Dona et al., 2018), here we have included only blended patients with a positive oral DPT to ASA to avoid potential differences in the intensity of the stimulus due to the administration route. Here we have showed for the first time that, after a positive DPT with ASA, patients with blended reactions also showed an increase in urinary LTE4 compared with their basal state, with no differences between basal state levels and those of controls. These results highlight an important difference between blended reactions and NERD for which high baseline LTE4 concentrations have been repeatedly reported (Christie et al., 1991; Kumlin et al., 1992; Oosaki et al., 1997; Higashi et al., 2002; Gaber et al., 2008; Higashi et al., 2010). Such difference may be a reflection of the underlying respiratory disease, as none of patients we labeled as blended presented nasal polyposis in their medical history (Bochenek et al., 2018). Blended reactions represent a heterogeneous group of entities which include cutaneous (urticaria/angioedema) and respiratory symptoms (rhinitis/asthma with or without nasal polyposis); cutaneous symptoms and glottis edema; cutaneous and respiratory symptoms accompanied with glottis edema; and a combination of cutaneous, respiratory and gastrointestinal symptoms (abdominal pain, diarrhea, nausea, vomiting) (Dona et al., 2018). Moreover, we cannot rule out that other phenotypes could be further included in this category (Dona et al., 2020), as described for asthma (Mastalerz et al., 2015) and NERD (Celejewska-Wojcik et al., 2020).

Despite of the participation of COX-1 inhibition and CysLTs in NSAID-hypersensitivity, the molecular basis of these reactions remains elusive. In addition to their inflammatory role in cardiovascular disease (Massberg et al., 2002) and allergen response in bronchial asthma (Moritani et al., 1998), platelet-adherent leukocytes have been shown to play a key role in NERD, as well as integrin subunits expression (Laidlaw et al., 2012). In fact, the frequency of $CD61^+$ neutrophils, eosinophils, and monocytes are increased in NERD compared with controls,

and these frequencies are correlated with systemic LTE4 levels (Laidlaw et al., 2012).

We have also found that $CD61^+$ leukocyte levels increase in the three groups of patients with cutaneous symptoms induced by cross-reactive NSAID-hypersensitivity after a positive DPT to ASA (acute phase) (**Figure 1**). However, we did not observe any increase in the frequency of $CD61^+$ leukocytes in any group of patients at the basal state compared to controls. In addition to dermal edema, the classic histopathological description of urticaria also includes a sparse perivascular infiltrate composed of neutrophils, eosinophils, macrophages and lymphocytes (Zuberbier et al., 2009), although some subgroups of urticaria may exist according to the predominance of neutrophils and lymphocytes (Barzilai et al., 2017). As proposed for NERD (Pitchford et al., 2005), platelets may prime leukocyte adhesion to the endothelium and amplify cutaneous inflammation during a hypersensitivity reaction to NSAIDs as a consequence of a pathogenic change in the homeostasis of this system. In fact, altered platelet function has been described in severe food-associated respiratory allergy (Obeso et al., 2018), and changes in platelet-related genes have been described in some types of chronic spontaneous urticaria (Gimenez-Arnau et al., 2017). Moreover, platelets have been associated with the etiology of a wide range of pathologies behind coagulation disorders (Gianazza et al., 2020), and some of platelet-related compounds may represent potential biomarkers (Duarte et al., 2013; Eguiluz-Gracia et al., 2018; Liao et al., 2018; Liao et al., 2020; Sokolowska et al., 2020).

Interestingly, we have also found that the percentage of $CD61^+$ leukocytes correlated with urinary LTE4 levels (neutrophils and monocytes in the three phenotypes, and eosinophils in blended reactions) during the acute phase (**Figure 3**). Nevertheless, no correlation was found between the frequencies of $CD61^+$ leukocytes and LTE4 levels at the basal state. In addition to the lack of differences in LTE4 levels between blended reactions in the basal state and controls described here, we have previously reported that differences do not existed between LTE4 basal levels in NIUA and NECD and LTE4 levels in controls (Dona et al., 2019). Although COX-1 inhibition and the dysregulation of LTE4 synthesis is thought to be shared by the different clinical entities induced by cross-reactive NSAID-hypersensitivity, our results suggest that a specific pattern exists for NERD and another one for the other three phenotypes as systemic LTE4 production does not exist in NIUA, NECD or blended reactions in our studies.

As adhesion to the endothelium has been reported to require up-regulation of integrins in neutrophils (Xu et al., 2007), eosinophils (Johansson and Mosher, 2011), and monocytes (da Costa Martins et al., 2006), we have also explored their expression in NIUA, NECD and blended reactions. As for NERD, we did not find any differences in PSGL-1 expression in our study. During the acute phase, CD18 and CD11a were significantly increased in $CD61^+$ leukocytes compared to $CD61^-$leukocytes in all patient groups, whereas CD11b was increased only in monocytes (**Figure 2**). We also found that CD18 and CD11a expression were significantly elevated in $CD61^+$ cells when in the acute phase compared to the basal state (**Table 3**). CD18 interacts with the other molecules to form β2 integrins in order to adhere leukocytes to endothelial and epithelial cells. Our results agree in general with those obtained for NERD (Laidlaw et al., 2012); however, we did not find any difference between $CD61^+$ and $CD61^-$leukocytes when evaluating integrin expression at the basal state (**Supplementary Figure S2**). These results agree with our previous findings reporting no increases in the frequencies of $CD61^+$ leukocytes at the basal state compared to control samples, as well as the lack of correlation with urinary LTE levels in such state. Unlike NERD, in the other three phenotypes induced by cross-hypersensitivity to NSAIDs there is no systemic imbalance for AA metabolism or in platelet-leukocytes interaction homeostasis.

In summary, we found that platelet-adherent leukocytes and integrin expression are increased in cutaneous cross-reactive NSAID-hypersensitivity, suggesting that a potential imbalance in the interaction of these leukocytes and endothelial and/or epithelial cells may participate in the underlying pathogenic mechanism, as suggested for NERD (Laidlaw et al., 2012). Primed platelets may bind to leukocytes before their migration to the skin and modify the homeostasis of this process. Platelet priming may be triggered by the inhibition of PGE2 synthesis due to COX-1 blockade as it is known that this prostaglandin usually increases the threshold for platelet activation (van der Meijden and Heemskerk, 2019). Although further studies are needed, which should include affected skin and isolated platelets for functional analyses, our results shed light on the molecular basis of non-immunological, cutaneous hypersensitivity to NSAIDs and open new treatment possibilities through the potential inhibition of platelet-leukocytes interactions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Malaga Regional University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ID and JAC-G designed the study. ID, NP-S, GB-H, MS, and MT recruited, evaluated and diagnosed patients. JL and RM-C revised all clinical data. RJ-E performed experiments, data analysis and drafted the article, and was supervised by JAC-G. CM and MT revised the article. ID and JAC-G are responsible for the final version. All authors revised and approved the submitted version of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.594427/full#supplementary-material

REFERENCES

Antczak, A., Montuschi, P., Kharitonov, S., Gorski, P., and Barnes, P. J. (2002). Increased exhaled cysteinyl-leukotrienes and 8-isoprostane in aspirin-induced asthma. *Am. J. Respir. Crit. Care Med.* 166 (3), 301–306. doi:10.1164/rccm.2101021

Barzilai, A., Sagi, L., Baum, S., Trau, H., Schvimer, M., Barshack, I., et al. (2017). The histopathology of urticaria revisited-clinical pathological study. *Am. J. Dermatopathol.* 39 (10), 753–759. doi:10.1097/DAD.0000000000000786

Bigby, T. D., and Meslier, N. (1989). Transcellular lipoxygenase metabolism between monocytes and platelets. *J. Immunol.* 143 (6), 1948–1954.

Bochenek, G., Stachura, T., Szafraniec, K., Plutecka, H., Sanak, M., Nizankowska-Mogilnicka, E., et al. (2018). Diagnostic accuracy of urinary LTE4 measurement to predict aspirin-exacerbated respiratory disease in patients with asthma. *J. Allergy Clin. Immunol. Pract.* 6 (2), 528–535. doi:10.1016/j.jaip.2017.07.001

Celejewska-Wojcik, N., Wojcik, K., Ignacak-Popiel, M., Cmiel, A., Tyrak, K., Gielicz, A., et al. (2020). Subphenotypes of nonsteroidal antiinflammatory disease-exacerbated respiratory disease identified by latent class analysis. *Allergy* 75 (4), 831–840. doi:10.1111/all.14141

Christie, P. E., Tagari, P., Ford-Hutchinson, A. W., Charlesson, S., Chee, P., Arm, J. P., et al. (1991). Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am. Rev. Respir. Dis.* 143 (5 Pt 1), 1025–1029. doi:10.1164/ajrccm/143.5_Pt_1.1025

Conaghan, P. G. (2012). A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity. *Rheumatol. Int.* 32 (6), 1491–1502. doi:10.1007/s00296-011-2263-6

da Costa Martins, P. A., van Gils, J. M., Mol, A., Hordijk, P. L., and Zwaginga, J. J. (2006). Platelet binding to monocytes increases the adhesive properties of monocytes by up-regulating the expression and functionality of beta1 and beta2 integrins. *J. Leukoc. Biol.* 79 (3), 499–507. doi:10.1189/jlb.0605318

Dona, I., Barrionuevo, E., Blanca-Lopez, N., Torres, M. J., Fernandez, T. D., Mayorga, C., et al. (2014). Trends in hypersensitivity drug reactions: more drugs, more response patterns, more heterogeneity. *J. Investig. Allergol. Clin. Immunol.* 24 (3), 143–153.

Dona, I., Barrionuevo, E., Salas, M., Laguna, J. J., Agundez, J., Garcia-Martin, E., et al. (2018). NSAIDs-hypersensitivity often induces a blended reaction pattern involving multiple organs. *Sci. Rep.* 8 (1), 16710. doi:10.1038/s41598-018-34668-1

Dona, I., Blanca-Lopez, N., Torres, M. J., Garcia-Campos, J., Garcia-Nunez, I., Gomez, F., et al. (2012). Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J. Investig. Allergol. Clin. Immunol.* 22 (5), 363–371.

Dona, I., Jurado-Escobar, R., Perkins, J. R., Ayuso, P., Plaza-Seron, M. C., Perez-Sanchez, N., et al. (2019). Eicosanoid mediator profiles in different phenotypes of nonsteroidal anti-inflammatory drug-induced urticaria. *Allergy* 74 (6), 1135–1144. doi:10.1111/all.13725

Dona, I., Perez-Sanchez, N., Eguiluz-Gracia, I., Munoz-Cano, R., Bartra, J., Torres, M. J., et al. (2020). Progress in understanding hypersensitivity reactions to nonsteroidal anti-inflammatory drugs. *Allergy* 75 (3), 561–575. doi:10.1111/all.14032

Duarte, D., Taveira-Gomes, T., Sokhatska, O., Palmares, C., Costa, R., Negrao, R., et al. (2013). Increased circulating platelet microparticles as a potential biomarker in asthma. *Allergy* 68 (8), 1073–1075. doi:10.1111/all.12190

Duong, M., Salvo, F., Pariente, A., Abouelfath, A., Lassalle, R., Droz, C., et al. (2014). Usage patterns of 'over-the-counter' vs. prescription-strength nonsteroidal anti-inflammatory drugs in france. *Br. J. Clin. Pharmacol.* 77 (5), 887–895. doi:10.1111/bcp.12239

Eguiluz-Gracia, I., Tay, T. R., Hew, M., Escribese, M. M., Barber, D., O'Hehir, R. E., et al. (2018). Recent developments and highlights in biomarkers in allergic diseases and asthma. *Allergy* 73 (12), 2290–2305. doi:10.1111/all.13628

Fosbol, E. L., Gislason, G. H., Jacobsen, S., Abildstrom, S. Z., Hansen, M. L., Schramm, T. K., et al. (2008). The pattern of use of non-steroidal anti-inflammatory drugs (NSAIDs) from 1997 to 2005: a nationwide study on 4.6 million people. *Pharmacoepidemiol. Drug Saf.* 17 (8), 822–833. doi:10.1002/pds.1592

Gaber, F., Daham, K., Higashi, A., Higashi, N., Gulich, A., Delin, I., et al. (2008). Increased levels of cysteinyl-leukotrienes in saliva, induced sputum, urine and blood from patients with aspirin-intolerant asthma. *Thorax* 63 (12), 1076–1082. doi:10.1136/thx.2008.101196

Gianazza, E., Brioschi, M., Baetta, R., Mallia, A., Banfi, C., and Tremoli, E. (2020). Platelets in healthy and disease states: from biomarkers discovery to drug targets identification by proteomics. *Int. J. Mol. Sci.* 21 (12), 4541. doi:10.3390/ijms21124541

Gimenez-Arnau, A., Curto-Barredo, L., Nonell, L., Puigdecanet, E., Yelamos, J., Gimeno, R., et al. (2017). Transcriptome analysis of severely active chronic spontaneous urticaria shows an overall immunological skin involvement. *Allergy* 72 (11), 1778–1790. doi:10.1111/all.13183

Higashi, N., Mita, H., Ono, E., Fukutomi, Y., Yamaguchi, H., Kajiwara, K., et al. (2010). Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers. *J. Allergy Clin. Immunol.* 125 (5), 1084–1091.e6. doi:10.1016/j.jaci.2009.12.977

Higashi, N., Taniguchi, M., Mita, H., Osame, M., and Akiyama, K. (2002). A comparative study of eicosanoid concentrations in sputum and urine in patients with aspirin-intolerant asthma. *Clin. Exp. Allergy* 32 (10), 1484–1490. doi:10.1046/j.1365-2745.2002.01507.x

Johansson, M. W., and Mosher, D. F. (2011). Activation of beta1integrins on blood eosinophils by P-selectin. *Am. J. Respir. Cell Mol. Biol.* 45 (4), 889–897. doi:10.1165/rcmb.2010-0402OC

Kowalski, M. L., Agache, I., Bavbek, S., Bakirtas, A., Blanca, M., Bochenek, G., et al. (2019). Diagnosis and management of NSAID-exacerbated respiratory disease (N-ERD)-a EAACI position paper. *Allergy* 74 (1), 28–39. doi:10.1111/all.13599

Kowalski, M. L., Asero, R., Bavbek, S., Blanca, M., Blanca-Lopez, N., Bochenek, G., et al. (2013). Classification and practical approach to the diagnosis and management of hypersensitivity to nonsteroidal anti-inflammatory drugs. *Allergy* 68 (10), 1219–1232. doi:10.1111/all.12260

Kowalski, M. L., Makowska, J. S., Blanca, M., Bavbek, S., Bochenek, G., Bousquet, J., et al. (2011). Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) - classification, diagnosis and management: review of the EAACI/ENDA(#) and GA2LEN/HANNA*. *Allergy* 66 (7), 818–829. doi:10.1111/j.1398-9995.2011.02557.x

Kumlin, M., Dahlen, B., Bjorck, T., Zetterstrom, O., Granstrom, E., and Dahlen, S. E. (1992). Urinary excretion of leukotriene E4 and 11-dehydro-thromboxane B2 in response to bronchial provocations with allergen, aspirin, leukotriene D4, and histamine in asthmatics. *Am. Rev. Respir. Dis.* 146 (1), 96–103. doi:10.1164/ajrccm/146.1.96

Laidlaw, T. M., Kidder, M. S., Bhattacharyya, N., Xing, W., Shen, S., Milne, G. L., et al. (2012). Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. *Blood* 119 (16), 3790–3798. doi:10.1182/blood-2011-10-384826

Lam, B. K., Penrose, J. F., Freeman, G. J., and Austen, K. F. (1994). Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. *Proc. Natl. Acad. Sci. U.S.A.* 91 (16), 7663–7667. doi:10.1073/pnas.91.16.7663

Liao, B., Liu, J. X., Guo, C. L., Li, Z. Y., Pan, L., and Liu, Z. (2020). A panel of clinical and biological markers predict difficult-to-treat chronic rhinosinusitis. *Allergy* 75 (4), 946–949. doi:10.1111/all.14049

Liao, B., Liu, J. X., Li, Z. Y., Zhen, Z., Cao, P. P., Yao, Y., et al. (2018). Multidimensional endotypes of chronic rhinosinusitis and their association with treatment outcomes. *Allergy* 73 (7), 1459–1469. doi:10.1111/all.13411

Maclouf, J., Antoine, C., Henson, P. M., and Murphy, R. C. (1994). Leukotriene C4 formation by transcellular biosynthesis. *Ann. N. Y. Acad. Sci.* 714, 143–150. doi:10.1111/j.1749-6632.1994.tb12038.x

Massberg, S., Brand, K., Gruner, S., Page, S., Muller, E., Muller, I., et al. (2002). A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J. Exp. Med.* 196 (7), 887–896. doi:10.1084/jem.20012044

Mastalerz, L., Celejewska-Wojcik, N., Wojcik, K., Gielicz, A., Cmiel, A., Ignacak, M., et al. (2015). Induced sputum supernatant bioactive lipid mediators can identify subtypes of asthma. *Clin. Exp. Allergy* 45 (12), 1779–1789. doi:10.1111/cea.12654

Mastalerz, L., Setkowicz, M., Sanak, M., and Szczeklik, A. (2004). Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. *J. Allergy Clin. Immunol.* 113 (4), 771–775. doi:10.1016/j.jaci.2003.12.323

Mastalerz, L., Tyrak, K. E., Ignacak, M., Konduracka, E., Mejza, F., Cmiel, A., et al. (2019). Prostaglandin E2 decrease in induced sputum of hypersensitive asthmatics during oral challenge with aspirin. *Allergy* 74 (5), 922–932. doi:10.1111/all.13671

Maugeri, N., Evangelista, V., Celardo, A., Dell'Elba, G., Martelli, N., Piccardoni, P., et al. (1994). https://pubmed.ncbi.nlm.nih.gov/7531878/. *Thromb. Haemost.* 72 (3), 450–456. doi:10.1055/s-0038-1648888

Mita, H., Endoh, S., Kudoh, M., Kawagishi, Y., Kobayashi, M., Taniguchi, M., et al. (2001). Possible involvement of mast-cell activation in aspirin provocation of aspirin-induced asthma. *Allergy* 56 (11), 1061–1067. doi:10.1111/j.1398-9995.2001.00913.x

Moritani, C., Ishioka, S., Haruta, Y., Kambe, M., and Yamakido, M. (1998). Activation of platelets in bronchial asthma. *Chest* 113 (2), 452–458. doi:10.1378/chest.113.2.452

Obeso, D., Mera-Berriatua, L., Rodriguez-Coira, J., Rosace, D., Fernandez, P., Martin-Antoniano, I. A., et al. (2018). Multi-omics analysis points to altered platelet functions in severe food-associated respiratory allergy. *Allergy* 73 (11), 2137–2149. doi:10.1111/all.13563

Oosaki, R., Mizushima, Y., Mita, H., Shida, T., Akiyama, K., and Kobayashi, M. (1997). Urinary leukotriene E4 and 11-dehydrothromboxane B2 in patients with aspirin-sensitive asthma. *Allergy* 52 (4), 470–473. doi:10.1111/j.1398-9995.1997.tb01032.x

Penrose, J. F., Spector, J., Lam, B. K., Friend, D. S., Xu, K., Jack, R. M., et al. (1995). Purification of human lung leukotriene C4 synthase and preparation of a polyclonal antibody. *Am. J. Respir. Crit. Care Med.* 152 (1), 283–289. doi:10.1164/ajrccm.152.1.7599836

Pitchford, S. C., Momi, S., Giannini, S., Casali, L., Spina, D., Page, C. P., et al. (2005). Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood* 105 (5), 2074–2081. doi:10.1182/blood-2004-06-2282

Reid, G. K., Kargman, S., Vickers, P. J., Mancini, J. A., Leveille, C., Ethier, D., et al. (1990). Correlation between expression of 5-lipoxygenase-activating protein, 5-lipoxygenase, and cellular leukotriene synthesis. *J. Biol. Chem.* 265 (32), 19818–19823.

Sala, A., Zarini, S., Folco, G., Murphy, R. C., and Henson, P. M. (1999). Differential metabolism of exogenous and endogenous arachidonic acid in human neutrophils. *J. Biol. Chem.* 274 (40), 28264–28269. doi:10.1074/jbc.274.40.28264

Sanak, M., Kielbasa, B., Bochenek, G., and Szczeklik, A. (2004). Exhaled eicosanoids following oral aspirin challenge in asthmatic patients. *Clin. Exp. Allergy* 34 (12), 1899–1904. doi:10.1111/j.1365-2222.2004.02123.x

Setkowicz, M., Mastalerz, L., Podolec-Rubis, M., Sanak, M., and Szczeklik, A. (2009). Clinical course and urinary eicosanoids in patients with aspirin-induced urticaria followed up for 4 years. *J. Allergy Clin. Immunol.* 123 (1), 174–178. doi:10.1016/j.jaci.2008.09.005

Sokolowska, M., Rovati, G. E., Diamant, Z., Untersmayr, E., Schwarze, J., Lukasik, Z., et al. (2020). Current perspective on eicosanoids in asthma and allergic diseases - EAACI Task Force consensus report, part I. *Allergy* [Epub ahead of print]. doi:10.1111/all.14295

Stevenson, D. D., Sanchez-Borges, M., and Szczeklik, A. (2001). Classification of allergic and pseudoallergic reactions to drugs that inhibit cyclooxygenase enzymes. *Ann. Allergy Asthma Immunol.* 87 (3), 177–180. doi:10.1016/S1081-1206(10)62221-1

Swierczynska, M., Nizankowska-Mogilnicka, E., Zarychta, J., Gielicz, A., and Szczeklik, A. (2003). Nasal versus bronchial and nasal response to oral aspirin challenge: clinical and biochemical differences between patients with aspirin-induced asthma/rhinitis. *J. Allergy Clin. Immunol.* 112 (5), 995–1001. doi:10.1016/s0091-6749(03)02015-3

Szczeklik, A., Gryglewski, R. J., and Czerniawska-Mysik, G. (1975). Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. *Br. Med. J.* 1 (5949), 67–69. doi:10.1136/bmj.1.5949.67

Szczeklik, A., Sladek, K., Dworski, R., Nizankowska, E., Soja, J., Sheller, J., et al. (1996). Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am. J. Respir. Crit. Care Med.* 154 (6 Pt 1), 1608–1614. doi:10.1164/ajrccm.154.6.8970343

van der Meijden, P. E. J., and Heemskerk, J. W. M. (2019). Platelet biology and functions: new concepts and clinical perspectives. *Nat. Rev. Cardiol.* 16 (3), 166–179. doi:10.1038/s41569-018-0110-0

Xu, T., Zhang, L., Geng, Z. H., Wang, H. B., Wang, J. T., Chen, M., et al. (2007). P-selectin crosslinks PSGL-1 and enhances neutrophil adhesion to fibrinogen and ICAM-1 in a Src kinase-dependent,but GPCR-independent mechanism. *Cell Adh. Migr.* 1 (3), 115–123. doi:10.4161/cam.1.3.4984

Zuberbier, T., Asero, R., Bindslev-Jensen, C., Walter Canonica, G., Church, M. K., Gimenez-Arnau, A., et al. (2009). EAACI/GA(2)LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy* 64 (10), 1417–1426. doi:10.1111/j.1398-9995.2009.02179.x

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MINI REVIEW
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Analysis of the Costs Associated With the Elective Evaluation of Patients Labelled as Allergic to Beta-Lactams or Nonsteroidal Antiinflamatory Agents

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Introduction: Being labelled as allergic to different drugs results in patients receiving other treatments, which are more toxic, less effective and more expensive. We aimed to analyze different studies of the costs of drug hypersensitivity assessment.

Methods: A bibliographic search on studies regarding this issue was performed, including the available scientific evidence up to June 2020. We searched three databases with terms related to costs and allergy testing in drug hypersensitivity reactions.

Results: Our search revealed 1,430 publications, of which 20 met the inclusion criteria. In the manuscript, prospective studies evaluating the costs of the evaluation of patients with suspected allergy to beta-lactams or non-steroidal anti-inflammatory drugs are analyzed. Also, comment is made on the costs associated with incorrect labeling as non-steroidal anti-inflammatory drug or penicillin hypersensitivity.

Conclusions: Taking all costs into account, the study of drug hypersensitivity is not expensive, particularly considering the economic and clinical consequences of labeling a patient with hypersensitivity to drugs.

Keywords: beta-lactam, cost, delabelling, drug hypersensitivity, non-steroidal anti-inflammatory drug, penicillin, drug allergy

INTRODUCTION

Drug allergy can affect 7–10% of the general population and constitutes a Public Health issue (Park et al., 2011; Macy and Ngor, 2013; Sagar and Katelaris, 2013). Nevertheless, most patients that claim to have drug hypersensitivity are not really allergic after an allergological study (Park et al., 2011; Macy and Ngor, 2013; Sagar and Katelaris, 2013).

Beta-lactams are one of the drugs most usually implicated in adverse immunological reactions (Bedolla-Barajas et al., 2018). An unverified penicillin allergy results in patients receiving broader-spectrum antibiotics that are frequently less clinically and economically effective. In addition, the unnecessary use of alternative antibiotics leads to more adverse reactions, treatment failures, and healthcare infections (MacLaughlin et al., 2000; Sade et al., 2003; Shehab et al., 2008; Picard et al., 2013; Macy and Contreras, 2014; McDanel et al., 2015; Barlam et al., 2016).

Regarding children, about 10% of parents state that their children are allergic to drugs, especially to beta-lactams, probably related to high prescription rates (Atanaskovic-Markovic et al., 2019; Calamelli et al., 2019; Roduit, 2019). Nevertheless, only a small proportion of them are true drug allergic reactions (Macy and Ngor, 2013). In this sense, different studies concluded that fewer than 10% of patients claiming to be allergic really are, so most children are mislabeled as drug allergic (Seitz et al., 2011; Abrams et al., 2016; Mill et al., 2016).

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequent drugs causing hypersensitivity reactions with a prevalence of 1–3%.This is higher in patients with chronic rhinosinusitis, nasal polyposis, asthma, or chronic urticaria, rising to 30% (Wöhrl, 2018). The importance of its evaluation is because the necessity of NSAIDs for analgesic/anti-inflammatory or antiplatelet therapy (Modena et al., 2017).

Nevertheless, the costs associated with the evaluation of patients claiming to be allergic to drugs should be considered. In this review, the costs of evaluating drug hypersensitivity in beta-lactam antibiotics and NSAIDs are analyzed.

Thus, the review aims were to analyze different studies of the costs of drug hypersensitivity assessment, particularly prospective studies of the evaluation of beta-lactam allergy in adults and children patients, NSAID hypersensitivity in adults, and other studies revealing the clinic and economic consequences and the importance of delabelling.

METHODS

Literature Search

A bibliographic search on studies regarding this issue was performed including the available scientific evidence up to June 2020. The primary sources for the search included PubMed, SCOPUS, and EMBASE.

The search terms for PubMed included ("costs and cost analysis" [MeSH Terms] OR "cost-benefit analysis" [MeSH Terms]) AND "allergy testing" [Other Term]) OR "allergy tests" [Other Term]) OR "allergy evaluation" [Other Term]) OR "delabelling" [Other Term]) AND "penicillin" [Other Term]) OR "beta-lactam" [Other Term]) OR "nsaid" [Other Term]. Similar terms and methods were used for the other databases.

Inclusion and Exclusion Criteria

Only original articles or systematic reviews were selected.
Non-systematic reviews, comments, and other types of articles were not selected.
Only articles in English were considered.
Only articles explicitly dealing with hypersensitivity reactions were included.
No age restriction was considered.
At least two blinded researchers independently reviewed titles and abstracts from the initial search, and eligibility criteria determined their inclusion or exclusion.

Prospective studies about the costs of evaluating patients labeled as allergic to different drug such as beta-lactam in adults (Blumenthal et al., 2018; Sobrino-García et al., 2019) and children (Sobrino et al., 2020) or NSAIDs (Sobrino-García et al., 2020) drive this field forward prioritized.

RESULTS

Firstly, database searches showed 1,430 results. After removing duplicates and articles without abstracts, 1,233 abstracts remained for screening. Articles not peer-reviewed, conference proceedings, editorials or commentaries to review articles were excluded after abstracts were evaluated. Other articles were excluded because they did not explicitly analyze costs in drug allergy. After applying inclusion and exclusion criteria, 20 articles were included in the review (**Figure 1**).

Assessment of Costs

We only found three prospective articles evaluating patients with suspected beta-lactam allergy, two of them performed in adults, and one in children. There was also a prospective study evaluating the costs of the evaluation of hypersensitivity to NSAIDs in adults. Direct and indirect costs as explained by Soto-Álvarez (2020) were systematically recorded in only a few studies(Blumenthal et al., 2018; Sobrino et al. 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020). Direct health costs were calculated by Blumenthal et al. (2018), who analyzed personnel costs, consumables and space costs. Moreover, other studies (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020) considered the number of consultations and all the diagnostic tests performed. Direct non-health costs (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020) were calculated taking into account the number of consultations and the distance from their homes to the Allergy service. Finally, indirect costs (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020) were calculated considering the absenteeism of patients.

In the US, Blumenthal et al. (2018) prospectively calculated the cost of evaluating beta-lactam allergy in outpatients and found a cost of $220 (€209.37) for a case that included penicillin skin tests and a drug provocation test (DPT) in one step with amoxicillin. In cases where more resources were used, assessment of penicillin allergy costs were around $540 (€482.45). In Europe (Spain, Sobrino-García et al., 2019) performed a prospective, one-year-long study in 296 patients (46 positives), obtaining an average cost of €187.49, with a maximum cost of €789.96.

In children, the only prospective study (Sobrino et al. 2020), performed in Europe and one year lasting, evaluated 40 children with suspected beta-lactam allergy (with only three children

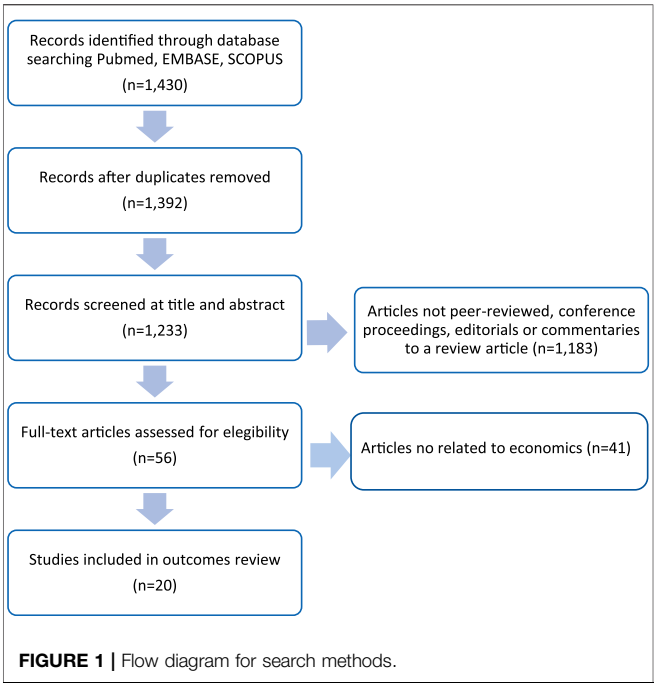

FIGURE 1 | Flow diagram for search methods.

finally diagnosed with beta-lactam allergy). The mean cost was €275.27 with a maximum cost of €746.78.

Concerning NSAIDs the only prospective elective study (Sobrino-García et al., 2020) included 233 patients (43 positives at the end of the study), with an average cost of €185.30 (maximum €1,055.96).

DISCUSSION

Prospective studies concerning the costs of the elective evaluation of drug allergy are scarce. Thus, two studies evaluated the costs of the elective evaluation of adult patients labeled with beta-lactam allergy (Blumenthal et al., 2018; Sobrino-García et al., 2019) Another study evaluated such costs in children (Sobrino et al. 2020) and yet another analyzed the costs of the elective evaluation of adults labeled as hypersensitive to NSAIDs (Sobrino-García et al., 2020). One of them was performed in the United States (Blumenthal et al., 2018) and the other three in Europe (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020).

Beta-Lactam Studies in Adults

European studies (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020) were performed following the ENDA/EAACI protocols (Torres et al., 2003; Kowalski et al., 2011; Moreno et al., 2016). According theses protocols in beta-lactam allergy evaluation included the anamnesis followed by STs. When negative, patients underwent DPT.

In one of the beta-lactam allergy studies (Sobrino-García et al., 2019) performed, additional visits for challenging with alternative beta-lactams were done. All consultations were prospectively collected. In the prospective beta-lactam study performed in the United States, Blumenthal et al. (2018) (United States), who studied 30 outpatients with suspected beta-lactam allergy, the mean cost was $220 (€209.37) for the base case (skin tests with beta-lactam and DPT with amoxicillin). In cases where more resources were used and testing was expanded, assessment of penicillin allergy would only cost about $540 (€482.45).

The results of both studies are not entirely comparable for several reasons (World Bank, 2019): i) per capita income of the United States and Spain are very different (€53,341 and €25,900, respectively, in 2018), ii) there are apparent differences between both countries regarding their National Health System (mainly private in the United States and predominantly public in Spain); iii) the differences between protocols, and iv) the exchange rate between the euro and the dollar, which means that amounts obtained require a conversion to be able to compare them (1 EUR = 1.1250 USD). Thus, another study (Sousa-Pinto et al., 2020a) designed an online questionnaire to study the practice and cost perceptions of diagnostic tests used in beta-lactam allergy evaluation. They concluded that there is a great deal of diversity in performing beta-lactam allergy studies and reported cost estimates, with median values ranging from €50 for SPT to €190 for DPT and providing information on the need for context-based cost assessments and when these studies can be economically effective. Sobrino-García et al. studied different factors that influenced the costs of their studies (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020). In the study of beta-lactams in adults (Sobrino-García et al., 2019), the costs in patients with immediate reactions (€152.64 ± 106.73) and delayed reactions (€220.48 ± 171.79), were significantly different ($p < 0.001$). This fact was mainly related to the mean number of visits (1.95 ± 0.90 and 2.76 ± 1.30, respectively, $p < 0.001$). For patients who worked and did not work for hire, wages were also different ($p < 0.001$), with a mean loss of income of €364.12 ± 156.38 and €121.28 ± 68.18, respectively.

Moreover, Sousa-Pinto et al. (2020b) concluded in a recent study that penicillin allergy evaluation was cost-saving in twenty-four base case decision models. For models evaluating the performance of skin tests and DPT, allergy testing involved an average savings of $657 for inpatients (USA: $1,444; Europe: $489) and $2,746 for outpatients (US: $256; Europe: $6,045).

The main advantages of conducting an elective study of drug allergy are delabelling false allergic patients, who are more numerous than true allergy sufferers, and the correct diagnosis of patients with true hypersensitivity. In addition, there are other advantages, apart from economics.

Regarding beta-lactam antibiotics, patients mislabeled as allergic to beta-lactams receive alternative drugs which are generally less effective and less efficient. In this sense, Picard et al. (2013) observed additional costs over a year in 1,738 patients with suspected allergy to beta-lactams because of the use of other antibiotics for more than $15,000. Sade et al. (2003) identified a 38% increase in costs in terms of antimicrobial prescribed at discharge. Similarly, (MacLaughlin et al., 2000) showed that the mean cost of antibiotic treatments prescribed to patients labeled as allergic to beta-lactams ($26.81) was significantly higher compared to patients without allergy to these antibiotics ($16.28). In Spain, (Sastre et al., 2012) evaluated 505 hospitalized patients with a history of drug hypersensitivity and concluded that changes in treatments increased the mean

TABLE 1 | Studies concerning the costs of the evaluation of drug allergy.

Study	Costs
Beta-Lactams (adults)	
Blumenthal et al. (2018)	$220 for the base case and $540 with more resources needed
Sobrino-García et al. (2019)	Mean cost of the elective evaluation of patients with suspected allergy to NSAIDs €187.49 ± 148.14, with a maximum of €789.96
Picard et al. (2013)	Additional costs over a year in 1,738 patients for more than $15,000
Sade et al. (2003)	38% increase in costs in terms of antimicrobial prescribed at discharge
MacLaughlin et al. (2000)	$26.81labeled as allergic vs. $16.28
Sousa-Pinto et al. (2020a)	Mean savings of $657 for inpatients and $2,746 for outpatients
Sousa-Pinto et al. (2020b)	Wide diversity in penicillin allergy testing practice (median values ranging from €50 for SPT to €190 DPT)
Sastre et al. (2012)	€273.47 per patient with a history of drug allergy per day of hospitalization
Rimawi et al. (2013)	Removing the label resulted in an annual savings of $82,000
Bermingham et al. (2020)	The cost of alternative antibiotics in patients with penicillin allergy labels was 2.61 times higher
Beta-Lactams (children)	
Sousa-Pinto et al. (2018)	Hospitalization costs were higher (2,071 vs. €1,798) in children with this label
Au et al. (2019)	Mean cost per patient was $8,171, compared to $6,278 in patients without this label
Sobrino-García et al. (2019)	Mean cost of the elective evaluation of patients with suspected allergy to BL: €275.27 ± €164.70, with a maximum of €746.78
NSAIDs (adults)	
Cubero et al. (2017)	Annual increase in the cost of using alternative drugs such as clopidogrel or trifusal instead of AAS: 218.13 vs. €17.64 and 134.56 vs. €17.64, respectively
Sobrino-García et al. (2020)	Mean cost of the elective evaluation of patients with suspected allergy to NSAIDs: €185.30 ± 146.77, with a maximum cost of €1,055.96

cost of treatment four-fold (€273.47 per patient per day of hospitalization). Macy and Ngor (2013)) concluded that patients labeled as allergic to beta-lactams required 9.9% more days of hospitalization (0.59 days: 95% CI, 0.47–0.71) compared to controls. In addition, Chen et al. (2017) evaluated 252 patients labeled as allergic to penicillin who were hospitalized for other reasons and concluded that, after the allergy evaluation, a penicillin allergy label was removed in 228 subjects (90.5%). Another cross-sectional case-control study of hospitalized patients also concluded that antibiotic costs doubled in patients labeled with penicillin allergy (Borch et al., 2006). Moreover a penicillin allergy (Rimawi et al., 2013). In the US, an antimicrobial administration program at a tertiary hospital observed that evaluating beta-lactam allergy, removing the label in 145 of the 146 cases, resulted in an annual savings of $82,000 (Rimawi et al., 2013). Mattingly et al. (2018) observed that patients with penicillin allergy had direct drug costs during inpatient admission ranging from no difference to an additional $609 per patient respect patients without penicillin allergy. Moreover, outpatient prescription costs were estimated from $14 to $193 per patient higher for penicillin allergic patients. Moreover, in the case of allergy to beta-lactams, patients with selective hypersensitivity have different degrees of cross-reactivity, being able in these cases to check tolerance to other beta-lactam antibiotics that can be used as an alternative in certain situations. In fact, Sobrino-García et al. (2019), showed that of patients with selective hypersensitivity to amoxicillin who underwent a DPT with cephalosporins and carbapenems (82.76%), all tolerated alternative beta-lactams. Therefore, most patients could benefit from treatment with other beta-lactams. In addition to the economic consequences, treatment with non-beta-lactam antibiotics has multiple clinical implications. These include a higher incidence of infection by *Clostridium difficile*, *Vancomycin*-resistant *Enterococcus*, or methicillin-resistant *Staphylococcus aureus*, which are associated with greater number of days of hospitalization (Macy and Contreras, 2014) and readmissions. Furthermore, alternative antibiotics are often less effective than beta-lactams (vancomycin treatment for methicillin-sensitive *S. aureus* bacteremia is associated with more significant frequency of worsening of the disease (Barlam et al., 2016; McDanel et al., 2015) and more frequently leads to adverse reactions, which may contribute to the readmission of patients (Shehab et al., 2008). Suspicion of a penicillin allergy already has a direct impact on the choice of alternative antibiotics and entails the use of broader-spectrum and less effective antimicrobials, often associated with antimicrobial resistance (Shehab et al., 2008; Torres et al., 2019). In this sense, Bermingham et al. (2020) investigated the impact of being labeled as allergic to penicillin in a cohort of patients with sepsis. Their results showed that these patients frequently receive second-line antibiotics. Furthermore, they observed that the cost of alternative antibiotics in patients with suspected penicillin

allergy was 2.61 times higher. Another aspect that influences the lower efficiency of antibiotic treatment in these patients is the fact that there is a delay in administering the first dose of the antibiotic (Conway et al., 2017). For its part, The United Kingdom Sepsis Trust estimates that there are about 250,000 episodes of sepsis in the United Kingdom per year. According to published data, around 20% (Mirakian et al., 2015) are associated with penicillin allergy labels that could be ruled out in 95% of cases (Shenoy et al., 2019).

Delabelling penicillin allergy is associated with greater use of penicillins and other beta-lactams. A systematic review and meta-analysis (Sacco et al., 2017) of inpatient penicillin allergy testing that included 24 studies demonstrated increase penicillin utilization (9.9–49%) after skin testing. Penicillin allergy testing in outpatient settings is also associated with significantly less health care utilization and greater use of penicillins and cephalosporins (Macy and Shu, 2017). There are regional differences in approaches for delabelling patients allergic to beta-lactams. A precise diagnosis is mainly based on skin tests and DPT tools that are time-consuming and are not without risks Torres et al., 2019. In the US, in recent years, there has been a growing interest in the development of risk stratification using a computerized clinical decision support system or a multidisciplinary antibiotic stewardship program with or without evaluation by an allergy specialist (Torres et al., 2019). It has been suggested performing DPT without previous skin tests in patients with low risk (Abrams et al., 2019;. In this sense, Li et al., 2019 concluded that penicillin allergy evaluation performing a DPT without previous STs might be feasible for adult patients with a history of type B reactions to penicillins without a history of anaphylaxis within the last ten years or a type 2, 3, or 4 (severe) hypersensitivity reaction. There is a consensus about this practice in children (Abrams et al., 2019; Stone et al., 2020).

Beta-Lactam Studies in Children

In the case of children, it is necessary to consider that the percentage of positive results for beta-lactam antibiotics after the allergological evaluation is less than 10% (prevalence of 6% (Ibáñez and Olaguibel, 2015)), so delabelling acquires more significant importance in this population group. In this sense, Abrams et al. (2016) proposed a diagnostic protocol for children who are labeled as allergic to beta-lactams and insisted on the importance of correctly labeling the allergy to beta-lactams in the pediatric population, given its low prevalence (Seitz et al., 2011; Abrams et al., 2016; Mill et al., 2016; Roduit, 2019). Sousa-Pinto et al. (2018) identified 1,718 hospitalizations corresponding to children with suspected allergy to beta-lactams. These children had more extended days of hospitalization and a higher comorbidity rate. Hospitalization costs were also higher (€2,071 vs. €1,798), nevertheless, in this case, there was not a significant difference. Also, Au et al. (2019) estimated the costs of antibiotics used throughout their lives by patients labeled as allergic to penicillin before ten years of age compared to those who were not allergic to penicillin. Thus, they found that in the first group, the mean cost per patient was $8,171, compared to $6,278 in patients without this label.

In the study of children with suspected beta-lactam allergy (Sobrino et al.), indirect costs were higher than those of adult studies (Sobrino-García et al., 2019; Sobrino-García et al., 2020) due to the significant number of legal guardians who went to the Allergy Service and were employed, reaching 60% of cases. In the prospective study of costs of beta-lactams in children (Sobrino et al.) who went to the Allergy Service with a legal guardian who worked for hire, total costs were significantly higher (€352.70 ± 167.98) than in those whose legal guardian did not work for hire (€159.13 ± 57.29), $p < 0.001$.

NSAIDs Studies

Concerning **NSAIDs**, Aspirin® and other drugs in this group represent one frequent cause of hypersensitivity reactions, which affect 1–3% of the population (Stevenson et al., 2001; Sánchez-Borges et al., 2010; Doña et al., 2012; Kowalski and Stevenson, 2013; Park et al., 2013; Demir et al., 2015; Kowalski and Makowska, 2015; Lipscomb et al., 2019). This percentage increases to 30% in patients with other pathologies, Szczeklik and Stevenson (2003) and there are even studies in which NSAIDs are the drugs most frequently involved in hypersensitivity reactions (Doña et al., 2011). Regarding the economic cost of hypersensitivity to NSAIDs, some studies have evaluated the costs associated with the use of alternative drugs and desensitization. In Spain, Cubero et al. (2017) confirmed that annual increase in the cost of using alternative antiplatelet agents such as clopidogrel was 1,142.12% (€218.13 vs. €17.64) and with trifusal was 662.76% (€134.56 vs. €17.64). In turn, Shaker et al. (2008) performed an economic analysis of desensitization to acetylsalicylic acid in aspirin-exacerbated respiratory disease (AERD), concluding that ambulatory desensitization is cost-effective in patients with moderate to severe AERD and that it continues to be a less expensive option for secondary cardiovascular prophylaxis.

In the only prospective study of costs of NSAID hypersensitivity (Sobrino-García et al., 2020), mean costs in patients with or without a final diagnosis of hypersensitivity were €239.53 ± 140.59 and €173.02 ± 145.71, respectively ($p = 0.004$). This difference was related to the mean number of visits necessary to reach the diagnosis: 4.23 ± 1.46 in patients diagnosed with hypersensitivity to NSAID and 3.34 ± 1.42 in whom allergy was discarded ($p < 0.001$).

In this case, being employed, or not, significantly increased the cost: €304.10 ± 172.55 in patients who worked for hire compared to €14.93 ± 62.55 in those who did not, $p < 0.001$.

Recently, the importance of correct labeling and delabelling of patients with a possible hypersensitivity to drugs (Macy, 2020; Solensky, 2020; Vyles et al., 2020) has also been highlighted in the context of SARS-CoV-2 Castells, 2020 infection. In health alert situations such as the present pandemic, it becomes more relevant to know when there is a real hypersensitivity to a drug that prevents its use, and when there is not. Thus, it is essential to study patients with a suspected reaction to a drug, carry out correct labeling and delabelling and, thus, reduce the risk for patients (Castells, 2020).

This review's main limitations were the paucity of prospective studies about the topic and the fact that the same group performed three of them (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020). Another limitation is that the studies' results are not comparable due to how the studies were performed. Thus, the American studyl (Blumenthal et al., 2018) did not include indirect costs, whereas the European studies (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020) did. **Table 1** shows a summary of the costs in the studies discussed in the review.

CONCLUSION

An allergy evaluation in patients with suspected drug hypersensitivity is essential to establishing a correct diagnosis. Allergy testing allows for delabelling in a substantial percentage of patients with suspected drug allergy. The elective evaluation of beta-lactams and NSAID hypersensitivity is affordable and permits using more effective first-line drugs, which generally involves cost savings. In prospective European studies (Sobrino et al., 2020; Sobrino-García et al., 2019; Sobrino-García et al., 2020), the average cost of evaluating beta-lactam allergy in adults and children was €187.49 and €275.70, respectively, and the average cost of NSAID evaluation hypersensitivity was €185.30. In the prospective American study21, the average cost was $220 (€209.37) for a case and $540 (€482.45) when more resources were used. In this sense, several recent studies have shown that drug allergy evaluation is cost-saving in patients with suspected hypersensitivity to beta-lactams or NSAIDs.

Finally, we believe that all patients labeled as allergic to beta-lactams or NSAIDs should undergo an allergy study due to critical clinical and economic consequences. However, more prospective studies are needed for comprehensive cost-effectiveness analyses of this crucial issue.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

Abrams, E. M., Atkinson, A. R., Wong, T., and Ben-Shoshan, M. (2019). The importance of delabeling β-lactam allergy in children. *J. Pediatr.* 204, 291–297. doi:10.1016/j.jpeds.2018.09.035

Abrams, E. M., Wakeman, A., Gerstner, T. V., Warrington, R. J., and Singer, A. G. (2016). Prevalence of beta-lactam allergy; a retrospective chart review of drug allergy assessment in a predominantly pediatric population. *Allergy Asthma Clin. Immunol.* 12, 59. doi:10.1186/s13223-016-0165-6

Atanaskovic-Markovic, M., Gomes, E., Cernadas, J. R., du Toit, G., Kidon, M., Kuyucu, S., et al. (2019). Diagnosis and management of drug-induced anaphylaxis in children: an EAACI position paper. *Pediatr. Allergy Immunol.* 30, 269–276. doi:10.1111/pai.13034

Au, L. Y. C., Siu, A. M., and Yamamoto, L. G. (2019). Cost and risk analysis of lifelong penicillin allergy. *Clin. Pediatr.* 58, 1309–1314. doi:10.1177/0009922819853014

Barlam, T. F., Cosgrove, S. E., Abbo, L. M., MacDougall, C., Schuetz, A. N., Septimus, E. J., et al. (2016). Implementing an antibiotic stewardship program: guidelines by the infectious diseases society of America and the society for healthcare epidemiology of America. *Clin. Infect. Dis.* 62, e51–e77. doi:10.1093/cid/ciw118

Bedolla-Barajas, M., Delgado-Figueroa, N., Pérez-Molina, J., Orozco-Alatorre, L., Bedolla-Pulido, T., Morales-Romero, J., et al. (2018). Self-reported drug hypersensitivity amongst late adolescents in Mexico: a population based study. *J Investig. Allergol. Clin. Immunol.* 28, 281–282. doi:10.18176/jiaci.0268

Bermingham, W. H., Hussain, A., Bhogal, R., Balaji, A., and Krishna, M. T. (2020). The adverse impact of penicillin allergy labels on antimicrobial stewardship in sepsis and associated pharmacoeconomics-an observational cohort study (IMPALAS Study). *J. Allergy Clin. Immunol. Pract.* 8, 1747–1749.e4. doi:10.1016/j.jaip.2019.12.030

Blumenthal, K. G., Li, Y., Banerji, A., Yun, B. J., Long, A. A., and Walensky, R. P. (2018). The cost of penicillin allergy evaluation. *J. Allergy Clin. Immunol. Pract.* 6, 1019–1027.

Borch, J. E., Andersen, K. E., and Bindslev-Jensen, C. (2006). The prevalence of suspected and challenge-verified penicillin allergy in a university hospital population. *Basic Clin. Pharmacol. Toxicol.* 98, 357–362. doi:10.1111/j.1742-7843.2006.pto_230.x

Calamelli, E., Caffarelli, C., Franceschini, F., Saretta, F., Cardinale, F., Bernardini, R., et al. (2019). A practical management of children with antibiotic allergy. *Acta Biomed.* 90, 11–19. doi:10.23750/abm.v90i3-S.8157

Castells, M. C. (2020). Drug allergy labeling and delabeling in the coronavirus disease 2019 era. *Ann. Allergy Asthma Immunol.* 124, 523–525. doi:10.1016/j.anai.2020.04.012

Chen, J. R., Tarver, S. A., Alvarez, K. S., Tran, T., and Khan, D. A. (2017). A proactive approach to penicillin allergy testing in hospitalized patients. *J. Allergy Clin. Immunol. Pract.* 5, 686–693. doi:10.1016/j.jaip.2016.09.045

Conway, E. L., Lin, K., Sellick, J. A., Kurtzhalts, K., Carbo, J., Ott, M. C., et al. (2017). Impact of penicillin allergy on time to first dose of antimicrobial therapy and clinical outcomes. *Clin. Therapeut.* 39, 2276–2283. doi:10.1016/j.clinthera.2017.09.012

Cubero, J. L., Simó Sánchez, B., Millán, P., and Colás, C. (2017). Desensibilización al ácido acetilsalicílico en pacientes con cardiopatía isquémica: ahorro de costes. *Med. Intensiva* 41, 446–447. doi:10.1016/j.medin.2016.09.012

Demir, S., Olgac, M., Unal, D., Gelincik, A., Colakoglu, B., and Buyukozturk, S. (2015). Evaluation of hypersensitivity reactions to nonsteroidal anti-inflammatory drugs according to the latest classification. *Allergy* 70, 1461–1467. doi:10.1111/all.12689

Doña, I., Blanca-López, N., Cornejo-García, J. A., Torres, M. J., Laguna, J. J., Fernández, J., et al. (2011). Characteristics of subjects experiencing hypersensitivity to non-steroidal anti-inflammatory drugs: patterns of response. *Clin. Exp. Allergy* 41, 86–95. doi:10.1111/j.1365-2222.2010.03651.x

Doña, I., Blanca-López, N., Torres, M. J., García-Campos, J., García-Núñez, I., Gómez, F., et al. (2012). Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J Investig. Allergol. Clin. Immunol.* 22, 363–371. PMID: 23101312.

Ibáñez, D. P., and Olaguibel, J. M. (2015). *Alergológica 2015, Factores epidemiológicos, clínicos y socioeconómicos de las enfermedades alérgicas en España en 2015. Capítulo 12. Alergia infantil*, 276–333.

Kowalski, M. L., and Makowska, J. S. (2015). Seven steps to the diagnosis of NSAIDs hypersensitivity: how to apply a new classification in real practice? *Allergy Asthma Immunol. Res.* 7, 312–320. doi:10.4168/aair.2015.7.4.312

Kowalski, M. L., Makowska, J. S., Blanca, M., Bavbek, S., Bochenek, G., Bousquet, J., et al. (2011). Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) - classification, diagnosis and management: review of the EAACI/ENDA# and GA2LEN/HANNA*. *Allergy* 66, 818–829. doi:10.1111/j.1398-9995.2011.02557.x

Kowalski, M. L., and Stevenson, D. D. (2013). Classification of reactions to nonsteroidal antiinflammatory drugs. *Immunol. Allergy Clin.* 33, 135–145. doi:10.1016/j.iac.2012.10.008

Li, J., Shahabi-Sirjani, A., Figtree, M., Hoyle, P., and Fernando, S. L. (2019). Safety of direct drug provocation testing in adults with penicillin allergy and association with health and economic benefits. *Ann. Allergy Asthma Immunol.* 123, 468–475. doi:10.1016/j.anai.2019.08.005

Lipscomb, J., Wong, M., and Birkel, M. (2019). Management of nonsteroidal anti-inflammatory drug-induced hypersensitivity reactions. *U.S. Pharm.* 44, 22–26.

MacLaughlin, E. J., Saseen, J. J., and Malone, D. C. (2000). Costs of beta-lactam allergies: selection and costs of antibiotics for patients with a reported beta-lactam allergy. *Arch. Fam. Med.* 9, 722–726. doi:10.1001/archfami.9.8.722

Macy, E. (2020). Addressing the epidemic of antibiotic "allergy" over-diagnosis. *Ann. Allergy Asthma Immunol.* 124, 550–557. doi:10.1016/j.anai.2019.12.016

Macy, E., and Contreras, R. (2014). Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: a cohort study. *J. Allergy Clin. Immunol.* 133, 790–796. doi:10.1016/j.jaci.2013.09.021

Macy, E., and Ngor, E. W. (2013). Safely diagnosing clinically significant penicillin allergy using only penicilloyl-poly-lysine, penicillin, and oral amoxicillin. *J. Allergy Clin. Immunol. Pract.* 1, 258–263. doi:10.1016/j.jaip.2013.02.002

Macy, E., and Shu, Y.-H. (2017). The effect of penicillin allergy testing on future health care utilization: a matched cohort study. *J. Allergy Clin. Immunol. Pract.* 5, 705–710. doi:10.1016/j.jaip.2017.02.012

Mattingly, T. J., II, Fulton, A., Lumish, R. A., Williams, A. M. C., Yoon, S., Yuen, M., et al. (2018). The cost of self-reported penicillin allergy: a systematic review. *J. Allergy Clin. Immunol. Pract.* 6 (5), 1649–1654.e4. doi:10.1016/j.jaip.2017.12.033

McDanel, J. S., Perencevich, E. N., Diekema, D. J., Herwaldt, L. A., Smith, T. C., Chrischilles, E. A., et al. (2015). Comparative effectiveness of beta-lactams versus vancomycin for treatment of methicillin-SusceptibleStaphylococcus aureusBloodstream infections among 122 hospitals. *Clin. Infect. Dis.* 61, 361–367. doi:10.1093/cid/civ308

Mill, C., Primeau, M.-N., Medoff, E., Lejtenyi, C., O'Keefe, A., Netchiporouk, E., et al. (2016). Assessing the diagnostic properties of a graded oral provocation challenge for the diagnosis of immediate and nonimmediate reactions to amoxicillin in children. *JAMA Pediatr.* 170, e160033. doi:10.1001/jamapediatrics.2016.0033

Mirakian, R., Leech, S. C., Krishna, M. T., Richter, A. G., Huber, P. A. J., Farooque, S., et al. (2015). Management of allergy to penicillins and other beta-lactams. *Clin. Exp. Allergy* 45, 300–327. doi:10.1111/cea.12468

Modena, B., White, A. A., and Woessner, K. M. (2017). Aspirin and nonsteroidal antiinflammatory drugs hypersensitivity and management. *Immunol. Allergy Clin.* 37, 727–749. doi:10.1016/j.iac.2017.07.008

Moreno, E., Laffond, E., Muñoz-Bellido, F., Gracia, M. T., Macías, E., Moreno, A., et al. (2016). Performance in real life of the European Network on Drug Allergy algorithm in immediate reactions to beta-lactam antibiotics. *Allergy* 71, 1787–1790. doi:10.1111/all.13032

Park, H-S., Kowalski, M. L., and Sanchez-Borges, M. (2013). "Hypersensitivity to aspirin and other non-steroidal antiinflammatory drugs," in *Middleton's allergy principles and practice*. 8th Edn. Philadelphia, PA: Elsevier, 1296–1309.

Park, M. A., McClimon, B. J., Ferguson, B., Markus, P. J., Odell, L., Swanson, A., et al. (2011). Collaboration between allergists and pharmacists increases β-lactam antibiotic prescriptions in patients with a history of penicillin allergy. *Int. Arch. Allergy Immunol.* 154, 57–62. doi:10.1159/000319209

Picard, M., Bégin, P., Bouchard, H., Cloutier, J., Lacombe-Barrios, J., et al. (2013). Treatment of patients with a history of penicillin allergy in a large tertiary-care academic hospital. *J. Allergy Clin. Immunol. Pract.* 1, 252–257. doi:10.1016/j.jaip.2013.01.006

Rimawi, R. H., Cook, P. P., Gooch, M., Kabchi, B., Ashraf, M. S., Rimawi, B. H., et al. (2013). The impact of penicillin skin testing on clinical practice and antimicrobial stewardship. *J. Hosp. Med.* 8, 341–345. doi:10.1002/jhm.2036

Roduit, C. (2019). Drug allergy in children: more often suspected than real. *Ther. Umsch.* 75, 29–31 [in German]. doi:10.1024/0040-5930/a001057

Sacco, K. A., Bates, A., Brigham, T. J., Imam, J. S., and Burton, M. C. (2017). Clinical outcomes following inpatient penicillin allergy testing: a systematic review and meta-analysis. *Allergy* 72 (9), 1288–129610. doi:1111/all.13168

Sade, K., Holtzer, I., Levo, Y., and Kivity, S. (2003). The economic burden of antibiotic treatment of penicillin-allergic patients in internal medicine wards of a general tertiary care hospital. *Clin. Exp. Allergy* 33, 501–506. doi:10.1046/j.1365-2222.2003.01638.x

Sagar, P. S., and Katelaris, C. H. (2013). Utility of penicillin allergy testing in patients presenting with a history of penicillin allergy. *Asia Pac. Allergy* 3, 115–119. doi:10.5415/apallergy.2013.3.2.115

Sánchez-Borges, M., Caballero-Fonseca, F., Capriles-Hulett, A., and González-Aveledo, L. (2010). Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs: an update. *Pharmaceuticals* 3, 10–18. doi:10.3390/ph3010010

Sastre, J., Manso, L., Sanchez-García, S., and Fernández-Nieto, M. (2012). Medical and economic impact of misdiagnosis of drug hypersensitivity in hospitalized patients. *J. Allergy Clin. Immunol.* 129, 566–567. doi:10.1016/j.jaci.2011.09.028

Seitz, C. S., Bröcker, E.-B., and Trautmann, A. (2011). Diagnosis of drug hypersensitivity in children and adolescents: discrepancy between physician-based assessment and results of testing. *Pediatr. Allergy Immunol.* 22, 405–410. doi:10.1111/j.1399-3038.2011.01134.x

Shaker, M., Lobb, A., Jenkins, P., O'Rourke, D., Takemoto, S. K., Sheth, S., et al. (2008). An economic analysis of aspirin desensitization in aspirin-exacerbated respiratory disease. *J. Allergy Clin. Immunol.* 121, 81–87. doi:10.1016/j.jaci.2007.06.047

Shehab, N., Patel, P. R., Srinivasan, A., and Budnitz, D. S. (2008). Emergency department visits for antibiotic-associated adverse events. *Clin. Infect. Dis.* 47, 735–743. doi:10.1086/591126

Shenoy, E. S., Macy, E., Rowe, T., and Blumenthal, K. G. (2019). Evaluation and management of penicillin allergy. *J. Am. Med. Assoc.* 321, 188–199. doi:10.1001/jama.2018.19283

Sobrino, M., Muñoz-Bellido, F. J., Macías, E., Lázaro-Sastre, M., de Arriba-Méndez, S., Dávila, I., et al. (2020). A prospective study of costs associated with the evaluation of β-lactam allergy in children. *J. Pediatr.* 223, 108–113.e2. doi:10.1016/j.jpeds.2020.04.018

Sobrino García, M., Muñoz Bellido, F. J., Moreno, E., Macías, E., Gracia Bara, M. T., Laffond, E., et al. (2019). A comprehensive prospective study of costs associated to the evaluation of beta-lactam allergy. *J. Investig. Allergol. Clin. Immunol.* 31 (1). Online ahead of print. doi:10.18176/jiaci.0457

Sobrino-García, M., Muñoz-Bellido, F. J., Moreno, E., Macías, E., Gracia-Bara, M. T., Laffond, E., et al. (2020). A prospective study of costs associated to the evaluation of nonsteroidal anti-inflammatory hypersensitivity reactions. *Allergy* 75 (6), 1495–1497. doi:10.1111/all.14169

Solensky, R. (2020). Doctor, I am allergic to penicillin; is this dangerous? *Ann. Allergy Asthma Immunol.* 124, 544–545. doi:10.1016/j.anai.2020.01.027

Soto-Álvarez, J. (2020). Estudios de farmacoeconomía, principios y práctica. Available at: http://www.academia.cat/files/425-3261 (Accessed de febrero de 12, 2020).

Sousa-Pinto, B., Araújo, L., Freitas, A., and Delgado, L. (2018). Hospitalizations in children with a penicillin allergy label: an assessment of healthcare impact. *Int. Arch. Allergy Immunol.* 176, 234–238. doi:10.1159/000488857

Sousa-Pinto, B., Blumenthal, K. G., Macy, E., Bavbek, S., Benić, M. S., Alves-Correia, M., et al. (2020a). Diagnostic testing for penicillin allergy: a survey of practices and cost perceptions. *Allergy* 75, 436–441. doi:10.1111/all.13951

Sousa-Pinto, B., Blumenthal, K. G., Macy, E., Pereira, A. M., Azevedo, L. F., Delgado, L., et al. (2020b). Penicillin allergy testing is cost-saving: an economic evaluation study. *Clin. Infect. Dis.*, ciaa194. doi:10.1093/cid/ciaa194

Stevenson, D. D., Sanchez-Borge, M., and Szczeklik, A. (2001). Classification of allergic and pseudoallergic reactions to drugs that inhibit cyclooxygenase enzymes. *Ann. Allergy Asthma Immunol.* 87, 177–180. doi:10.1016/s1081-1206(10)62221-1

Stone, C. A., Trubiano, J., Coleman, D. T., Rukasin, C. R. F., and Phillips, E. J. (2020). The challenge of de-labeling penicillin allergy. *Allergy* 75, 273–288. doi:10.1111/all.13848

Szczeklik, A., and Stevenson, D. D. (2003). Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. *J. Allergy Clin. Immunol.* 111, 913–921. doi:10.1067/mai.2003.1487

Torres, M. J., Adkinson, N. F., Jr., Caubet, J.-C., Khan, D. A., Kidon, M. I., Mendelson, L., et al. (2019). Controversies in drug allergy: beta-lactam hypersensitivity testing. *J. Allergy Clin. Immunol. Pract.* 7 (1), 40–45. doi:10.1016/j.jaip.2018.07.051

Torres, M. J., Blanca, M., Fernández, J., Romano, A., Weck, A., Aberer, W., et al. (2003). Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 58, 961–972. doi:10.1034/j.1398-9995.2003.00280.x

Torres, M. J., Moreno, E., Fernandez-Santamaría, R., Doña, I., and Fernández, T. D. (2019). Diagnostic approximation to delabeling beta-lactam allergic patients. *Curr. Treat. Options Allergy* 6, 56–70. doi:10.1007/s40521-019-0202-z

Vyles, D., Antoon, J. W., Norton, A., Stone, C. A., Trubiano, J., Radowicz, A., et al. (2020). Children with reported penicillin allergy. *Ann. Allergy Asthma Immunol.* 124, 558–565. doi:10.1016/j.anai.2020.03.012

Wöhrl, S. (2018). NSAID hypersensitivity - recommendations for diagnostic work up and patient management. *Allergo J. Int.* 27, 114–121. doi:10.1007/s40629-018-0064-0

World Bank (2019). GPD per capita (current US dollar). World Bank national accounts data, and OECD National Accounts data files. Available at: https://data.worldbank.org/indicator/ny.gdp.pcap.cd (Accessed November 17, 2019).

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MINI REVIEW
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Mechanisms of Drug Desensitization: Not Only Mast Cells

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Drug desensitization (DD) allows transient clinical tolerance to the drug in reactive patients and it is frequently and successfully used in the management of both IgE and non IgE-mediated hypersensitivity reactions (HRs). The underlying mechanisms behind this process is not well understood. The desensitization procedure is associated with the inhibition of mast cells degranulation and cytokine production, that, is attributable, at least partially, to the abrogation of Ca2+ mobilization; *in vitro* findings and *in vivo* mouse models of rapid desensitization show that the organization and spatial distribution of actin is critical for Ca2+ mobilization. Some clinical observations may suggest the induction of a longer memory of tolerance by DD and they raise the suspicion that other cells and mechanisms are involved in DD. Some data are emerging about the modifications of immune responses during DD in patients with previous immediate HRs. In particular, an increase of regulatory cytokines, mainly represented by IL-10, has been shown, and more importantly, the appearance of IL-35 producing T regulatory cells has been described during DD. The release of controlled cellular mediators by mast cells over time and the development of the antigen-specific regulation of adaptive response allow to safely and successfully reach the target dose of a first line drug during DD.

Keywords: desensetisation, allergy (hypersensitive anaphylaxis), mast cells, T reg cell, IL-10, IL-35

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INTRODUCTION

The treatment of many disorders including cancer and autoimmune diseases can be complicated by hypersensitivity reactions (HRs). Clinical manifestations vary considerably, ranging from mild to severe and life-threatening reactions leading to drug discontinuation, which in turn can decrease patients' quality of life and/or life expectancy (Vultaggio et al., 2011; Sala-Cunill et al., 2019). Phenotypes in drug allergy focus on symptoms and timing, classifying the reactions as immediate or delayed, depending on the time between treatment administration and the onset of symptoms (Bonamichi-Santos and Castells, 2016). The most frequently involved culprit drugs are represented by antibiotics, aspirin, chemotherapeutics (mainly platinum compounds and taxanes) and biological agents (Vultaggio and Castells, 2014).

Management of HRs, beyond an allergological work-up aimed to define the pathogenic mechanism of the reaction, may include drug desensitization (DD) when there is no alternative therapy available. The culprit drug is usually avoided in order to prevent future reactions and DD was developed as a treatment option to maintain patients on first line therapy (Castells, 2017).

The activation of mast cells (MC) plays a critical role in HRs, not only limited to the immediate release of an array of preformed inflammatory mediators including histamine, tryptase, serotonin,

chymases, cytokines, and growth factors, but also *de novo* synthesis of lipid mediators such as leukotrienes. In addition to the classical IgE-mediated MC activation, other mechanisms may be involved. Some medications can directly activate MC via the recently identified Mas-related G Protein Coupled Receptor-X2 (MRGPRX2) transmembrane protein, as in the case of fluoroquinolones, neuromuscular blocking agents, and vancomycin (McNeil et al., 2015; Boyce, 2019). Others may further activate mast cells via complement activation leading to the production of anaphylotoxins C3a and C5a (Jimenez-Rodriguez et al., 2018). The direct activation of membrane receptors as in the case of opioids and estrogens, represent a non-immunological pathway of MC activation possibly involved in the induction of HRs (Spoerl et al., 2017). Finally, cyclooxygenase-1 inhibition [as in the case of Aspirin Exacerbated Respiratory disease (AERD)] may occur.

The aim of this review is to evaluate the mechanisms involved in successful DD, highlighting the role of regulatory cells and cytokines in the modulation of a drug-specific immune response.

DRUG DESENSITIZATION: GENERAL CONCEPTS

Management of HRs in patients without treatment alternatives is based on the DD procedure, able to induce a temporary hyporesponsive state by incremental escalation of sub-optimal doses of the offending drug, until reaching required dosage (De las Vecillas Sanchez et al., 2017). Drug desensitization was developed due to the pressing need to reintroduce drugs in a safe fashion in patients who had developed both IgE-and/or non IgE-mediated HRs to critical drugs. Because DD is able to induce a temporary tolerance to the culprit drug, and considering that some medications (chemotherapy, biologic agents) have prolonged dosing intervals, subsequent administrations must be preceded by a DD procedure in order to overcome the loss of tolerance.

Desensitization is conceptually dedicated to patients in which an IgE-mediated mechanism is demonstrated by positive skin testing or serum IgE for culprit drug, however, patients who suffered immediate reactions to taxanes and other chemotherapies in which the IgE mechanisms cannot be demonstrated have also been successfully desensitized (Madrigal-Burgaleta et al., 2019).

Two types of DD protocols are available: rapid drug desensitization which addresses type I reactions with mast cells/basophils/IgE involvement, and slow drug desensitization which addresses delayed type IV reactions with T-cell involvement (Castells, 2015). Mixed reactions however have become more frequent, so DD protocols have slowly changed the segregation paradigm of DD vs. slow drug desensitization (Pyle et al., 2014). Desensitization is contraindicated in patients whose reaction suggests a history of severe cutaneous reactions, such as Stevens-Johnsons syndrome, toxic epidermal necrolysis, drug induced hypersensitivity syndrome, drug reaction (rash) with eosinophilia and systemic symptoms and acute generalized exanthematous pustulosis. Desensitization is also not considered appropriate for reactions of serum sickness or haemolytic anemia (Castells, 2017). Omalizumab is a humanized IgG1 monoclonal antibody, initially approved for the treatment of severe allergic asthma and more recently, for the treatment of chronic idiopathic urticaria. In several case reports it has been applied to control the reactions occurring during DD, for aspirin (Waldram et al., 2018), insulin (Mishra et al., 2018), elosulfasi α (Guvenir et al., 2017), carboplatin (Oude Elberink et al., 2020), and oxaliplatin (Prieto-Garcia et al., 2019).

MECHANISMS INVOLVED IN DRUG DESENSITIZATION: MAST CELLS

Regardless of whether the reaction is the consequence of an IgE- or non IgE-mediated mechanism, MC are key effector cells in the majority of immediate drug reactions, and the desensitization procedure is associated with the inhibition of MC degranulation and cytokine production.

Both *in vivo* and *in vitro* studies have been used to understand the cellular and molecular pathways influencing the function of MC and basophils during DD.

Several observations displayed negative skin testing after desensitization, indicating inhibition of the MC activation. These data have been extensively described in DD for chemotherapeutics and more recently for biological agents (Lee et al., 2004). By using sensitized bone marrow-derived MC under physiologic calcium conditions and by administrating incremental doses of the drug at fixed time intervals, cells were shown to become unresponsive (Sancho-Serra et al., 2011). Recent data obtained in a subject sensitized to infliximab (IFX) and grass pollen, who experienced an immediate HR to IFX, showed that skin testing for IFX was positive before cycles of DD but negativized after each procedure, while skin testing for grass pollen remained positive before and after each cycle (Vultaggio et al., 2020). These data obtained in humans were confirmed by *in vitro* results. In fact, challenging with the culprit drug after being desensitized did not induce *in vitro* activation of MC that could still be activated by different antigen stimulation, supporting the concept that DD is an antigen-specific process (Gladys et al., 2016).

To understand the mechanisms by which DD procedures impact MC mediator release, it is useful to define sequential events starting from IgE/FcεRI cross-linking to the intracellular signals.

Phosphorylation of subunit ITAMs (immune-receptor tyrosine-based activation motif) is important in initiating and inducing downstream propagation of intracellular signaling (Phong et al., 2015). Activated Lyn initiates signal transduction through phosphorylation of the β and γ ITAM chain. In the first phase of the process, phospholipase Cγ phosphorylates and then hydrolyzes phosphatidyl inositol bisphosphate to yield inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 induces an increase in cytosolic calcium ion (Ca2+) concentration, by binding to its receptor in the endoplasmic reticulum and rapidly inducing the process of

calcium mobilization. In the subsequent phase, a prolonged Ca2+ influx occurs (Nam and Kim, 2020).

Although several studies have attempted to examine the underlying mechanisms regarding the effects of DD on MC, a general consensus is not yet reached in the literature.

Initial studies suggested that MCs became unresponsive after DD as the consequence of internalization of FcεRI through progressive cross-linking at low antigen concentrations (Shalit and Levi-Schaffer, 1995; Morales et al., 2005). More recent studies have shown that antigen/IgE/FcεRI may remain on the surface during DD (Gladys et al., 2016). In particular, it has been shown that surface IgE was not completely internalized, leaving enough IgE bound on the cell surface to bind Ag and potentially cause degranulation.

It would seem that MCs' hypo-responsiveness is attributable, at least partially, to the abrogation of Ca2+ mobilization, a critical determinant of degranulation and cytokine production in mast cells (Gladys et al., 2016). Specifically, the organization and spatial distribution of actin is critical for Ca2+ mobilization in several cell types, including MCs, as demonstrated by *in vitro* findings and *in vivo* mouse models of rapid desensitization.

Of note, MRGPRX2-related MC degranulation are probably to DD preocedure as MRGPRX2 receptor does not undergo internalization.

OTHER MECHANISMS INVOLVED IN DRUG DESENSITIZATION: NOT ONLY MAST CELLS

It is currently assumed that drug tolerance induced by DD is not a permanent state and that it is sustained by a pharmacologic, and not immunologic, tolerance. However, some clinical observations may suggest the induction of a longer memory of tolerance by DD.

Firstly, the rate of reactions during DD procedures progressively reduces over increasing number of desensitisations. As shown by Sloane et al., the percentage of patients with any breakthrough reaction during the DD procedures to chemotherapy and monoclonal antibodies decreased in time with a corresponding increase in the percentage of patients who tolerated the desensitization procedure during repeated cycles (Sloane et al., 2016). Patients initially presenting with anaphylaxis and desensitized in the intensive care setting proceeded to repeated successful desensitisations in the outpatient setting.

Secondly, although protocols are mostly empirical and the best and safest protocol is unknown, it has been reported that after two successful DD cycles, patients may tolerate even shorter subsequent protocols (Sloane et al., 2016).

Finally, DD is widely used in the management of immediate reactions, whereas in non immediate reactions where a T cell-mediated mechanism predominates the role of DD is still limited. However, in mild clinical conditions such as macula-papular exanthemas and fixed drug eruptions, some DD protocols are successfully applied (Castells et al., 2012; Scherer et al., 2013).

Overall, these observations raise the suspicion that other cells and mechanisms are involved in DD.

Modulation of Adaptive Immune Response to the Drug During DD

The adaptive immune response sustained by drug-specific T cells and its modification during DD procedures has been scarcely evaluated until now. One study, focused on aspirin DD in patients suffering from aspirin-exacerbated respiratory disease (AERD), showed that one month after beginning of DD, no difference was detectable in the percentage of $CD4^+$ T cells and their cytokine production (IL-2, IL-4 and IFN-g) in comparison with baseline (Atkas et al., 2013). However, the lack of effects on T cells described do not exclude long-term effects of DD.

Concerning other immediate hypersensitivity reactions (HRs), such as those induced by biological agents (BA), we have recently shown that drug-specific T cell proliferation to infliximab (IFX) was progressively reduced during DD procedures in a patient suffering from allergic asthma with grass sensitization who had experienced an IFX-induced anaphylaxis. Accordingly, the humoral response to the drug (anti-IFX antibodies titer) showed a parallel decrease over successful DD cycles (Vultaggio et al., 2020). These DD-induced modifications of both cellular and humoral response to IFX were drug-specific, as anti-grass pollen IgE remained positive during the entire protocol as well as the cellular response to Phl p5 was consistently positive in all tested samples over DD cycles.

Some data are emerging about the modifications of immune response toward biological agents (BA) during DD in patients with delayed reactions. Teraki and Shiohara have shown a decrease in the percentage of CD8+T cells infiltrating the lesion in allopurinol fixed drug eruptions during DD procedure (Teraki and Shiohara, 2004). Overall, these studies provide limited and controversial information, not allowing any significant understanding of the cellular immune mechanisms operating during DD.

Drug Desensitization Increases Regulatory Cytokines

The impairment of effector responses observed during DD procedures suggested the involvement of regulatory mechanisms operating in successful DD, in a similar way to what happens during allergen immunotherapy. The effects of DD on regulatory cytokine levels in patients desensitized have been evaluated in both immediate and delayed drug hypersensitivity reactions. Gelincik and coworkers have described a significant increase in IL-10 serum levels 24 h within the end of DD procedures in 24 patients who underwent successful DD for several oral or parental culprit drugs. In the same case series, no changes of IL-4, IL-5 and IFN-γ levels were observed. The authors observed a greater increase in IL-10 levels in patients desensitized for chemotherapeutic drugs (Gelincik et al., 2019). An additional study focused on platinum desensitized patients for immediate HRs has confirmed the increase of IL-10 serum levels after DD, with a tendency to reach higher levels of IL-10 after

multiple cycles (Tüzer et al. 2020). Regarding delayed HRs, in a case report about DD to allopurinol after a fixed drug eruption, an increase of IL-10 (and IL-6) production by peripheral blood mononuclear cells, was observed. Intracellular IL-10 contents in T cells, but not serum levels, have been analyzed in one study involving patients during desensitization to aspirin in AERD. A decrease of IL-10 (and IFN-γ) intracellular expression in $CD4^+$ T cells, was observed after 1 month of desensitization (Aksu et al, 2014). This discrepancy may be caused by the type of reaction where DD has been applied (pathogenesis of AERD) and by the fact that serum levels were not evaluated in this study.

IL-10 is an important regulatory and anti-inflammatory cytokine, largely studied and involved in successful allergen immunotherapy (AIT) (Ni et al., 2015). Studies involving AIT showed the role for IL-10 production by T cells (Treg) and B cells (Breg) in inducing tolerance (Akdis et al., 2005, van de Veen, 2017). It important to note that before Treg and Breg cells appear, early desensitization effect of AIT seems to be associated with IL-10 produced by other cells, such as basophils and MCs (van de Veen et al., 2017). In fact, different cells may produce IL-10, such as cells of adaptive (T cells, B cells) and innate immunity (dendritic cells, natural killer T cells, eosinophils, neutrophils, basophils, MC) and keratinocytes (Saraiva and O'Garra et al., 2010). The cellular source of IL-10 production during DD has to be defined as yet, and further studies are required in this field, however we might speculate that the immunological effects of DD are similar to those observed during AIT.

IL-35 is the newest member of IL-12 family. It is a dimeric protein consisting of two separate subunits, an IL-12 subunit α chain (P35) and IL-27 subunit Epstein-Barr virus-induced gene 3 (EBI3) β chain; IL-35 has manifested suppressive actions on the immune system. It is secreted by a variety of cells, and then activates its receptors through JAK/STAT signaling to exert its anti-inflammatory and immunosuppressive effects (Zhang, et al., 2019). In a patient desensitized to IFX, serum IL-35 was highly increased after each DD cycle and a progressive increase of baseline values in serum samples collected before each cycle was observed (Vultaggio et al., 2020). Such response to high antigen dose during DD is likely comparable to that described for AIT in which IL-35 has been recently described to play a relevant role (Shamji and Durham, 2017; Shamji et al., 2018) and confirm the involvement of regulatory cytokines in the DD-related immunological mechanisms.

Drug Desensitization Induces Drug-SpecificTreg Cells

Regulatory T (Treg) cells are a subset of $CD4^+$ αβT cells that play a major role for maintaining self tolerance and preventing autoimmunity, limiting chronic inflammatory diseases, dampening homeostatic lymphocyte expansion, and suppressing immune responses to parasites and viruses and tumors, including that induced by therapeutic vaccines. The manipulation of Treg functions is an important goal of AIT, since a successful AIT is sustained by the generation of allergen-specific Tregcells (Palomares et al., 2010), and, as recently shown, in particular by T cells producing the regulatory cytokine IL-35 (called Tr35) (Shamji et al. 2018).

The involvement of Treg cells in DD mechanisms has been analyzed in few studies until now, mainly regarding DD after HRs to monoclonal antibodies. An increase of $CD4^+CD25^+$ cells and $CD4^+CD25$ + FoxP3+ Treg cells in peripheral blood after DD for rituximab has been described in a successful procedure in a patient suffering from nephrotic syndrome (Aydogan et al., 2013). In addition, during DD for IFX, PBMC upon *in vitro* stimulation with IFX were able to produce IL-35 in a MHC-Class II-restricted manner, suggesting that the production of this regulatory cytokine is sustained by the presence of drug-specific Tr35. Notably these cells constitutively express check point molecules, including PD1 (Turnis et al., 2016), and accordingly, in the same case report, increased proportion of circulating $CD3^+CD4$+PD1+ and $CD3^+CD4$+Foxp3+ T cells were observed after the second and third cycle of desensitization. Regarding delayed reactions induced by allopurinol, Teraki and Shiohara showed that the number of $CD4^+CD25^+$ T cells increased in skin lesions after the beginning of DD, suggesting that Treg cells may migrate from blood to skin, where they might act to suppress effector T cells, that conversely decreased in proportion (from 91% to 35%) (Teraki and Shiohara, 2004). Even though these data obtained in single case reports must be considered preliminary, overall, they suggest a possible role of Tregulatory cells in the drug tolerance induced by DD.

DISCUSSION

Drug desensitization allows a transient clinical tolerance to the culprit drug by administering, in a short time, increasing amounts of the drug until reaching the therapeutic dose. Such procedure is to be applied mainly in reactive patients with no alternative treatment options.

Besides a profound change of MC reactivity with inhibition of their activation pathways and mediators' release (only partially known until now), some reports strongly indicate that tolerance induced by DD implies the modulation of drug-specific response by regulatory mechanisms, confirming that this type of procedure deeply impacts on the immune response, more than that has been demonstrated to date. Of note, this regulatory activity is transient and lasts a short and variable period after stopping the treatment.

Drug-specific immune response to BAs is down-regulated by a panel of regulatory cytokines, including the traditional IL-10 and a new molecule belonging to IL-12 family, IL-35. In particular, the activation/expansion of drug-specific Tr35 cells, occurring during the DD procedure to some BAs, may have a particular relevance in the mechanisms of DD tolerance, since IL-35 orchestrates other regulatory cells and cytokines. **Figure 1** illustrates the immunological modifications of both umoral and cellular adaptive immune response to BA during DD.

It would be desirable to establish in the near future which regulatory cells (Treg, Breg, ILCreg, DCreg etc) are involved in each drug treatment, when they become operative during the DD procedure, and how long they last after stopping the DD. In addition, we cannot exclude that the type of drug, the route of administration, the dose and the scheme could influence the mechanisms operating in successful DD.

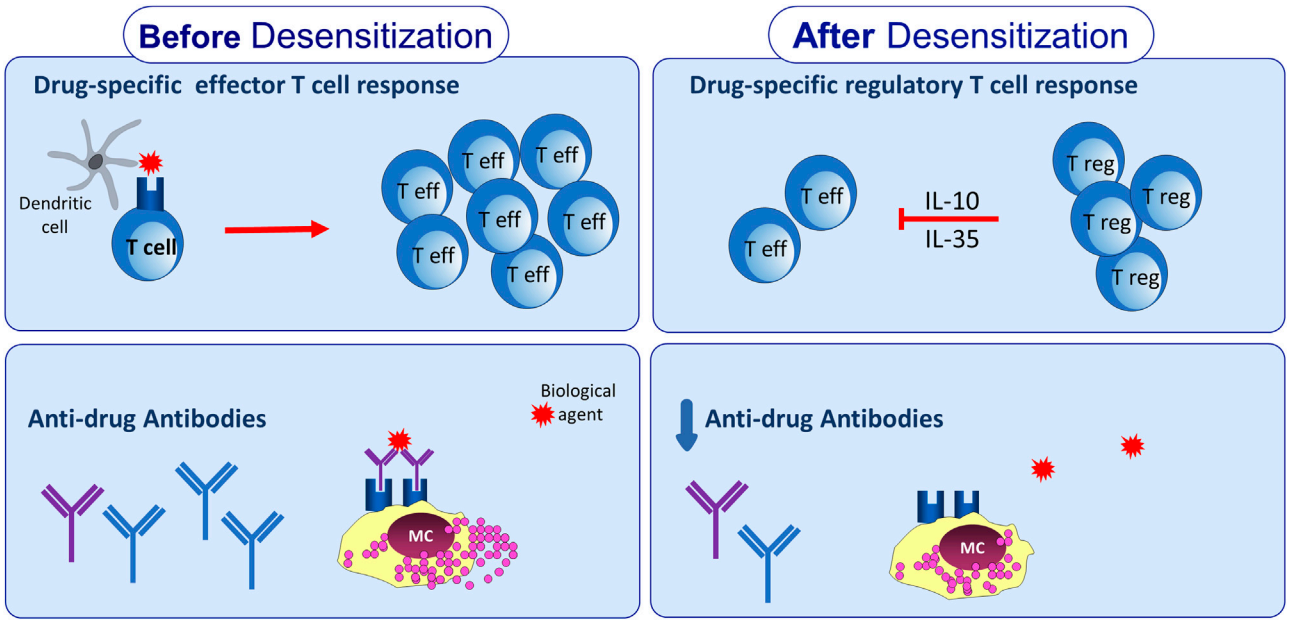

Figure 1 | Adaptive immunological changes induced by drug desensitization. Drug desensitization may impact on adaptive immune response leading to reduction of anti-drug antibodies levels. In addition, drug-specific T cell response seems to be affected by drug desensitization, due to expansion of T regulatory cells able to produce IL-10 and IL-35.

In conclusion, DD procedure induces two independent antigen-specific mechanisms: the release of controlled cellular mediators by MC over time and the development of the antigen-specific regulation of adaptive response. These mechanisms allow to safely and successfully reach the target dose of the drug.

AUTHOR CONTRIBUTIONS

AV and AM wrote the manuscript. FN designed the figure. EM, SB and EV revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

Akdis, M., Blaser, K., and Akdis, C. A. (2005). T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. *J. Allergy Clin. Immunol.* 116, 961–969. doi:10.1016/j.jaci.2005.09.004

Aksu, K., Kurt, E., Alatas, Ö., and Gülbas, Z. (2014). Effect of aspirin desensitization on T-cell cytokines and plasma lipoxins in aspirin-exacerbated respiratory disease. *Allergy Asthma Proc.* 35, 148–155. doi:10.2500/aap.2014.35.3726

Aktas, A., Kurt, E., and Gulbas, Z. (2013). Cytokine expression before and after aspirin desensitization therapy in aspirin-exacerbated respiratory disease. *Inflammation* 36, 1553–1559. doi:10.1007/s10753-013-9699-7

Aydogan, M., Yologlu, N., Gacar, G., Uyan, Z. S., Eser, I., and Karaoz, E. (2013). Successful rapid rituximab desensitization in an adolescent patient with nephrotic syndrome: increase in number of Treg cells after desensitization. *J. Allergy Clin. Immunol.* 132, 478–480. doi:10.1016/j.jaci.2013.02.004

Bonamichi-Santos, R., and Castells, M. (2016). Diagnoses and management of drug hypersensitivity and anaphylaxis in cancer and chronic inflammatory diseases: reactions to taxanes and monoclonal antibodies. *Clin. Rev. Allergy Immunol.* 54, 375–385. doi:10.1007/s12016-016-8556-5

Boyce, J. A. (2019). Aspirin sensitivity: lessons in the regulation (and dysregulation) of mast cell function. *J. Allergy Clin. Immunol.* 144, 875–881. doi:10.1016/j.jaci.2019.08.022

Castells, M. (2017). Drug hypersensitivity and anaphylaxis in cancer and chronic inflammatory diseases: the role of desensitizations. *Front. Immunol.* 8, 1472. doi:10.3389/fimmu.2017.01472

Castells, M., Sancho-Serradel, M. C., and Simarro, M. (2012). Hypersensitivity to antineoplastic agents: mechanisms and treatment with rapid desensitization. *Cancer Immunol. Immunother.* 61, 1575–1584. doi:10.1007/s00262-012-1273-x

Castells, M. C. (2015). A new era for drug desensitizations. *J. Allergy Clin. Immunol. Pract.* 3, 639–640. doi:10.1016/j.jaip.2015.05.006

Castells, M. C. (2017). Diagnosis and management of anaphylaxis in precision medicine. *J. Allergy Clin. Immunol.* 140, 321. doi:10.1016/j.jaci.2017.06.011 doi:10.1016/j.jaci.2017.06.012

De las Vecillas Sánchez, L., Alenazy, L., Garcia-Neuer, M., and Castells, M. (2017). Drug hypersensitivity and desensitizations: mechanisms and new approaches. *Int. J. Mol. Sci.* 18, 1316. doi:10.3390/ijms18061316

Gelincik, A., Demir, S., Şen, F., Bozbey, U. H., Olgaç, M., Ünal, D., et al. (2019). Interleukin-10 is increased in successful drug desensitization regardless of the hypersensitivity reaction type. *Asia Pac Allergy* 28, 9. doi:10.5415/apallergy.2019.9.e9.

Gladys, A. W. X., Church, A. M., Kulis, M., Choi, H. W., Abraham, S. N., and Abraham, S. N. 2016). Mast cell desensitization inhibits calcium flux and aberrantly remodels actin. *J. Clin. Invest.* 126, 4103–4118. doi:10.1172/JCI87492

Guvenir, H., Dibek Misirlioglu, E., Capanoglu, M., Buyuktiryaki, B., Unal, O., Toyran, M., et al. (2017). Successful desensitization of elosulfase alfa-induced anaphylaxis in a pediatric patient with Morquio syndrome. *J. Allergy Clin. Immunol. Pract.* 5, 1156–1157. doi:10.1016/j.jaip.2017.02.020

Jimenez-Rodriguez, T., Garcia-Neuer, M., Alenazy, L. A., and Castells, M. (2018). Anaphylaxis in the 21st century: phenotypes, endotypes, and biomarkers. *J. Asthma Allergy* 11, 121–142. doi:10.2147/jaa.s159411

Lee, C. W., Matulonis, U. A., and Castells, M. C. (2004). Carboplatin hypersensitivity: a 6-h 12-step protocol effective in 35 desensitizations in patients with gynecological malignancies and mast cell/IgE-mediated reactions. *Gynecol. Oncol.* 95, 370–376. doi:10.1016/j.ygyno.2004.08.002

Madrigal-Burgaleta, R., Bernal-Rubio, L., Berges-Gimeno, M. P., Carpio-Escalona, L. V., Gehlhaar, P., and Alvarez-Cuesta, E. (2019). A large single hospital experience using drug provocation testing and rapid drug desensitization in

hypersensitivity to antineoplastic and biological agents. *J. Allergy Clin. Immol. Pract.* 7, 618–632. doi:10.1016/j.jaip.2018.07.031

McNeil, B. D., Pundir, P., Meeker, S., Han, L., Undem, B. J., Kulka, M., , et al.Dong, X. (2015). Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 519, 237–241. doi:10.1038/nature14022

Mishra, S., Connors, L., and Tugwell, B. (2018). Role of omalizumab in insulin hypersensitivity: a case report and review of the literature. *Diabet. Med.* 35, 663–666. doi:10.1111/dme.13591

Morales, A. R., Shah, N., and Castells, M. (2005). Antigen-IgE desensitization in signal transducer and activator of transcription 6-deficient mast cells by suboptimal doses of antigen. *Ann. Allergy Asthma Immunol.* 94, 575–580. doi:10.1016/s1081-1206(10)61136-2

Nam, J. H., and Kim, W. K. (2020). The role of TRP channels in allergic inflammation and its clinical relevance. *Curr. Med. Chem.* 27, 1446–1468. doi:10.2174/0929867326666181126113015

Ni, G., Wang, T., Walton, S., Zhu, B., Chen, S., Wu, X., et al. (2015). Manipulating IL-10 signalling blockade for better immunotherapy. *Cell. Immunol.* 293, 126–129. doi:10.1016/j.cellimm.2014.12.012

Oude Elberink, H. N. G., Jalving, M., Dijkstra, H., and van de Ven, A. A. J. M. (2020). Modified protocol of omalizumab treatment to prevent carboplatin-induced drug hypersensitivity reactions: a case study. *Clin. Transl. Allergy* 10 (5). doi:10.1186/s13601-020-0309-0

Palomares, O., Yaman, G., Azkur, A. K., Akkoc, T., Akdis, M., and Akdis, C. A. (2010). Role of Treg in immune regulation of allergic diseases. *Eur. J. Immunol.* 40, 1232–1240. doi:10.1002/eji.200940045

Phong, B. L., Avery, L., Sumpter, T. L., Gorman, J. V., Watkins, S. C., Colgan, J. D., et al. (2015). Tim-3 enhances FcεRI-proximal signaling to modulate mast cell activation. *J. Exp. Med.* 212, 2289–2304. doi:10.1084/jem.20150388

Prieto-García, A., Noguerado, B., Rojas, P., Torrado, I., Rodríguez-Fernández, A., and Tornero, P. (2019). Unexpected anaphylaxis after completing a desensitization protocol to oxaliplatin: successful adjuvant use of omalizumab. *J Investig. Allergol. Clin. Immunol.* 29, 53–55. doi:10.18176/jiaci.0326

Pyle, R. C., Butterfield, J. H., Volcheck, G. W., Podjasek, J. C., Rank, M. A., Li, J. T., et al. (2014). Successful outpatient graded administration of trimethoprim-sulfamethoxazole in patients without HIV and with a history of sulfonamide adverse drug reaction. *J. Allergy Clin. Immunol. Pract.* 2, 52–58. doi:10.1016/j.jaip.2013.11.002

Sala-Cunill, A., Luengo, O., and Cardona, V. (2019). Biologics and anaphylaxis. *Curr. Opin. Allergy Clin. Immunol.* 19, 439–446. doi:10.1097/aci.0000000000000550

Sancho-Serra, M.. C., Simarro, M., and Castells, M. (2011). Rapid IgE desensitization is antigen specific and impairs early and late mast cell responses targeting FcεRI internalization. *Eur. J. Immunol.* 41, 1004–1013. doi:10.1002/eji.201040810

Saraiva, M., and O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10, 170–181. doi:10.1038/nri2711

Scherer, K., Brockow, K., Aberer, W., Gooi, J. H., Demoly, P., Romano, A., et al. (2013). Desensitization in delayed drug hypersensitivity reactions—an EAACI position paper of the Drug Allergy Interest Group. *Allergy* 68, 844–852. doi:10.1111/all.12161

Shalit, M., and Levi-Schaffer, F. (1995). Challenge of mast cells with increasing amounts of antigen induces desensitization. *Clin. Exp. Allergy* 25, 896–902. doi:10.1111/j.1365-2222.1995.tb00033.x

Shamji, M. H., and Durham, S. R. (2017). Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. *J. Allergy Clin. Immunol.* 140, 1485–1498. doi:10.1016/j.jaci.2017.10.010

Shamji, M. H., Kappen, J., Abubakar-Waziri, H., Zhang, J., Steveling, E., Watchman, S., et al. (2018). Nasal allergen-neutralizing IgG. *J. Allergy Clin. Immunol.* 143, 1067–1076. doi:10.1016/j.jaci.2018.09.039

Sloane, D., Govindarajulu, U., Harrow-Mortelliti, J., Barry, W., Hsu, F. I., Hong, D., et al. (2016). Safety, costs, and efficacy of rapid drug desensitizations to chemotherapy and monoclonal antibodies. *J. Allergy Clin. Immunol. Pract.* 4, 497–504. doi:10.1016/j.jaip.2015.12.019

Spoerl, D., Nigolian, H., Czarnetzki, C., and Harr, T. (2017). Reclassifying anaphylaxis to neuromuscular blocking agents based on the presumed patho-mechanism: IgE-mediated, pharmacological adverse reaction or "innate hypersensitivity"?. *Int. J. Mol. Sci.* 18, 1223. doi:10.3390/ijms18061223

Teraki, Y., and Shiohara, T. (2004). Successful desensitization to fixed drug eruption: the presence of CD25+CD4+ T cells in the epidermis of fixed drug eruption lesions may Be involved in the induction of desensitization. *Dermatology* 209, 29–32. doi:10.1159/000078583

Turnis, M. E., Sawant, D. V., Szymczak-Workman, A. L., Andrews, L. P., Delgoffe, G. M., Yano, H., et al. (2016). Interleukin-35 limits anti-tumor immunity. *Immunity* 44, 316–329. doi:10.1016/j.immuni.2016.01.013

Tüzer, C., Sari, M., AktaşÇetin, E., Ak, N., Büyüköztürk, S., Çolakoğlu, B., et al. (2020). Rapid drug desensitization for platinum-based chemotherapy drugs significantly increases peripheral blood IL-10 levels. *Allergy*. doi:10.1111/all.14311

Van de Veen, W. (2017). The role of regulatory B cells in allergen immunotherapy. *Curr. Opin. Allergy Clin. Immunol.* 17, 447–452. doi:10.1097/ACI.0000000000000400

Van de Veen, W., Wirz, O. F., Globinska, A., and Akdis, M. (2017). Novel mechanisms in immune tolerance to allergens during natural allergen exposure and allergen-specific immunotherapy. *Curr. Opin. Immunol.* 48, 74–81. doi:10.1016/j.coi.2017.08.012

Vultaggio, A., and Castells, M. C. (2014). Hypersensitivity reactions to biologic agents. *Immunol. Allergy Clin.* 34, 615–632. doi:10.1016/j.iac.2014.04.008

Vultaggio, A., Maggi, E., and Matucci, A. (2011). Immediate adverse reactions to biologicals: from pathogenic mechanisms to prophylactic management. *Curr. Opin. Allergy Clin. Immunol.* 11, 262–268. doi:10.1097/aci.0b013e3283464bcd

Vultaggio, A., Nencini, F., Bormioli, S., Dies, L., Vivarelli, E., Maggi, E., et al. (2020). Desensitization modulates humoral and cellular immune response to infliximab in a patient with an immediate hypersensitivity reaction. *J. Allergy Clin. Immunol. Pract.* 8, 1764–1767. doi:10.1016/j.jaip.2019.12.040

Waldram, J., Walters, K., Simon, R., Woessner, K., Waalen, J., and White, A. (2018). Safety and outcomes of aspirin desensitization for aspirin exacerbated respiratory disease: a single center study. *J. Allergy Clin. Immunol.* 141, 250–256. doi:10.1016/j.jaci.2017.05.006

Zhang, J., Zhang, Y., Wang, Q., Li, C., Deng, H., Si, C., et al. (2019). Interleukin 35 in immune-related diseases: protection, or destruction. *Immunology* 157, 13–20. doi:10.1111/imm.13044

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Drug Allergy Profile From a National Drug Allergy Registry

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Background: Drug hypersensitivity reactions (DHRs) are among the most frequent reasons for consultation in allergy departments and are becoming more common due to increasing prevalence and case complexity.

Objective: To describe the most common drugs associated with clinical reactions, diagnostic methods used, and outcomes of allergic evaluations of a national drug allergy registry over a 12-year period were used.

Methods: An observational, prospective, patient's data registry-based study was conducted to analyze all referrals to the drug allergy outpatient clinics at Al-Rashed Allergy Center, Kuwait, between 2007 and 2019. Demographics, description of DHRs, and results of allergy tests to potential causative medications were reviewed. Diagnostic methods were focused mainly on skin tests (STs) and drug provocation test (DPT), when indicated.

Results: We evaluated 1,553 patients with reported DHRs. The mean age of the population was 41.52 ± 16.93 years, and the study population consisted of 63.7% female patients. Hypersensitivity was finally confirmed in 645 (41.5%) of patients, probable in 199 (12.8%), and not confirmed/nonallergic in 709 (45.6%) patients. Anti-inflammatory drugs and analgesics contributed to 39.22% of all confirmed drug allergies, followed by antibiotics 38.1% (β-lactam antibiotics (BLs) constituted 73.98% of all antibiotics and 28.21% of all drugs), anesthetics 1.8%, and radio-contrast media 0.31%. The majority of reactions were non-immediate 51.44%. The most commonly presenting symptoms among confirmed patients were urticaria 57.80%, angioedema 42.50%, respiratory symptoms 47.60%, and erythema 33.60%. Symptoms of anaphylaxis/anaphylactic shock were reported by 284 patients (44.00%) among confirmed cases. The most common method of diagnosis was a positive clinical history (54.4% in BLs and 90.45% in nonsteroidal anti-inflammatory drugs (NSAIDs). Among confirmed allergy to BLs, a positive ST was obtained in 31.9% of patients and positive DPT in 13.7%.

Conclusion: NSAIDs and antibiotics, mainly BLs, are the most commonly implicated in confirmed allergy. In both confirmed and not confirmed/nonallergic cases, BLs are the most frequently involved DHRs which are mainly immediate, and the most commonly presenting symptoms were urticaria, angioedema, and respiratory symptoms. Diagnosis was confirmed mainly by a positive clinical history and when indicated, by positive STs or a DPT.

Keywords: drug allergy, registry, hypersensitivity, allergy, NSAIDs, penicillin

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INTRODUCTION

A drug hypersensitivity reaction (DHR) can be defined as an adverse drug reaction (ADR), with an immunological etiology, to an otherwise safe and effective therapeutic agent (Park and Demoly, 2012; Böhm and Cascorbi, 2016). Type I hypersensitivity (IgE-mediated) reactions are the most studied among other DHRs that were described by Gell and Coombs (Demoly et al., 2008). DHRs are of significant concern for clinicians and patients as suspected cases may result in avoidance of first-line medications like in cases of suspected β-lactam antibiotic (BL) allergy that leads to worse outcomes and increased cost (Macy and Contreras, 2014; Su et al., 2017; Sousa-Pinto et al., 2018), and in consequence, both under- and overdiagnosis of DHRs are potential challenges in everyday practice. Although *in vivo* and *in vitro* testing including the gold standard drug provocation test (DPT) can confirm the diagnosis, and clinicians have to challenge problems such as the lack of standardized tests to most of the medications, the contraindication for DPT in severe cases, or patient refusal to undergo a DPT with the culprit drug. These problems push clinicians to accept the diagnosis of drug allergy based on clinical history alone on the cases that there is no standardized test or DPT is not as suitable option. Throughout the years, two main groups of drugs have consistently remained prevalent worldwide, BLs and nonsteroidal anti-inflammatory drugs (NSAIDs), with different clinical presentations like cutaneous symptoms of urticaria, angioedema, and respiratory symptoms, among others (Demoly et al., 2014). Many factors affect the DHRs, some are related to the drug itself as the ability to act as a hapten, prohapten, or binding to immune receptors and others to patient factors like female sex, age, history of drug reactions, concomitant infections, or genetics (HLA genotypes) (Gamboa, 2009; Thong and Tan, 2011).

Despite the fact that many studies utilize data from patient's database and electronic medical records, there are not many publications on specifically drug allergy databases, and none of the previously published ones belong to the Middle East region. The reasons might be related to the difficulties and challenges of maintaining and following up patients in a registry-based format. This might be due to the need of a specific database on drug allergy using common standardized procedures (Bousquet et al., 2009). The most remarkable existing database from Europe is the Drug Allergy and Hypersensitivity Database (DAHD) that has provided information regarding cross-reactivity with cephalosporins in confirmed allergic patients to BL (Sidoroff et al., 2010), and of other BLs in proven allergy to cefazolin (Pipet et al., 2011), the need for DPT after negative skin testing (Bousquet et al., 2008), risk of systemic reactions during skin testing (Co Minh et al., 2006), the accuracy of clinical history in patients presenting with reactions to BL (Chiriac et al., 2018), comparison of DHR prevalence in children and adults (Demoly et al., 2012), and NSAIDs patterns of reactions and possible classifications (Caimmi et al., 2012). The objective of this study, based on a national drug allergy registry, was to determine the prevalence, clinical presentation, and drug distribution of DHRs in a country from the Middle East.

METHODS AND MATERIALS

Al-Rashed Allergy Center is a tertiary public center in Kuwait, and it is a referral center for all drug allergy evaluation in the country, covering both public and private health systems. An initial drug allergy evaluation is performed on all patients referred to our clinic for suspected DHRs, and patients presenting with a suggestive history of DHRs from July 2007 to June 2019 were included in this study. The following data were collected: patient demographics (age and gender), drug(s) involved in the clinical reaction, signs and symptoms of DHRs (as reported by the patient and/or obtained from their medical records), time of onset of DHRs after drug(s) exposure, results of DPT when indicated, and results of the final evaluation. All patients were evaluated by a detailed clinical history related to ADR or DHR including physical examination (Demoly et al., 1999).

Regarding symptoms, different clinical categories were established; anaphylaxis was defined as per the WHO criteria as a serious allergic reaction that is rapid in onset and might cause death (Simons et al., 2011) and anaphylactic shock, defined as those with anaphylaxis and signs of critical organ hypoperfusion (Thong et al., 2006). Urticaria was defined as hives, angioedema as swelling of the skin, erythema as redness of the skin or mucous membranes, and respiratory symptoms as shortness of breath from upper or lower airways.

Patients were included in the group "multiple" when they refer to the same symptoms upon exposure to three or more different groups of medication. In an attempt to include all groups of drugs that were reported by the patients in our registry, patients were included in the group "others" when they were the only patient in our registry reporting a reaction to a specific group of drugs, and on the contrary, on those cases where more than a single patient refers to symptoms of a specific group of drugs, the group was named by the name of the drug itself (i.e., NSAIDs).

All patients were asked to determine the approximate time elapsed since the intake of the drug and the start of the reaction. Immediate reaction was defined when presenting symptoms, compatible with hypersensitivity reaction, appear till 1 h after drug administration and nonimmediate reaction was defined when presenting symptoms appear after 1 h.

In our cohort, the cultural background of Middle Eastern patients had a strong influence on the way we based our allergy testing. Our patients are usually less prone to assume DPT risks, and they are favoring a safe testing with alternatives, whenever possible.

Patients were grouped according to the following three categories:

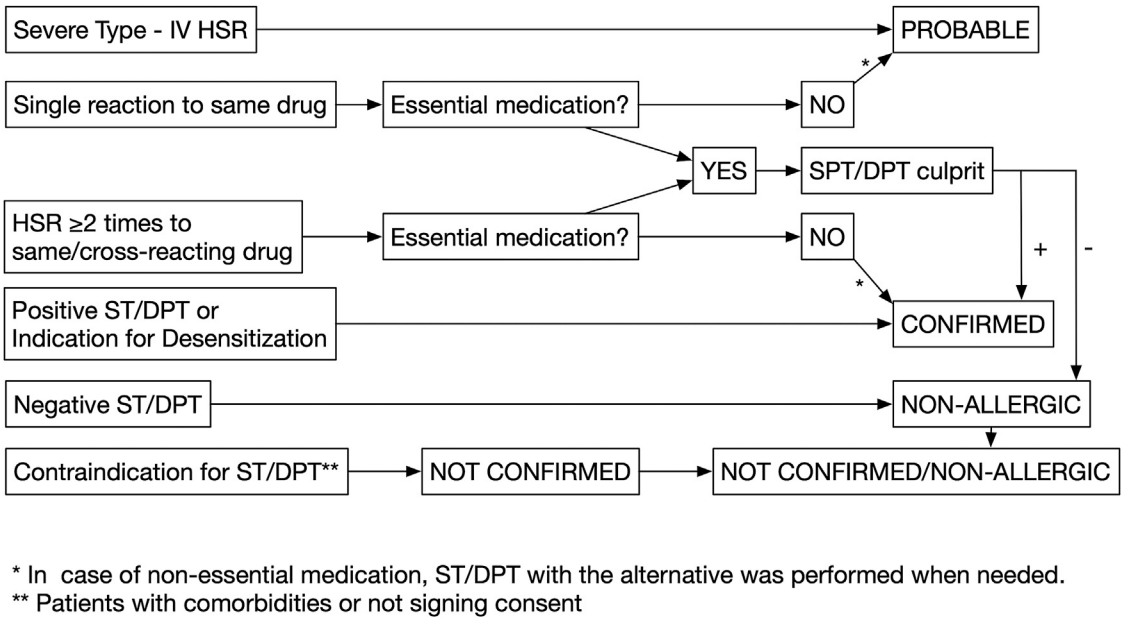

FIGURE 1 | Diagnostic methods flowchart.

(1) Confirmed drug allergy: When patients had a positive clinical history alone, defined as symptoms compatible with type I hypersensitivity reactions (immediate), including pruritus, urticaria/angioedema, shortness of breath, on two or more occasions to the same or cross-reacting drugs (graph1), or positive ST/DPT, or if they had an indication for desensitization (Decker et al., 2010).
(2) Probable drug allergy: When patients had a single reaction to the offending drug, or in those presenting with severe cutaneous reactions such as SJS/TEN (Stevens–Johnson syndrome/toxic epidermal necrolysis), AGEP (acute generalized exanthematous pustulosis), and DRESS/DIHS/HSS (drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome/hypersensitivity syndrome).
(3) Not confirmed/nonallergic: Patients are defined as not confirmed when they did not consent for DPT, despite being indicated and in case of contraindication for DPT due to comorbidities or other factors: acute infections; cardiac, hepatic, or renal diseases; pregnancy; breastfeeding; or receiving beta-blockers. Patients are defined as nonallergic when they had negative DPT.

An allergy workup was performed on patients with the following drug categories (**Figure 1**):

(1) Essential medications: Antibiotic, NSAIDs, monoclonal antibodies, chemotherapy, proton pump inhibitor, corticosteroids, antidiabetic drugs, antihypertensive drugs, anticoagulants, general anesthesia, anticonvulsants, allopurinol, supplemental drugs (iron and vitamin D), and interferon. Skin tests (STs) and DPT with the culprit drug were used to confirm the diagnosis in these cases. However, when a suitable same efficacy alternative drug is available on those cases with a positive DPT with the culprit drug, additional tests, STs, and DPTs with the suitable alternative were considered in case of cross-reactivity.
(2) Nonessential medications: Other supplemental drugs, hyoscine, antihistamines, and local anesthesia. DPT with the culprit drug was not performed, and instead patients were tested with STs and DPTs to a suitable alternative in case of possible cross-reactivity.

On those patients reporting reactions to radio-contrast media (RCM), the diagnosis was confirmed by STs. If STs were negative, a premedication prior to next infusion was recommended (Pichler, 2010 and American Academy of Allergy; Demoly et al., 2014) and desensitization was performed, if premedication fails (Al-Ahmad and Bouza, 2017).

Patients presenting with erythema alone were tested to the offending medication. A risk assessment was performed by the staff for each individual patient presenting with DHRs, and the decision to proceed with STs/DPTs was decided on an individual basis.

SKIN TESTING

STs were performed according to the European Network of Drug Allergy (ENDA) (Brockow et al., 2002) guidelines. STs were performed using the dilutions shown in **Table 1**. The prick test was initially performed, and in those with negative results, was followed by intradermal testing. Intradermal testing is done

TABLE 1 | Concentrations of the different drugs used for skin prick testing (SPT) and intradermal testing (ID).

Drug	SPT	ID	DPT
PPL	0.04 mg/ml	0.0004–0.04 mg/ml	Amoxicillin/clavulanate 875/125 mg
MDM	0.5 mg/ml	0.005–0.5 mg/ml	Same as above
Amoxicillin	20 mg/ml	0.2 mg/ml	Same as above
Clavulanic acid	5 m-20 mg/ml	0.05–20 mg/ml	Same as above
Ampicillin	25 mg/ml	0.025–25 mg/ml	Same as above
Penicillin G	10,000 U/ml	10–1,000 U/ml	Same as above
Meropenem	1 mg/ml	0.1–1 mg/ml	1 gm
Cephalosporins	2 mg/ml	0.002, 2 mg/ml	Ceftriaxone 2 gm I.V or Cefuroxime 500 mg oral
Hydrocortisone	2, 20 mg/ml	0.2, 2 mg/ml	Dexamethasone 5 mg
Methylprednisolone	2, 20 mg/ml	0.2, 2 mg/ml	
Iohexol	350 mg I/ml	35, 350 mg I/mL	NA
Iodixanol	320 mg/ml	3.2, 320 mg/ml	
Lidocaine	20 mg/ml	2 mg/ml	Lidocaine 2 ml 0f 20 mg/ml

Abbreviations: *PPL, penicilloyl-polylysine; MDM, minor determinant mixture; amoxicillin/clavulanic acid (diater Madrid, Spain), iohexol (GE Health care), ampicillin sodium equivalent to 500 mg ampicillin activity (Bristol-Myers Squibb, United States), Hymox Forte in powder form (Biocheme Spimaco, Saudi Arabia), or amoxicillin commercial kit or clavulanic acid commercial kit (cephalosporin), Penicillin G (Sandoz Gmb H, Kundl-Astria/ Autriche Sanduz), meropenem (AstraZeneca, UK), cefuroxime (Glaxo, Italy), or ceftriaxone (Sandoz, Austria).*

by marking the bleb created by the injection of 0.3 ml. An immediate positive response was considered when an increase in the diameter of the wheal area was greater than 3 mm than the saline control and accompanied by erythema that is read 15–20 min after testing (Demoly and Bousquet, 2002). A reading was done after 24–48 h in the case of nonimmediate reactions. Patch testing was performed in suspicion of type IV reactions.

DPT

If STs were negative or not available, DPT with the suspected drug was performed (Aberer et al., 2003; Torres et al., 2003). Single-blind placebo-controlled DPT was performed following the ENDA general guidelines (Torres et al., 2003), with slight modifications in some cases. Drugs were administered at increasing doses every 30–90 min until the full therapeutic dose was reached. In patients with reactions induced by NSAIDs, DPT was performed as previously described (Doña et al., 2011). When patient-reported symptoms (e.g., skin and respiratory) or changes in vital signs were observed (heart rate and blood pressure) or a decrease in the peak expiratory flow rate (PEFR), the procedure was stopped, and patients' symptoms were evaluated and treated. If patients tolerated the given drug, they were advised to report any nonimmediate reactions and were considered negative DPT.

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

ETHICS COMMITTEE

All patients were informed about the risk and outcomes of the procedure and provided informed consent. Ethical clearance was granted by Kuwait Research Ethics Committee at the Ministry of Health (Research study number 808/2018).

STATISTICAL ANALYSIS

Nonparametric and parametric methods are used to calculate statistical significance. The distribution value is determined by D'Agostino and Pearsonomnibus test normality. Student's t-test, Mann–Whitney test, Fisher's test, and $\chi 2$ test were used for calculating the difference between the groups. The ANOVA test was used to calculate the relative difference distribution variance between variables. The statistical hypotheses were tested at a level of $\alpha = 0.05$, and the difference between the groups in the sample was considered significant when $p < 0.05$ or less. Statistical significance was depicted as $p < 0.05$, $p < 0.01$, and $p < 0.001$. All data were analyzed using GraphPad Prism version 7 (San Diego, California, United States). All the percentages in the tables were calculated from the pooled group of patients, and the difference between pooled groups was calculated using the $\chi 2$ test.

RESULTS

Description of the Total Sample

We have evaluated 1,553 patients with a history compatible of DHRs, with 65.58% females with a mean age of 41.52 ± 16.93 years. Among all episodes, 42.18% were attributed to antibiotics (32.13% to BLs) and 28.65% to anti-inflammatory drugs and analgesics (24.66% to NSAIDs). Drug allergy was confirmed in 645 (41.5%), probable in 199 (12.8%), and not

TABLE 2 | Clinical characteristics among patients with confirmed and not confirmed/nonallergic.

Clinical characteristic		Confirmed (n = 645; 41.5%)		Probable (n = 199; 12.8%)		Not confirmed/ nonallergic (n = 709; 45.6%)		*p* value
Age (years)		42.6 ± 16.02		40.7 ± 17.3		43.1 ± 17.6		0.0625
Females (n; %)		432	66.98	126	63.3	463	65.03	0.6014
Culprit drug	Allopurinol	2	0.3	4	2.0	2	0.3	0.0069[a]
	Anti-inflammatory and analgesics	253	39.2	30	15.1	162	22.8	<0.0001[a]
	Anesthetic	12	1.9	1	0.5	47	6.6	<0.0001[a]
	Antibiotics	246	38.1	72	36.2	337	47.5	0.0004[a]
	Anticholinergic	1	0.2	0	0.0	1	0.1	0.8609
	Anticoagulants	4	0.6	2	1.0	4	0.6	0.7859
	Anticonvulsants	3	0.5	7	3.5	0	0.0	<0.0001[a]
	Antidiabetics	6	0.9	0	0.0	4	0.6	0.3351
	Antihistamines	4	0.6	3	1.5	5	0.7	0.4406
	Antihypertensive	4	0.6	0	0.0	2	0.3	0.3890
	Chemotherapy	4	0.6	3	1.5	2	0.3	0.1299
	Corticosteroids	8	1.2	2	1.0	14	2.0	0.4415
	Hormones	6	0.9	2	1.0	2	0.3	0.2615
	Hyoscine	4	0.6	0	0.0	0	0.0	0.0594
	Interferon	1	0.2	1	0.5	0	0.0	0.2112
	Monoclonal antibodies	7	1.1	3	1.5	3	0.4	0.2209
	Multiple[b]	30	4.7	33	16.6	38	5.4	<0.0001[a]
	Others[c]	32	5.0	25	12.6	16	2.3	<0.0001[a]
	Proton pump inhibitors	8	1.2	2	1.0	2	0.3	0.1221
	Prostaglandin inhibitors	0	0.0	2	1.0	0	0.0	0.0011[a]
	Radio-contrast media	2	0.3	1	0.5	60	8.5	<0.0001[a]
	Supplementals	8	1.2	6	3.0	8	1.1	0.1221
	β lactams[##]	182	28.2	21	10.6	296	41.7	<0.0001[a]
	Quinolones	23	3.6	16	8.0	19	2.7	0.0019[a]
	Macrolides	18	2.8	12	6.0	15	2.1	0.0142[a]
	Sulphomides	13	2.0	7	3.5	3	0.4	0.0021[a]
	Metronidazole	3	0.5	2	1.0	1	0.1	0.2027
	NSAIDs	220	34.1	22	11.1	141	19.9	<0.0001[a]
	Paracetamol	28	4.3	7	3.5	20	2.8	0.3190
	Opioids	5	0.8	1	0.5	1	0.1	0.0282[a]
Clinical symptoms	Urticaria	373	57.8	119	59.8	283	39.9	<0.0001[a]
	Angioedema	274	42.5	132	66.3	226	31.9	<0.0001[a]
	Erythema	217	33.6	173	86.9	285	40.2	<0.0001[a]
	Respiratory	307	47.6	109	54.8	142	20.0	<0.0001[a]
	Anaphylaxis/anaphylactic shock	284	44.0	99	49.7	77	10.9	<0.0001[a]
Timing	Immediate	425	65.9	25	12.6	304	42.9	<0.0001[a]
	Nonimmediate	220	34.1	174	87.4	405	57.1	
Time elapsed between reaction and study	≤1 year	435	67.4	162	81.4	423	59.7	<0.0001[a]
	>1 year	210	32.6	37	18.6	286	40.3	

[a]*Difference was significant statistically.*

[b]Multiple*: When there was a patient in our registry reporting the same symptoms upon exposure to three or more different groups of medication.*

[c]Others: *When there was only one patient in our registry reporting a reaction to a specific group of drugs.*

confirmed/nonallergic in 709 (45.6%) of patients. Of the total studied patients for each drug, confirmation was obtained in 38.10% of the patients for antibiotics (28.20% for BLs) and 39.2% for anti-inflammatory drugs and analgesics (34.10% for NSAIDs) (**Table 2**).

Age and Gender

Patients with confirmed, probable, and not confirmed/nonallergic, with immediate and nonimmediate reactions, and with time elapsed between the reaction and study ≤1 and >1 year showed a similar age and gender distribution ($p > 0.05$) (**Table 3**).

Comparison of Immediate vs. Nonimmediate Reactions

Patients with confirmed allergy showed more frequently immediate reaction (65.90%) than probable drug allergy (12.6%) and not confirmed/nonallergic (42.9%) $p < 0.0001$ (**Table 2**). Timing of reactions was immediate, ≤1 h, in 48.55% of the patients, and nonimmediate, >1 h, in 51.44%. The ratio of frequency of immediate and nonimmediate reaction was 0.94. In patients with confirmed drug allergy, the frequency ratio of immediate and nonimmediate reactions was 1.93 (**Table 4**). In patients with confirmed drug allergy, allergy to antibiotics and BLs was more common in patients with

TABLE 3 | Clinical characteristics among *all patients* in regard to immediate and nonimmediate drug allergy reactions.

Clinical characteristic		Immediate (n = 754; 48.55%)		Nonimmediate (n = 799; 51.45%)		*p* value
Age(years)		41.3 ± 16.4		40.7 ± 17.4		0.1402
Females (n; %)		497	65.9	524	65.6	0.9148
Drug involved	Antibiotics	308	40.8	347	43.4	0.3045
	Analgesics	267	35.4	178	22.3	<0.0001[a]
	β lactams	251	33.3	248	31.0	0.3556
	NSAIDs	225	29.8	158	19.8	<0.0001[a]
Clinical symptoms	Urticaria	388	51.5	387	48.4	0.2431
	Angioedema	304	40.3	328	41.1	0.7962
	Erythema	237	31.4	438	54.8	<0.0001[a]
	Respiratory	359	47.6	199	24.9	<0.0001[a]
	Anaphylaxis/anaphylactic shock	308	40.8	152	19.0	<0.0001[a]
Time elapsed between reaction and study	≤1 year	506	67.1	514	64.3	0.2616
	>1 year	248	32.9	285	35.7	

[a]*Difference was significant statistically.*

TABLE 4 | Clinical characteristics among patients with confirmed drug allergy in regard to immediate and nonimmediate drug allergy reactions.

Clinical characteristic		Immediate (n = 425; 65.9%)		Non-immediate (n = 220; 34.1%)		*p* value
Age (years)		42.7 ± 15.7		41.1 ± 16.6		0.8995
Females (n; %)		293	68.9	139	63.2	0.1577
Drug involved	Antibiotics	171	40.2	59	26.8	0.0007[a]
	Analgesics	194	45.6	75	34.1	0.0054[a]
	β lactams	134	31.5	48	21.8	0.0098[a]
	NSAIDs	167	39.3	53	24.1	0.0001[a]
Clinical symptoms	Urticaria	249	58.6	124	56.4	0.6142
	Angioedema	194	45.6	80	36.4	0.0288[a]
	Erythema	126	29.6	91	41.4	0.0037[a]
	Respiratory	267	62.8	40	18.2	<0.0001[a]
	Anaphylaxis/anaphylactic shock	248	58.4	36	16.4	<0.0001[a]
Time elapsed between reaction and study	≤1 year	286	67.3	149	67.7	0.9296
	>1 year	139	32.7	71	32.3	

[a]*Difference was significant statistically.*

TABLE 5 | Frequency of symptoms and reactions on specific drug allergy is done in confirmed allergy only.

		Antibiotics (n = 246)		Analgesics (n = 253)		*p* value	β lactams (n = 182)		NSAIDs (n = 220)		*p* value
		n	%	N	%		n	%	n	%	
Symptoms	Urticaria	161	65.4	130	51.4	0.0015[a]	119	65.4	108	49.1	0.0012[a]
	Angioedema	103	41.9	117	46.2	0.3672	75	41.2	102	46.4	0.3142
	Erythema	84	34.1	75	29.6	0.2918	56	30.8	61	27.7	0.5103
	Respiratory symptoms	123	50.0	140	55.3	0.2446	101	55.5	124	56.4	0.9197
	Anaphylaxis/anaphylactic shock	125	50.8	107	42.3	0.0599	103	56.6	91	41.4	0.0026[a]
Timing	Immediate reaction	171	69.5	194	76.7	0.0857	134	73.6	167	75.9	0.6445
	Nonimmediate reaction	75	30.5	59	23.3		48	26.4	53	24.1	

[a]*Difference was significant statistically.*
Note: These symptoms do overlap.

immediate reaction, while in patients with nonimmediate reaction, hypersensitivity to analgesics and NSAIDs was more common (**Table 4**). Among patients with confirmed drug allergy, immediate and nonimmediate reactions were similarly distributed between allergy to antibiotics and analgesics (**Table 5**).

TABLE 6 | Clinical characteristics of patients with confirmed drug allergy in regard to anaphylaxis.

Clinical characteristic		With anaphylaxis/ anaphylactic shock (n = 284; 44.03%)		Without anaphylaxis/ anaphylactic shock (n = 361; 55.97%)		*p* value
Age (years)		43.4 ± 15.7		46.2 ± 16.3		0.6409
Females (n; %)		206	72.5	226	62.6	0.0089[a]
Drug involved	Antibiotics	125	44.0	121	33.5	0.0071[a]
	Analgesics	107	37.7	146	40.4	<0.0001[a]
	β lactams	103	36.3	79	21.9	0.3576
	NSAIDs	91	32.0	129	35.7	<0.0001[a]
Timing	Immediate	248	87.3	177	49.0	<0.0001[a]
	Nonimmediate	36	12.7	184	51.0	
	≤1 year	198	69.7	237	65.7	0.3098
	>1 year	86	30.3	124	34.3	

[a]*Difference was significant statistically.*

Time Since Reaction to Study

Patients with confirmed drug allergy showed more frequent time elapsed between the reaction and study ≤1 year than not confirmed/nonallergic patients, but less frequent than patients with probable allergy (**Table 2**). The time elapsed between reaction and study >1 year was similar to the ones without anaphylaxis/anaphylactic shock (**Table 6**).

Comparison of Confirmed and Not Confirmed Cases

Patients with confirmed drug allergy showed more frequent ($p < 0.05$) allergy to analgesics, NSAIDs, and opioids than patients with probable drug allergy and not confirmed/nonallergic. However, patients with probable drug allergy showed more frequent allergy to allopurinol, anticonvulsants, multiple drugs, other drugs, prostaglandin inhibitors, quinolones, macrolides, and sulphomides than patients with confirmed and not confirmed/nonallergic. Furthermore, not confirmed/nonallergic patients showed more frequent allergy to anesthetics, antibiotics, radiocontrast media, and β lactams than patients with probable and confirmed drug allergy (**Table 2**).

All symptoms (urticaria, angioedema, respiratory symptoms, and anaphylaxis) were more common in patients with confirmed and probable drug allergy, rather than in not confirmed/nonallergic patients, with the exception of erythema, which was most common in patients with probable allergy (**Table 2**). Anaphylaxis was shown in 44.00% of confirmed patients, which was 18.28% of the total population (**Table 2**). In this group of patients, angioedema, erythema, respiratory symptoms, and anaphylaxis were similarly distributed in allergy to antibiotics and analgesics. However, urticaria was more frequent in allergy to antibiotics than in analgesics allergy. Urticaria and anaphylaxis were more common in BL than NSAID hypersensitivity, while angioedema, erythema, respiratory symptoms, and immediate and nonimmediate reactions were similarly distributed between BL and NSAID hypersensitivity (**Table 5**). In patients with confirmed drug allergy, patients with anaphylaxis were younger than those without anaphylaxis, but these differences were not statistically significative ($p = 0.6409$) (**Table 6**). Patients with anaphylaxis/anaphylactic shock showed more common allergy to antibiotics, but less common to analgesics and NSAIDs, than patients without anaphylaxis/anaphylactic shock (**Table 6**). Among the anaphylactic cases, antibiotics were the culprit in 44% of cases, whereas anti-inflammatory drugs and analgesics as a group was responsible in 37.7% (<0.0001) (**Table 6**).

In regard to diagnosis, the most common method was a positive clinical history (54.4% in BLs and 90.45% in nonsteroidal anti-inflammatory drugs (NSAIDs)). Among confirmed allergy to BLs, positive ST was obtained in 31.9% of patients and positive DPT in 13.7% (**Table 7**). Among patients with confirmed drug allergy, allergy diagnosis was made more frequently by positive history alone for the following drugs: BLs, quinolones, macrolides, metronidazole, sulphomides, NSAIDs, paracetamol, opioids, and RCM (**Table 7**).

DISCUSSION

To our knowledge, this is the first published drug allergy database in the Middle East region. We hypothesize that this might be due

TABLE 7 | Diagnostic methods in confirmed drug allergy.

Drug	Positive by history only		Positive by skin prick test		Positive by DPT		*p* value
	n	%	n	%	n	%	
β lactams (n = 182)	99	54.4	58	31.9	25	13.7	<0.0001[a]
Quinolones (n = 23)	20	86.9	0	0.0	3	13.1	<0.0001[a]
Macrolides (n = 18)	18	100.0	0	0.0	0	0.0	<0.0001[a]
Metronidazole (n = 3)	3	100.0	0	0.0	0	0.0	<0.0001[a]
Sulphomides (n = 13)	13	100	0	0.0	0	0.0	<0.0001[a]
NSAIDs (n = 220)	199	90.45	0	0.0	9	9.54	<0.0001[a]
Paracetamol (n = 28)	25	89.29	0	0.0	3	10.71	<0.0001[a]
Opioids (n = 5)	5	100	0	0.0	0	0.0	<0.0001[a]
RCM (n = 2)[b]	0	0.0	0	0.0	0	0.0	—

[a]*Difference was significant statistically.*
[b]*Desensitization was done for 12 patients who had reaction to NSAIDs.*
[c]*Desensitization was done for two patients who had reaction to RCM.*
DPT, drug provocation test.

not only to the complexity and time-consuming task of developing a standardized database in a registry-based format (Bousquet et al., 2009) but also to other factors as the recently developed electronic databases, the relatively recent increased development of the health systems in Middle East compared with those in Europe and North America. This is a drug allergy registry–based study that was done over 12-year duration. The diagnosis in our study was confirmed in 41.5% of cases, and this compares to other European studies where drug allergy was confirmed in 37.4% and not confirmed/nonallergic in 62.6% (including 13.4% with contraindications for testing) (Doña et al., 2012), and American studies, where at least one drug allergy was confirmed in 19.66% of patients (Blanca et al., 2020). This difference can be attributed to the confirmation criteria that were adapted in our study, which specifically include patients who had a positive clinical history alone. This is a key factor to understand some of the diagnostic differences with other studies.

Our sex and age distribution is similar to other studies that report 64.58–71.9% of females, with a mean age of 43.7–48.9 years (Doña et al., 2012; Gabrielli et al., 2018; Blanca et al., 2020). In our cohort, reactions occurred ≤1 h in 48.55% of all the patients and ≥1 h in 51.44%. Interestingly, these results are similar to those of a study by Bousquet PJ et al. (Bousquet et al., 2008), which excluded type IV reactions and found that reactions occurring ≤1 h after drug intake in 36.6% of patients. However, other studies (Ben-Shoshan et al., 2018) focused on BL reactions and found a reaction ≤1 h after drug intake in 19.9% and after 24 h in 34.4%.

Many studies have evaluated the timing since reaction; interestingly, the average delay was 299.7 months in a large study, which was reduced to 43 months on those confirmed for one drug and 76.9 months for multiple drugs (Blanca et al., 2020). On the other hand, a study with BLs showed an average of 54.7 months for not confirmed/nonallergic and 25.8 months for confirmed patients (Bousquet et al., 2008). These results are consistent with ours, with confirmed and probable patients presenting earlier to our clinic for consultation.

The number of confirmed and probable patients showing a time elapse since reaction <1 year was significantly higher than for those not confirmed/nonallergic, and this is consistent with other studies following the decrease in positive ST responses after 1, 3, and 5 years which showed a decrease of 68.1, 50, and 36.1% for cephalosporins (Demoly et al., 2003) or 80.6, 78.3, and 70.6% for patients presenting positive STs to benzylpenicilloyl (BPO) or minor determinant mixture (MDM) or 50, 54, and 0% for patients reacting to amoxicillin side chains (Blanca et al., 1999). This decrease in sensitivity over time has also been reported in NSAIDs for NIUA (NSAID-induced urticaria/angioedema) and SNIUAA (single NSAID-induced urticaria/angioedema and anaphylaxis) (Doña et al., 2020).

Of the total studied patients for each drug, confirmation was obtained in 42.17% of the patients with antibiotics (32.13% for BLs) and 28.65% with anti-inflammatory drugs and analgesics (24.66% for NSAIDs). In other studies, hypersensitivity to NSAIDs was confirmed in 19.6–27% and BLs in 18.4–6.99% (Bourke et al., 2015; Cornejo-García et al., 2019; Blanca et al., 2020). Confirmation was reached for BLs in 45.6% of the patients by means of STs or DPTs, and the remaining by clinical history alone. When compared with American studies of patients allergic to BLs, one study showed that 7.35% of tested individuals had positive penicillin ST results, with only 1.6% of the negative ST patients had a reaction to the DPT (Macy et al., 2009). Another study (Blanca et al., 2020) showed that 14.14% of tested individuals had positive ST or DPT results. In an Australian study that evaluated the effectiveness of penicillin allergy delabeling of 341 patients, a positive ST was found in 42 (12.3%) of patients, which was similar to our findings, in which 58 of 499 BL patients (11.62%) had positive STs (Bourke et al., 2015). In comparison to European studies, our results compare with a multicenter study that included patients with reactions to BLs only (Chiriac et al., 2018; Ben-Shoshan et al., 2018), in which 23.6% of the studied patients were confirmed as allergic by means of STs or DPTs only. Furthermore, the number of confirmed patients in these studies was lower than ours as positive testing was generally required for confirmation. Previous studies from our group showed that our data compare more with European than with American studies (Al-Ahmad et al., 2014; Al-Ahmad and Rodriguez-Bouza, 2018); this multicenter study can provide an idea of additional patient's number required to be positive using our criteria of positive BL allergy based on clinical history alone.

The frequency of drug allergy types varied among different studies. In some studies (Doña et al., 2012; Çelik et al., 2014), 31.9–37% of the episodes were attributed to NSAIDs and 20.4–28.1% to BL antibiotics (Doña et al., 2012; Çelik et al., 2014; Gabrielli et al., 2018), and the most frequent drug allergy was to multiple NSAIDs 47.29%, followed by immediate reactions to BLs 18.12% (Doña et al., 2012), and these findings are similar to ours. However, another study (Gabrielli et al., 2018) reported 20.3% of patients were confirmed to NSAIDs, which was lower than our study, and 57.8% of the reactions were due to antibiotics, which was higher than ours.

In the study by Doña et al. (Doña et al., 2012), the diagnosis was established by clinical history in 742 patients (44%), by SPTs in 246 patients (14.6%), by *in vitro* testing in 176 patients (10.4%), and by DPT in 519 patients (30.8%). This was different from our results. We had about 79.58% patients diagnosed by clinical history alone, 12.08% by SPT, and 8.33 by DPT. These differences are explained by the escalating preferences of patients for more conservative approaches including alternative treatments, rather than performing DPT with the culprit drug.

STs or serum-specific IgE antibodies were used as methods of diagnosis in BL reactions in 70–82% of patients and DPT in 18–30% (Bousquet et al., 2008; Kalyoncu et al., 2016), and this was in concordance with our findings, where the method to confirm diagnosis in BLs was STs in 31% of patients and a DPT in 13.7%. The observed difference was likely due to not performing STs in patients with anaphylactic reactions to BLs, and instead performing drug testing with suitable alternatives (Al-Ahmad et al., 2014; Al-Ahmad and Rodriguez-Bouza, 2018). Of the five confirmed patients for opioids reactions, the diagnosis was elucidated from a positive history only (100% of patients), compared to other studies, where they used DPT as the main diagnostic method. These differences are due to a more

conservative approach where a suitable alternative could be found (Li et al., 2017; Powell et al., 2019).

We are aware of some limitations in our study. The most important limitation of the study is the mixing of unconfirmed and nonallergic patients in the same category. Our group was forced to do so because we were using real-life data from a registry, and even if the outcome of the test is very likely to be negative, patients who rejected or had contraindication for DPT cannot be called nonallergic, and patients with negative DPT are simply "nonallergic" and cannot be called not confirmed. The use of clinical history alone as a positive criterion should not be used if we rely on other diagnostic testing. Another limitations of the study included that some patients were unsure on which drug caused the reaction, the temporal correlation after drug exposure due to recall bias, the relatively small study population, especially for less common drug reactions, and that atopy was not routinely assessed in all patients, and therefore, atopy could not be studied as a risk factor. However, this is a prospective data-based registry, and ongoing data collection might address some of these issues in the near future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Kuwait Research Ethics Committee at the Ministry of Health (Research study number 808/2018). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

Aberer, W., Bircher, A., Romano, A., Blanca, M., Campi, P., Fernandez, J., et al. (2003). Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. *Allergy* 58, 854–863. doi:10.1034/j.1398-9995.2003.00279.x

Al-Ahmad, M., and Bouza, T. R. (2017). Successful desensitization to radiocontrast media in two high-risk cardiac patients. *Ann. Saudi Med.* 37, 333–335. doi:10.5144/0256-4947.2017.333

Al-Ahmad, M., and Rodriguez-Bouza, T. (2018). Drug allergy evaluation for betalactam hypersensitivity: cross-reactivity with cephalosporines, carbapenems and negative predictive value. *Asian Pac. J. Allergy Immunol.* 36, 27–31. doi:10.12932/AP0853

Al-Ahmad, M., Rodriguez Bouza, T., and Arifhodzic, N. (2014). Penicillin allergy evaluation: experience from a drug allergy clinic in an Arabian Gulf Country, Kuwait. *Asia Pac Allergy* 4, 106–112. doi:10.5415/apallergy.2014.4.2.106

Ben-Shoshan, A. M., Wang, Y., Schrijvers, R., Bousquet, P. J., Mura, T., Molinari, N., et al. (2018). Designing predictive models for beta-lactam allergy using the drug allergy and hypersensitivity database. *J. Allergy Clin. Immunol. Pract.* 6, 139–148.e2. doi:10.1016/j.jaip.2017.04.045

Blanca, M., Torres, M. J., García, J. J., Romano, A., Mayorga, C., de Ramon, E., et al. (1999). Natural evolution of skin test sensitivity in patients allergic to beta-lactam antibiotics. *J. Allergy Clin. Immunol.* 103, 918–924. doi:10.1016/s0091-6749(99)70439-2

Blanca, Q., Zhang, S., Ferrando, L., Bourrain, J. L., Demoly, P., and Chiriac, A. M. (2020). Multiple drug hypersensitivity syndrome in a large database. *J. Allergy Clin. Immunol. Pract.* 8, 258–266.e1. doi:10.1016/j.jaip.2019.06.009

Böhm, R., and Cascorbi, I. (2016). Pharmacogenetics and predictive testing of drug hypersensitivity reactions. *Front. Pharmacol.* 7, 396. doi:10.3389/fphar.2016.00396

Bourke, J., Pavlos, R., James, I., and Phillips, E. (2015). Improving the effectiveness of penicillin allergy de-labeling. *J. Allergy Clin. Immunol. Pract.* 3, 365–34.e1. doi:10.1016/j.jaip.2014.11.002

Bousquet, P. J., Demoly, P., Romano, A., Aberer, W., Bircher, A., Blanca, M., et al. (2009). Pharmacovigilance of drug allergy and hypersensitivity using the ENDA-DAHD database and the GALEN platform. The Galenda project. *Allergy* 64, 194–203. doi:10.1111/j.1398-9995.2008.01944.x

Bousquet, P. J., Pipet, A., Bousquet-Rouanet, L., and Demoly, P. (2008). Oral challenges are needed in the diagnosis of beta-lactam hypersensitivity. *Clin. Exp. Allergy* 38, 185–190. doi:10.1111/j.1365-2222.2007.02867.x

Brockow, K., Romano, A., Blanca, M., Ring, J., Pichler, W., and Demoly, P. (2002). General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 57, 45–51. doi:10.1034/j.1398-9995.2002.13027.x

Caimmi, S., Caimmi, D., Bousquet, P. J., and Demoly, P. (2012). How can we better classify NSAID hypersensitivity reactions?--validation from a large database. *Int. Arch. Allergy Immunol.* 159, 306–312. doi:10.1159/000337660

Çelik, G. E., Karakaya, G., Öztürk, A. B., Gelincik, A., Abadoğlu, O., Sin, A., et al. (2014). Drug allergy in tertiary care in Turkey: results of a national survey. The ADAPT study: adult drug allergy perception in Turkey. *Allergol. Immunopathol.* 42, 573–579. doi:10.1016/j.aller.2013.07.007

Chiriac, A. M., Wang, Y., Schrijvers, R., Bousquet, P. J., Mura, T., Molinari, N., et al. (2018). Designing predictive models for beta-lactam allergy using the drug allergy and hypersensitivity database. *J Allergy Clin. Immunol. Pract.* 6, 139–148.e2. doi:10.1016/j.jaip.2017.04.045

Co Minh, H. B., Bousquet, P. J., Fontaine, C., Kvedariene, V., and Demoly, P. (2006). Systemic reactions during skin tests with beta-lactams: a risk factor analysis. *J. Allergy Clin. Immunol.* 117, 466–468. doi:10.1016/j.jaci.2005.10.020

Cornejo-García, F., Atanaskovic-Markovic, M., Blanca-Lopez, N., Gomes, E., Gaeta, F., Sarti, L., et al. (2019). A multicenter retrospective study on hypersensitivity reactions to nonsteroidal anti-inflammatory drugs (NSAIDs) in children: a report from the European Network on drug allergy (ENDA) group. *J. Allergy Clin. Immunol. Pract.* 8, 1022–1031.e1. doi:10.1016/j.jaip.2019.10.049

Decker, J. R., Brockow, K., Romano, A., Aberer, W., Torres, M. J., Bircher, A., et al. (2010). General considerations on rapid desensitization for drug hypersensitivity – a consensus statement. *Allergy* 65, 1357–1366. doi:10.1111/j.1398-9995.2010.02441.x

Demoly, P., and Bousquet, J. (2002). Drug allergy diagnosis work up. *Allergy* 57 (Suppl. 72), 37–40. doi:10.1034/j.1398-9995.57.s72.7.x

Demoly, A., Mondino, C., Viola, M., and Montuschi, P. (2003). Immediate allergic reactions to beta-lactams: diagnosis and therapy. *Int. J. Immunopathol. Pharmacol.* 16, 19–23. doi:10.1177/039463200301600103

Demoly, M., Bousquet, P. J., Gomes, E., Romano, A., and Demoly, P. (2012). Results of drug hypersensitivity evaluations in a large group of children and adults. *Clin. Exp. Allergy* 42, 123–130. doi:10.1111/j.1365-2222.2011.03887.x

Demoly, P., Adkinson, N. F., Brockow, K., Castells, M., Chiriac, A. M., Greenberger, P. A., et al. (2014). International consensus on drug allergy. *Allergy* 69, 420–437. doi:10.1111/all.12350

Demoly, P., Kropf, R., Bircher, A., and Pichler, W. J. (1999). Drug hypersensitivity: questionnaire. EAACI interest group on drug hypersensitivity. *Allergy* 54, 999–1003. doi:10.1034/j.1398-9995.1999.00247.x

Demoly, P., Pichler, W., Pirmohamed, M., and Romano, A. (2008). Important questions in allergy: 1–drug allergy/hypersensitivity. *Allergy* 63, 616–619. doi:10.1111/j.1398-9995.2008.01693.x

Doña, I., Blanca-López, N., Cornejo-García, J. A., Torres, M. J., Laguna, J. J., Fernández, J., et al. (2011). Characteristics of subjects experiencing hypersensitivity to non-steroidal anti-inflammatory drugs: patterns of response. *Clin. Exp. Allergy* 41, 86–95. doi:10.1111/j.1365-2222.2010.03651.x

Doña, I., Blanca-López, N., Torres, M. J., García-Campos, J., García-Núñez, I., Gómez, F., et al. (2012). Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J. Investig. Allergol. Clin. Immunol.* 22, 363–371.

Doña, I., Pérez-Sánchez, N., Eguiluz-Gracia, I., Muñoz-Cano, R., Bartra, J., Torres, M. J., et al. (2020). Progress in understanding hypersensitivity reactions to nonsteroidal anti-inflammatory drugs. *Allergy* 75, 561–575. doi:10.1111/all.14032

Gabrielli, S., Clarke, A. E., Eisman, H., Morris, J., Joseph, L., La Vieille, S., et al. (2018). Disparities in rate, triggers, and management in pediatric and adult cases of suspected drug-induced anaphylaxis in Canada. *Immun. Inflamm. Dis.* 6, 3–12. doi:10.1002/iid3.201

Gamboa, P. M. (2009). The epidemiology of drug allergy-related consultations in Spanish Allergology services: alergológica-2005. *J. Investig. Allergol. Clin. Immunol.* 19 (Suppl. 2), 45–50.

Kalyoncu, I., Gaspar, Â., Chambel, M., Piedade, S., and Morais-Almeida, M. (2016). Hypersensitivity to beta-lactam antibiotics: a three-year study. *Eur. Ann. Allergy Clin. Immunol.* 48, 212–219.

Li, P. H., Ue, K. L., Wagner, A., Rutkowski, R., and Rutkowski, K. (2017). Opioid hypersensitivity: predictors of allergy and role of drug provocation testing. *J. Allergy Clin. Immunol. Pract.* 5, 1601–1606. doi:10.1016/j.jaip.2017.03.035

Macy, E., and Contreras, R. (2014). Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: a cohort study. *J. Allergy Clin. Immunol.* 133, 790–796. doi:10.1016/j.jaci.2013.09.021

Macy, E., Schatz, M., Lin, C., and Poon, K. Y. (2009). The falling rate of positive penicillin skin tests from 1995 to 2007. *Perm. J.* 13, 12–18. doi:10.7812/tpp/08-073

Park, B. N. D., and Demoly, P. (2012). *Allergy*. 4th Edn, Editors M. K. C. Stephen, T. Holgate, D. H. Broide, and F. D. Martinez, 321–330.

Pichler, P. (2010). Drug allergy: an updated practice parameter. *Ann. Allergy Asthma Immunol.* 105, 259–273. doi:10.1016/j.anai.2010.08.002

Pipet, A., Veyrac, G., Wessel, F., Jolliet, P., Magnan, A., Demoly, P., et al. (2011). A statement on cefazolin immediate hypersensitivity: data from a large database, and focus on the cross-reactivities. *Clin. Exp. Allergy* 41, 1602–1608. doi:10.1111/j.1365-2222.2011.03846.x

Powell, M. Z., Mueller, S. W., and Reynolds, P. M. (2019). Assessment of opioid cross-reactivity and provider perceptions in hospitalized patients with reported opioid allergies. *Ann. Pharmacother.* 53, 1117–1123. doi:10.1177/1060028019860521

Sidoroff, S., Galéra, C., Bousquet-Rouanet, L., Arnoux, B., Demoly, P., and Bousquet, P. J. (2010). Safety of cefuroxime as an alternative in patients with a proven hypersensitivity to penicillins: a DAHD cohort survey. *Int. Arch. Allergy Immunol.* 153, 53–60. doi:10.1159/000301579

Simons, F. E., Ardusso, L. R., Bilò, M. B., El-Gamal, Y. M., Ledford, D. K., Ring, J., et al. (2011). World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J.* 4, 13–37. doi:10.1097/WOX.0b013e318211496c

Sousa-Pinto, B., Cardoso-Fernandes, A., Araújo, L., Fonseca, J. A., Freitas, A., and Delgado, L. (2018). Clinical and economic burden of hospitalizations with registration of penicillin allergy. *Ann. Allergy Asthma Immunol.* 120, 190–194.e2. doi:10.1016/j.anai.2017.11.022

Su, T., Broekhuizen, B. D. L., Verheij, T. J. M., and Rockmann, H. (2017). The impact of penicillin allergy labels on antibiotic and health care use in primary care: a retrospective cohort study. *Clin. Transl. Allergy* 7, 18. doi:10.1186/s13601-017-0154-y

Thong, B. Y., and Tan, T. C. (2011). Epidemiology and risk factors for drug allergy. *Br. J. Clin. Pharmacol.* 71, 684–700. doi:10.1111/j.1365-2125.2010.03774.x

Thong, H. A., Muñoz-Furlong, A., Campbell, R. L., Adkinson, N. F., Jr., Bock, S. A., Branum, A., et al. (2006). Second symposium on the definition and management of anaphylaxis: summary report–second national institute of allergy and infectious disease/food allergy and anaphylaxis network symposium. *Ann. Emerg. Med.* 47, 373–380. doi:10.1016/j.annemergmed.2006.01.018

Torres, M. J., Blanca, M., Fernandez, J., Romano, A., Weck, A., Aberer, W., et al. (2003). Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 58, 961–972. doi:10.1034/j.1398-9995.2003.00280.x

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REVIEW
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An Updated Review of the Diagnostic Methods in Delayed Drug Hypersensitivity

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Delayed drug hypersensitivity reactions are clinically diverse reactions that vary from isolated benign skin conditions that remit quickly with no or symptomatic treatment, drug discontinuation or even continued drug treatment, to the other extreme of severe cutaneous adverse reactions (SCARs) that are associated with presumed life-long memory T-cell responses, significant acute and long-term morbidity and mortality. Diagnostic "in clinic" approaches to delayed hypersensitivity reactions have included patch testing (PT), delayed intradermal testing (IDT) and drug challenges for milder reactions. Patch and IDT are, in general, performed no sooner than 4–6 weeks after resolution of the acute reaction at the maximum non-irritating concentrations. Functional *in vitro* and *ex vivo* assays have largely remained the province of research laboratories and include lymphocyte transformation test (LTT) and cytokine release enzyme linked ImmunoSpot (ELISpot) assay, an emerging diagnostic tool which uses cytokine release, typically IFN-γ, after the patient's peripheral blood mononuclear cells are stimulated with the suspected drug(s). Genetic markers such as human leukocyte antigen have shown recent promise for both pre-prescription screening as well as pre-emptive and diagnostic testing strategies.

Keywords: delayed hypersensitivity reaction, drug allergy, severe cutaneous adverse reactions, T cells, skin testing, lymphocyte transformation test (LTT), enzyme linked ImmunoSpot (ELISpot), HLA

INTRODUCTION

In this review, we will address the immune mechanisms of delayed hypersensitivity and how they have formed the premise for diagnostic methods used in the clinic and research laboratory such as intradermal skin testing (IDT), patch testing (PT) and new and investigational laboratory-based methods such as the lymphocyte transformation test (LTT) and the enzyme linked ImmunoSpot (ELISpot) assay. In addition, the role of genetic markers such as human leukocyte antigen (HLA) in screening, early diagnosis and diagnosis will be discussed.

DELAYED HYPERSENSITIVY REACTIONS

Delayed drug hypersensitivities are predominantly the result of T-cell mediated reactions of varying severity and clinical diagnosis such as maculopapular exanthema (MPE), fixed drug eruption (FDE), symmetrical drug-related intertriginous and flexural exanthema (SDRIFE), single organ disease (e.g., drug induced liver injury (DILI) and kidney diseases), acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).

MPE or morbilliform drug eruption is the most common of the self-limiting reactions to drugs characterized by erythematous macules and papules that can become generalized and confluent and are associated with pruritis and/or mild eosinophilia (Peter et al., 2017). FDE is characterized by red dark lesions localized in the same area after drug re-exposure that might be accompanied by a burning or itchy sensation (Rive et al., 2013). SDRIFE is characterized by a well-demarcated macular eruption involving the flexural or intertriginous folds, inguinal and peri-genital as well as gluteal and peri-anal areas (Wolf and Tuzun, 2015). DILI generally manifests as an isolated hepatitis with multiple metabolic, immune and genetic factors considered causal (Rive et al., 2013). However, some patients can present with features of hypersensitivity such as fever and skin eruption as well as pruritus with secondary excoriations. AGEP is a non-follicular sterile pustular eruption over widespread erythema, with a predilection for the flexural folds, and accompanied by fever and/or biological abnormalities (Peter et al., 2017). A validation score from the EuroSCAR group criteria can be used to confirm the clinical diagnostic for AGEP cases (Sidoroff et al., 2001). The main clinical features of DRESS or drug-induced hypersensitivity syndrome (DIHS) are erythematous urticaria-like plaques or violaceous skin eruption that can progress to exfoliative dermatitis, facial and extremity edema, lymphadenopathy, fever, biological abnormalities and internal organ involvement. The European Registry of Severe Cutaneous Adverse Reactions (RegiSCAR) score is calculated using clinical and laboratory data to determine the likelihood of disease (definite, probable, possible or no case) (Kardaun et al., 2007). Another multisystem disease related to drug exposure is the abacavir hypersensitivity syndrome (AB HS) that is characterized by constitutional symptoms including fever, gastrointestinal manifestations and skin eruption (Clay, 2002; Phillips et al., 2002).

SJS and TEN are characterized by skin detachment and full-thickness epidermal necrosis of various severities depending of the body surface area (BSA) affected (1–10% for SJS, 10–30% for SJS/TEN overlap and >30% for TEN) as well as blistering of mucous membranes accompanied by other serious systemic manifestations (Peter et al., 2017). As the mortality can reach 30–50% (Rive et al., 2013), a validated clinical score of toxic epidermal necrosis (SCORTEN), can be calculated at admission to predict mortality (Bastuji-Garin et al., 2000). Drug causality can be assessed with the algorithm of drug causality for epidermal necrolysis (ALDEN) score, an algorithm that helps identify the most likely causal drug(s) based on criteria such as type of drug, timing and possible alternative causes (Shear and Dodiuk-Gad, 2019). The time from the drug exposure to the development of symptoms can vary from 4 to 28 days and, in one third of cases, no causal agent is identified (Duong et al., 2017). The spectrum of T-cell mediated phenotypic scoring tools are outlined in **Table 1**.

TABLE 1 | Clinical diagnosis and described scoring algorithms.

Clinical diagnosis	AGEP	DRESS	SJS/TEN
Disease likelihood	AGEP validation score	RegiSCAR score	None
Drug causality	Naranjo score	Naranjo score	ALDEN score Naranjo score
Mortality	None	None	SCORTEN

AGEP, acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; SJS/TEN, SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis. ALDEN, algorithm of drug causality for epidermal necrolysis; Naranjo score: The Adverse Drug Reaction (ADR) Probability Scale; RegiSCAR, european registry of severe cutaneous adverse reactions; SCORTEN, score of toxic epidermal necrosis.

Mechanisms of Immune Response in Delayed Hypersensitivities

With a better understanding of the pharmacogenomic and pathogenesis of drug reactions, newer classifications of adverse drug reactions that enhance our understanding of the drug hypersensitivity framework have been suggested. The on-target/off-target model categorizes adverse drug reactions by describing the interactions between drugs and their known targets for the desired pharmacological effect as well as the known or unknown mechanism for an off-target effect (White et al., 2015; Phillips, 2016). On-target reactions are generally non-immunologically mediated, dose-dependent and related to the primary pharmacologic mechanism of action of the drug. Off-target effects can relate to a number of known toxic, non-immunological and immunological mechanisms and can be subclassified in 1) dose dependent interactions with off-target receptors and pharmacological interactions such as non-IgE mediated mast cell activation or cellular toxicity and 2) drug allergy with immunological memory of variable duration such as delayed T-cell mediated reactions or IgE-mediated reactions (White et al., 2015). This is illustrated in **Figure 1**.

A well-established classification of historical relevance is the Gell and Coombs criteria of T-cell mediated hypersensitivity where type IVa is marked by T helper 1 (Th1) cells, macrophages and a secretion of IFN-γ, TNF-α, IL-18; type IVb by Th1 and other components such as B cells, IgE, IgG4, mast cells, eosinophils with a marked secretion of IL-4, IL-5 and IL-13; type IVc characterized by cytotoxic T cells that secrete granzyme, B perforin and granulysin and type IVd, where Th1/Th17 cells and neutrophils act through cytokine mediators such as GM-CSF, IL-8 and CXCL8 (Pichler and Hausmann, 2016). As the different phenotypes of delayed T-cell mediated reactions have different effector cells and cytokines, they have been portrayed under one of these subcategories with SJS/TEN probably related with $CD8^+$ T-cell infiltrates (type IVc) and DRESS with a $CD4^+$ dominant T-cell infiltration (type IVb) (Hari et al., 2001). The clear divergence in predominant cytokine signature between T-cell subsets provided indication

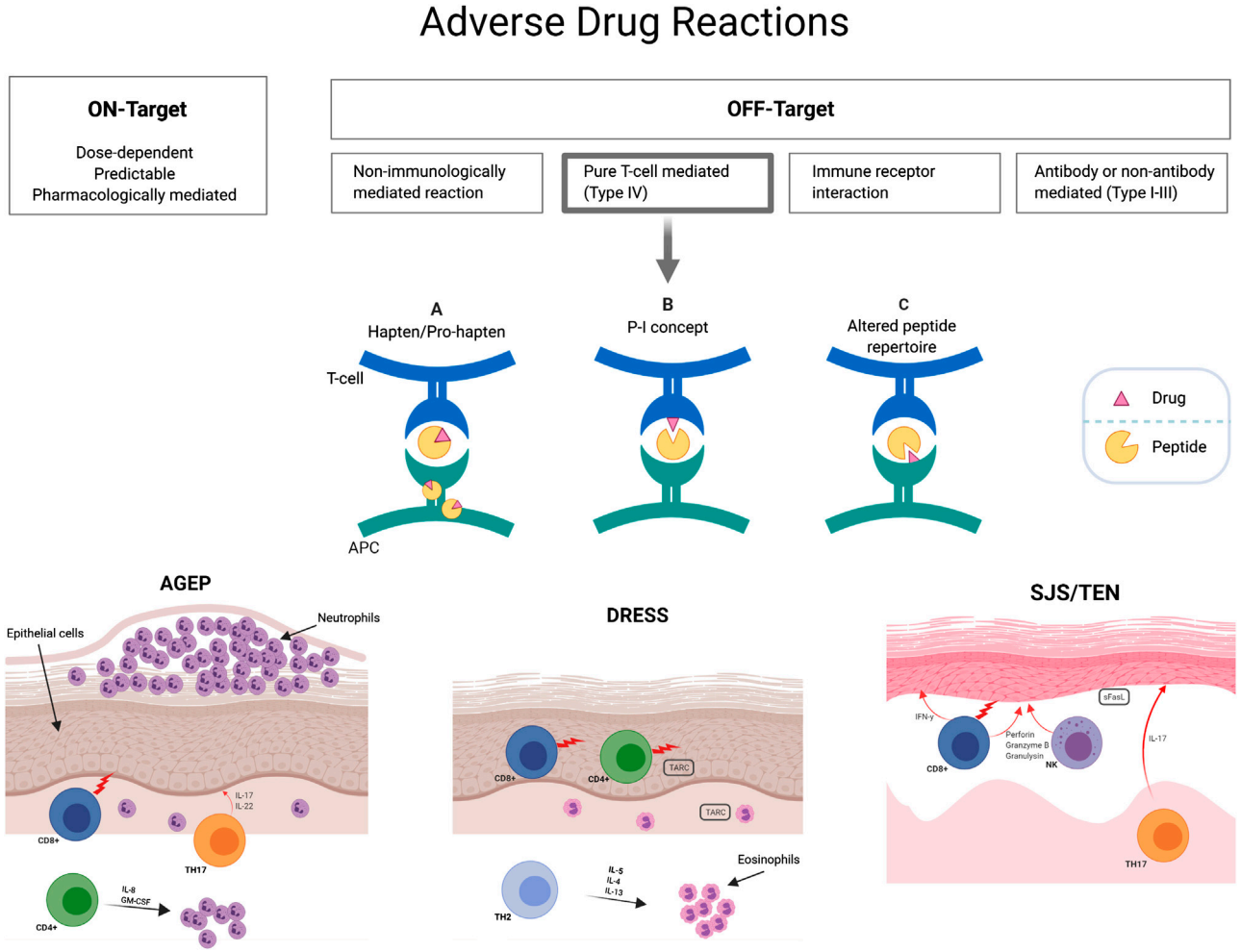

FIGURE 1 | T-cell mediated delayed hypersensitivity mechanistic hypotheses. Three non-mutually exclusive hypotheses have been described to clarify drug triggered T-cell activation: **(A)** The hapten/prohapten model describes how an antigen (drug) that covalently binds to a self-peptide is intracellularly processed and then presented by MHC to T cells. **(B)** The p-i concept (pharmacological interaction with immune receptor) is based upon non-covalent binding of antigens to HLA or TCR without immune processing. **(C)** The altered repertoire model indicates that drugs can occupy positions in the peptide binding groove of the MHC, altering the binding cleft and the specificity of MHC binding. HLA, human leukocyte antigens; MHC, major histocompatibility complex; TCR, T-cell receptor.

for their detection to drive response categorization in each patient (see ELISpot section below).

This classification only partially accounts for underlying immunological mechanisms and does not explain the specific mechanism by which drugs may activate T cells. Three non-mutually exclusive hypotheses have been described to clarify drug triggered T-cell activation: 1) the p-i concept, 2) the hapten/pro-hapten model and 3) the altered peptide repertoire model (**Figure 1**). The pharmacological-interaction (p-i) model suggests that the offending drug can rapidly stimulate T cells by directly binding non-covalently to either T-cell receptor (TCR) or HLA (without antigen processing) (Pichler and Hausmann, 2016). This concept was proposed after observation that protein-unreactive drugs can stimulate T cells (Pichler and Watkins, 2014; White et al., 2015). In the hapten/pro-hapten model, novel antigens are generated from endogenous proteins that covalently bind the culprit drug or its metabolites, forming a neoantigen that then triggers T-cell response (Pichler and Hausmann, 2016). Haptens are small reactive molecule that become antigenic by covalent binding to high-molecular-weight autologous extracellular or cytoplasmic proteins. The resultant "haptenated" product undergoes presentation by APC on HLA molecules with subsequent activation of T cells. In this setting, re-exposure will generate rapid memory T-cell proliferation and inflammatory response. A classic example is the binding of penicillin metabolites to serum albumin (Padovan et al., 1996). Finally, in the altered peptide repertoire model, the causal drug occupies a position in the HLA peptide binding groove altering the binding cleft and the specificity of self-peptides able to bind to the HLA molecule (Illing et al., 2012). This model has only been established for abacavir hypersensitivity with the crystal structure of abacavir bound to peptide and HLA-B*57:01 having been described. It has hence been elucidated through this structure, peptide binding studies

and peptide elution studies that abacavir binds non-covalently within the F pocket of the peptide-binding cleft of HLA-B*57:01 and alters the normal C9 peptide specificity from aromatic aliphatic amino acids, such as phenylalanine, to linear aliphatic amino acids, such as leucine, isoleucine and valine (Illing et al., 2012; Ostrov et al., 2012).

DIAGNOSTIC METHODS

Drug Challenge

In the context of drug allergy, drug challenge in a patient with suspected drug-induced hypersensitivity remains the gold standard for determining tolerance (Aberer et al., 2003). For immediate reactions, such as IgE mediated reactions, a negative drug challenge has a 100% negative predictive value. However, in the case of a severe delayed reaction, re-challenge with a single dose of a drug may not reproduce the reaction and, hence, it has a lower sensitivity than a prolonged challenge (3–5 days), particularly with a remote reaction (Bousquet et al., 2008; Hjortlund et al., 2012). In particular settings such as childhood non-specific delayed mild exanthem associated with antibiotics in the context of a possible viral infection, there is increase evidence that direct oral challenge is a safe diagnostic tool (Mill et al., 2016; Trubiano et al., 2017a).

In addition, with high severity reactions, drug challenge carries an inherent risk and the benefit of re-challenge has to be carefully weighed against the risk of a serious reaction. In cases of severe cutaneous adverse reactions or severe organ involvement, challenges are contraindicated because of the risk of a life-threatening clinical reaction (Rive et al., 2013; Trubiano and Phillips, 2013). In this context, investigational tools have been developed to aid drug evaluation. *In vivo* testing such as PT and delayed IDT and *ex vivo* assays such as the LTT and ELISpot have been described for various drugs and phenotypes but lack international validation. Combining *in vivo* and *ex vivo* methods in delayed hypersensitivity reactions can increase the diagnostic yield, although this has been shown in only small cohort studies (Trubiano et al., 2018).

Skin Testing

In vivo testing (PT and delayed IDT) is usually performed to the implicated drug(s) at least 4–6 weeks after delayed hypersensitivity resolution at the recommended non-irritating concentrations (Phillips et al., 2019).

Patch Testing

The main types of reactions where PT is used with high specificity are MPE, AGEP, DRESS, SJS/TEN and FDE (Ozkaya-Bayazit et al., 1999; Barbaud et al., 2001; Barbaud et al., 2013). The sensitivity of this investigational tool varies depending on the clinical setting, the causal drug, the drug concentrations used and the phenotype with typical figures for AGEP at 58–64% (Wolkenstein et al., 1996; Barbaud et al., 2013), DRESS between 32 and 80% (Barbaud et al., 2013; Barbaud, 2014) and SJS/TEN, 9–24% (Wolkenstein et al., 1996; Barbaud et al., 2013). Drugs like antiepileptics, contrast media, beta-lactams, tetrazepam and pristinamycin increase the sensitivity of PT (Johansen et al., 2015), while allopurinol or its active metabolite, oxypurinol, appear to never provide clinical utility.

TABLE 2 | Score—Interpretation of patch testing reactions.

Score	Interpretation
–	Negative reaction
? or +/–	Doubtful reaction, faint erythema
+	Weak reaction, erythema, slight infiltration
++	Strong reaction, erythema, infiltration, papules or vesicles (bullae) Reaction may extend beyond the margins of the patch
+++	Extreme, bullous, ulcerative
IR	Irritant reaction: Follicular, pustular, bullous or necrotic
NT	Not tested

The testing should be performed at least one month after the resolution of the reaction or after discontinuation of oral steroids, as immunosuppressants can decrease T-cell mediated immunity, and preferably during the first year after the reaction. The European Network on drug allergy (ENDA) and the European Academy of Allergy and Clinical Immunology (EAACI) recommend timing between 3 weeks and 3 months and describe drug concentrations between 5 and 30% with most antimicrobials diluted at 20% (Brockow et al., 2002) or 30% (Barbaud et al., 2001) in petrolatum vehicle and the retained vehicle alone as negative control (Barbaud et al., 2001; Brockow et al., 2013). For DRESS, patch testing may be further delayed because of the concomitant dosing of topical or systemic steroids or other immunosuppressants and to avoid confusion with DRESS relapse. Available literature suggests that the yield from patch testing for SJS/TEN is in general low but dependent on the drug and class of drugs. Sensitivities will vary from 0% for allopurinol to >50% for aromatic antiepileptic drugs such as carbamazepine (Konvinse et al., 2016).

The two forms of PT described are the extemporaneous, involving the local preparation of the PT by the pharmacy or the drug allergy staff with commercially available drugs and petrolatum or water, and the conventional PT implying use of a limited number of ready-to-use commercialized PT products at 10% concentration in petrolatum (Chemotechnique, Sweden). In a retrospective study, 21/75 (23.3%) patients with MPE, FDE, AGEP, DRESS, SJS/TEN tested simultaneously with both methods had positive results, indicating that both methods are as valuable and reliable (Assier et al., 2017). PT is usually applied in the upper back regions for practical reasons with the exception of FDE in which the PT is applied on the region of the previous reaction. The International Contact Dermatitis Research Group have published an interpretation score for the patch test reactions (**Table 2**) (Barbaud et al., 2001).

In a large multi-center patch testing cohort, only one patient (1/134) presented a relapse of his skin condition (AGEP) following patch testing (Barbaud et al., 2013) indicating that this diagnostic method carries low morbidity. In a retrospective review including 826 patients, PT showed promising results for drug challenge outcomes with 82.3% (14/17) with positive PT having a positive challenge and 90.4% (207/229) patients with

TABLE 3 | Role of diagnostic/screening tests in delayed drug hypersensitivity reactions.

	In vivo			*Ex vivo*		
Clinical diagnosis	Patch testing	Delayed IDT	Oral challenge	LTT	ELISpot	HLA
MPE	Yes ♣	Yes ♣	Yes	No	No	No
AGEP	Yes	Yes	No	Equivocal	Equivocal	No
DRESS/DIHS	Yes	Yes	No	Yes	Yes	Yes ψ
SJS/TEN	Yes	No	No	Yes	Yes	Yes ψ
FDE	Yes ω	No	Equivocal	No	No	No
SDRIFE	Yes ω	Equivocal	Equivocal	No	No	No

AGEP, acute generalized exanthematous pustulosis; DIHS, Drug-induced Hypersensitivity syndrome; DRESS, Drug reaction with eosinophilia and systemic symptoms; ELISpot, enzyme-linked immunospot; FDE, fixed drug eruption; HLA, human leukocyte antigen; LTT, Lymphocyte transformation test; SDRIFE, symmetrical drug-related intertriginous and flexural exanthema, SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis. ♣ As the sensitivity for PT and IDT is poor, drug challenge of the implicated drug can be considered. PT/IDT may give information on cross-reactivity ω PT should be applied on the region of the previous reaction ψ HLA screening is not routinely used globally in clinical practice. Please refer to ***Table 4*** *for details.*

negative PT presenting no reaction to challenge (Lammintausta and Kortekangas-Savolainen, 2005).

PT is a quick and safe investigational method clinically relevant when testing is conclusive, a negative PT not excluding the possibility that the drug is causal. There is need to re-challenge negative testing in less severe clinical phenotypes. This method should be homogenized, as to resolve current inconsistencies, by comparing the outcomes in large multicenter studies, determining concentration thresholds and avoiding false negative and false positive results.

Intradermal Testing

Intradermal testing is done on the volar aspect of the forearm with 0.02–0.05 ml of antibiotic reagent or normal 0.9% serum saline (negative control) (Empedrad et al., 2003a; Brockow et al., 2013). The use of IDT is limited to drugs available in liquid sterile formulations. The positive control normally used is a skin prick test with histamine 10 mg/ml (Heinzerling et al., 2013). In terms of drug concentrations, expert consensus advises the use of the highest non-irritating concentration described for immediate reactions (Phillips et al., 2019). However, recent work for drugs with non-IgE mast cell activation determined that higher concentrations that might initially be irritating are needed for improved sensitivity (i.e., ciprofloxacin, vancomycin) (Brockow et al., 2002; Brockow et al., 2013; Konvinse et al., 2016). An IDT result is considered positive when the dermal induration and erythema at the injection site exceeded 5 mm from baseline (Empedrad et al., 2003b; Brockow et al., 2013). Delayed reading is performed at 24, 48 h and up to 1 week (Empedrad et al., 2003b; Brockow et al., 2013). IDT with delayed reading has been described in reactions such as MPE, AGEP and DRESS with potential risk in SJS/TEN and unknown utility in FDE (**Table 3**). This investigational tool was previously considered potentially harmful in SCAR phenotypes but actually few reports describe severe systemic reactions following IDT (Makris et al., 2010; Sala Cunill et al., 2011; Syrigou et al., 2016; Watts, 2017). For SJS/TEN, based on the current available literature, the benefit of IDT does not outweigh the risk. For DRESS, it is recommended that testing generally be deferred 6 months following the acute reaction.

In terms of cross-reactivity between beta-lactams in the context of delayed hypersensitivities, 18.7–31.2% of the patients tested presented a reaction to amino-penicillins and amino-cephalosporins (Dash, 1975) predicted by the presence of shared R1 and R2 side chains (Buonomo et al., 2014; Romano et al., 2016). Also, in patients with a delayed penicillin type reaction, delayed IDT to beta-lactams has allowed to confirm tolerance to cephalosporins (Picard et al., 2019; Trubiano et al., 2020), carbapenems (Gaeta et al., 2015; Picard et al., 2019) and monobactams (Buonomo et al., 2011). Other classes of interest are currently being studies with no evidence of cross-reactivity such as glycopeptides (Empedrad et al., 2003a), antibiotic and non-antibiotic sulfonamides (Empedrad et al., 2003a; Lammintausta and Kortekangas-Savolainen, 2005), drugs in the rifampin class (Lammintausta and Kortekangas-Savolainen, 2005) and aromatic and non-aromatic anticonvulsants (Heinzerling et al., 2013).

In the setting of a severe delayed reaction, PT is related to lower adverse reactions but IDT has been described as more sensitive in non-SJS/TEN reactions (Osawa et al., 1990; Barbaud et al., 2001; Cabanas et al., 2014) while some recommendations only suggest proceeding to IDT after negative PT (Brockow et al., 2002). In a cohort study of 21 patients with delayed reactions to penicillin and 30 controls with no allergic history, no false positives were reported and 20/21 were positive for IDT compared to 18/21 for patch testing (Torres et al., 2004).

Widespread implementation of IDT for delayed hypersensitivities still carries some barriers such as the lack of available sterile preparation for all drugs, generally low negative predictive value (NPV) and limited data in some reactions.

Ex Vivo Diagnostic Tools

In vitro/ex vivo diagnostics, such as the LTT and the ELISpot assay, while having the advantage of carrying no risk of drug re-exposure for the patient, are not available for routine diagnostic use in most centers. A practical management approach for delayed T-cell mediated hypersensitivity reactions is illustrated in **Figure 2**.

Lymphocyte Transformation Test

LTT has been extensively studied as a diagnostic method for delayed hypersensitivity reactions. Lymphocytes are isolated from the patient's peripheral blood mononuclear cells (PBMC) and cultured with pharmacological concentrations of the

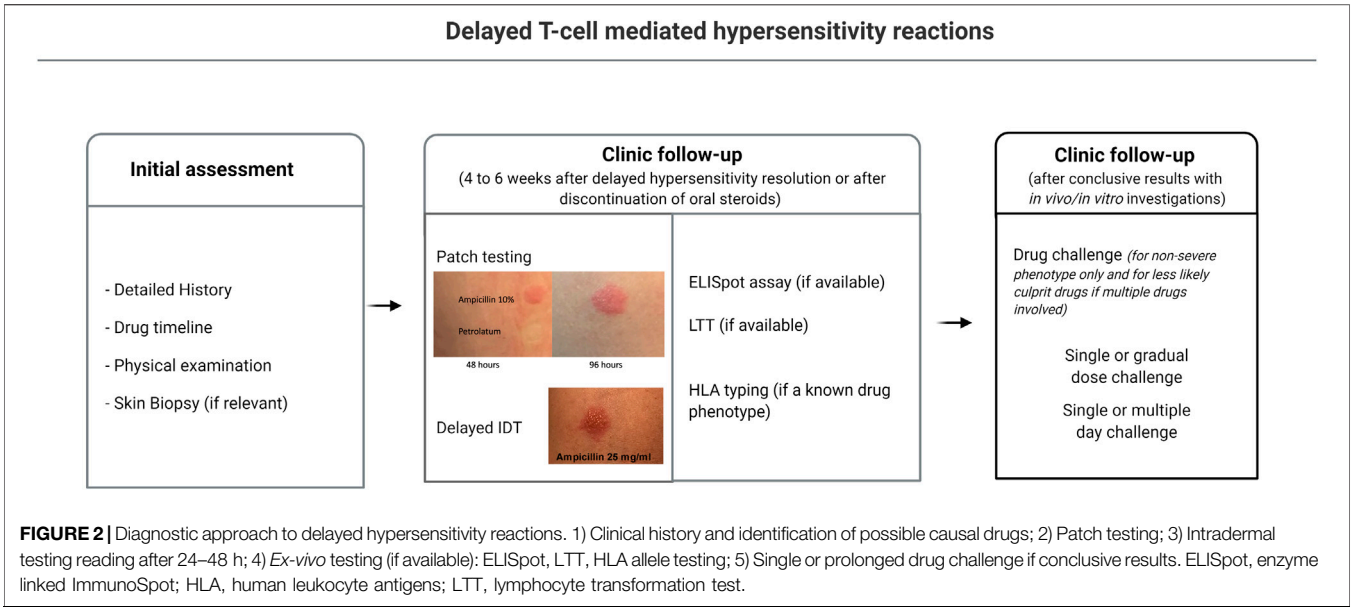

FIGURE 2 | Diagnostic approach to delayed hypersensitivity reactions. 1) Clinical history and identification of possible causal drugs; 2) Patch testing; 3) Intradermal testing reading after 24–48 h; 4) *Ex-vivo* testing (if available): ELISpot, LTT, HLA allele testing; 5) Single or prolonged drug challenge if conclusive results. ELISpot, enzyme linked ImmunoSpot; HLA, human leukocyte antigens; LTT, lymphocyte transformation test.

suspected drugs for 5–7 days. LTT responses are measured by the stimulation index (SI, average proliferation of drug-exposed cultures/average proliferation of negative control cultures), with typically an SI > 2+ confirming response, which is calculated based on the radioactive thymidine (H-thymidine) uptake, a marker directly proportional to the degree of T-cell proliferation in response to a drug antigen (Pichler and Tilch, 2004). This enhanced response is interpreted as a T-cell sensitization and has produced positive responses in different clinical settings and with various implicated drugs (Rive et al., 2013). However, one might keep in mind that lymphocyte stimulation can occur not only by immunological mechanisms but also pharmacological ones and some drugs may cause false positive results as was observed in some patients that presented positive responses to drugs they had tolerated (Pichler and Tilch, 2004).

The reported sensitivity of LTT in delayed hypersensitivity reactions ranges from 27% (Porebski et al., 2013) to 74% (Nyfeler and Pichler, 1997) and specificity was quoted as 85% (Nyfeler and Pichler, 1997; Rozieres et al., 2009) to 100% (Porebski et al., 2013; Porebski et al., 2015). Putting aside the demanding and time-consuming laboratory manipulations and the use of radioactivity and specialist equipment, the LTT can be an interesting support in drug hypersensitivity diagnosis but is still only used as a research tool (Pichler and Tilch, 2004; Nagao-Dias et al., 2009). The largest study describes LTT in 923 patients with suspected hypersensitivity among which only 100 patients had a confirmed drug hypersensitivity reaction and 58/78 penicillin allergy labeled patients presented a positive LTT (Picard et al., 2019). In the last 10 years, aside from case reports or small cases series (Kim et al., 2013; Cabanas et al., 2014; Dias de Castro et al., 2015; Tomida et al., 2016), very few studies have focused solely on the LTT method for diagnosis.

Enzyme Linked ImmunoSpot

The ELISpot technique quantifies the secretion and activation of drug-specific cells by determining the number of spot-forming units (SFU) or spot-forming cells (SFC) that release cytokine markers or cytolytic molecules after the patient's PBMC is activated with the suspected drug(s) (**Figure 3**). The patient's cells are added to a 96-well plate coated with specific anti-cytokine antibody depending on the expected measured T-cell response. In drug-induced delayed hypersensitivity, interferon gamma (IFN-γ), a key Th1-type cytokine, is released from activated T cells, while granzyme B (GrB), a serine protease, is released from cytoplasmic granules within cytotoxic T cells and natural killer cells. Anti-CD3 antibodies, a mix of viral peptides CEF (cytomegalovirus (CMV), Epstein-Bar Virus (EBV) and influenza (FLU)) and tetanus toxoid can be used as positive controls as they stimulate INF-γ release from $CD8^+$ T cells. The background immunological activation can be assessed with negative controls (cells and media). Cytokine secretion is captured by the anti-cytokine antibodies in the next 24–48 h with detection antibody and enzyme substrate being added just before reading the plate. The SFU representing cells that secrete cytokines are then identified and counted. As the incubation time is shorter than for LTT and T-cell activation occurs after 48–72 h, this could be a promising technique. However, one must consider the often-diverse response between replicates and the researcher intensive laboratory manipulations.

In a recent study, the sensitivity of this technique in patients with SCAR was 52%, 10/19 patients presenting a positive IFN-γ ELISpot (>50 SFU/10^6), with a specificity of 100% (Trubiano et al., 2018). The GrB ELISpot has a lower sensitivity (33%; 5/15 positive patients (>20 SFU/well) (Porebski et al., 2013) up to 55%; 13/23 positive patients (>0 SFU) (Porebski et al., 2015)) and similar specificity. However, when compared to LTT, ELISpot seems to have a better sensitivity (Rozieres et al., 2009). Depending on the positive ELISpot assay definition, the

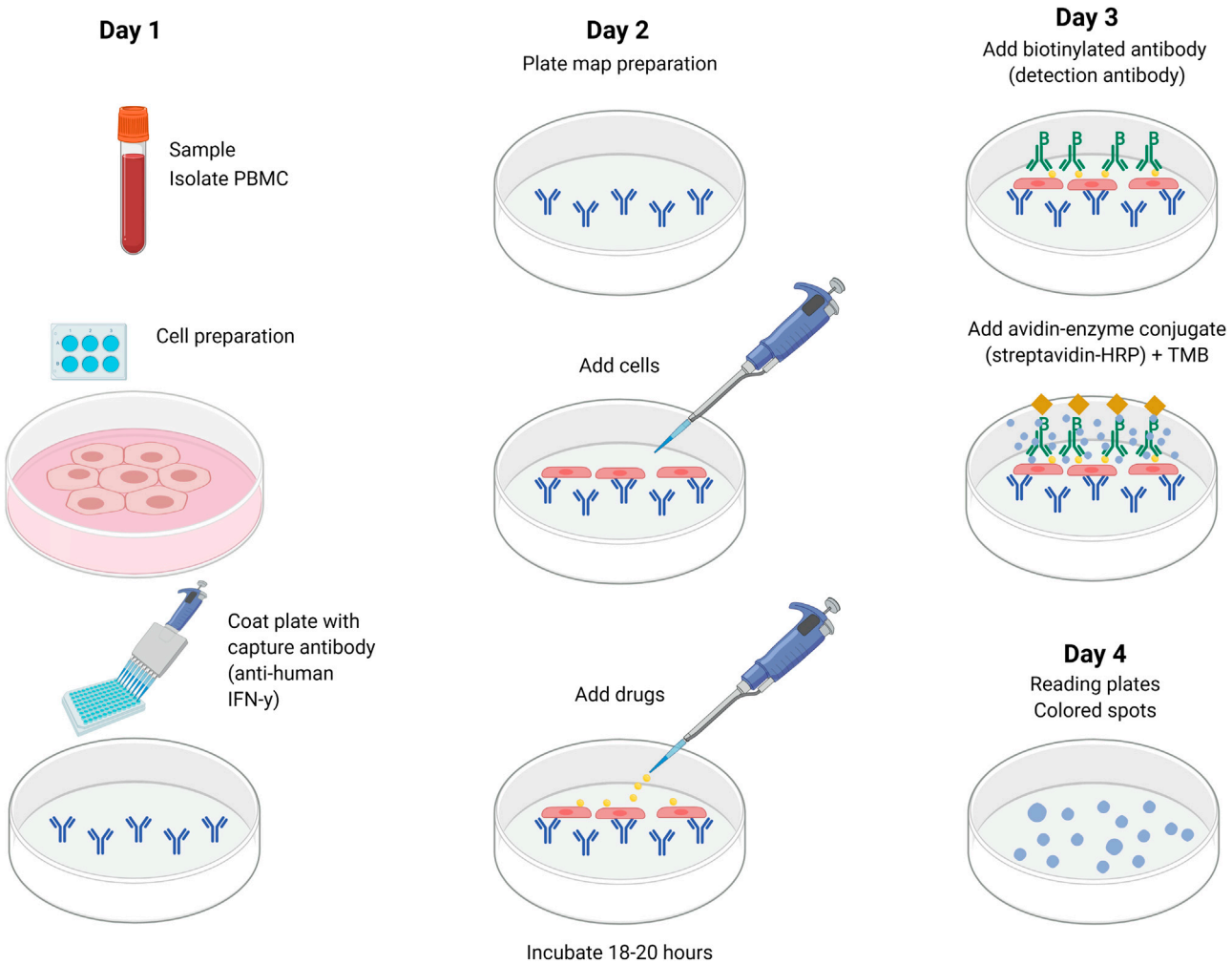

FIGURE 3 | Detection of cytokine secretion using ELISpot. Cytokine-specific coating antibody is added and incubated overnight at 4°C. The plate is washed and PBMCs and drug(s) are added and incubated for 18–20 h at 37°C. The following day, the plate is washed again and biotin-conjugated detection antibody is added and incubated for 2 h at room temperature. After another wash, streptavidin-bound enzyme is added and incubated for 1 h at room temperature. After the last wash, substrate is added (BCIP-NBT or TMB). Spot development is monitored for ~15 min (in dark). The plate is washed and left to dry overnight for a final reading on the fourth day. BCIP-NBT, 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT); ELISpot, enzyme linked ImmunoSpot; PBMC, peripheral blood mononuclear cells; TMB, tetramethyl benzidine.

number of confirmed cases varies with several studies considering the unique presence of SFU as sufficient for a positive test (Khalil et al., 2008; Porebski et al., 2015; Castagna et al., 2018). A recent study from our group reported 5/12 positive ELISpot among SCAR patients with a 50 SFU/10^6 cut-off (Trubiano et al., 2020). However, in a cohort of 22 patients with amoxicillin MPE, the sensitivity of the IFN-γ ELISpot was 91% (15/22) when the cut-off used was more than 30 SFU/10^6 (Rozieres et al., 2009). Using this same reference value, the sensitivity was 87.5% (7/8) in a study involving eight patients with hypersensitivity to piperacillin (El-Ghaiesh et al., 2012). Finally, some authors determine a positive value based solely on the SFU detection level in controls (Suthumchai et al., 2018). Because of the current controversy in the literature, our definition of a positive response is equal or greater than 50 spot-forming unit (SFU)/million cells after background (unstimulated control) (Keane et al., 2012).

As discussed, LTT and ELISpot do not have a good sensitivity especially when the blood is collected during the acute reaction. One hypothesis is that the reactive cells are not found in the circulation or that overstimulated lymphocytes could be exhausted. Thus, a cytokine or cytolytic marker panel could help delineate the implicated mediators. While alternative cell viability and proliferative assays have been developed in recent years including several variants of the MTT and carboxyfluorescein succinimidyl ester (CFSE) staining assays, these have not widely been applied for diagnostic investigation due to issues surrounding potential drug-inhibition of

metabolism-dependant colorimetric conversion and flow cytometer access, and difficulties in staining, respectively.

Biomarkers in Adverse Drug Reactions

As the most severe reactions but also those with the most varied clinical presentations are SJS/TEN and DRESS/DiHS, research efforts have been concentrated to develop new biomarkers with a particular interest in cytokines and chemokines released from activated T cells.

Studies on cytokine measurements after clinical drug challenge in patients with the generalized form of FDE clinically and histologically mimicking SJS/TEN have reported an initial increase in serum TNF-α and IL-8 followed by elevation in IFN-γ, IL-6 and IL-10 levels (Kauppinen, 1991; Correia et al., 2002; Shiohara et al., 2015). Similarly, dosage of levels for multiple cytokines/chemokines in order to identify essential markers has also been attempted with studies identifying a significant increase in IL-6 and interferon gamma-produced protein 10 (IP-10) in SJS/TEN and DRESS as well as IL-16 in FDE, SJS and DRESS but not TEN (Shiohara et al., 2015). These authors go to recommend the use of IL-6 and IL-10 as diagnostic and predictive tools in monitoring adverse drug reactions (Shiohara et al., 2015). On a cautionary note, these markers may be elevated in other conditions such as acute infection and sepsis.

Further, serum soluble Fas-ligand (sFasL) levels (Posadas et al., 2002; Abe et al., 2003; Murata et al., 2008), granulysin (Chung et al., 2008; Porebski et al., 2013; Cho et al., 2014; Chung et al., 2015; Weinborn et al., 2016), IL-15 (Su et al., 2017), CD137 (Trubiano et al., 2017b) and the proapoptotic factor galectin-7 (Hama et al., 2019) have been described in the pathological processes of SJS/TEN with sFasL and galectin-7 being considered as biomarkers able to predict TEN progression but not SJS (Shiohara et al., 2015; Hama et al., 2019) and granulysin serum levels correlating with disease severity and mortality (Chung et al., 2008; Chung et al., 2015). In DRESS/DiHS, several markers were reported as indicators of disease progression and activity such as the serum thymus and activation-regulated chemokine (TARC) (Ogawa et al., 2013; Komatsu-Fujii et al., 2017; Komatsu-Fujii et al., 2018) and granulysin (Saito et al., 2012). Other markers such IL-2, IL-4, IL-5, IL-13, IFN-γ and granzyme-B have been described in T-cell drug hypersensitivity (Lochmatter et al., 2009; Zawodniak et al., 2010; Polak et al., 2013). Measurement of these markers was reported using the ELISpot, intracellular cytokine staining, ELISA, rapid immunochromatographic tests (Su et al., 2016), plex bead-based immunoassay kits (Lochmatter et al., 2009) and flow cytometry (Porebski et al., 2013). Controversial markers are also important to underline such as IL-17 with some studies reporting a negative correlation with adverse drug reactions (Shiohara et al., 2015) while others described an increase of this cytokine in SJS/TEN (Teraki et al., 2013). Similarly, procalcitonin has been described as a marker for bacterial infection that could benefit the differential diagnostic that includes delayed hypersensitivity (Yoon et al., 2013).

In the early stages of severe delayed hypersensitivity disease, laboratory tests that can be used in clinical routine are needed to predict disease progression and to monitor treatment responses.

High-Resolution Human Leukocyte Antigen Class I and II Typing

The association between particular class I HLA alleles and specific phenotypes such as allopurinol SJS/TEN and DRESS (HLA-B*58:01), carbamazepine SJS/TEN (HLA-B*15:02) and abacavir hypersensitivity reaction and flucloxacillin drug-induced liver injury (HLA*57:01) has allowed a better understanding of the immunopathogenesis of severe T-cell mediated delayed hypersensitivity reactions and the implementation of guidelines and screening programs in the case of HLA-B*57:01 and abacavir and HLA-B*15:02 and carbamazepine in Southeast Asian populations in particular (**Table 4**).

DNA from patients with drug reactions can be obtained by a routine blood draw and extracted from whole blood or extracted from saliva collected into, for instance, a gene collection kit. DNA can then be used to perform high resolution HLA class I and II typing with next generation sequencing methods. To facilitate HLA testing with rapid turnaround times, cost-effective single allele assays have been developed for many class I HLA alleles such as HLA-B*57:01, HLA-B*15:02 and HLA-A*32:01 with parallel allele specific quality assurance programs which was crucial for the widespread global implementation of HLA-B*57:01 screening programs. HLA genes encode cell-surface protein receptors that present antigenic peptides to T cells. Class I MHC molecules (HLA-A, B and C) are expressed on most nucleated cells and are responsible for presenting peptides to $CD8^+$ cytotoxic T lymphocytes. Class II MHC molecules (HLA-DP, DQ and DR) are expressed only on antigen presenting cells (B cells, macrophages and dendritic cells) and stimulate $CD4^+$ helper T lymphocytes. The association between HLA and disease confers explanations on disease susceptibility with HLA polymorphisms playing a crucial role in T-cell repertoire and auto-reactive T cells, immune system presentation, recognition and antigen processing and the adaptability of the immune system (Shiina et al., 2004). Also, HLA allele have a different prevalence in different ethnic groups and this might explain the increased drug reactions in specific populations (Rive et al., 2013). The global epidemiology of severe cutaneous adverse drug reactions is illustrated in **Figure 4**.

Currently, screening for HLA-B*57:01 prior to abacavir prescription is the standard of care in HIV clinical practice across the developed world. When screening occurs and is acted upon, it eliminates abacavir hypersensitivity (Mallal et al., 2008). Another example is screening for HLA-B*15:02 before initiating treatment with carbamazepine to avert SJS/TEN in some Southeastern Asian countries with increased prevalence. The xanthine oxidase inhibitor, allopurinol, was also associated with SJS/TEN and DRESS and HLA-B*58:01genotyping in Han Chinese showed 100% NPV and 3%

TABLE 4 | HLA associations in SCAR and DILI with possible clinical implications.

Reference	Reaction type	Drug	HLA	Ethnicity	Screening	NPV (%)	PPV (%)	NNT
(Konvinse et al. (2019))	DRESS	Vancomycin	A*32:01	European ancestry (6.8%) African American (4%) Southeast Asian (<1.5%)	Pre-emptive♣	99.99	0.51	75
(Daly et al., (2009))	DILI	Flucloxacillin	B*57:01	European ancestry (5–8%) African American (2.5%) African/Asia (<1%)	None	99.99	0.14	13,819
(Mallal et al. (2002))	AB HS	Abacavir	B*57:01	Caucasian (5–8%)	HIV positive patients	100	55	13
(Hung et al. (2005))	SJS/TEN DRESS	Allopurinol	B*58:01	Han Chinese (9–11%) Caucasian (1–6%)	None	100	3	250
(Zhang et al. (2013))	SJS/TEN	Carbamazepine	B*15:02ψ	Han Chinese (10–15%)	Routine in southeast Asian countries	100	3	1,000
(Zhang et al. (2013))	DRESS	Dapsone	B*13:01	Papuans/Australian aborigines (28%) Chinese (2–20%) Japanese (1.5%) Indian (1–12%)	Leprosy patients in countries with increased prevalence	99.8	7.8	84

AB HS, abacavir hypersensitivity syndrome; DILI, drug-induced liver injury; DRESS, drug reaction with eosinophilia and systemic symptoms; HIV, human immunodeficiency virus; NNT, numbers needed to test (to prevent one case); NPV, negative predictive value; PPV, positive predictive value; SJS/TEN, SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis.
*♣ HLA-A*32:01 testing could have a role in determining the culprit drug (vancomycin) when multiple drugs are implicated in a delayed hypersensitivity reaction.ψ Other described alleles: HLA-B*15:21, HLA-B*15:11, and HLA-B*15:18.*

PPV (Hung et al., 2005), however it has incomplete negative predictive value in European and African populations where 50–60% of patients with allopurinol DRESS/SJS/TEN do not carry HLA-B*58:01 (Lonjou et al., 2006).

Many of the described HLA alleles associations have a close to 100% negative predicting value, however this is highly dependent on the prevalence of the HLA allele in the population and the risk allele(s) in different populations. For instance, for allopurinol SJS/TEN and DRESS and HLA-B*58:01, it has almost a 100% NPV in Southeast Asian population however explains only 50–60% of allopurinol DRESS/SJS/TEN in European and African populations. The number needed to test to prevent one case of disease is thus population specific. However, as the prevalence of these diseases in the general population is reduced, more targeted populations could benefit from screening.

Recommendations

- Patch testing and intradermal testing can be used in the clinical setting for specific clinical diagnosis of T-mediated delayed hypersensitivity reactions while appreciating that preparations and drug concentration should be standardized to optimize their use.
- While skin testing (patch testing and intradermal testing) has a high drug specificity, both have phenotype and drug dependent sensitivity and incomplete NPV and, in the setting of severe delayed drug reaction, clinical history is the main determinant of drug safety that guides the decision for drug challenge or future drug use.
- Different non-irritating drug concentrations have been described for intradermal and patch testing. However, global consensus is lacking and clinicians are encouraged to follow the most recent drug allergy guidelines.
- Intradermal testing can be safety performed for non-SJS/TEN delayed reactions. Patch testing is the initial *in vivo* investigational tool that can be used for severe delayed reactions such as SJS/TEN.
- *Ex vivo* tools such as the lymphocyte transformation test and the enzyme-linked ImmunoSpot assay are currently not available for routine clinical practice and are used solely in specialized center. Collaborating with such a center will not only improve patient care but could benefit research in this field.
- In the early stages of severe delayed hypersensitivity disease, laboratory tests that can be used in clinical routine are needed to predict disease progression and to monitor treatment responses. There are currently no tests that should be order on a routine basis.
- Strong HLA associations with delayed T-cell mediated hypersensitivity reactions have enlightened our understanding of their immunopathogenesis and, in combination with availability of cost-effective single HLA testing, have provided a pathway for pre-prescription screening strategies. In the future, HLA testing may be increasingly relevant for pre-emptive testing and diagnosis.
- There is currently no diagnostic tool that offers a 100% NPV for the delayed hypersensitivity reactions and any decision to reintroduce a drug in the treatment setting should weigh the risk benefit ratio.

CONCLUSION

Identifying culprit drugs implicated in delayed T-cell mediated hypersensitivity with the use of exemplary clinical

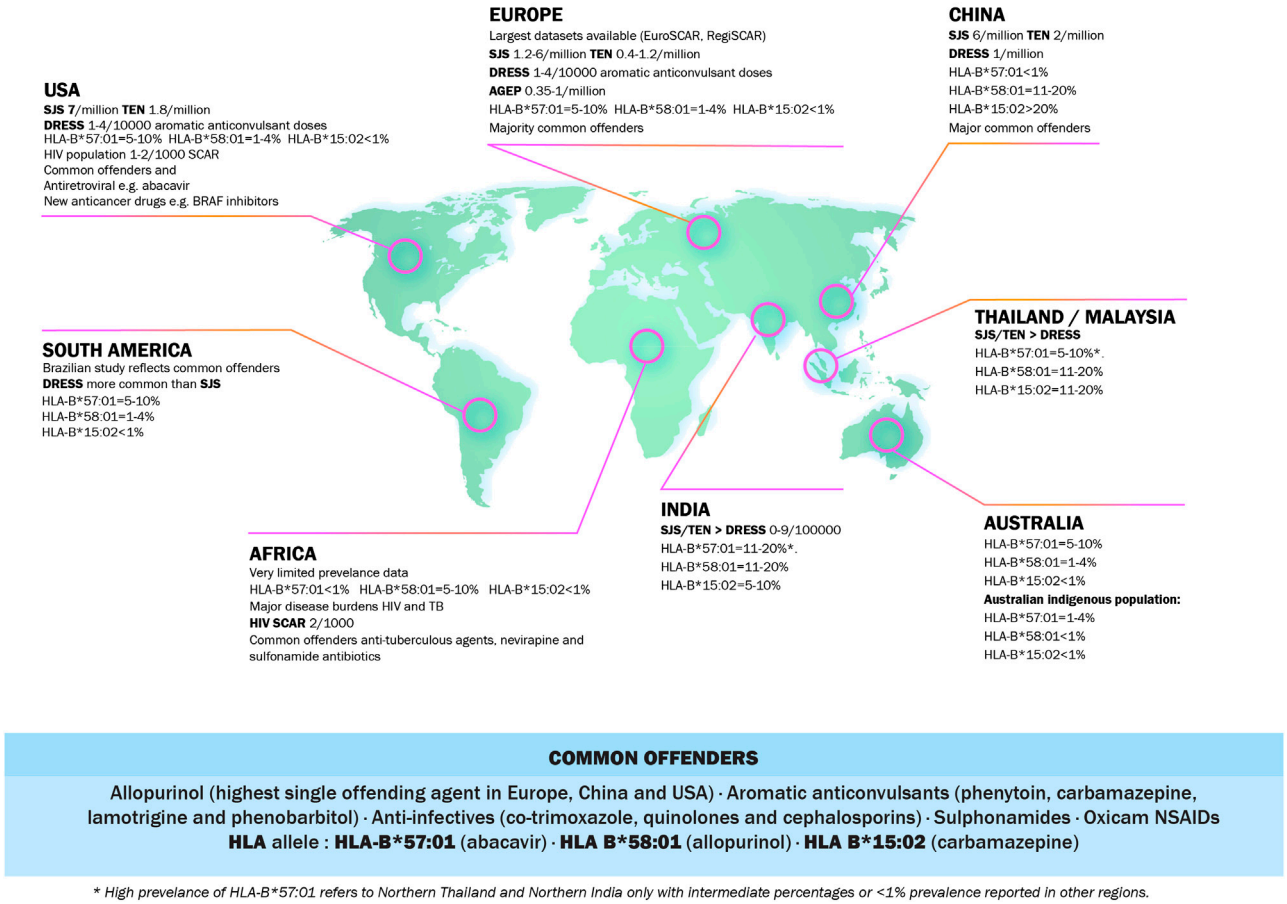

FIGURE 4 | Global epidemiology of severe cutaneous adverse drug reactions.

phenotyping, clinical drug causality assessment and adjunctive *in vivo* and *ex vivo* testing including HLA-typing is increasingly useful to guide safe and optimal future treatment.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

Abe, R., Shimizu, T., Shibaki, A., Nakamura, H., Watanabe, H., and Shimizu, H. (2003). Toxic epidermal necrolysis and Stevens-Johnson syndrome are induced by soluble Fas ligand. *Am. J. Pathol.* 162 (5), 1515–1520. doi:10.1016/S0002-9440(10)64284-8

Aberer, W., Bircher, A., Romano, A., Blanca, M., Campi, P., Fernandez, J., et al. (2003). Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. *Allergy.* 58 (9), 854–863. doi:10.1034/j.1398-9995.2003.00279.x

Assier, H., Valeyrie-Allanore, L., Gener, G., Verlinde Carvalh, M., Chosidow, O., and Wolkenstein, P. (2017). Patch testing in non-immediate cutaneous adverse drug reactions: value of extemporaneous patch tests. *Contact Dermatitis.* 77 (5), 297–302. doi:10.1111/cod.12842

Barbaud, A., Gonçalo, M., Bruynzeel, D., and Bircher, A., European Society of Contact Dermatitis (2001). Guidelines for performing skin tests with drugs in the investigation of cutaneous adverse drug reactions. *Contact Dermatitis*. 45 (6), 321–328. doi:10.1034/j.1600-0536.2001.450601.x

Barbaud, A., Collet, E., Milpied, B., Assier, H., Staumont, D., Avenel-Audran, M., et al. (2013). A multicentre study to determine the value and safety of drug patch tests for the three main classes of severe cutaneous adverse drug reactions. *Br. J. Dermatol*. 168 (3), 555–562. doi:10.1111/bjd.12125

Barbaud, A. (2014). Skin testing and patch testing in non-IgE-mediated drug allergy. *Curr. Allergy Asthma Rep*. 14 (6), 442. doi:10.1007/s11882-014-0442-8

Bastuji-Garin, S., Fouchard, N., Bertocchi, M., Roujeau, J. C., Revuz, J., and Wolkenstein, P. (2000). SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. *J. Invest. Dermatol*. 115 (2), 149–153. doi:10.1046/j.1523-1747.2000.00061.x

Bousquet, P. J., Pipet, A., Bousquet-Rouanet, L., and Demoly, P. (2008). Oral challenges are needed in the diagnosis of beta-lactam hypersensitivity. *Clin. Exp. Allergy*. 38 (1), 185–190. doi:10.1111/j.1365-2222.2007.02867.x

Brockow, K., Romano, A., Blanca, M., Ring, J., Pichler, W., and Demoly, P. (2002). General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy*. 57 (1), 45–51. doi:10.1046/j.0105-4538.2001.00001.x-i8

Brockow, K., Garvey, L. H., Aberer, W., Atanaskovic-Markovic, M., Barbaud, A, Bilo, M. B., et al. (2013). Skin test concentrations for systemically administered drugs–an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy*. 68 (6), 702–712. doi:10.1111/all.12142

Buonomo, A., Nucera, E., De Pasquale, T., Pecora, V., Lombardo, C., Sabato, V., et al. (2011). Tolerability of aztreonam in patients with cell-mediated allergy to β-lactams. *Int. Arch. Allergy Immunol*. 155 (2), 155–159. doi:10.1159/000318844

Buonomo, A., Nucera, E., Pecora, V., Rizzi, A., Aruanno, A., Pascolini, L., et al. (2014). Cross-reactivity and tolerability of cephalosporins in patients with cell-mediated allergy to penicillins. *J Investig. Allergol. Clin. Immunol*. 24 (5), 331–337.

Cabanas, R., Calderon, O., Ramirez, E., Fiandor, A., Prior, N., Caballero, T., et al. (2014). Piperacillin-induced DRESS: distinguishing features observed in a clinical and allergy study of 8 patients. *J Investig. Allergol. Clin. Immunol*. 24 (6), 425–430. .

Castagna, J., Nosbaum, A., Vial, T., Rozieres, A., Hacard, F., Vocanson, M., et al. (2018). Drug-induced aseptic meningitis: a possible T-cell-mediated hypersensitivity. *J. Allergy Clin. Immunol. Pract*. 6 (4), 1409–1411. doi:10.1016/j.jaip.2017.11.034

Cho, Y. T., Lin, J. W., Chen, Y. C., Chang, C. Y., Hsiao, C. H., Chung, W. H., et al. (2014). Generalized bullous fixed drug eruption is distinct from Stevens-Johnson syndrome/toxic epidermal necrolysis by immunohistopathological features. *J. Am. Acad. Dermatol*. 70 (3), 539–548. doi:10.1016/j.jaad.2013.11.015

Chung, W. H., Hung, S. I., Yang, J. Y., Su, S. C., Huang, S. P., Wei, C. Y., et al. (2008). Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat. Med*. 14 (12), 1343–1350. doi:10.1038/nm.1884

Chung, W. H., Chang, W., Stocker, S. L., Juo, C. G., Graham, G. G., Lee, M. H., et al. (2015). Insights into the poor prognosis of allopurinol-induced severe cutaneous adverse reactions: the impact of renal insufficiency, high plasma levels of oxypurinol and granulysin. *Ann. Rheum. Dis*. 74 (12), 2157–2164. doi:10.1136/annrheumdis-2014-205577

Clay, P. G. (2002). The abacavir hypersensitivity reaction: a review. *Clin. Ther*. 24 (10), 1502–1514. doi:10.1016/s0149-2918(02)80057-1

Correia, O., Delgado, L., Barbosa, I. L., Campilho, F., and Fleming-Torrinha, J. (2002). Increased interleukin 10, tumor necrosis factor alpha, and interleukin 6 levels in blister fluid of toxic epidermal necrolysis. *J. Am. Acad. Dermatol*. 47 (1), 58–62. doi:10.1067/mjd.2002.120473

Daly, A. K., Donaldson, P. T., Bhatnagar, P., Shen, Y., Pe'er, I., Floratos, A., et al. (2009). HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat. Genet*. 41 (7), 816–819. doi:10.1038/ng.379

Dash, C. H. (1975). Penicillin allergy and the cephalosporins. *J. Antimicrob. Chemother*. 1 (Suppl. 3), 107–118. doi:10.1093/jac/1.suppl_3.107

Dias de Castro, E., Leblanc, A., Sarmento, A., and Cernadas, J. R. (2015). An unusual case of delayed-type hypersensitivity to ceftriaxone and meropenem. *Eur. Ann. Allergy Clin. Immunol*. 47 (6), 225–227. .

Duong, T. A., Valeyrie-Allanore, L., Wolkenstein, P., and Chosidow, O. (2017). Severe cutaneous adverse reactions to drugs. *Lancet*. 390 (10106), 1996–2011. doi:10.1016/S0140-6736(16)30378-6

El-Ghaiesh, S., Monshi, M. M., Whitaker, P., Jenkins, R., Meng, X., Farrell, J., et al. (2012). Characterization of the antigen specificity of T-cell clones from piperacillin-hypersensitive patients with cystic fibrosis. *J. Pharmacol. Exp. Ther*. 341 (3), 597–610. doi:10.1124/jpet.111.190900

Empedrad, R., Darter, A. L., Earl, H. S., and Gruchalla, R. S. (2003a). Nonirritating intradermal skin test concentrations for commonly prescribed antibiotics. *J. Allergy Clin. Immunol*. 112 (3), 629–630. doi:10.1016/s0091-6749(03)01783-4

Empedrad, R., Darter, A. L., Earl, H. S., and Gruchalla, R. S. (2003b). Nonirritating intradermal skin test concentrations for commonly prescribed antibiotics. *J. Allergy Clin. Immunol*. 112 (3), 629–630. doi:10.1016/s0091-6749(03)01783-4

Gaeta, F., Valluzzi, R. L., Alonzi, C., Maggioletti, M., Caruso, C., and Romano, A. (2015). Tolerability of aztreonam and carbapenems in patients with IgE-mediated hypersensitivity to penicillins. *J. Allergy Clin. Immunol*. 135 (4), 972–976. doi:10.1016/j.jaci.2014.10.011

Hama, N., Nishimura, K., Hasegawa, A., Yuki, A., Kume, H., Adachi, J., et al. (2019). Galectin-7 as a potential biomarker of Stevens-Johnson syndrome/toxic epidermal necrolysis: identification by targeted proteomics using causative drug-exposed peripheral blood cells. *J. Allergy Clin. Immunol. Pract*. 7 (8), 2894–2897.e7. doi:10.1016/j.jaip.2019.05.002

Hari, Y., Frutig-Schnyder, K., Hurni, M., Yawalkar, N., Zanni, M. P., Schnyder, B., et al. (2001). T cell involvement in cutaneous drug eruptions. *Clin. Exp. Allergy*. 31 (9), 1398–1408. doi:10.1046/j.1365-2222.2001.01164.x

Heinzerling, L., Mari, A., Bergmann, K.-C., Bresciani, M., Burbach, G., Darsow, U., et al. (2013). The skin prick test–European standards. *Clin. Transl. Allergy*. 3 (1), 3. doi:10.1186/2045-7022-3-3

Hjortlund, J., Mortz, C., Skov, P., Eller, E., Poulsen, J. M., Borch, J. E., et al. (2012). One-week oral challenge with penicillin in diagnosis of penicillin allergy. *Acta Derm. Venereol*. 92 (3), 307–312. doi:10.2340/00015555-1254

Hung, S. I., Chung, W. H., Liou, L.-B., Chu, C. C., Lin, M., Huang, H. P., et al. (2005). HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc. Natl. Acad. Sci. U.S.A*. 102 (11), 4134–4139. doi:10.1073/pnas.0409500102

Illing, P. T., Vivian, J. P., Dudek, N. L., Kostenko, L., Chen, Z., Bharadwaj, M., et al. (2012). Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature*. 486 (7404), 554–558. doi:10.1038/nature11147

Johansen, J. D., Aalto-Korte, K., Agner, T., Andersen, K. E., Bircher, A., Bruze, M., et al. (2015). European Society of Contact Dermatitis guideline for diagnostic patch testing-recommendations on best practice. *Contact Dermatitis*. 73 (4), 195–221. doi:10.1111/cod.12432

Kardaun, S. H., Sidoroff, A., Valeyrie-Allanore, L., Halevy, S., Davidovici, B. B., Mockenhaupt, M., et al. (2007). Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br. J. Dermatol*. 156 (3), 609–611. doi:10.1111/j.1365-2133.2006.07704.x

Kauppinen, K. (1991). Fixed drug eruptions and oral rechallenge. *Cleve. Clin. J. Med*. 58 (1), 64–65. doi:10.3949/ccjm.58.1.64

Keane, N. M., Roberts, S. G., Almeida, C. A., Krishnan, T., Chopra, A., Demaine, E., et al. (2012). High-avidity, high-IFNgamma-producing CD8 T-cell responses following immune selection during HIV-1 infection. *Immunol. Cell Biol*. 90 (2), 224–234. doi:10.1038/icb.2011.34

Khalil, G., El-Sabban, M., Al-Ghadban, S., Azzi, S., Shamra, S., Khalifé, S., et al. (2008). Cytokine expression profile of sensitized human T lymphocytes following *in vitro* stimulation with amoxicillin. *Eur. Cytokine Netw*. 19 (3), 131–141. doi:10.1684/ecn.2008.0132

Kim, J. Y., Sohn, K. H., Song, W. J., and Kang, H. R. (2013). A case of drug reaction with eosinophilia and systemic symptoms induced by ethambutol with early features resembling Stevens-Johnson syndrome. *Acta Derm. Venereol*. 93 (6), 753–754. doi:10.2340/00015555-1600

Komatsu-Fujii, T., Kaneko, S., Chinuki, Y., Suyama, Y., Ohta, M., Niihara, H., et al. (2017). Serum TARC levels are strongly correlated with blood eosinophil count in patients with drug eruptions. *Allergol. Int*. 66 (1), 116–122. doi:10.1016/j.alit.2016.06.003

Komatsu-Fujii, T., Chinuki, Y., Niihara, H., Hayashida, K., Ohta, M., Okazaki, R., et al. (2018). The thymus and activation-regulated chemokine (TARC) level in serum at an early stage of a drug eruption is a prognostic biomarker of severity

of systemic inflammation. *Allergol. Int.* 67 (1), 90–95. doi:10.1016/j.alit.2017.06.001

Konvinse, K. C., Phillips, E. J., White, K. D., and Trubiano, J. A. (2016). Old dog begging for new tricks: current practices and future directions in the diagnosis of delayed antimicrobial hypersensitivity. *Curr. Opin. Infect. Dis.* 29 (6), 561–576. doi:10.1097/QCO.0000000000000323

Konvinse, K. C., Trubiano, J. A., Pavlos, R., James, I., Shaffer, C. M., Bejan, C. A., et al. (2019). HLA-A*32:01 is strongly associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms. *J. Allergy Clin. Immunol.* 144 (1), 183–192. doi:10.1016/j.jaci.2019.01.045

Lammintausta, K., and Kortekangas-Savolainen, O. (2005). The usefulness of skin tests to prove drug hypersensitivity. *Br. J. Dermatol.* 152 (5), 968–974. doi:10.1111/j.1365-2133.2005.06429.x

Lochmatter, P., Beeler, A., Kawabata, T. T., Gerber, B. O., and Pichler, W. J. (2009). Drug-specific *in vitro* release of IL-2, IL-5, IL-13 and IFN-gamma in patients with delayed-type drug hypersensitivity. *Allergy*. 64 (9), 1269–1278. doi:10.1111/j.1398-9995.2009.01985.x

Lonjou, C., Thomas, L., Borot, N., Ledger, N., de Toma, C., LeLouet, H., et al. (2006). A marker for Stevens-Johnson syndromeethnicity matters. *Pharmacogenomics J.* 6 (4), 265–268. doi:10.1038/sj.tpj.6500356

Makris, M. P., Koulouris, S., and Kalogeromitros, D. (2010). Nonimmediate systemic hypersensitivity reaction to beta-lactam intradermal tests. *J Investig. Allergol. Clin. Immunol.* 20 (7), 630–631. .

Mallal, S., Nolan, D., Witt, C., Masel, G., Martin, A. M., Moore, C., et al. (2002). Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet*. 359 (9308), 727–732. doi:10.1016/s0140-6736(02)07873-x

Mallal, S., Phillips, E., Carosi, G., Molina, J. M., Workman, C., Tomazic, J., et al. (2008). HLA-B*5701 screening for hypersensitivity to abacavir. *N. Engl. J. Med.* 358 (6), 568–579. doi:10.1056/NEJMoa0706135

Mill, C., Primeau, M. N., Medoff, E., Lejtenyi, C., O'Keefe, A., Netchiporouk, E., et al. (2016). Assessing the diagnostic properties of a graded oral provocation challenge for the diagnosis of immediate and nonimmediate reactions to amoxicillin in children. *JAMA Pediatr.* 170 (6), e160033. doi:10.1001/jamapediatrics.2016.0033

Murata, J., Abe, R., and Shimizu, H. (2008). Increased soluble Fas ligand levels in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis preceding skin detachment. *J. Allergy Clin. Immunol.* 122 (5), 992–1000. doi:10.1016/j.jaci.2008.06.013

Nagao-Dias, A. T., Teixeira, F. M., and Coelho, H. L. (2009). Diagnosing immune-mediated reactions to drugs. *Allergol. Immunopathol.* 37 (2), 98–104. doi:10.1016/s0301-0546(09)71112-7

Nyfeler, B., and Pichler, W. J. (1997). The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin. Exp. Allergy.* 27 (2), 175–181.

Ogawa, K., Morito, H., Hasegawa, A., Daikoku, N., Miyagawa, F., Okazaki, A., et al. (2013). Identification of thymus and activation-regulated chemokine (TARC/CCL17) as a potential marker for early indication of disease and prediction of disease activity in drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS). *J. Dermatol. Sci.* 69 (1), 38–43. doi:10.1016/j.jdermsci.2012.10.002

Osawa, J., Naito, S., Aihara, M., Kitamura, K., Ikezawa, Z., and Nakajima, H. (1990). Evaluation of skin test reactions in patients with non-immediate type drug eruptions. *J. Dermatol.* 17 (4), 235–239. doi:10.1111/j.1346-8138.1990.tb01631.x

Ostrov, D. A., Grant, B. J., Pompeu, Y. A., Sidney, J., Harndahl, M., Southwood, S., et al. (2012). Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc. Natl. Acad. Sci. U.S.A.* 109 (25), 9959–9964. doi:10.1073/pnas.1207934109

Ozkaya-Bayazit, E., Bayazit, H., and Ozarmagan, G. (1999). Topical provocation in 27 cases of cotrimoxazole-induced fixed drug eruption. *Contact Dermatitis.* 41 (4), 185–189. doi:10.1111/j.1600-0536.1999.tb06127.x

Padovan, E., Mauri-Hellweg, D., Pichler, W. J., and Weltzien, H. U. (1996). T cell recognition of penicillin G: structural features determining antigenic specificity. *Eur. J. Immunol.* 26 (1), 42–48. doi:10.1002/eji.1830260107

Peter, J. G., Lehloenya, R., Dlamini, S., Risma, K., White, K. D., Konvinse, K. C., et al. (2017). Severe delayed cutaneous and systemic reactions to drugs: a global perspective on the science and art of current practice. *J. Allergy Clin. Immunol. Pract.* 5 (3), 547–563. doi:10.1016/j.jaip.2017.01.025

Phillips, E. J., Sullivan, J. R., Knowles, S. R., and Shear, N. H. (2002). Utility of patch testing in patients with hypersensitivity syndromes associated with abacavir. *AIDS.* 16 (16), 2223–2225. doi:10.1097/00002030-200211080-00017

Phillips, E. J., Bigliardi, P., Bircher, A. J., Broyles, A., Chang, Y. S., Chung, W. H., et al. (2019). Controversies in drug allergy: testing for delayed reactions. *J. Allergy Clin. Immunol.* 143 (1), 66–73. doi:10.1016/j.jaci.2018.10.030

Phillips, E. J. (2016). Classifying ADRs–does dose matter? *Br. J. Clin. Pharmacol.* 81 (1), 10–12. doi:10.1111/bcp.12749

Picard, M., Robitaille, G., Karam, F., Daigle, J. M., Bédard, F., Biron, É., et al. (2019). Cross-reactivity to cephalosporins and carbapenems in penicillin-allergic patients: two systematic reviews and meta-analyses. *J. Allergy Clin. Immunol. Pract.* 7 (8), 2722–2738. doi:10.1016/j.jaip.2019.05.038

Pichler, W. J., and Hausmann, O. (2016). Classification of drug hypersensitivity into allergic, p-i, and pseudo-allergic forms. *Int. Arch. Allergy Immunol.* 171 (3–4), 166–179. doi:10.1159/000453265

Pichler, W. J., and Tilch, J. (2004). The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy.* 59 (8), 809–820. doi:10.1111/j.1398-9995.2004.00547.x

Pichler, W. J., and Watkins, S. (2014). Interaction of small molecules with specific immune receptors: the p-i concept and its consequences. *Curr. Immunol. Rev.* 10 (1), 7–18. doi:10.2174/1573395510666140407212357

Polak, M. E., Belgi, G., McGuire, C., Pickard, C., Healy, E., Friedmann, P. S., et al. (2013). *In vitro* diagnostic assays are effective during the acute phase of delayed-type drug hypersensitivity reactions. *Br. J. Dermatol.* 168 (3), 539–549. doi:10.1111/bjd.12109

Porebski, G., Pecaric-Petkovic, T., Groux-Keller, M., Bosak, M., Kawabata, T. T., and Pichler, W. J. (2013). *In vitro* drug causality assessment in Stevens-Johnson syndrome–alternatives for lymphocyte transformation test. *Clin. Exp. Allergy.* 43 (9), 1027–1037. doi:10.1111/cea.12145

Porebski, G., Czarnobilska, E., and Bosak, M. (2015). Cytotoxicbased assays in delayed drug hypersensitivity reactions induced by antiepileptic drugs. *Pol. Arch. Med. Wewn.* 125 (11), 823–834. doi:10.20452/pamw.3160

Posadas, S. J., Padial, A., Torres, M. J., Mayorga, C., Leyva, L., Sanchez, E., et al. (2002). Delayed reactions to drugs show levels of perforin, granzyme B, and Fas-L to be related to disease severity. *J. Allergy Clin. Immunol.* 109 (1), 155–161. doi:10.1067/mai.2002.120563

Rive, C. M., Bourke, J., and Phillips, E. J. (2013). Testing for drug hypersensitivity syndromes. *Clin. Biochem. Rev.* 34 (1), 15–38. .

Romano, A., Gaeta, F., Valluzzi, R. L., Maggioletti, M., Caruso, C., and Quaratino, D. (2016). Cross-reactivity and tolerability of aztreonam and cephalosporins in subjects with a T cell-mediated hypersensitivity to penicillins. *J. Allergy Clin. Immunol.* 138 (1), 179–186. doi:10.1016/j.jaci.2016.01.025

Rozieres, A., Hennino, A., Rodet, K., Gutowski, M. C., Gunera-Saad, N., Berard, F., et al. (2009). Detection and quantification of drug-specific T cells in penicillin allergy. *Allergy.* 64 (4), 534–542. doi:10.1111/j.1398-9995.2008.01674.x

Saito, N., Abe, R., Yoshioka, N., Murata, J., Fujita, Y., and Shimizu, H. (2012). Prolonged elevation of serum granulysin in drug-induced hypersensitivity syndrome. *Br. J. Dermatol.* 167 (2), 452–453. doi:10.1111/j.1365-2133.2012.10921.x

Sala Cunill, A., Labrador-Horrillo, M., Guilarte, M., Luengo, O., and Cardona, V. (2011). Generalised delayed desquamative exanthema after intradermal testing with betalactam antibiotics. *Allergy.* 66 (5), 702–703. doi:10.1111/j.1398-9995.2010.02495.x

Shear, N. H., and Dodiuk-Gad, R. P. (2019). *Advances in diagnosis and management of cutaneous adverse drug reactions*., Vol. 1. Singapore: ADIS, 307.

Shiina, T., Inoko, H., and Kulski, J. K. (2004). An update of the HLA genomic region, locus information and disease associations: 2004. *Tissue Antigens.* 64 (6), 631–649. doi:10.1111/j.1399-0039.2004.00327.x

Shiohara, T., Mizukawa, Y., and Aoyama, Y. (2015). Monitoring the acute response in severe hypersensitivity reactions to drugs. *Curr. Opin. Allergy Clin. Immunol.* 15 (4), 294–299. doi:10.1097/ACI.0000000000000180

Sidoroff, A., Halevy, S., Bavinck, J. N. B., Vaillant, L., and Roujeau, J. C. (2001). Acute generalized exanthematous pustulosis (AGEP)–a clinical reaction pattern. *J. Cutan. Pathol.* 28 (3), 113–119. doi:10.1034/j.1600-0560.2001.028003113.x

Su, S. C., Hung, S. I., Fan, W. L., Dao, R. L., and Chung, W. H. (2016). Severe cutaneous adverse reactions: the pharmacogenomics from research to clinical implementation. *Int. J. Mol. Sci.* 17 (11), 1890. doi:10.3390/ijms17111890

Su, S. C., Mockenhaupt, M., Wolkenstein, P., Dunant, A., Le Gouvello, S., Chen, C. B., et al. (2017). Interleukin-15 is associated with severity and mortality in Stevens-Johnson syndrome/toxic epidermal necrolysis. *J. Invest. Dermatol.* 137 (5), 1065–1073. doi:10.1016/j.jid.2016.11.034

Suthumchai, N., Srinoulprasert, Y., Thantiworasit, P., Rerknimitr, P., Tuchinda, P., Chularojanamontri, L., et al. (2018). The measurement of drug-induced interferon gamma-releasing cells and lymphocyte proliferation in severe cutaneous adverse reactions. *J. Eur. Acad. Dermatol. Venereol.* 32 (6), 992–998. doi:10.1111/jdv.14890

Syrigou, E., Zande, M., Grapsa, D., and Syrigos, K. (2016). Severe delayed skin reaction during intradermal testing with beta-lactam antibiotics. *J. Allergy Clin. Immunol. Pract.* 4 (1), 158–159. doi:10.1016/j.jaip.2015.07.018

Teraki, Y., Kawabe, M., and Izaki, S. (2013). Possible role of TH17 cells in the pathogenesis of Stevens-Johnson syndrome and toxic epidermal necrolysis. *J. Allergy Clin. Immunol.* 131 (3), 907–909. doi:10.1016/j.jaci.2012.08.042

Tomida, E., Kato, Y., Ozawa, H., Hasegawa, H., Ishii, N., Hashimoto, T., et al. (2016). Causative drug detection by drug-induced lymphocyte stimulation test in drug-induced linear IgA bullous dermatosis. *Br. J. Dermatol.* 175, 1106–1108. doi:10.1111/bjd.14069

Torres, M. J., Sánchez-Sabaté, E., Alvarez, J., Mayorga, C., Fernández, J., Padial, A., et al. (2004). Skin test evaluation in nonimmediate allergic reactions to penicillins. *Allergy*. 59 (2), 219–224. doi:10.1046/j.1398-9995.2003.00308.x

Trubiano, J. A., Adkinson, N. F., and Phillips, E. J. (2017a). Penicillin allergy is not necessarily forever. *J. Am. Med. Assoc.* 318 (1), 82–83. doi:10.1001/jama.2017.6510

Trubiano, J. A., Redwood, A., Strautins, K., Pavlos, R., Woolnough, E., Chang, C. C., et al. (2017b). Drug-specific upregulation of CD137 on CD8+ T cells aids in the diagnosis of multiple antibiotic toxic epidermal necrolysis. *J. Allergy Clin. Immunol. Pract.* 5 (3), 823–826. doi:10.1016/j.jaip.2016.09.043

Trubiano, J. A., Strautins, K., Redwood, A. J., Pavlos, R., Konvinse, K. C., Aung, A. K., et al. (2018). The combined utility of ex vivo IFN-gamma release enzyme-linked ImmunoSpot assay and in vivo skin testing in patients with antibiotic-associated severe cutaneous adverse reactions. *J. Allergy Clin. Immunol. Pract.* 6 (4), 1287–1296 e1. doi:10.1016/j.jaip.2017.09.004

Trubiano, J. A., Chua, K. Y. L., Holmes, N. E., Douglas, A. P., Mouhtouris, E., Goh, M., et al. (2020). Safety of cephalosporins in penicillin class severe delayed hypersensitivity reactions. *J Allergy Clin Immunol Pract.* 8 (3), 1142–1146.e4. doi:10.1016/j.jaip.2019.10.005

Trubiano, J., and Phillips, E. (2013). Antimicrobial stewardship's new weapon? A review of antibiotic allergy and pathways to 'de-labeling'. *Curr. Opin. Infect. Dis.* 26 (6), 526–537. doi:10.1097/QCO.0000000000000006

Watts, T. J. (2017). Severe delayed-type hypersensitivity to chloramphenicol with systemic reactivation during intradermal testing. *Ann. Allergy Asthma Immunol.* 118 (5), 644–645. doi:10.1016/j.anai.2017.03.004

Weinborn, M., Barbaud, A., Truchetet, F., Beurey, P., Germain, L., and Cribier, B. (2016). Histopathological study of six types of adverse cutaneous drug reactions using granulysin expression. *Int. J. Dermatol.* 55 (11), 1225–1233. doi:10.1111/ijd.13350

White, K. D., Chung, W. H., Hung, S. I., Mallal, S., and Phillips, E. J. (2015). Evolving models of the immunopathogenesis of T cell-mediated drug allergy: the role of host, pathogens, and drug response. *J. Allergy Clin. Immunol.* 136 (2), 219–235; quiz 235. doi:10.1016/j.jaci.2015.05.050

Wolf, R., and Tuzun, Y. (2015). Baboon syndrome and toxic erythema of chemotherapy: fold (intertriginous) dermatoses. *Clin. Dermatol.* 33 (4), 462–465. doi:10.1016/j.clindermatol.2015.04.008

Wolkenstein, P., Chosidow, O., Fléchet, M. L., Robbiola, O., Paul, M., Dumé, L., et al. (1996). Patch testing in severe cutaneous adverse drug reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis. *Contact Dermatitis*. 35 (4), 234–236. doi:10.1111/j.1600-0536.1996.tb02364.x

Yoon, S. Y., Baek, S. H., Kim, S., Lee, Y. S., Lee, T., Bae, Y. J., et al. (2013). Serum procalcitonin as a biomarker differentiating delayed-type drug hypersensitivity from systemic bacterial infection. *J. Allergy Clin. Immunol.* 132 (4), 981–983. doi:10.1016/j.jaci.2013.04.038

Zawodniak, A., Lochmatter, P., Yerly, D., Kawabata, T., Lerch, M., Yawalkar, N., et al. (2010). *In vitro* detection of cytotoxic T and NK cells in peripheral blood of patients with various drug-induced skin diseases. *Allergy*. 65 (3), 376–384. doi:10.1111/j.1398-9995.2009.02180.x

Zhang, F. R., Liu, H., Irwanto, A., Fu, X. A., Li, Y., Yu, G. Q., et al. (2013) HLA-B*13:01 and the dapsone hypersensitivity syndrome. *N. Engl. J. Med.* 369 (17), 1620–1628. doi:10.1056/NEJMoa1213096

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GLOSSARY

ADR: adverse drug reactions

AGEP: acute generalized exanthematous pustulosis

ALDEN: algorithm of drug causality for epidermal necrolysis

CEF: cytomegalovirus (CMV) Epstein-Bar Virus (EBV) and influenza (FLU)

CFSE: carboxyfluorescein succinimidyl ester

DILI: drug induced liver injury

DRESS: drug reaction with eosinophilia and systemic symptoms

ELISpot: enzyme linked ImmunoSpot

FBS: fetal bovine serum

FDE: fixed drug eruption

GrB: granzyme B

HLA: human leukocyte antigens

IFN-γ: Interferon gamma

IP-10: interferon gamma-produced protein 10

LTT: lymphocyte transformation test

MHC: major histocompatibility complex

MPE: maculopapular exanthema

MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

NPV: negative predictive value

PBMC: peripheral blood mononuclear cells

PPV: positive predictive value

SCAR: severe cutaneous adverse reactions

SCORTEN: SCORe of toxic epidermal necrosis

SDRIFE: symmetrical drug-related intertriginous and flexural exanthema

sFasL: soluble Fas-ligand

SFC: spot-forming cells

SI: stimulation index

SJS: Stevens-Johnson syndrome

TARC: thymus and activation-regulated chemokine

TCR: T-cell receptor

TEN: toxic epidermal necrolysis

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The Role of Benzylpenicilloyl Epimers in Specific IgE Recognition

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The high prevalence of allergy to β-lactam antibiotics is a worldwide issue. Accuracy of diagnostic methods is important to prove tolerance or allergy, with skin test considered the best validated *in vivo* method for diagnosing immediate reactions to β-lactams. Although drug provocation test is the reference standard, it cannot be performed in highly risk reactions or in those with positive skin tests. For skin tests, the inclusion of major and minor determinants of benzylpenicillin (BP) is recommended. Commercial skin test reagents have changed along time, including as minor determinants benzylpenicillin, benzylpenicilloate (BPO), and benzylpenilloate (PO). Major determinants consists of multivalent conjugates of benzylpenicilloyl coupled through amide bond to a carrier polymer, such as penicilloyl-polylysine (PPL) or benzylpenicilloyl-octalysine (BP-OL). The chemical stability of such reagents has influenced the evolution of the composition of the commercial kits, as this requirement is necessary for improving the quality and standardization of the product. In this work, we provide a detailed study of the chemical stability of BP determinants. We observed that those structures suffer from an epimerization process in C-5 at different rates. Butylamine-Benzylpenicilloyl conjugates (5*R*,6*R*)-Bu-BPO and (5*S*,6*R*)-Bu-BPO were selected as a simple model for mayor determinant to evaluate the role of the different epimers in the immunoreactivity with sera from penicillin-allergic patients. *In vitro* immunoassays indicate that any change in the chemical structure of the antigenic determinant of BP significantly affects IgE recognition. The inclusion of stereochemically pure compounds or mixtures may have important implications for both the reproducibility and sensitivity of *in vivo* and *in vitro* diagnostic tests.

Keywords: antigenic determinant, diagnostic test, drug allergy, penicillin, specific IgE

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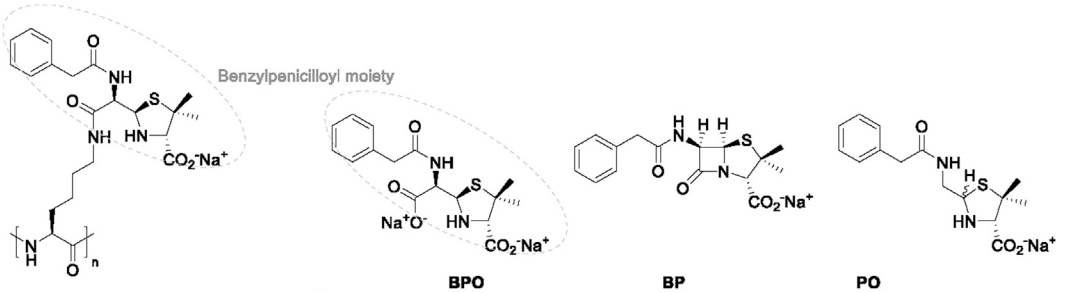
INTRODUCTION

β-lactam antibiotics (BLs) family is nowadays the first choice for the treatment of a large number of bacterial infections. Its extended use probably explains why they are the drugs most frequently involved in drug hypersensitivity reactions, which have important consequences in terms of safety, durability and effectiveness of treatment. Alternative antibiotics may be less effective, more toxic and expensive, and lead to increased bacterial resistance (Doña et al., 2017). Therefore, a correct diagnosis of drug allergy is very important for an adequate prescription of the drug in order to avoid risks for



Commercial kits composition	Validity time			
	1974-2004	1980-2005	2004-2011	2011-currently
Major determinant	PPL	PPL	PPL	BP-OL
Minor determinant mixture (MDM)		BP, BPO, PO	BP, BPO	PO

FIGURE 1 | Mayor (BP-OL and PPL) and minor (BPO, BP and PO) determinants of BP traditionally used in skin tests. Evolution of the commercial kits composition over the time.

the patient (Montañez et al., 2015). The first approach for evaluating the patients is a detailed clinical history, which is often very difficult to obtain. After that, the initial choice is frequently skin test (Doña et al., 2019), and *in vitro* tests which are mainly recommended in patients with dermatological problems, for whom skin test can produce equivocal results, or in high risk patients to reduce the risk of systemic reactions (Mayorga et al., 2016a; Doña et al., 2017). However, *in vitro* tests are less sensitive than skin test to diagnose penicillin allergy. Although many factors could be involved, one of the most important ones could be the drug or drug metabolite included in the test, in which IgE from patients shows specific recognition (Torres et al., 2010; Mayorga et al., 2016a). Because of patient safety, above described methods are the preferred for diagnosis of drug allergy (Demoly et al., 2014; Doña et al., 2019). However if skin test and *in vitro* tests are negative, drug provocation tests, also called oral challenge, is the reference standard test required to confirm diagnosis (Torres et al., 2017; Demoly et al., 2014).

The most representative drug model nowadays in the study of immediate (or IgE-mediated) reactions to BLs continues to be Benzylpenicillin (BP) (Levine and Ovary 1961; Batchelor et al., 1965). The main reason is its well-known reactivity based on the nucleophilic attack of free amino groups of proteins to the extremely reactive β-lactam ring. The opening of the high strain four member ring is an efficient process that leads to the formation of the benzylpenicilloyl determinant. The benzylpenicilloyl amide linked to protein constitutes the reaction product of the 95% of the penicillin molecules that reacts with proteins under physiological conditions, and it is thus considered the major antigenic determinant of BP. The remaining BP molecules react in a different way, resulting in other structures considered as minor determinants, such as BPO acid form and benzylpenilloic acid (PO) (Martin-Serrano et al., 2016). Many of these structures can be recognized in a different way by IgE from allergic patients, and therefore they are used with diagnostic purposes in *in vivo* and *in vitro* tests.

In the case of skin testing, BPO acid, BPO amide forms and PO have been used, showing in some cases higher sensitivity than when using BP itself, probably because the two forms are able to bind better the polyclonal IgE. Extended studies have been carried out since 1977, when the suitability of the major determinant and the different minor determinants was evaluated. In fact, commercial kits have continuously changed over the time. The first commercial kit, in 1974, contained only penicilloyl-poly-L-lysine (PPL) as major determinant. In 1980s, commercial kit included the traditional BP reagents: PPL and a minor determinant mixture (MDM), containing BP, BPO and PO, **Figure 1**. Such kits were removed from the market between 2004 and 2005 (Ariza et al., 2015). In 2004 a different composition mixture was commercialized, including PPL as major determinant, and only BP and BPO as MDM. In 2011, this formulation was substituted by the more stable benzylpenicilloyl-octa-L-lysine (BP-OL) as major determinant and PO as minor determinant (Fernández et al., 2013; Fernandez et al., 2017). Indeed the diagnosis guidelines from Europe (Maria J. Torres et al., 2003) and North America (Joint Task Force on Practice Parameters et al., 2010) recommended the use of these antigenic determinants of penicillin in skin testing. Nowadays, the commercial determinants in skin test reagents used are BPO-lysine polymer conjugates (BP-OL or PPL, in Europe and United States respectively), together with PO (only in Europe) (Ariza et al., 2015; Martin-Serrano et al., 2016). However, it should be noted that the commercial determinants available depend on the country.

In the case of *in vitro* tests, immunoassays, which are based on the determination of specific IgE, have been the most widely employed technique. The amide form of BPO covalently bound to PLL is included in solid phase of both commercial and homemade

immunoassays (Montañez et al., 2011a). In fact, the ImmunoCAP tests available for several penicillins (BP, amoxicillin, ampicillin and penicillin V) is based on the penicilloyl-PLL conjugate attached to cellulose solid phase (Fontaine et al., 2007). The homemade RAST (Radio Allergo Sorbent Test) also employs BPO-PLL conjugates attached to a solid phase which, in this case, is a cellulose paper disc. The main reason for the endorse use of such compounds in diagnosis is their high specific recognition by sIgE from penicillin-allergic patients, together with the straightforward reaction of BP with amine nucleophiles and the stability of the penicilloyl determinants formed. This has allowed modifications in homemade RAST assays, in which different carriers and solid phases have been successfully tested (Montañez et al., 2008; Ruiz-Sanchez et al., 2012; Vida et al., 2013; Mayorga et al., 2016b). However, the IgE recognition with BP determinants different from BPO amide structure has not been studied in detail.

Immunoassays are a valuable tool for evaluating the IgE recognition of the different structures, and in this study we have used them to address the immunological recognition of antigenic determinants and very related chemical structures derived from their storage conditions. In fact, the chemical stability of BP skin test reagents has influenced the evolution of the composition of the commercial kits, as this requirement is necessary for improving the quality and standardization of the product.

Experimental Nuclear Magnetic Resonance (NMR) studies and theorical calculations are suitable methods to get insight into the stability of the determinants and the structural mechanisms concerned. Herein, we report a detailed study of the chemical stability of the BP determinants traditionally used in skin tests, elucidating the chemical process involved, the resulting isomers formed as well as their structural immunoreactivity (or immunological recognition).

MATERIALS AND METHODS

Standard chemicals were obtained from Aldrich or VWR and used without further purification. Phosphate buffer saline (PBS, pH~7.4) was prepared as described elsewhere (Blanca et al., 1992), by dissolving 40 mg of NaCl, 1 mg of KH_2PO_4, 4.5 mg of Na_2HPO_4 and 1 mg of KCl in 5 ml of either H_2O or D_2O (99.96% D, from VWR). Carbonate buffer (pH~10.2) was prepared by disolving 14.5 mg of Na_2CO_3 and 9.5 mg of $NaHCO_3$ in 5 ml of either H_2O or D_2O. BP sodium salt, sodium benzylpenicilloate and sodium benzylpenilloate were supplied by DIATER, SA. Benzylpenicilloyl-Butylamine (Bu-BPO) was prepared as previously described (Sánchez-Sancho et al., 2002). NMR samples were prepared by dissolving 8 mg of the corresponding determinant (BP, BPO, PO or Bu-BPO) in 0.75 ml of the corresponding deuterated solvent and solutions were kept at the indicated temperature until the spectra were recorded. ^{1}H-NMR spectra were measured in the indicated deuterated solvent on a Bruker Ascend 400 MHz spectrometer. Proton chemical shifts (δ) are reported with the solvent resonance employed as the internal standard (D_2O δ 4.79).

Computational Studies

All calculations were performed with the Gaussian 16 package (Frisch et al., 2016). In all simulations, the solvent effect was considered including the polarizable continuum model (PCM) (Tomasi et al., 2005), and water as solvent. The potential energy scans were done for compounds BPO, PO and Bu-BPO at DFT/B3LYP/6-311G (2 days,p) level of theory using the dihedral angle (H_6-C_6-C_5-H_5) as variable. The minima obtained in the potential energy scan was used as the initial point for the optimization of their structures at B3LYP/6-311++G (2 days,p) level or theory. The absence of a negative frequency in analytical Hessian calculations confirmed that all the geometries found were minima. To study the intramolecular hydrogens bonds in these molecules, a topological analysis was done using the Bader's atoms in molecules (AIM) theory (Bader 1991), and the Multiwfn 3.6 program (Lu and Chen 2012; Lu 2020).

Selection of Patients

Patients with a clinical history of an immediate allergic reaction to BP diagnosed following European Academy of Allergy and Clinical Immunology (EAACI) and European Network of Drug Allergy (ENDA) guidelines (Doña et al., 2019; Romano et al., 2020). The studied group was obtained from the Regional University Hospital of Málaga Drug Allergy database, from which we selected eleven cases with a positive skin test and *in vitro* detection of sIgE to the BP greater than 4.5%, measured by direct RAST. Data from the patients included in the study are displayed in **Supplementary Table S1** (ESI).

The study was approved by the institutional review board, and informed consent for all procedures was obtained from all patients.

Skin Test

Skin prick tests and, if negative, intradermal tests were performed as described (Doña et al., 2019; Romano et al., 2020), using PPL (DAP, Diater, Leganés, Spain) at $1.07{\cdot}10^{-2}$ M, minor determinant mixture (MDM: BP, BPO and PO) at 1.5 M. Since May 2011 DAP composition has changed and includes the major determinant BP-OL at 0.04 mg/ml, equivalent to $8.64{\cdot}10^{-5}$ M concentration of the benzylpenicilloyl moiety, and the minor determinant (MD) at 0.5 mg/ml, equivalent to $1.5{\cdot}10^{-3}$ M concentration of PO.

Readings were done after 20 min and considered positive: 1) In skin prick test, if a wheal larger than 3 mm surrounded by erythema appeared, with a negative response to the control saline; 2) In intradermal tests, if the increase in diameter of the wheal area marked initially was greater than 3 mm surrounded by erythema. Positive data expressed as two diameters being one of them the straight line connecting the two most distant points of the wheal and the other the one at 180° (Brockow et al., 2002).

In vitro Specific IgE Determination

RAST was done using BP conjugated to PLL functionalized-cellulose discs resulting in BPO-PLL in the solid phase, as described (Antunez et al., 2006; Ariza et al., 2016), and radiolabeled anti-IgE antibody (kindly provided by Thermo Fisher Scientific and radiolabeled in our laboratory) (Martín-

Serrano et al., 2020). Results were expressed as percentage from a maximum counts and samples were considered positive if the percentage was higher than 2.5% of label uptake, which was the mean + 2SD of a negative control group.

Competitive Inhibition Immunoassay

Solution of inhibitors were prepared as follows. Inhibitor 1: (5*R*,6*R*)-Bu-BPO was freshly dissolved in PBS; Inhibitor 3: (5*R*,6*R*)-Bu-BPO in carbonate buffer for 7 days afforded the mixture including 55% of its epimer, (5*S*,6*R*)-Bu-BPO; Inhibitor 2: a mixture with equivalent volumes of previous samples (inhibitors 1 and 3) resulted in the mixture containing 22.5% of C-5 epimer. RAST inhibition assay was done as described (Antunez et al., 2006; Ariza et al., 2016), incubating sera from patients and the Bu-BPO determinants (as inhibitors) in three ten-fold decreasing concentrations (100 mM–1 mM) for 18 h at room temperature. After this, the BPO-PLL discs were added, and RAST procedure was performed as described above. The results were expressed as percentage inhibition with respect to the serum incubated only with PBS (non-inhibited serum). Comparison of the inhibition capacity of the different inhibitors was performed at 50% inhibition using the IC50 and statistically analyzed for differences among the distributions of the three inhibitors by Friedman test, Wilcoxon test was used to make comparisons between two pair groups. All assays were performed at room temperature.

RESULTS

Stability of the Minor Determinants

NMR Studies were performed to evaluate the stability of the minor determinant reagents used along the last years in solution, in a 7–12 pH range. Solutions of (3*S*,5*R*,6*R*)-BP resulted in one pure product. In the ^{1}H-NMR it can be clearly distinguished the signal corresponding to H-5, H-6 and H-3, bonded to the β–lactam moiety (Busson and Vanderhaeghe 1976). The compound was completely stable for several days, in both D_2O and physiological conditions (PBS, pH~7.4) (**Supplementary Figure S1**, ESI).

The obtainment of the BPO determinant from BP is a well described procedure (Munro et al., 1978). The formation of BPO is a spontaneous and efficient process when dissolving BP in aqueous basic media (Pajares et al., 2020). Such reaction could be easily followed by ^{1}H-NMR spectroscopy, resulting in a unique compound with a well defined stereochemistry derived from the original BP (**Supplementary Figure S2**, ESI). (3*S*,5*R*,6*R*)-benzylpenicilloate can be easily distinguished from BP by NMR. Signals corresponding to H-5 and H-6 suffer a displacement toward high fields and appear more separated from each other when the β-lactam ring is opened. Additionally, signal corresponding to H-3 displaces from 4.20 to 3.91 ppm. The separation between the signal of both methyl groups of the thiazolidine ring, namely $CH_3(\alpha)$ and $CH_3(\beta)$, increased too.

To evaluate the stability of BPO aqueous solution at different conditions, ^{1}H-NMR spectra were recorded over time. We observed important changes, indicating the formation of a new product. Signal displacements seem to indicate the formation of a diastereoisomer, in particular the epimer (3*S*,5*S*,6*R*)-benzylpenicilloate (Ghebre-Sellassie et al., 1984; Haginaka and Wakai 1985). To get more insight into the C-5 epimerization process, the stability of (3*S*,5*R*,6*R*)-BPO has been tested in aqueous media at different pH and temperatures. First, a solution of the compound in PBS/D_2O (pH~7.4) at room temperature was monitored over time (**Figure 2**).

Appearance of new signals was appreciable after 3 h (**Figure 2B**). The gap between the chemical shifts of the signals corresponding to the geminal methyl groups increased. Signal corresponding to H-5 displaced to higher field, whereas signal corresponding to H-6 displaced to lower field (from 4.55 to 4.75 ppm). The coupling constant between H-6 and H-5 also decreased from 5.3 Hz to 3.8 Hz. All these data confirm the formation of the (5*S*,6*R*)-BPO epimer. The process evolves until it reaches an equilibrium state after ~30 h, in which approximately 70% of the (5*S*,6*R*)-BPO epimer is formed, while a 30% of the original (5*R*,6*R*)-BPO isomers remains in solution (**Figure 3**). To evaluate the effect of pH in the process, a solution of (5*R*,6*R*)-BPO in D_2O was prepared and its evolution was monitored. The appearance of the same signal can be clearly observed after 15 h (**Supplementary Figure S3**, ESI). However, the equilibrium state was not reached until seven days after. The influence of temperature in the process was evaluated. When a solution of (5*R*,6*R*)-BPO in PBS/D_2O (pH~7.4) was monitored cooling at 4°C, we observed that the process is even slower, taking 7 days to reach a conversion of the 60% (**Figure 3**). The same effect was observed when monitoring the process in D_2O at 4°C.

Similar studies were made to evaluate the stability in solution of commercial PO, which included (3*S*,5*R*)- and (3*S*,5*S*)-Benzylpenilloate diastereomers. Their solutions at physiological conditions were analyzed both at room (37°C) and low (4°C) temperature conditions. ^{1}H-NMR spectra of the freshly prepared solutions show two products (**Supplementary Figure S4**, ESI), corresponding to 50:50 mixture of the PO diastereomers in C-5. The solution is stable, observing the same mixture (50:50 ratio) without any degradation product, after 48 h at room temperature, or 7 days at 4°C, at least. Similar behavior is observed when the solution is prepared using D_2O as solvent (**Supplementary Figure S5**, ESI).

Table 1 shows the conversion rates for the epimerization of (5*R*,6*R*)-BPO and the equimolar mixture of (5*R*)-PO and (5*S*)-PO. Differences can be clearly observed between both compounds. For BPO, it is also noticeable the influence of the pH and temperature on the process, which is accelerated in basic media and high temperature.

Stability of the Major Determinants

As previously exposed, the mayor determinants currently used in skin tests are BPO-lysine polymer conjugates (BP-OL or PPL). In order to simplify the study, a low molecular weight amine functionalized compound was used instead of amine functionalized polymers to generate the amide-BPO derivatives. We chose benzylpenicilloyl-butylamine (Bu-BPO)

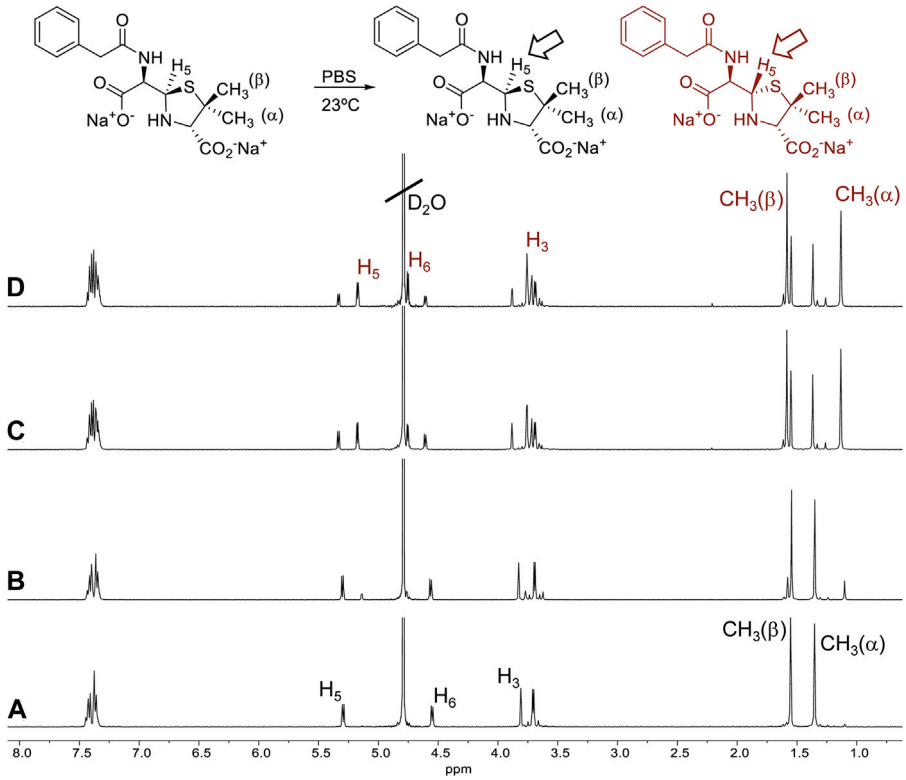


FIGURE 2 | ^{1}H-NMR spectra of a solution of BPO in PBS/D_2O, pD~7.4 at 23°C, **(A)** freshly prepared and after **(B)** 3 h, **(C)** 24 h and **(D)** 48 h.

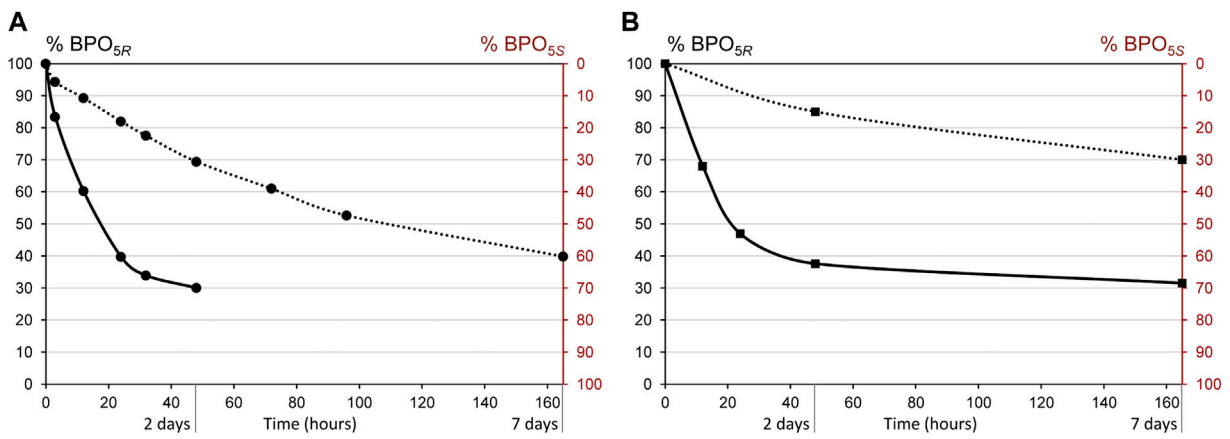


FIGURE 3 | Stability of BPO in **(A)** PBS/D_2O, pD~7.4 and **(B)** D_2O, pD = 6, at 23°C (solid line) and 4°C (dotted line).

as model monomer compound. The reaction between butylamine and BP in aqueous media yielded the product corresponding to the aminolysis of the β-lactam ring (Bu-BPO) instantaneously (Sánchez-Sancho et al., 2002: Montañez et al., 2011b). The stability of Bu-BPO has been tested in similar conditions to those studied for minor determinants.

Solutions of Bu-BPO in aqueous media and PBS resulted perfectly stable at room temperature for 7 days (**Supplementary Figures S6, S7**, ESI), since signals corresponding to Bu-BPO in ^{1}H-NMR spectra do not change in these conditions. To evaluate the stability of the determinant in basic media, solutions of Bu-BPO in carbonate buffer (pH~10.2) at room temperature were evaluated. The formation of the (5*S*,6*R*)-Bu-BPO epimer can be observed in the NMR spectrum after 48 h (**Supplementary Figure S8**, ESI). The process evolves

TABLE 1 | Conversion rates of the minor determinants epimerization process.

	PBS/D_2O (pH~7.4)		D_2O (pH~6)	
Time/T (°C)	**23 ± 2°C**	**4 ± 1°C**	**23 ± 2°C**	**4 ± 1°C**
% (5*R*,6*R*)-BPO remaining in solution				
0 h	100	100	100	100
24 h	40	82	47	
48 h	30	69	38	85
7 days		40	30	70
% (5*R*)-PO remaining in solution				
0 h	50	50	50	50
48 h	50		50	50
7 days		50		

TABLE 2 | Conversion rates of Bu-BPO epimerization process.

Time	D_2O (pH~6)	PBS/D_2O (pH~7.4)	Carbonate buffer/D_2O (pH = 10.2)
% (5*R*,6*R*)-Bu-BPO remaining in solution			
0 h	100	100	100
48 h	100	100	70
7 days	100	100	45

until approximately 55% of the (5*S*,6*R*)-Bu-BPO epimer is formed, while a 45% of the original (5*R*,6*R*)-Bu-BPO isomers remain in solution after 7 days (**Table 2**).

Immunological Studies

The ability of IgE in sera from BP-allergic patients to recognize BP determinants bearing different stereochemistry was studied by RAST inhibition. This assay consists in competitive serum IgE recognition between the solid phase (PLL-BPO conjugate attached to cellulose) and the different inhibitors (Bu-BPO 1) and its mixture with different percentage of its epimer at C-5 (2 (22.5%) and 3, (55%)) at different concentrations in the fluid phase.

BPO-Bu compound was selected because it presents the higher stability in aqueous solution at neutral pH, which permits controlling the precise chemical structures of inhibitors. Moreover, inhibitor 3, consisting of a mixture containing a 55% of its epimer in C-5 (45:55, *5R6R*:*5S6R*) obtained when reached equilibrium in basic aqueous media, was evaluated. In addition, inhibitor 2, a mixture containing midpoint of above concentrations of Bu-BPO and its epimer in C-5 (77.5:22.5, *5R6R*: *5S6R*) was also included.

The immunological evaluation of Bu-BPO, and its mixtures with its epimer at C-5, by RAST inhibition (**Figure 4A**) showed, in most cases, a concentration dependent inhibition of BP-specific sera, with inhibitors following similar patterns in each serum independently of the BP-specific IgE levels, with the best recognition obtained with the inhibitor 1, with higher content of (*5R,6R*)-Bu-BPO.

In vitro IgE recognition is normally considered meaningful and positive when the inhibition percentage is higher than 50%. In general, at the maximum concentration of the determinants (100 mM) there was a positive inhibition of 100% of sera for inhibitors one and two and of 81.8% for the inhitor 3. This inhibition dropped at 1 mM concentration, being positive in three out of 11 cases (27%) for inhitor one and one out 11 cases (9%) for inhibitors 2 and 3, which contain the epimer.

In order to evaluate the role of the epimer in sIgE recognition from allergic patients, we calculated the half-maximal inhibitory concentration (IC50) for each inhibitor represented in **Figure 4B**. The median and interquartilic ranges of IC50 were 3.32 (IQ: 0.96–7.7), 7.19 (IQ:2.14–9.57) and 15.11 (IQ: 7.64–329.3) for inhibitors 1, two and three respectively, indicanting a decrease of recognition with the increase of the epimer concentration. Statistical comparisons by Friedman test showed significant differences for the three inhibitors (p = 0.0002). The concentration to get 50% of inhibition was significantly lower for the inhibitor 1, which contains only (*5R,6R*)-Bu-BPO compared with the inhibitors 2 and 3, which contain 22.5 (p = 0.037) and 55% (p = 0.004) of C-5 epimer, respectively. Moreover, we found significant differences in the IC50 between the inhibitors 2 and 3, which contain 22.5 and 55% of the epimer, respectively (p = 0.002) (**Figure 4B**).

DISCUSSION

BP consumption has decreased over the three last decades, mainly substituted by amoxicillin (with or without clavulanic acid) and cephalosporins. However, skin test to BP determinants is still recomended in the BL allergy work up diagnosis (Doña et al., 2017). Different compositions have been used over time, including as minor determinants: BP (active principle), BPO (the hydrolysis product), and PO (the decarboxilated BPO); and as major determinants conjugates of lysine polymers: PPL and BP-OL. The change in compositions can be explained by stability issues (which must overcome quality and standardization requirements of the product in different countries) and diagnostic results. In fact, a recent study concluded that skin testing with BP can induce false-positive results in patients with a history of BL allergy, and that the addition of BP does not increase the skin test sensitivity obtained with classic BP determinants (Lacombe-Barrios et al., 2016). Moreover, false positive results issues have also been reported in commercial *in vitro* tests, ImmunoCAP, in patients with suspected IgE-mediated hypersensitivity to penicillins and a positive penicillin ImmunoCAP due to the presence of IgE antibodies to phenylethylamine. This is a common allergenic structure that shares structural fragments (benzyl group) of the penicillin determinants and inappropriately, is potentially present in the ImmunoCAP test (Johansson et al., 2013). In addition, experts from the United States have reported that ImmunoCAP to quantify sIgE to BP shows suboptimal sensitivity and low concordance with *in vivo* tests, probably because this immunoassay only identifies IgE to the major determinant (Macy et al., 2010).

Although BPO is no longer the most relevant hapten in immediate reactions to penicillins, major and minor determinants of BP continue to play a key role in drug allergy diagnosis (Fernández et al., 2013). These determinants consist of the precise chemical structures involved in the specific IgE molecular recognition. Therefore the stability of these molecules is an important factor for diagnosis evaluation, as a small change in

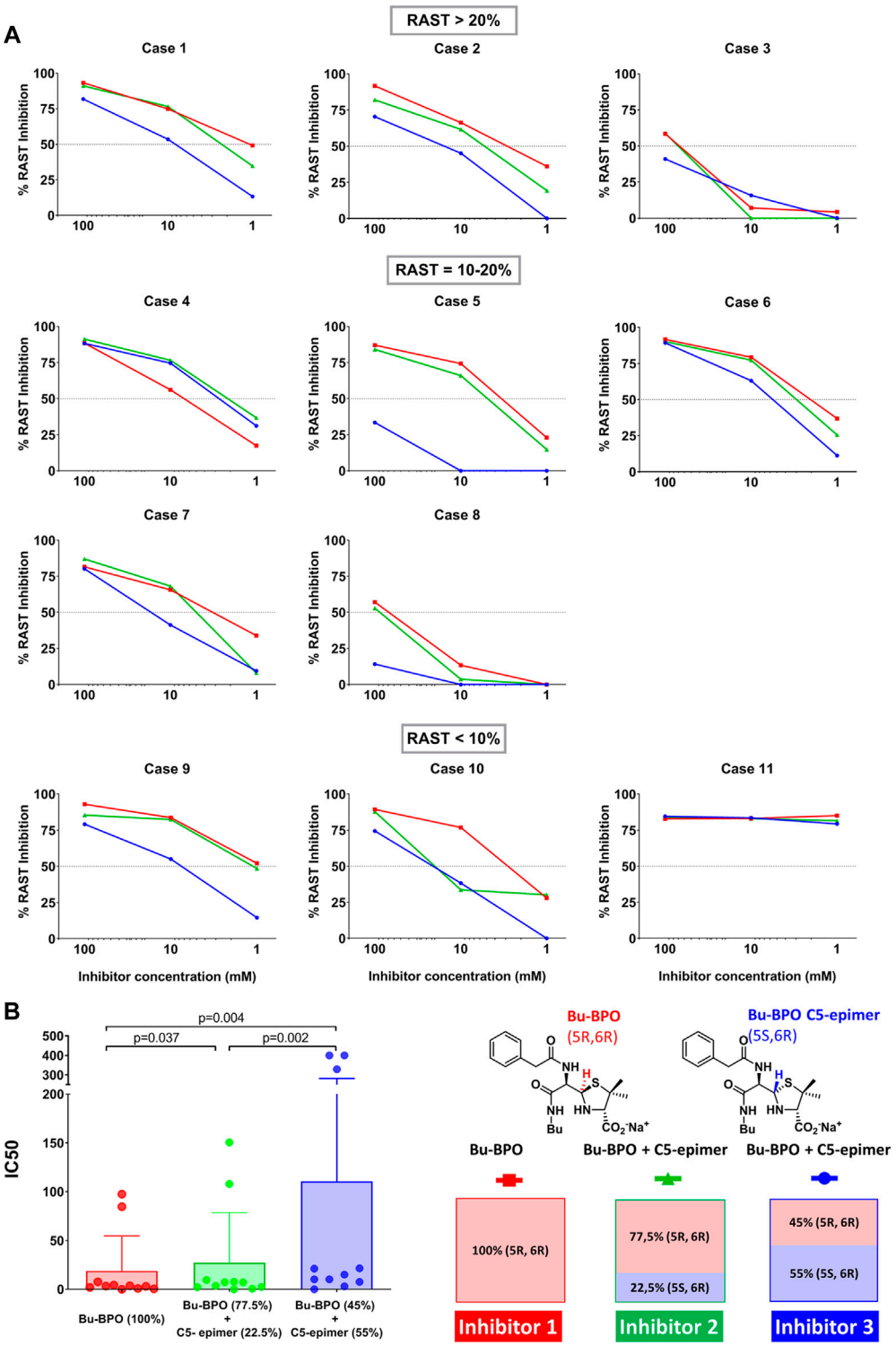


FIGURE 4 | RAST inhibition assays. **(A)** Results obtained with eleven serum samples from patients allergic to penicillins with different BP-sIgE levels. The Bu-BPO and the mixtures including its epimer at C-5, at different proportion, were used as fluid phase inhibitors at three concentrations. Dots lines indicate 50% of inhibition in which sIgE recognition is considered. **(B)** IC50 for the three kinds of inhibitors (Bu-BPO and the two mixtures with its epimer in C-5) from the eleven sera included. Friedman test indicated statistically significant differences between the distributions of the three inhibitors (p = 0.0002). Wilcoxon test was used to make comparisons between two pair groups.

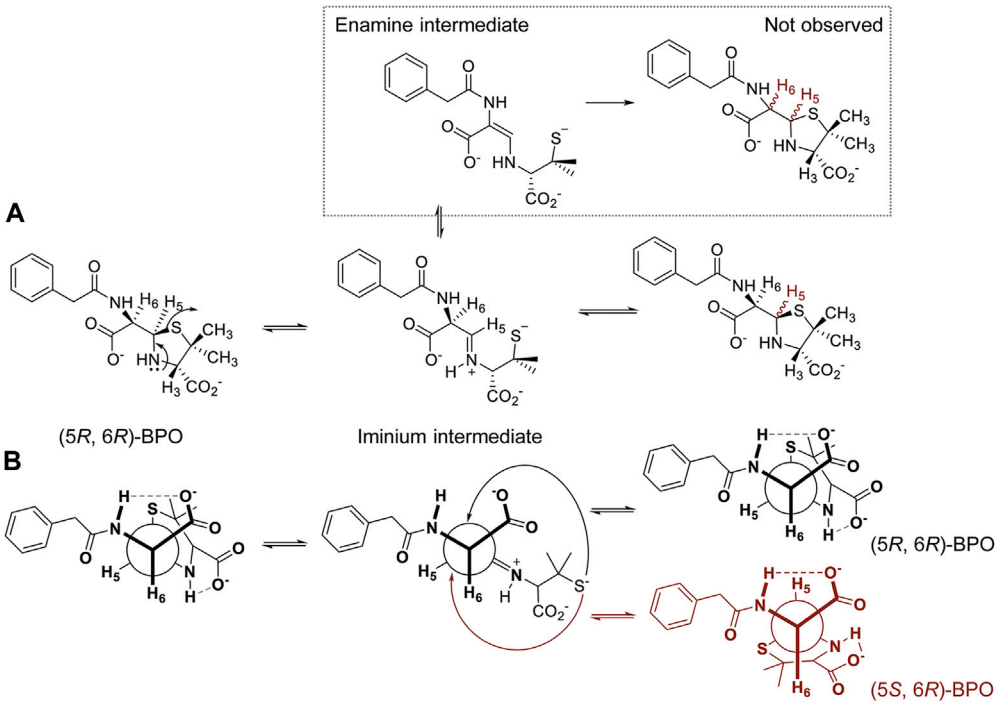


FIGURE 5 | Epimerization process of (5*R*,6*R*)-BPO. **(A)** Proposed mechanism through an iminium intermediate and **(B)** same mechanism represented by the C6-C5 bond Newman projection.

the structure can affect the immunological recognition. Moreover, the stability of the determinants can also affect the reproducibility of the tests, which is a crucial point in diagnosis. In that sense, the stability of the determinants used has been evaluated in aqueous solutions in different pH conditions, ranging from pH 7 to 10.2. BP resulted stable enough in 6–7.2 pH range, however, by increasing basicity the β-lactam ring opens, resulting in the formation of BPO (Munro et al., 1978).

According to previous studies, BP degradation products, formed *in vitro* when the drug is longer in aqueous solution, are the sensitization agents rather than the BP molecule itself. Although authors did not identify these degradation products, we could assume that BPO and its epimer are involved (Neftel et al., 1984; Neftel et al., 1982).

In the present study, (5*R*,6*R*)-BPO initially formed resulted not completely stable in solution. While maintaining the carbon skeleton of the structure, the stereochemistry of one of the three stereocenters is altered (Ghebre-Sellassie et al., 1984; Haginaka and Wakai 1985). Although the formation of different degradation products had been previously proposed (Davis et al., 1991), epimerization rate is considerably greater and the formation of other products is almost negligible. The compound evolved reaching an equilibrium in which 70% of the (5*S*,6*R*)-BPO epimer is formed. The mechanism involved has been proposed through both the formation of an emanime (Davis and Page 1985) or the formation of an iminum intermediate (Ghebre-Sellassie et al., 1984; Branch et al., 1987; Llinás et al., 2001). However, the enamine pathway has been proposed to occur at pH above 12 (Davis et al., 1991), and has not been considered in this study, since the epimerization of C-6 is not observed experimentally (**Figure 5**).

In 7–7.4 pH range, the formation of an iminium intermediate promotes the opening of the thiazolidine ring. This implies breaking the covalent bond between C-5 and the sulfur atom in position 1, and therefore the free rotation between C-3, C-2 and S-1. This is a reversible process in which the ring closure through the intramolecular nucleophilic attach from the sulfur atom to the iminium C-5 can occur from the two different faces of the iminium, forming thus the two diastereoisomers observed. This closing process could be expected to occur faster than the conformational change, however, as the ring closure is a 5-*endo-trig* process, which is assumed to be unfavorable (Baldwin 1976), it may allow sufficient time for a conformational change. This process is described to be faster at higher pH, being almost immediate in strong basic media (Davis et al., 1991). We also observed a strong influence of temperature, reaching the equilibrium in 48 h in PBS/D_2O at room temperature but taking 7 days at 4°C. More than one week is necessary to reach the equilibrium when the solution is in D_2O. Experimentally, no hydrolysis of the iminium intermediate or intramolecular reaction between C-3 carboxylate and C-5 has been observed.

PO is the current available minor determinant reagent for skin test. Its formation may involve C-5 epimerization process, since the two epimers (50:50%) can be observed in freshly prepared solutions. These are very stable compounds in aqueous solutions, as they contain 50% of the product retaining original stereochemistry of BP for the studied time (seven days).

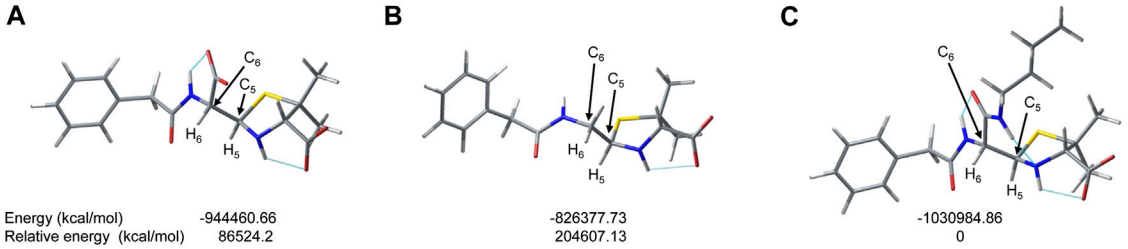


FIGURE 6 | DFT Simulation at PCM(H_2O)/B3LYP/6-311++(2 days,*p*)//PCM(H_2O)/B3LYP/6-311 (2 days,*p*) of **(A)** (5*R*,6*R*)-BPO, **(B)** (5*R*)-PO and **(C)** (5*R*,6*R*)-Bu-BPO and their relative energies.

Bu-BPO was used as model compound to evaluate the stability of the major determinats reagents for skin test. Bu-BPO resulted completely stable in aqueous solutions and PBS at room temperature. Epimerization was oserved when increasing basic conditions to pH = 10.2, where the C-5 epimerization ocurrs, but with a slower rate that in the case of BPO. Seven days were necessary to observe the formation of 55% of the (5*S*,6*R*)-Bu-BPO epimer. This is consistent with previous results described in the bibliography in which the esterification of the carboxy group reduced considerably the rate of spontaneous thiazolidine ring opening (Davis et al., 1991).

To deepen the stability of the different compounds, DFT calculations were made. In **Figure 6** the most stable conformations of (5*R*,6*R*)-BPO, (5*R*)-PO and (5*R*,6*R*)-Bu-BPO are shown.

We can observe the formation of intramolecular hydrogen bonds that could contribute to the stabilization of the molecules. In all cases, a hydrogen bond is observed between the carboxylic group in position three and the hydrogen bonded to N-4 of the thiazolidine ring (cyane lines in **Figure 6**). In the case of BPO, the formation of another hydrogen bond is observed between the carboxylic acid and the amide moiety bonded to C-6. This extra interaction is not possible in the case of PO, since this carboxylic group is not present in the PO structure, and this could be translated into a more efficient epimerization process. In contrast, in the case of Bu-BPO, we can also observe the formation of a third intramolecular hydrogen bond, formed between the amide moiety of the butyl substituent and the N-4 of the thiazolidine ring. In this case, the formation of an extra hydrogen bond could make the epimerization process for Bu-BPO less successful. Electronic effects can also contribute to this effect, since the formation of the third hydrogen bong involves that N-4 of the thiazolidine ring does not have its electron pair available for the iminium formation, minimizing thus the epimerization rate. Althougth the formation of the hydrogen bonds is the main difference observed between PO, BPO and Bu-BPO molecules, we can not exclude other effects when molecules are in saline solutions. It is noteworthy that the DFT calculations using a polarizable continuum model (PCM) results in a negligible relative energy difference between the epimers ((5*R*)-PO/(5*S*)-PO; (5*R*,6*R*)-BPO/(5*S*,6*R*)-BPO and (5*R*,6*R*)-Bu-BPO/(5*S*,6*R*)-Bu-BPO), see ESI), so the driving force for the epimerization process is not clear.

To gain insight into how the relative spatial arrangement of a simple carbon (C-5) in the determinant structure can affect the interaction with the immune system, we selected a determinant model that allows the *in vitro* evaluation of the product retaining original BP stereochemistry, as well as constant proportions of its epimer. Running RAST inhibition experiments requires the inhibitors to be in neutral pH aqueous solution for 18 h. Since BPO is not stable at this conditions, due to epimerization; and PO is already supplied as mixture of epimers (50:50), Bu-BPO was selected as model determinant that allows evaluating both epimers as controling the content of epimers is possible. Therefore, we studied the correlation between Bu-BPO chemical structures and their recognition by sIgE from patients allergic to BP. From the analysis of data, we have observed that, as a general trend, sIgE recognizes Bu-BPO determinants with a lower degree of recognition with increasing proportion content of the C-5 epimer. The way in which the stereochemistry of C-5 of Bu-BPO affects IgE recognition is independent on the patient and the sIgE levels. This higher IgE specificity to (5*R*,6*R*)-Bu-BPO, which preserves the initial stereochemistry of BP, is better visualized with IC50 values. Significant lower concentrations to get the same 50% of inhibition were needed for the inhibitor one compared with the inhibitors two and three to get the same 50% of inhibition. These findings indicate, as a general rule, that both epimers of Bu-BPO determinants are specifically recognized by sera from BP-allergic patients, although the tridimensional conformation of C-5 seems to refine the extent of recognition in a high percentage of cases.

These stability and sIgE recognition data could be somewhat extrapolated about their implications into current skin reagents of BP, and their diagnostic implications, as follows: 1) the major determinant, BP-OL, is stable enough in solution to perform the *in vivo* assay, as no epimerization of this benzylpencilloyl amide form (Bu-BPO) occurs at neutral pH; 2) the minor determinant, PO, includes both epimers at 50:50 proportion, which is stable and constant to perform the *in vivo* assay; 3) regarding the BPO minor determinant ((5*R*,6*R*)-BPO (acid)), although it would contain higher amount of the one with sterochemistry better recognized *in vitro* (inhibitor 1), since it epimerizes in neutral pH solution, the reagent present in the skin test would always be a mixture of compounds in different proportions, impairing its sensitivity and impeding its reproducibility.

CONCLUSION

The chemical stability of BP skin test reagents has influenced the evolution of the composition of the commercial available kits available, as this requirement is necessary for improving the quality and standardization of the product. Althought the epimerization of (5*R*,6*R*)-BPO at C-5 position in aqueous solutions is a well-known process, we provide a detailed study of the chemical stability of BP determinants at pH conditions normally used in order to further understand this progress. Our findings indicate that the epimerization rate is influenced by the structure of the determinant, changing dramatically also the kinetics of the process. *In vitro* immunoassays results show the importance of the spatial configuration of C-5 of benzylpenicilloyl determinants in the IgE recognition, with the original (5*R*,6*R*)-configuration as the best recognized. Any change, albeit small, in the chemical structure of the antigenic determinant of BP significantly affects IgE recognition. Therefore, the inclusion of stereochemically pure compounds or mixtures may have important implications for the sensitivity of both *in vivo* and *in vitro* diagnostic tests.

The conclusions drawn for the BP determinants in this study could serve as basis for the evaluation of the determinants derived from the rest of penicillins. In addition, the conditions of pH and temperature in which these reagents can be handled, for avoiding degradation or epimerization, is crucial to properly use standardized reagents that lead to reproducible results.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board, and informed consent for all procedures was obtained from all patients (Regional University Hospital of Málaga). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EP-I, MIM, and YV conceived and designed the experiments; MIM performed the NMR experiments; FN performed DFT calculations; CM performed *in vitro* tests and analyzed RAST data; TDF made statistical analysis of the results of Competitive Inhibition immunoassay; GB and MJT evaluated and selected patients and controls; YV and MIM analyzed all the data and wrote the paper with input from CM and EP-I.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.585890/full#supplementary-material.

REFERENCES

Antunez, C., Fernandez, T., Blanca-Lopez, N., Torres, M. J., Mayorga, C., Canto, G., et al. (2006). IgE antibodies to betalactams: relationship between the triggering hapten and the specificity of the immune response. *Allergy* 61 (8), 940–946. doi:10.1111/j.1398-9995.2006.01120.x

Ariza, A., Mayorga, C., Fernández, T. D., Barbero, N., Martín-Serrano, A., Pérez-Sala, D., et al. (2015). Hypersensitivity reactions to β-lactams: relevance of hapten-protein conjugates. *J Investig. Allergol. Clin. Immunol.* 25 (1), 12–25.

Ariza, A., Mayorga, C., Salas, M., Doña, I., Martín-Serrano, Á., Pérez-Inestrosa, E., et al. (2016). The influence of the carrier molecule on amoxicillin recognition by specific IgE in patients with immediate hypersensitivity reactions to betalactams. *Sci. Rep.* 6 (1), 35113–35210. doi:10.1038/srep35113

Bader, R. F. W. (1991). A quantum theory of molecular structure and its applications. *Chem. Rev.* 91 (5), 893–928. doi:10.1021/cr00005a013

Baldwin, J. E. (1976). Rules for ring closure. *J. Chem. Soc., Chem. Commun.* 18, 734–736. doi:10.1039/C39760000734

Batchelor, F. R., Dewdney, J. M., and Gazzard, D. (1965). Penicillin allergy: the Formation of the penicilloyl determinant. *Nature* 206 (4982), 362–364. doi:10.1038/206362a0

Blanca, M., Mayorga, C., Perez, E., Suau, R., Juarez, C., Vega, J. M., et al. (1992). Determination of IgE antibodies to the benzyl penicilloyl determinant. A comparison between poly-l-lysine and human serum albumin as carriers. *J. Immunol. Methods* 153 (1–2), 99–105. doi:10.1016/0022-1759(92)90311-g

Branch, S. K., Casy, A. F., and Ominde, E. M. (1987). Application of 1H nuclear magnetic resonance spectroscopy to the analysis of beta-lactam antibiotics and their common degradation products. *J. Pharmaceut. Biomed. Anal.* 5, 73. doi:10.1016/0731-7085(87)80011-0

Brockow, K., Romano, A., Blanca, M., Ring, J., Pichler, W., and Demoly, P. (2002). General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 57 (1), 45–51. doi:10.1046/j.0105-4538.2001.00001.x-i8

Busson, R., and Vanderhaeghe, H. (1976). Preparation and stereochemical analysis of 5-epibenzylpenicillin (S)- and (R)-sulfoxide esters. *J. Org. Chem.* 41 (18), 3054–3056. doi:10.1021/jo00880a039

Davis, A. M., Jones, M., and Page, M. I. (1991). Thiazolidine ring opening in penicillin derivatives. Part 1. Imine formation. *Journal of the Chemical Society, Perkin Transactions* 2 (8), 1219–1223. doi:10.1039/P29910001219

Davis, A. M., and Page., M. I. (1985). Opening of the thiazolidine ring of penicillin derivatives. *J. Chem. Soc., Chem. Commun.* 23 (January), 1702–1704. doi:10.1039/c39850001702

Demoly, P., Adkinson, N. F., Brockow, K., Castells, M., Chiriac, A. M., Greenberger, P. A., et al. (2014). International consensus on drug allergy. *Allergy* 69 (4), 420–437. doi:10.1111/all.12350

Doña, I., Romano, A., and Torres, M. J. (2019). Algorithm for betalactam allergy diagnosis. *Allergy* 74 (9), 1817–1819. doi:10.1111/all.13844

Doña, I., Torres, M. J., Montañez, M. I., and Fernández, T. D. (2017). *In Vitro* diagnostic testing for antibiotic allergy. *Allergy, Asthma and Immunology Research* 9 (4), 288–298. doi:10.4168/aair.2017.9.4.288

Fernández, J., Torres, M. J., Campos, J., Arribas-Poves, F., Blanca, M., and on behalf of the Dap-Diater Group (2013). Prospective, multicenter clinical trial to validate new products for skin tests in the diagnosis of allergy to penicillin. *J. Invest. Allergol. Clin. Immunol.* 23 (6), 398–408. Available at: http://www.jiaci.org/summary/vol23-issue6-num1052.

Fernandez, T. D., Mayorga, C., Salas, M., Barrionuevo, E., Posadas, T., Ariza, A., et al. (2017). Evolution of diagnostic approaches in betalactam hypersensitivity. *Expet Rev. Clin. Pharmacol.* 10, 671. doi:10.1080/17512433.2017.1313110

Fontaine, C., Mayorga, C., Bousquet, P. J., Arnoux, B., Torres, M. J., Blanca, M., et al. (2007). Relevance of the determination of serum-specific IgE antibodies in the diagnosis of immediate beta-lactam allergy. *Allergy* 62 (1), 47–52. doi:10.1111/j.1398-9995.2006.01268.x

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., et al. (2016). *Gaussian 09, revision A.02*. Wallingford, CT: Gaussian, Inc. Available at: http://gaussian.com/g09citation/.

Ghebre-Sellassie, I., Knevel, A. M., and Hem, S. L. (1984). Epimerization of benzylpenicilloic acid in alkaline media. *J. Pharmaceut. Sci.* 73 (1), 125–128. doi:10.1002/jps.2600730135

Haginaka, J., and Wakai, J. (1985). Epimerization of benzylpenicilloate in alkaline aqueous solutions. *Chem. Pharm. Bull.* 33 (6), 2605–2608. doi:10.1248/cpb.33.2605

Johansson, S. G. O., Adédoyin, J., Van Hage, M., Grönneberg, R., and Nopp, A. (2013). . False-positive penicillin immunoassay: an unnoticed common problem. *J. Allergy Clin. Immunol.* 132 (1), 235–237. doi:10.1016/j.jaci.2012.11.017

Joint Task Force on Practice ParametersAmerican Academy of AllergyAsthma and ImmunologyAmerican College of AllergyAsthma and ImmunologyJoint Council of Allergy, et al. (2010). Drug allergy: an updated Practice parameter. *Ann. Allergy Asthma Immunol.* 105 (4), 259–273. doi:10.1016/j.anai.2010.08.002

Lacombe-Barrios, J., Salas, M., Gómez, F., Doña, I., Ariza, A., Mayorga, C., et al. (2016). The addition of benzylpenicillin does not increase the skin test sensitivity obtained with classic β-lactam determinants. *J Investig. Allergol. Clin. Immunol.* 26, 52. doi:10.18176/jiaci.0009

Levine, B. B., and Ovary, Z. (1961). Studies on the mechanism of the formation of the penicillin antigen. III. The N-(D-alpha-benzylpenicilloyl) group as an antigenic determinant responsible for hypersensitivity to penicillin G. *J. Exp. Med.* 114 (6), 875–904. doi:10.1084/jem.114.6.875

Llinás, A., Vilanova, B., Muñoz, F., and Donoso, J. (2001). The role of a β-proton transfer donor in the degradation of benzylpenicillin. *J. Mol. Catal. Chem.* 175 (1–2), 3–16. doi:10.1016/S1381-1169(01)00203-5

Lu, T., and Chen, F. (2012). Multiwfn: a multifunctional wavefunction analyzer. *J. Comput. Chem.* 33 (5), 580–592. doi:10.1002/jcc.22885

Lu, T. (2020). *Multiwfn manual, version 3.6(dev), section 3.14Multiwfn manual, version 3.6(dev)*. Available at: http://sobereva.com/multiwfn/.

Macy, E., Goldberg, B., and Poon, K. Y. (2010). Use of commercial anti-penicillin IgE fluorometric enzyme immunoassays to diagnose penicillin allergy. *Ann. Allergy Asthma Immunol.* 105 (2), 136–141. doi:10.1016/j.anai.2010.06.014

Martin-Serrano, A., Barbero, N., Agundez, J. A., Vida, Y., Perez-Inestrosa, E., and Montanez, M. I. (2016). New advances in the study of IgE drug recognition. *Curr. Pharmaceut. Des.* 22 (45), 6759–6772. doi:10.2174/1381612822666160921142231

Martín-Serrano, A., Mayorga, C., Barrionuevo, E., Pérez, N., Romano, A., Moreno, E., et al. (2020). Design of an antigenic determinant of cefaclor: chemical structure–IgE recognition relationship. *J. Allergy Clin. Immunol.* 145 (4), 1301–1304. doi:10.1016/j.jaci.2019.11.036

Mayorga, C., Celik, G., Rouzaire, P., Whitaker, P., Bonadonna, P., Rodrigues-Cernadas, J., et al. (2016a). *In Vitro* tests for drug hypersensitivity reactions: an ENDA/EAACI drug allergy interest group position paper. *Allergy* 71 (8), 1103–1134. doi:10.1111/all.12886

Mayorga, C., Perez-Inestrosa, E., Molina, N., Montañez, M. I., and Montañez (2016b). Development of nanostructures in the diagnosis of drug hypersensitivity reactions. *Curr. Opin. Allergy Clin. Immunol.* 16 (4), 300–307. doi:10.1097/ACI.0000000000000282

Montañez, M. I., Ariza, A., Mayorga, C., Diana Fernandez, T., and Torres, M. J. (2015). Cross-reactivity in betalactam allergy: alternative treatments. *Current Treatment Options in Allergy* 2 (2), 141–154. doi:10.1007/s40521-015-0050-4

Montañez, M. I., Mayorga, C., Torres, M. J. M. B., and Perez-Inestrosa, E. (2011a). Methodologies to anchor dendrimeric nanoconjugates to solid phase: toward an efficient *in Vitro* detection of allergy to β-lactam antibiotics. *Nanomed. Nanotechnol. Biol. Med.* 7 (6), 682–685. doi:10.1016/j.nano.2011.07.008

Montañez, M. I., Perez-Inestrosa, E., Suau, R., Mayorga, C., Torres, M. J., and Blanca, M. (2008). Dendrimerized cellulose as a scaffold for artificial antigens with applications in drug allergy diagnosis. *Biomacromolecules* 9 (5), 1461–1466. doi:10.1021/bm701380a

Montañez, M. I., Najera, F., and Perez-Inestrosa, E. (2011b). NMR studies and molecular dynamic simulation of synthetic dendritic antigens. *Polymers* 3 (3), 1533–1553. doi:10.3390/polym3031533

Munro, A. C., Chainey, M. G., and Woroniecki, S. R. (1978). Preparation and immunological cross-reactions of penicilloic and penilloic acids. *J. Pharmacol. Sci.* 67 (9), 1197–1204. doi:10.1002/jps.2600670903

Neftel, K. A., Wälti, M., Schulthess, H. K., and Gubler, J. (1984). Adverse reactions following intravenous penicillin-G relate to degradation of the drug *in Vitro*. *Klin. Wochenschr.* 62 (1), 25–29. doi:10.1007/BF01725189

Neftel, K. A., Wälti, M., Spengler, H., and De Weck, A. L. (1982). Effect of storage of penicillin-G solutions on sensitisation to penicillin-G after intravenous administration. *Lancet* 1 (8279), 986–988. doi:10.1016/S0140-6736(82)91991-2

Pajares, M. A., Zimmerman, T., Sánchez-Gómez, F. J., Sánchez-Gómez, F. J. A., Ariza, A., Torres, M. J., et al. (2020). Amoxicillin inactivation by thiol-catalyzed cyclization reduces protein haptenation and antibacterial potency. *Front. Pharmacol.* 11, 189. doi:10.3389/fphar.2020.00189

Romano, A., Atanaskovic-Markovic, M., Barbaud, A., Bircher, A. J., Brockow, K., Caubet, J. C., et al. (2020). Towards a more precise diagnosis of hypersensitivity to beta-lactams - an EAACI position paper. *Allergy* 75 (6), 1300–1315. doi:10.1111/all.14122

Ruiz-Sanchez, A. J., Montañez, M. I., Mayorga, C., Torres, M. J., Kehr, N. S., Vida, Y., et al. (2012). Dendrimer-modified solid supports: nanostructured materials with potential drug allergy diagnostic applications. *Curr. Med. Chem.* 19 (29), 4942–4954. doi:10.2174/0929867311209024942

Sánchez-Sancho, F., Pérez-Inestrosa, E., Suau, R., Mayorga, C., Torres, M. J., and Blanca, M. (2002). Dendrimers as carrier protein mimetics for IgE antibody recognition. Synthesis and characterization of densely penicilloylated dendrimers. *Bioconjugate Chem.* 13 (3), 647–653. doi:10.1021/bc0155824

Tomasi, J., Mennucci, B., and Cammi, R. (2005). Quantum mechanical continuum solvation models. *Chem. Rev.*, 105, 2999. doi:10.1021/cr9904009

Torres, M. J., Ariza, A., Fernández, J., Moreno, E., Laguna, J. J., Montañez, M. I., et al. (2010). Role of minor determinants of amoxicillin in the diagnosis of immediate allergic reactions to amoxicillin. *Allergy* 65 (5), 590–596. doi:10.1111/j.1398-9995.2009.02245.x

Torres, M. J., Blanca, M., Fernandez, J., Romano, A., Weck, A., Aberer, W., et al. (2003). Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 58 (10), 961–972. doi:10.1034/j.1398-9995.2003.00280.x

Torres, M. J., Romano, A., Celik, G., Demoly, P., Khan, D. A., Macy, E., et al. (2017). Approach to the diagnosis of drug hypersensitivity reactions: similarities and differences between Europe and North America. *Clin. Transl. Allergy* 7, 7. doi:10.1186/s13601-017-0144-0

Vida, Y., Montañez, M. I., Collado, D., Najera, F., Ariza, A., Blanca, M., et al. (2013). Dendrimeric antigen-silica particle composites: an innovative approach for IgE quantification. *J. Mater. Chem. B* 1 (24), 3044–3050. doi:10.1039/c3tb20548g

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REVIEW
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Viral Infections and Cutaneous Drug-Related Eruptions

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In the general population, up to 10% of children treated by antibiotics have cutaneous adverse drug reaction, but allergy is confirmed in less than 20% of patients. Most of the non-allergic reactions are probably due to virus, such as enterovirus acute infection or Ebstein-Barr Virus (EBV) acute infection or reactivation. Especially in children, viruses have the propensity to induce skin lesions (maculopapular rash, urticaria) due to their skin infiltration or immunologic response. In drug-related skin eruptions, a virus can participate by activating an immune predisposition. The culprit antibiotic is then the trigger for reacting. Even in severe drug-induced reactions, such as Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) syndrome, viruses take part in immune phenomena, especially herpes viruses. Understanding the mechanisms of both virus- and drug-induced skin reaction is important to develop our clinical reflection and give an adaptive care to the patient. Our aim is to review current knowledge on the different aspects and potential roles of viruses in the different type of drug hypersensitivity reactions (DHR). Although major advances have been made those past year, further studies are needed for a better understanding of the link between viruses and DHR, to improve management of those patients.

Keywords: drug, hypersensitivity, allergy, virus, mechanism

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INTRODUCTION

Drug allergy is a major public health problem, associated with a high morbidity and mortality, as well as elevated medical costs (Macy, 1998; MacLaughlin et al., 2000; Solensky, 2013; Solensky, 2014; van Dijk et al., 2016). The clinical pictures, and the underlying mechanisms are very heterogeneous (Macy, 1998; MacLaughlin et al., 2000; Solensky, 2013; Solensky, 2014; van Dijk et al., 2016). Thus, diagnosis of drug allergies is difficult and a challenge for the treating physician (Macy, 1998;

Abbreviations: ADR, adverse drug reaction; APC, antigen presenting cells; BL, betalactam; COX, cyclooxygenase; CYP, cytochrome P; DRESS, drug rash with eosinophilia and systemic symptoms; HIV, human immunodeficiency virus; HSV, herpes simplex virus; DIHS, drug induced hypersensitivity syndrome; NSAID, nonsteroidal anti-inflammatory drugs; PGE2, prostaglandin E2; SJS, Stevens-Johnson syndrome; SMX, sulfamethoxazole; n-SMX, nitrososulfamethoxazole; TMP, trimethoprim; TEN, toxic epidermal necrolysis; EBV, Epstein-Barr virus; EV, enteroviruses; RSV, respiratory sincitial virus; GCS, Gianotti-Crosti syndrome; MI, mononucleosis infectious; NRTI, nucleoside reverse transcriptase inhibitor; HR, homing receptor; CLA, cutaneous lymphocyte-associated antigen; SAg, superantigen; PRR, pattern recognition receptor; SCAR, severe cutaneous adverse reactions syndrome; DPT, drug provocation test.

MacLaughlin et al., 2000; Solensky, 2013; Solensky, 2014; van Dijk et al., 2016). A further problem is overdiagnosis. It is common, particularly during childhood, as the drug allergy may be transient and allergy tests are difficult, cumbersome, of limited sensitivity and expensive. One of these confounding factors are virus infections, as they constitute the major cause of skin eruptions in childhood and represent an important differential diagnosis in patients with a suspicion of drug allergy (Goodyear et al., 1991). Indeed, common clinical manifestations of drug allergy i.e., maculopapular exanthema and urticaria, are similar to viral-induced rashes. Some viral infections are name-giving for drug-induced exanthemas (rubeola like or measles like exanthemas) and distinction is difficult during the acute phase. Avoidance of the potential incriminated drug is usually recommended, although "threating through" can be considered as an option with close monitoring of the patient.

In addition, viral infections may be involved by providing a co-factor for immune stimulation. Numerous clinical observations suggest that viral infections promote or aggravate drug-related skin rashes (Ponvert et al., 1999; Shiohara and Kano, 2007; Caubet et al., 2011). Epstein Barr Virus (EBV) is one of the best known examples with a higher rate of skin eruptions in EBV-infected patients treated by betalactams (BL) antibiotics (Chovel-Sella et al., 2013). Another example is the apparent role of herpes viruses in the pathogenesis of severe drug-related reactions, particularly in the Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), which is increasingly discussed in the literature (Descamps et al., 2001; Kano et al., 2006; Shiohara et al., 2006).

Based on a selection of best quality papers, the aim of this manuscript is to review current knowledge on the different aspects and potential roles of viruses in the different types of drug hypersensitivity reactions (DHR).

PATHOMECHANISMS

DHR Classification

The traditional classification of Rawlings and Thompson proposed a sub-classification of adverse drug reactions (ADR) into type A reactions, which are due to the pharmacological activity of the drug (80% of all ADR). Type B reactions comprise about 15–20% of all ADR: they involve DHR (Rawlins, 1981).

The DHR have been shown to be induced by different and distinct mechanisms. The drug or drug metabolite usually acts as a hapten, which is able to bind by covalent bonds to a protein and thus forms an antigen that is able to induce IgE- or T cell-mediated allergic reactions (White et al., 2015). Drugs can also stimulate the immune system directly, namely by binding by non-covalent bonds (pharmacological interaction) to immune receptors like HLA or T-cell receptor (TCR); this so-called p-i mechanism stimulate exclusively T-cells (Pichler et al., 2002).

The third mechanism is summarized as "pseudo-allergy," term that is controversial, where the drug interferes with inflammatory mechanisms or activates inflammatory cells like mast cells, eosinophils, neutrophils, etc. without involving the specific immune system. Such pseudo-allergic reactions manifest as clinical pictures mimicking allergy, depending on the cells/mediators involved: e.g., the mast cells with urticarial/anaphylaxis are involved in off-target pharmacological activities of certain drugs on mast cells receptors (MRGPRX2); the blocking of enzymes like cyclooxygenase in nonsteroidal anti-inflammatory drugs (NSAID) can lead to exacerbated asthma or urticaria; and blocking the degradation of bradykinin by angiotensin converting enzyme (ACE) inhibitors may lead to angioedema.

Mechanisms of Viral-Induced Skin Eruptions

Skin eruptions are among the most common causes of consultations at primary care physicians, particularly paediatricians: it has been found that up to 17% of paediatric emergency consultations are motivated by occurrence of a skin eruption (Kramkimel et al., 2010; Landolt et al., 2013). The major causes are infections, most notably viruses. Despite the relatively high frequency of this problem, epidemiologic data are scarce (Folster-Holst and Kreth, 2009a). The estimated prevalence of maculopapular virus-linked exanthemas is estimated to be 158.3/10,000 (CI: 142.3–174.4) (Vega Alonso et al., 2003). Based on typical morphological feature, six classical exanthemas have been described at the beginning of the 20th century, i.e., measles or rubeola, scarlet fever, rubella, Filatow–Dukes disease (fourth disease), erythema infectiosum (fifth disease), and exanthem subitum (sixth disease) (Keighley et al., 2015). Exanthemas not included in the previous list are referred to "atypical exanthemas" (Drago et al., 2012). The majority of exanthema are caused by non-polio enteroviruses, respiratory viruses (adenoviruses, rhinoviruses, parainfluenza viruses, respiratory syncytial virus, influenza viruses), acute EBV, human herpes viruses (HHV) 6 and 7, parvovirus B-19 and norovirus (Hogan, 1996; Leiste et al., 2008). Among enterovirus, the most commonly involved are Coxsackie virus A16 and EV71, responsible for hand, foot and mouth disease, typically in children (He et al., 2017). Different clinical aspects have been described based on the morphological aspects of primary lesions (i.e., erythematous, papular, vesicular, urticarial-like, pustular, or petechial) and the most common types are maculopapular exanthema and maculovesicular exanthema (Schneider et al., 2013).

The mechanisms by which a virus leads to the development of skin eruption have been explored since the 60s (Mims, 1964; Mims, 1966). They are complex and are still not well defined in many aspects. The occurrence of a rash induced by a virus may depend on virus ability to grow in dermal and epidermal cells. Indeed, viruses are able to infiltrate skin and infect tissue cells, via fixation to cellular receptors or intracellular penetration (Laksono et al., 2016). Particularly, it has been shown that skin manifestations can be induced in part by a direct viral cytopathic effect (inclusions, ballooning, vacuolation and necrosis) which may lead to macroscopical modification such as edema and hemorrhage, generating the skin lesions (Geck et al., 1964; Agol, 2012). Theoretically, any circulating virus, free or cell-associated, which localizes in a skin blood vessel can infect the vessel wall (or pass through) and grow in extravascular tissues, giving rise to a skin eruption (Mims, 1966). Skin cell lesions induce discharge of pro-inflammatory products,

especially damage (or danger) signals, cytokines and chemokines (Smith, 1972; Folster-Holst and Kreth, 2009b). Keratinocytes are probably important actors of non-specific inflammation, through the fixation of the virus and the secretion of different signals (Strittmatter et al., 2016). In addition to the direct effect of the virus, immunologic mechanisms induced by the virus can also be involved in the development of a skin lesion. Indeed, viral-induced cell-mediated responses might be responsible for damage through a nonspecific inflammatory reaction (Parham and Janeway, 2009). Recruitment of adaptive immune cells is permitted by the interaction between inflamed endothelium receptors and skin-addressing markers on the lymphocyte surface, for example the CLA (Cutaneous Lymphocyte Antigen) (Schon et al., 2003; Clark, 2010).

From another point of view, viruses can also lead to exanthema by a local delayed (type 4) hypersensitivity reaction within the dermis to various pathogens, such as in Gianotti-Crosti syndrome, where exanthema is typically papulo-vesicular, but neither viral particles nor antigens have been demonstrated in the skin lesions (Gianotti, 1979). This syndrome would results from an immunologic response rather than a primary manifestation of an infection (Lowe et al., 1989; Magyarlaki et al., 1991; Hofmann et al., 1997; Folster-Holst and Kreth, 2009b).

However, it is unknown why skin rashes are seen in only a small proportion of all generalized virus diseases, and the characteristic distribution of skin lesions in different virus exanthema remains unclear (Mims, 1966). Genetic and individual susceptibility may play an important role to the development of skin lesions and should be taken into account to understand the complexity of the problem. Non-immune mechanisms (i.e., sensitivity to histamine, antigen-antibody complexes clearing by reticuloendothelial system) may be involved as personal immunological factors necessary to develop an allergic reaction (Levine, 1965).

Potential Interaction Between Virus and Drug

The interaction between virus immunity and drug hypersensitivity are multiple and complex (White et al., 2015) (**Figure 1**). The heterologous immunity models is an enlarged vision that takes into account the specific HLA-restriction and the minimal co-stimulatory signals observed in drug-related Severe Cutaneous Adverse Reactions (SCARs) (White et al., 2015). In this model, drug is supposed to induce the formation of a neo-antigen recognized by virus-specific memory T cells. Those T cells were earlier sensitized by life-long infecting viruses, which periodically sort out of latency and turn on transcriptional programs (White et al., 2015). This intermittent viral replication stimulates a substantial anti-viral specific T cell proliferation, without developing the functional unresponsiveness which normally follows recurrent infections (Virgin et al., 2009). In this model, memory T cell are generated following pathogen exposure and reside at specific anatomic sites. These memory T cells may cross react with haptenated endogenous peptides presented in the context of the HLA risk allele, or drugs that bind the TCR and/or MHC in a non-covalent manner following the p-i model, or an altered repertoire of endogenous peptides following drug binding to MHC (Todd, 2006).

Another theory that explain this interplay between drug and infection is the danger hypothesis which was firstly proposed by Matzinger since the early 1990s (Das et al., 2011). This model states that the primary driving force of the immune system is to protect against danger (Anderson and Matzinger, 2000). Presentation of an antigen in the absence of danger results in tolerance, while the presence of a danger signal will result in a full-blown immune response. Indeed, three different elements are needed to elicit an immune response. Signal 1 represents the interaction between the MHC-restricted antigen and the T-cell receptor. Signal 2 is represented by the co-stimulatory molecule–receptor interactions and a series of pro-inflammatory cytokines such as IL-2, TNF-α, and IFN-γ that act indirectly on antigen presenting cells to up-regulate the expression of co-stimulatory molecules. Signal 3 represents polarizing cytokines that act directly on T-cells, and lead to either TH1 or TH2 immune responses. The danger signal can result from chemical, physical or viral stress. This theory was proposed to partially explain the reactions in HIV patients.

Regarding IgE-mediated hypersensitivity reactions, there is no data in the literature indicating a link between viruses and IgE mediated drug reactions. However, the implication of viruses in IgE-mediated food allergy is well-known and similarly, a potential role of viruses in these reactions is probable (Muraro et al., 2014). Further studies are needed to explore this important aspect.

ROLE OF VIRUS IN BENIGN NONIMMEDIATE REACTION

Viral Infection as a Differential Diagnosis

A common situation in clinical practice, and particularly in pediatric, is the appearance of a benign exanthema or urticaria (i.e., without any danger signs) in patients treated by antibiotics, mainly BL, and NSAID (Bigby, 2001; Thong and Tan, 2011).

It is difficult to distinguish urticaria-like exanthemas from "classical" urticaria, which is characterized by wheal and flare reactions: in "classical" urticaria, the manifestation is acute after drug intake (min to hours) vs. urticaria-like exanthemas, which appear after days, often together with macular exanthematic lesions. Classical urticarial lesions last <24 h, while some form of the urticaria-like exanthemas (some linked to drug intake) persists longer (e.g., as maculopapular exanthems). In clinical practice, most of these patients are labeled as drug allergic without appropriate testing, mainly due to fear of a life-threatening reaction, leading to an overdiagnosis of drug allergy. However, it has been found that an allergy will be confirmed by a complete allergy workup in only 7–20% of those patients (Caubet et al., 2011; Ponvert et al., 2011; Rubio et al., 2012; Demoly et al., 2014).

The cause of those non-allergic eruptions in patients with a negative allergy workup has been poorly investigated until recently, particularly in the paediatric population. By including 88 children who had developed an exanthema during a BL treatment, Caubet et al. detected a virus by PCR or serology in

65.9% of the children with a negative drug provocation test (DPT), the most frequent being enteroviruses (Picornavirus) (Caubet et al., 2011). Similarly, Atanaskovic-Markovic et al. found that 333 children (22%) tested positive for a virus or *Mycoplasma pneumoniae* infection among 1,026 children with a suspicion of nonimmediate hypersensitivity reactions (Atanaskovic-Markovic et al., 2016). Only two of them were confirmed to be allergic to the culprit drug (Atanaskovic-Markovic et al., 2016). This suggests that in patients developing an exanthema or delayed-appearing urticaria while taking concomitantly a drug, viral infection is frequent; and that reaction to the drug taken can be detected only rarely. Possibly the combination of viral infection—facilitating the drug reaction, is transient, and the single drug may be tolerated. The virus infections would represent the co-stimulatory factor enhancing drug reactions.

However, in these studies, a virus has not been found in all patients with a skin eruption during a BL treatment. It can be explained by the fact that not all viruses have been tested in those studies. From another point of view, we cannot exclude that the positivity of PCR or serology was due to a previous infection or an acute infection without any link with the current rash.

Clinically it is very difficult, and often impossible to differentiate a rash of viral origin or secondary to a drug allergy. Although blood tests are not routinely performed in our current clinical practice for exanthema or urticaria, it has been recently suggested that some tests could be helpful to distinguish between viral- and drug-induced skin eruptions. As an example, Hari Y et al. have shown that in viral exanthemas, IFN-γ is increased in most serum samples from different acute viral diseases, while in drug-induced exanthemas, IL-5 alone or in combination with granzyme B and perforin are often found to be increased – together with some eosinophilia (Hari et al., 1999; Bellini et al., 2013). Another example is the potential role of thymus and activation-regulated chemokine (TARC/CCL17) which plays an important in TH2 immune responses. Thus, a link between serum TARC levels and HHV-6 reactivation in patient with DRESS has been found and serum TARC levels have been suggested to be a useful indicator to differentiate DRESS/DIHS with HHV-6 reactivation from other drug eruptions (Ogawa et al., 2014).

The EBV Example as a Co-Factor for Drug-Induced Skin Eruptions

The best illustration for the drug-related exanthemas during a viral infection is those occurring after antibiotic administration in patients with an acute EBV infection. Indeed, it has been shown that the incidence of skin rash is higher in EBV patients treated by antibiotic (typically ampicillin) compared to EBV patients without associated antibiotic treatment (i.e., 27.8–90% and 3–10%, respectively) (Pullen et al., 1967; Copeman and Scrivener, 1977; Luzuriaga and Sullivan, 2010). No association with age, gender, ethnicity or allergic history appears to be correlated with rash development after antibiotic treatment in EBV patients (Chovel-Sella et al., 2013).

One of the hypothesis regarding the mechanisms for the development of skin eruption occurring in patients with infectious mononucleosis and concomitantly treated by antibiotics, appears to be a transient virus-mediated immune alteration (Thompson and Ramos, 2017). In patients with EBV infection, the $CD8^+$ T cell population is typically expanded, leading to the secretion of INF-γ and interleukine-2 (IL-2). This has been shown to inhibit the TH2-response (IL-4, 5, 6, 9, 13) (Schissel et al., 2000; Banerjee et al., 2014) and the anti-inflammatory IL-10 secretion, while the TH1-response is activated (Onodi-Nagy et al., 2015). These alterations could set the stage for a loss of antigenic tolerance and the development of a reversible DHR (Shiohara and Kano, 2007). Thus, the administration of an antibiotic, especially ampicillin, would then be the trigger for activation of this anti-IL-10 pro-TH1 response, leading to the maculopapular rash (Thompson and Ramos, 2017).

Conversely, recent studies suggest that a true long lasting antibiotic hypersensitivity might be a lot more prevalent than previously thought, during the acute EBV infection in patients treated by amoxicillin (Renn et al., 2002; Onodi-Nagy et al., 2015). Some authors found positive lymphocyte transformation tests (LLTs) to the incriminated antibiotic (Renn et al., 2002), as well as positive delayed intradermal and patch-tests in those patients (Jappe, 2007; Onodi-Nagy et al., 2015). Authors also described positive DPT or severe DHR upon re-exposure to the beta-lactam at distance of the initial reaction (Jappe, 2007). Thus, it is recommended to assess these reactions with a complete allergic workup, and discuss a DPT.

Long lasting HS may be supported by EBV which continuously co-activates immune response and prevents apoptosis of drug specific T-cell, as it has been found in EBV-induced malignant diseases (Chen, 2011). This anti-apoptotic capacity of EBV could be responsible to the maintenance of lymphocytes, which will then be activated by antibiotic administration (Chen, 2011; Lindsey et al., 2016).

Interestingly, it has been suggested that ampicillin can directly induce the reactivation of EBV, leading to a skin eruption. Thus, Saito-Katsuragi et al. reported the case of a 23-year-old woman with a Still's disease, who developed a maculopapular rash after an ampicillin treatment. She developed serum IgG antibody against EBV-VCA 1 week after. The authors performed two DPT with intravenous ampicillin, resulting in a recurrence of the maculopapular rash 24–48 h after the treatment intake. They monitored the concentration of EBV DNA in blood and found a significant increase of EBV DNA levels after the injection of ampicillin and just before the appearance of the skin rash. Further studies are needed to confirm the hypothesis by which ampicillin would be responsible for a reactivation of EBV, which would then trigger the skin eruption.

EBV continues to be one of the most important models to understand interaction between drugs and concomitant acute or chronic viral infections. Lymphocyte stimulation and direct stimulation of the virus appears to be the most likely hypotheses. However, further researches are needed for a better understanding of the mechanisms involved in the dysregulation of the immune system, leading to a reaction.

ROLE OF VIRUS IN SEVERE NONIMMEDIATE REACTIONS

A variety of severe, rare, potentially life-threatening, drug reactions are described, for which recent evidences suggest an intimate relationship with reactivation of specific virus: the DRESS syndrome, the Stevens-Johnson syndrome (SJS) as well as the Toxic epidermal necrolysis (TEN) and transitional forms (Tohyama and Hashimoto, 2011).

DRESS Syndrome

The DRESS syndrome is a drug-induced delayed reaction with an estimated incidence ranging from one case among 1,000 to 10,000 drug exposures (Fiszenson-Albala et al., 2003). It is most frequently associated with administration of aromatic anticonvulsants, antidepressants, sulfonamides and sulfones, anti-inflammatory drugs, antibiotics, angiotensin-converting enzyme inhibitors and beta-blockers (Kardaun et al., 2013). It has been suggested that viruses play an important role in the physiopathology of DRESS (Redwood and et al., 2018). Hypotheses are based on the evidence of virus replication (primo-infection or reactivation) during the development of disease (Descamps et al., 2001; Ichiche et al., 2003; Picard et al., 2010). Human herpes virus 6 (HHV-6) was the first chronic persistent virus incriminated in the pathology of DRESS (Descamps et al., 1997), being now considered, for some, as a specific and sensitive diagnostic criteria (Shiohara et al., 2007; Watanabe, 2018).

However, the role of HHV replication remains controversial as a study did not find a significant correlation between HHV DNA load and DRESS diagnosis (Ushigome et al., 2012). Several studies reported that HHV replication does not occur early in the clinical course of DRESS and generally, viremia is observed greater than 2 weeks following symptoms onset (White et al., 2015). These data suggest that viral reactivation itself is not involved in the onset of DRESS, but rather than some viruses, in particular of the herpes group, may be involved in the prolonged clinical course of DRESS (Ishida et al., 2014).

The expansion of $CD4^+$ T cells and $CD8^+$ T cells during HHV-6 reactivation seems to be an important feature in many patients with DRESS's multiple organ failure (Pritchett et al., 2012). In addition, it has been found that patients with HHV-6 reactivation have significant higher serum levels of TNF-α, compared to patients without HHV-6 reactivation. *In vitro* and *in vivo* studies showed that TNF-α and other cytokines participate in reactivation of CMV through the induction of CMV immediate early gene expression, leading to the initiation of the viral replication. CMV IE gene has a high level of homology with HHV-6 U95 gene and it is possible that TNF-α interacts identically with it (Watanabe, 2018). The serum thymus and activation-regulated chemokine (TARC) levels are also found to be higher in DRESS patients with HHV-6 replication than those without. TARC may be able to directly activate HHV-6 through a TARC receptor, or induce a relative immunosuppression through the activation of regulatory T cells (Tregs) (Watanabe, 2018). This is in accordance with some observations of dysfunction of Tregs and plasmacytoid dendritic cells in the DRESS syndrome (Takahashi et al., 2009). Thus, there are some evidence that HHV-6-related mechanisms exist to explain at least partially the complications of DRESS.

The importance of drug exposure could be integrated with those of viral interplay in a recent model: the heterologous immunity model. Furthermore, active viral replication is not required in this abovementioned model, so the evidences of viral reactivation highlighted during SCARs development may just represent a tangential event. There is still a need of further studies to highlight differences between patients with or without viral reactivation. In this context, a retrospective case series of 29 pediatric patient with DRESS, reported that those who were HHV-6 positive experimented a significantly greater severity and a longer hospitalization compared to HHV-6 negative subjects (11.5 days vs. 5 days, $p = 0.039$) (Ahluwalia et al., 2015). Even in adults, patients with HHV-6 reactivation showed longer course and more severe organ involvement than others, suggesting a possibly prognostic significance of HHV-6 (Tohyama et al., 2007; Asano et al., 2009).

Further researches should also emphasize on reactivation of other latent viruses too. Apparently, viral activation follows an identifiable chronological pathway and seems to implicate several viruses in the present order: firstly EBV and/or HHV-6, followed by HHV-7 and soon after CMV (Cho et al., 2017). The simultaneous appearance of multiple concomitant viral reactivations would be explained by the ability of herpes virus to reactivate others virus. The role of the EBV in the development of multi-organ involvement of DRESS is discussed particularly because infectious mononucleosis-like symptoms are observed during the early phase of DRESS (Tohyama and Hashimoto, 2011). Furthermore, Mardivirin et al. investigate the possibility of a drug-induced flare-up of DRESS due to antibiotic prescription. Amoxicillin seemed to be an aggravating factor, probably due to the same pathomechanism of amoxicillin-induced rash in EBV infected patients (Mardivirin et al., 2010).

Finally, hypothesis for DRESS syndrome pathophysiology include interaction between different factors: 1) genetic susceptibility factors, such as HLA type or cytochrome p450 polymorphism (Cho et al., 2017); 2) viral infection (primo-infection or replication) inducing a particular pre-activated immune state; and 3) drug as a final trigger for the immune reaction. Virus reactivation could also be the trigger for relapse of DRESS syndrome (Tan and Chan, 2016), as seen in chronic diseases. Besides, it is interesting to note that similarities are highlighted between DRESS and autoimmune disease mechanisms (Michels and Ostrov, 2015).

SJS and TEN

Similar observations have been made in SJS and TEN. These syndromes are most commonly caused by DHR rather than viruses (such as EBV, CMV, HHV-6, HSV, Varicella zoster virus, hepatitis A virus and HIV) (Stutman, 1987; Werblowsky-Constantini et al., 1989; Lam et al., 2004; Bay et al., 2005; Pereira et al., 2007; Cruz et al., 2010; Wetter and Camilleri, 2010; Khalaf et al., 2011; Kunimi et al., 2011; Kim et al., 2012; Sotelo-Cruz, 2012; Ferrandiz-Pulido and Garcia-Patos, 2013; Irungu et al., 2017). In about 30% of cases of SJS and

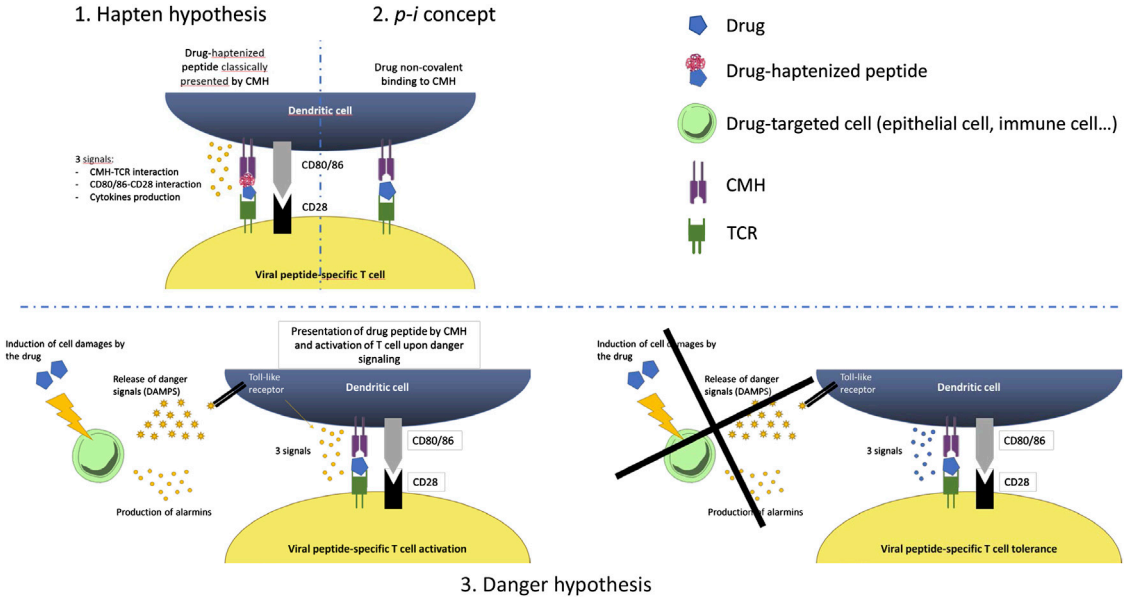

FIGURE 1 | Potential immune mechanisms involved in the interactions between viruses and drug.

TEN, no causative drug is identified, and in 15%, drug responsibility is deemed unlikely (Duong et al., 2017). Since now, over 200 drugs have been associated with SJS/TEN, most commonly sulfonamides and BL antibiotics (Roujeau et al., 1995; Forman et al., 2002; Sheridan et al., 2002).

To date it is still not clear if the virus is a potential co-factor or trigger. Expression of viral DNA fragments in the keratinocyte layer could lead to activation of $CD4^+$ T-helper cells, which induce various reactions, including cytokines production and subsequent inflammatory responses (McDermott et al., 2013). Furthermore, infections activate systemic host inflammatory pathways, as consequence, a perturbation of the natural defense mechanisms of oxidase enzymes could occur and multisystem damages may follow (Bay et al., 2005). Despite everything, F. Brunet-Possenti reports a case of SJS during a primary EBV infection in a 17-year-old adolescent. A 10 years retrospective study presented by Forman confirmed it, founding as the most commonly incriminated infectious agent the herpes simplex virus (19.7%) (Forman et al., 2002). However, while HHV-6 reactivation is primary related to DRESS, it is rare in SJS/TEN (Neuman et al., 2013), sometimes observed in patients treated with anticonvulsant (Peppercorn et al., 2010; Teraki et al., 2010). Actually, researchers are still arguing if "drug-induced" SJS/TEN and "infection-related" SJS/TEN are two separate entities.

HIV Example

Human immunodeficiency virus (HIV) infection is a long-life latent virus hosted by CD4 T cells and macrophages (Zack et al., 1990). This viral infection is associated with important immune deregulations and higher rates of conditions requiring drug administration. It has been found that frequency of DHR in HIV-infected patients is particularly high, up to 100 times more common compared to HIV-negative subjects (Coopman et al., 1993; Rzany et al., 1993; Temesgen and Beri, 2004). The pathogenesis and the reason for the greater propensity for HIV-infected patients to develop DHR to a great variety of drugs that can be particularly severe, remain unknown. It may be related to their greater exposition to medication compared to general population and/or to a higher incidence of co-infection with EBV and CMV (Cytomegalovirus) (Smith et al., 1997; Todd, 2006; Hoosen and et al., 2019). Since many different drugs are involved, the viral infection appears to enhance drug reactivity in general, not only for specific drugs.

This infection itself leads to apparent decrease and loss function of T cells in the blood and skin, in addition to dysregulation of tolerance to self-antigens (Todd, 2006). Interestingly, the incidence of severe DHR in the HIV-infected population has also been reported to increase with increasing stage of the disease, i.e., decreasing $CD4^+$ T cells counts and CD4/CD8 ratio (Coopman et al., 1993; Arp et al., 2005). An interesting example is the hypersensitivity reaction to Trimethoprim-Sulfamethoxazole (TMP-SMX), which occurs in 40–80% of HIV infected individuals (Meyer et al., 2015). The patients with uncontrolled HIV replication have a decrease reduction capacity and a depletion of glutathion in the CD4 cells, leading to an increased toxicity of nitrososulfamethoxazole (n-SMX), a reactive and toxic metabolites of SMX (Correia et al., 2002). This modification in redox balance may be related to the Tat protein, an HIV-specific protein essential for the viral replication (Das et al., 2011). The Tat protein would be secreted by infected cells, in relation to the viral load and disease progression, and promotes drug reactions, increasing oxidation status (Meyer et al., 2015). This strong predisposition to drug reactions is clearly dependent to multiple factors linked to the immune deregulation associated to the primary infection (Todd, 2006). But our understanding of the exact pathomechanisms remains limited and requires further studies.

The higher frequency of allergic drugs reactions in this viral infection may be the result of increased levels of cytokines and cell-surface markers and thereby acting in concert with the drug antigen, amplifying the potential of a drug to cause an immune reaction (Pirmohamed et al., 2002). Although an attractive hypothesis when applied to the pathogenesis of DHRs, there are many questions that remain unanswered. Indeed, the lack of direct experimental evidence has led to heavy criticism of the danger hypothesis (Jozefowski, 2016).

ROLE OF VIRUS IN OTHER TYPE OF DHR

The NSAID Example

It has recently been reported that NSAID could be the most common cause of DHR in children (Woessner et al., 2002; Morales et al., 2015). Prevalence of self-reported hypersensitivity to NSAID has been shown to range from 0.6 to 5.7% in the general population (Dona et al., 2011). NSAIDs, including aspirin, are a group of drugs sharing the capability of inhibiting the cyclooxygenase (COX) enzymes responsible for the prostaglandin synthetase pathway of arachidonic acid metabolism. The pathogenesis of hypersensitivity reactions owing to cross-intolerance has been hypothesized to be related to COX-1 inhibition, although it has not been clearly demonstrated (Macy, 1998).

Interestingly, it has been suggested that blocking prostaglandin synthesis could also allow specific cytotoxic lymphocytes to produce asthma attacks during respiratory tract viral infections (Szczeklik, 1988). Correlation between viral illness and NSAIDs hypersensitivity was first theorized by Szczeklik (1988). As cytotoxic lymphocyte activity is normally inhibited by prostaglandin E2 (PGE2); in case of aspirin and other NSAIDs treatment, COX enzyme is blocked and PGE2 production decrease allowing cytotoxic lymphocytes to attack and eliminate the respiratory tract cells infected by the virus. As a result, lysosomal enzymes and mediators are released and this could precipitate a NSAIDs reaction. These acute attacks can be prevented by avoidance of all drugs with anti-cyclooxygenase activity. However, asthma continues to run a protracted course because of chronic viral infection (Szczeklik, 1988). Nakagawa et al. suspected an acquired analgesic idiosyncrasy secondary to viral infection. They observed anti-Herpes simplex virus (HSV) IgG antibodies titers and hypothesized a relationship between the serological evidence of HSV infection and positive bronchial hyperresponsiveness provocation tests (Nakagawa et al., 2001). Contrariwise, several studies have showed that NSAID can inhibit viral replication (Newton, 1979; Pereira et al., 2003; Reynolds and Enquist, 2006; Zimmermann and Curtis, 2017), yielding more difficult the interpretation of virus and NSAID interaction.

CONCLUSION

In addition to be a major differential diagnosis of DHR, viruses might interact in different ways in different types of DHR to unmask a latent drug allergy. Particularly, viruses have been shown to cause cellular damages, to increase the inflammatory response, to induce the production of specific antibodies, to provoke a change in antigenic expression and to stimulate T-cell replication. From another point of view, the drug might enhance viral replication, leading secondarily to skin eruption. Pathomechanism of viral-induced skin lesions has been poorly studied. However, a better understanding is of major importance, as it can provide major insight in the understanding of drug induced skin rashes. Further studies are urgently needed to clarify the role of viruses in drugs HSRs, to improve the management of patients presenting skin eruptions during treatments and to avoid useless drug avoidance, related with increased morbidity and mortality.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

Agol, V. I. (2012). Cytopathic effects: virus-modulated manifestations of innate immunity? *Trends Microbiol.* 20 (12), 570–576. doi:10.1016/j.tim.2012.09.003

Ahluwalia, J., Abuabara, K., Perman, M. J., and Yan, A. C. (2015). Human herpesvirus 6 involvement in paediatric drug hypersensitivity syndrome. *Br. J. Dermatol.* 172 (4), 1090–1095. doi:10.1111/bjd.13512

Anderson, C. C., and Matzinger, P. (2000). Danger: the view from the bottom of the cliff. *Semin. Immunol.* 12 (3), 231–238. doi:10.1006/smim.2000.0236

Arp, J., Rieder, M. J., Urquhart, B., Freeman, D., Tucker, M. J., Krizova, A., et al. (2005). Hypersensitivity of HIV-1-infected cells to reactive sulfonamide metabolites correlated to expression of the HIV-1 viral protein tat. *J. Pharmacol. Exp. Therapeut.* 314 (3), 1218–1225. doi:10.1124/jpet.105.085050

Asano, Y., Kagawa, H., Kano, Y., and Shiohara, T. (2009). Cytomegalovirus disease during severe drug eruptions: report of 2 cases and retrospective study of 18 patients with drug-induced hypersensitivity syndrome. *Arch. Dermatol.* 145 (9), 1030–1036. doi:10.1001/archdermatol.2009.195

Atanaskovic-Markovic, M., Gaeta, F., Medjo, B., Gavrovic-Jankulovic, M., Cirkovic Velickovic, T., Tmusic, V., et al. (2016). Non-immediate hypersensitivity reactions to beta-lactam antibiotics in children - our 10-year experience in allergy work-up. *Pediatr. Allergy Immunol.* 27 (5), 533–538. doi:10.1111/pai.12565

Banerjee, S., Lu, J., Cai, Q., Sun, Z., Jha, H. C., and Robertson, E. S. (2014). EBNA3C augments pim-1 mediated phosphorylation and degradation of p21 to promote B-cell proliferation. *PLoS Pathog.* 10 (8), e1004304. doi:10.1371/journal.ppat.1004304

Bay, A., Akdeniz, N., Calka, O., Kösem, M., Faik Oner, A., and Doğan, M. (2005). Primary varicella infection associated with stevens-johnson syndrome in a Turkish child. *J. Dermatol.* 32 (9), 745–750. doi:10.1111/j.1346-8138.2005.tb00836.x

Bellini, V., Pelliccia, S., and Lisi, P. (2013). Drug- and virus- or bacteria-induced exanthems: the role of immunohistochemical staining for cytokines in differential diagnosis. *Dermatitis* 24 (2), 85–90. doi:10.1097/DER.0b013e318280cbe5

Bigby, M. (2001). Rates of cutaneous reactions to drugs. *Arch. Dermatol.* 137 (6), 765–770.

Caubet, J. C., Kaiser, L., Lemaître, B., Fellay, B., Gervaix, A., and Eigenmann, P. A. (2011). The role of penicillin in benign skin rashes in childhood: a prospective study based on drug rechallenge. *J. Allergy Clin. Immunol.* 127 (1), 218–222. doi:10.1016/j.jaci.2010.08.025

Chen, M. R. (2011). Epstein-barr virus, the immune system, and associated diseases. *Front. Microbiol.* 2, 5. doi:10.3389/fmicb.2011.00005

Cho, Y. T., Yang, C. W., and Chu, C. Y. (2017). Drug reaction with eosinophilia and systemic symptoms (DRESS): an interplay among drugs, viruses, and immune system. *Int. J. Mol. Sci.* 18 (6), 1243. doi:10.3390/ijms18061243

Chovel-Sella, A., Ben Tov, A., Lahav, E., Mor, O., Rudich, H., Paret, G., et al. (2013). Incidence of rash after amoxicillin treatment in children with infectious mononucleosis. *Pediatrics* 131 (5), e1424–e1427. doi:10.1542/peds.2012-1575

Clark, R. A. (2010). Skin-resident T cells: the ups and downs of on site immunity. *J. Invest. Dermatol.* 130 (2), 362–370. doi:10.1038/jid.2009.247

Coopman, S. A., Johnson, R. A., Platt, R., and Stern, R. S. (1993). Cutaneous disease and drug reactions in HIV infection. *N. Engl. J. Med.* 328 (23), 1670–1674. doi:10.1056/NEJM199306103282304

Copeman, P. W., and Scrivener, R. (1977). Amoxycillin rash. *Br. Med. J.* 1 (6072), 1354. doi:10.1136/bmj.1.6072.1354-b

Correia, O., Delgado, L., Roujeau, J. C., Le Cleach, L., and Fleming-Torrinha, J. A. (2002). Soluble interleukin 2 receptor and interleukin 1alpha in toxic epidermal necrolysis: a comparative analysis of serum and blister fluid samples. *Arch. Dermatol.* 138 (1), 29–32. doi:10.1001/archderm.138.1.29

Cruz, M. J., Mota, A., Baudrier, T., Gil-da-Costa, M. J., and Azevedo, F. (2010). Stevens-Johnson syndrome associated with cytomegalovirus infection in a child with ependymoma. *J. Dermatol. Case. Rep.* 4 (1), 11–14. doi:10.3315/jdcr.2010.1043

Das, A. T., Harwig, A., and Berkhout, B. (2011). The HIV-1 Tat protein has a versatile role in activating viral transcription. *J. Virol.* 85 (18), 9506–9516. doi:10.1128/JVI.00650-11

Demoly, P., Tanno, L. K., Akdis, C. A., Lau, S., Calderon, M. A., Santos, A. F., et al. (2014). Global classification and coding of hypersensitivity diseases-an EAACI-WAO survey, strategic paper and review. *Allergy* 69 (5), 559–570. doi:10.1111/all.12386

Descamps, V., Bouscarat, F., Laglenne, S., Aslangul, E., Veber, B., Descamps, D., et al. (1997). Human herpesvirus 6 infection associated with anticonvulsant hypersensitivity syndrome and reactive haemophagocytic syndrome. *Br. J. Dermatol.* 137 (4), 605–608. doi:10.1111/j.1365-2133.1997.tb03795.x

Descamps, V., Valance, A., Edlinger, C., Fillet, A. M., Grossin, M., Lebrun-Vignes, B., et al. (2001). Association of human herpesvirus 6 infection with drug reaction with eosinophilia and systemic symptoms. *Arch. Dermatol.* 137 (3), 301–304.

Dona, I., Blanca-López, N., Jagemann, L. R., Torres, M. J., Rondón, C., Campo, P., et al. (2011). Response to a selective COX-2 inhibitor in patients with urticaria/angioedema induced by nonsteroidal anti-inflammatory drugs. *Allergy* 66 (11), 1428–1433. doi:10.1111/j.1398-9995.2011.02684.x

Drago, F., Paolino, S., Rebora, A., Broccolo, F., Drago, F., Cardo, P., et al. (2012). The challenge of diagnosing atypical exanthems: a clinico-laboratory study. *J. Am. Acad. Dermatol.* 67 (6), 1282–1288. doi:10.1016/j.jaad.2012.04.014

Duong, T. A., Valeyrie-Allanore, L., Wolkenstein, P., and Chosidow, O. (2017). Severe cutaneous adverse reactions to drugs. *Lancet* 390 (10106), 1996–2011. doi:10.1016/S0140-6736(16)30378-6

Ferrandiz-Pulido, C., and Garcia-Patos, V. (2013). A review of causes of Stevens-Johnson syndrome and toxic epidermal necrolysis in children. *Arch. Dis. Child.* 98 (12), 998–1003. doi:10.1136/archdischild-2013-303718

Fiszenson-Albala, F., Auzerie, V., Mahe, E., Farinotti, R., Durand-Stocco, C., Crickx, B., et al. (2003). A 6-month prospective survey of cutaneous drug reactions in a hospital setting. *Br. J. Dermatol.* 149 (5), 1018–1022. doi:10.1111/j.1365-2133.2003.05584.x

Folster-Holst, R., and Kreth, H. W. (2009a). Viral exanthems in childhood–infectious (direct) exanthems. Part 1: classic exanthems. *J. Dtsch. Dermatol Ges.* 7 (4), 309–316. doi:10.1111/j.1610-0387.2008.06868.x

Folster-Holst, R., and Kreth, H. W. (2009b). Viral exanthems in childhood. Part 3: parainfectious exanthems and those associated with virus-drug interactions. *J. Dtsch. Dermatol. Ges.* 7 (6), 506–510. doi:10.1111/j.1610-0387.2008.06870.x

Forman, R., Koren, G., and Shear, N. H. (2002). *Erythema multiforme*, Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a review of 10 years' experience. *Drug Saf.* 25 (13), 965–972. doi:10.2165/00002018-200225130-00006

Geck, P., Dan, P., and Nasz, I. (1964). Examination of the cytopathic effect of adenoviruses by immunofluorescence. *Acta Microbiol. Acad. Sci. Hungar.* 11, 19–22.

Gianotti, F. (1979). Papular acrodermatitis of childhood and other papulo-vesicular acro-located syndromes. *Br. J. Dermatol.* 100 (1), 49–59. doi:10.1111/j.1365-2133.1979.tb03569.x

Goodyear, H. M., Laidler, P. W., Price, E. H., Kenny, P. A., and Harper, J. I. (1991). Acute infectious erythemas in children: a clinico-microbiological study. *Br. J. Dermatol.* 124 (5), 433–438. doi:10.1111/j.1365-2133.1991.tb00621.x

Hari, Y., Urwyler, A., Hurni, M., Yawalkar, N., Dahinden, C., Wendland, T., et al. (1999). Distinct serum cytokine levels in drug- and measles-induced exanthema. *Int. Arch. Allergy Immunol.* 120 (3), 225–229. doi:10.1159/000024271

He, S. Z., Chen, M. Y., Xu, X. R., Yan, Q., Niu, J. J., Wu, W. H., et al. (2017). Epidemics and aetiology of hand, foot and mouth disease in Xiamen, China, from 2008 to 2015, *Epidemiol. Infect.*, 145, 1865–1874. doi:10.1017/S0950268817000309

Hofmann, B., Schuppe, H. C., Adams, O., Lenard, H. G., Lehmann, P., and Ruzicka, T. (1997). Gianotti-Crosti syndrome associated with Epstein-Barr virus infection. *Pediatr. Dermatol.* 14 (4), 273–277. doi:10.1016/s0190-9622(89)70041-4

Hogan, P. A. (1996). Viral exanthems in childhood. *Australas. J. Dermatol.* 37 (Suppl. 1), S14–S16. doi:10.1111/j.1440-0960.1996.tb01071.x

Hoosen, K., Mosam, A., Cordelia Dlova, N., and Grayson, W. (2019). An update on adverse cutaneous drug reactions in HIV/AIDS. *Dermatopathology (Basel)* 6 (2), 111–125. doi:10.1159/000496389

Ichiche, M., Kiesch, N., and De Bels, D. (2003). DRESS syndrome associated with HHV-6 reactivation. *Eur. J. Intern. Med.* 14 (8), 498–500. doi:10.1016/j.ejim.2003.09.004

Irungu, K., Nyamu, D., and Opanga, S. (2017). Characterization of stevens-johnson syndrome and toxic epidermal necrolysis among patients admitted to Kenyatta national hospital: a retrospective cross-sectional study. *D. Real World Out.* 4 (2), 79–85. doi:10.1007/s40801-017-0105-x

Ishida, T., Kano, Y., Mizukawa, Y., and Shiohara, T. (2014). The dynamics of herpesvirus reactivations during and after severe drug eruptions: their relation to the clinical phenotype and therapeutic outcome. *Allergy* 69 (6), 798–805. doi:10.1111/all.12410

Jappe, U. (2007). Amoxicillin-induced exanthema in patients with infectious mononucleosis: allergy or transient immunostimulation? *Allergy* 62 (12), 1474–1475. doi:10.1111/j.1398-9995.2007.01518.x

Jozefowski, S. (2016). The danger model: questioning an unconvincing theory. *Immunol. Cell Biol.* 94 (5), 525. doi:10.1038/icb.2016.29

Kano, Y., Hiraharas, K., Sakuma, K., and Shiohara, T. (2006). Several herpesviruses can reactivate in a severe drug-induced multiorgan reaction in the same sequential order as in graft-versus-host disease. *Br. J. Dermatol.* 155 (2), 301–306. doi:10.1111/j.1365-2133.2006.07238.x

Kardaun, S. H., Sekula, P., Valeyrie-Allanore, L., Liss, Y., Chu, C. Y., Creamer, D., et al. (2013). Drug reaction with eosinophilia and systemic symptoms (DRESS): an original multisystem adverse drug reaction. Results from the prospective RegiSCAR study. *Br. J. Dermatol.* 169 (5), 1071–1080. doi:10.1111/bjd.12501

Keighley, C. L., Saunderson, R. B., Kok, J., and Dwyer, D. E. (2015). Viral exanthems. *Curr. Opin. Infect. Dis.* 28 (2), 139–150. doi:10.1097/QCO.0000000000000145

Khalaf, D., Toema, B., Dabbour, N., and Jehani, F. (2011). Toxic epidermal necrolysis associated with severe cytomegalovirus infection in a patient on regular hemodialysis. *Mediterr. J. Hematol. Infect. Dis.* 3 (1), e2011004. doi:10.4084/MJHID.2010.004

Kim, H. I., Kim, S. W., Park, G. Y., Kwon, E. G., Kim, H. H., Jeong, J. Y., et al. (2012). Causes and treatment outcomes of Stevens-Johnson syndrome and toxic epidermal necrolysis in 82 adult patients. *Korean J. Intern. Med.* 27 (2), 203–210. doi:10.3904/kjim.2012.27.2.203

Kramkimel, N., Soussan, V., Beauchet, A., Duhamel, A., Saiag, P., Chevallier, B., et al. (2010). High frequency, diversity and severity of skin diseases in a paediatric emergency department. *J. Eur. Acad. Dermatol. Venereol.* 24 (12), 1468–1475. doi:10.1111/j.1468-3083.2010.03672.x

Kunimi, Y., Hirata, Y., Aihara, M., Yamane, Y., and Ikezawa, Z. (2011). Statistical analysis of Stevens-Johnson syndrome caused by *Mycoplasma pneumonia* infection in Japan. *Allergol. Int.* 60 (4), 525–532. doi:10.2332/allergolint.11-OA-0309

Laksono, B. M., de Vries, R. D., McQuaid, S., Duprex, W. P., and de Swart, R. L. (2016). Measles virus host invasion and pathogenesis. *Viruses* 8 (8). doi:10.3390/v8080210

Lam, N. S., Yang, Y. H., Wang, L. C., Lin, Y. T., and Chiang, B. L. (2004). Clinical characteristics of childhood erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis in Taiwanese children. *J. Microbiol. Immunol. Infect.* 37 (6), 366–370.

Landolt, B., Staubli, G., Lips, U., and Weibel, L. (2013). Skin disorders encountered in a Swiss pediatric emergency department. *Swiss Med. Wkly.* 143, w13731. doi:10.4414/smw.2013.13731

Leiste, A., Skaletz-Rorowski, A., Venten, I., Altmeyer, P., and Brockmeyer, N. H. (2008). Urticaria associated with Norovirus infection: report of two cases. *J. Dtsch. Dermatol. Ges.* 6 (7), 563–565. doi:10.1111/j.1610-0387.2007.06501.x

Levine, B. B. (1965). Immunochemical mechanisms involved in penicillin hypersensitivity in experimental animals and in human beings. *Fed. Proc.* 24, 45–50.

Lindsey, J. W., deGannes, S. L., Pate, K. A., and Zhao, X. (2016). Antibodies specific for Epstein-Barr virus nuclear antigen-1 cross-react with human heterogeneous nuclear ribonucleoprotein L. *Mol. Immunol.* 69, 7–12. doi:10.1016/j.molimm.2015.11.007

Lowe, L., Hebert, A. A., and Duvic, M. (1989). Gianotti-Crosti syndrome associated with Epstein-Barr virus infection. *J. Am. Acad. Dermatol.* 20 (2 Pt 2), 336–338. doi:10.1016/s0190-9622(89)70041-4

Luzuriaga, K., and Sullivan, J. L. (2010). Infectious mononucleosis. *N. Engl. J. Med.* 362 (21), 1993–2000. doi:10.1056/NEJMcp1001116

MacLaughlin, E. J., Saseen, J. J., and Malone, D. C. (2000). Costs of beta-lactam allergies: selection and costs of antibiotics for patients with a reported beta-lactam allergy. *Arch. Fam. Med.* 9 (8), 722–726. doi:10.1001/archfami.9.8.722

Macy, E. (1998). Elective penicillin skin testing and amoxicillin challenge: effect on outpatient antibiotic use, cost, and clinical outcomes. *J. Allergy Clin. Immunol.* 102 (2), 281–285. doi:10.1016/s0091-6749(98)70097-1

Magyarlaki, M., Drobnitsch, I., and Schneider, I. (1991). Papular acrodermatitis of childhood (Gianotti-Crosti disease). *Pediatr. Dermatol.* 8 (3), 224–227. doi:10.1111/j.1525-1470.1991.tb00865.x

Mardivirin, L., Valeyrie-Allanore, L., Branlant-Redon, E., Beneton, N., Jidar, K., Barbaud, A., et al. (2010). Amoxicillin-induced flare in patients with DRESS (drug reaction with eosinophilia and systemic symptoms): report of seven cases and demonstration of a direct effect of amoxicillin on human Herpesvirus 6 replication *in vitro*. *Eur. J. Dermatol.* 20 (1), 68–73. doi:10.1684/ejd.2010.0821

McDermott, A. J., Taylor, B. M., and Bernstein, K. M. (2013). Toxic epidermal necrolysis from suspected Mycoplasma pneumoniae infection. *Mil. Med.* 178 (9), e1048–e1050. doi:10.7205/MILMED-D-13-00139

Meyer, C., Behm, N., Brown, E., Copeland, N. K., and Sklar, M. J. (2015). An adverse drug reaction to trimethoprim-sulfamethoxazole revealing primary HIV: a case report and literature review. *Case Rep. Infect. Dis.* 2015, 691010. doi:10.1155/2015/691010

Michels, A. W., and Ostrov, D. A. (2015). New approaches for predicting T cell-mediated drug reactions: a role for inducible and potentially preventable autoimmunity. *J. Allergy Clin. Immunol.* 136 (2), 252–257. doi:10.1016/j.jaci.2015.06.024

Mims, C. A. (1964). Aspects of the pathogenesis of virus diseases. *Bacteriol. Rev.* 28, 30–71.

Mims, C. A. (1966). Pathogenesis of rashes in virus diseases. *Bacteriol. Rev.* 30 (4), 739–760.

Morales, D. R., Guthrie, B., Lipworth, B. J., Jackson, C., Donnan, P. T., and Santiago, V. H. (2015). NSAID-exacerbated respiratory disease: a meta-analysis evaluating prevalence, mean provocative dose of aspirin and increased asthma morbidity. *Allergy* 70 (7), 828–835. doi:10.1111/all.12629

Muraro, A., Werfel, T., Hoffmann-Sommergruber, K., Roberts, G., Beyer, K., Bindslev-Jensen, C., et al. (2014). EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy* 69 (8), 1008–1025. doi:10.1111/all.12429

Nakagawa, H., Yoshida, S., Nakabayashi, M., Akahori, K., Shoji, T., Hasegawa, H., et al. (2001). Possible relevance of virus infection for development of analgesic idiosyncrasy. *Respiration* 68 (4), 422–424. doi:10.1159/000050540

Neuman, M. G., McKinney, K. K., Nanau, R. M., Kong, V., Malkiewicz, I., Mazulli, T., et al. (2013). Drug-induced severe adverse reaction enhanced by human herpes virus-6 reactivation. *Transl. Res.* 161 (5), 430–440. doi:10.1016/j.trsl.2012.12.012

Newton, A. A. (1979). Inhibitors of prostaglandin synthesis as inhibitors of herpes simplex virus replication. *Adv. Ophthalmol.* 38, 58–63.

Ogawa, K., Morito, H., Hasegawa, A., Miyagawa, F., Kobayashi, N., Watanabe, H., et al. (2014). Elevated serum thymus and activation-regulated chemokine (TARC/CCL17) relates to reactivation of human herpesvirus 6 in drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS). *Br. J. Dermatol.* 171 (2), 425–427. doi:10.1111/bjd.12948

Onodi-Nagy, K., Kinyó, Á., Meszes, A., Garaczi, E., Kemény, L., and Bata-Csörgő, Z. (2015). Amoxicillin rash in patients with infectious mononucleosis: evidence of true drug sensitization. *Allergy Asthma Clin. Immunol.* 11 (1), 1. doi:10.1186/1710-1492-11-1

Parham, P., and Janeway, C. (2009). *The immune system*. 3rd Edn. New York, NY, United States: Garland Science.

Peppercorn, A. F., Miller, M. B., Fitzgerald, D., Weber, D. J., Groben, P. A., and Cairns, B. A. (2010). High-level human herpesvirus-6 viremia associated with onset of Stevens-Johnson syndrome: report of two cases. *J. Burn Care Res.* 31 (2), 365–368. doi:10.1097/BCR.0b013e3181d0f48b

Pereira, C. F., Paridaen, J. T., Rutten, K., Huigen, M. C., van de Bovenkamp, M., Middel, J., et al. (2003). Aspirin-like molecules that inhibit human immunodeficiency virus 1 replication. *Antivir. Res.* 58 (3), 253–263. doi:10.1016/s0166-3542(03)00006-8

Pereira, F. A., Mudgil, A. V., and Rosmarin, D. M. (2007). Toxic epidermal necrolysis. *J. Am. Acad. Dermatol.* 56 (2), 181–200. doi:10.1016/j.jaad.2006.04.048

Picard, D., Janela, B., Descamps, V., D'Incan, M., Courville, P., Jacquot, S., et al. (2010). Drug reaction with eosinophilia and systemic symptoms (DRESS): a multiorgan antiviral T cell response. *Sci. Transl. Med.* 2 (46), 46ra62. doi:10.1126/scitranslmed.3001116

Pichler, W., Yawalkar, N., Schmid, S., and Helbling, A. (2002). Pathogenesis of drug-induced exanthems. *Allergy* 57 (10), 884–893. doi:10.1034/j.1398-9995.2002.02161.x

Pirmohamed, M., Naisbitt, D. J., Gordon, F., and Park, B. K. (2002). The danger hypothesis--potential role in idiosyncratic drug reactions. *Toxicology* 181–182, 55–63. doi:10.1016/s0300-483x(02)00255-x

Ponvert, C., Le Clainche, L., de Blic, J., Le Bourgeois, M., Scheinmann, P., and Paupe, J. (1999). Allergy to beta-lactam antibiotics in children. *Pediatrics* 104 (4), e45. doi:10.1542/peds.104.4.e45

Ponvert, C., Perrin, Y., Bados-Albiero, A., Le Bourgeois, M., Karila, C., Delacourt, C., et al. (2011). Allergy to betalactam antibiotics in children: results of a 20-year study based on clinical history, skin and challenge tests. *Pediatr. Allergy Immunol.* 22 (4), 411–418. doi:10.1111/j.1399-3038.2011.01169.x

Pritchett, J. C., Nanau, R. M., and Neuman, M. G. (2012). The link between hypersensitivity syndrome reaction development and human herpes virus-6 reactivation. *Int. J. Hepatol.* 2012, 723062. doi:10.1155/2012/723062

Pullen, H., Wright, N., and Murdoch, J. M. (1967). Hypersensitivity reactions to antibacterial drugs in infectious mononucleosis. *Lancet* 2 (7527), 1176–1178. doi:10.1016/s0140-6736(67)91893-4

Rawlins, M. D. (1981). Clinical pharmacology. Adverse reactions to drugs. *Br. Med. J.* 282 (6268), 974–976. doi:10.1136/bmj.282.6268.974

Redwood, A. J., Pavlos, R. K., White, K. D., and Phillips, E. J. (2018). HLAs: key regulators of T-cell-mediated drug hypersensitivity. *HLA* 91 (1), 3–16. doi:10.1111/tan.13183

Renn, C. N., Straff, W., Dorfmüller, A., Al-Masaoudi, T., Merk, H. F., and Sachs, B. (2002). Amoxicillin-induced exanthema in young adults with infectious mononucleosis: demonstration of drug-specific lymphocyte reactivity. *Br. J. Dermatol.* 147 (6), 1166–1170. doi:10.1046/j.1365-2133.2002.05021.x

Reynolds, A. E., and Enquist, L. W. (2006). Biological interactions between herpesviruses and cyclooxygenase enzymes. *Rev. Med. Virol.* 16 (6), 393–403. doi:10.1002/rmv.519

Roujeau, J. C., Kelly, J. P., Naldi, L., Rzany, B., Stern, R. S., Anderson, T., et al. (1995). Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N. Engl. J. Med.* 333 (24), 1600–1607. doi:10.1056/NEJM199512143332404

Rubio, M., Bousquet, P. J., Gomes, E., Romano, A., and Demoly, P. (2012). Results of drug hypersensitivity evaluations in a large group of children and adults. *Clin. Exp. Allergy* 42 (1), 123–130. doi:10.1111/j.1365-2222.2011.03887.x

Rzany, B., Mockenhaupt, M., Stocker, U., Hamouda, O., and Schöpf, E. (1993). Incidence of Stevens-Johnson syndrome and toxic epidermal necrolysis in patients with the acquired immunodeficiency syndrome in Germany. *Arch. Dermatol.* 129 (8), 1059. doi:10.1001/archderm.1993.01680290135026

Schissel, D. J., Singer, D., and David-Bajar, K. (2000). Azithromycin eruption in infectious mononucleosis: a proposed mechanism of interaction. *Cutis* 65 (3), 163–166.

Schneider, H., Adams, O., Weiss, C., Merz, U., Schroten, H., and Tenenbaum, T. (2013). Clinical characteristics of children with viral single- and co-infections and a petechial rash. *Pediatr. Infect. Dis. J.* 32 (5), e186–e191. doi:10.1097/INF.0b013e318280618d

Schon, M. P., Zollner, T. M., and Boehncke, W. H. (2003). The molecular basis of lymphocyte recruitment to the skin: clues for pathogenesis and selective therapies of inflammatory disorders. *J. Invest. Dermatol.* 121 (5), 951–962. doi:10.1046/j.1523-1747.2003.12563.x

Sheridan, R. L., Schulz, J. T., Ryan, C. M., Schnitzer, J. J., Lawlor, D., Driscoll, D. N., et al. (2002). Long-term consequences of toxic epidermal necrolysis in children. *Pediatrics* 109 (1), 74–78. doi:10.1542/peds.109.1.74

Shiohara, T., Iijima, M., Ikezawa, Z., and Hashimoto, K. (2007). The diagnosis of a DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations. *Br. J. Dermatol.* 156 (5), 1083–1084. doi:10.1111/j.1365-2133.2007.07807.x

Shiohara, T., Inaoka, M., and Kano, Y. (2006). Drug-induced hypersensitivity syndrome (DIHS): a reaction induced by a complex interplay among herpesviruses and antiviral and antidrug immune responses. *Allergol. Int.* 55 (1), 1–8. doi:10.2332/allergolint.55.1

Shiohara, T., and Kano, Y. (2007). A complex interaction between drug allergy and viral infection. *Clin. Rev. Allergy Immunol.* 33 (1–2), 124–133. doi:10.1007/s12016-007-8010-9

Smith, H. (1972). Mechanisms of virus pathogenicity. *Bacteriol. Rev.* 36 (3), 291–310.

Smith, K. J., Skelton, H. G., Yeager, J., Ledsky, R., Ng, T. H., and Wagner, K. F.; The Military Medical Consortium for the Advancement of Retroviral Research (MMCARR). (1997). Increased drug reactions in HIV-1-positive patients: a possible explanation based on patterns of immune dysregulation seen in HIV-1 disease. *Clin. Exp. Dermatol.* 22 (3), 118–121. doi:10.1111/j.1365-2230.1997.tb01038.x

Solensky, R. (2013). The time for penicillin skin testing is here. *J. Allergy Clin. Immunol. Pract.* 1 (3), 264–265. doi:10.1016/j.jaip.2013.03.010

Solensky, R. (2014). Penicillin allergy as a public health measure. *J. Allergy Clin. Immunol.* 133 (3), 797–798. doi:10.1016/j.jaci.2013.10.032

Sotelo-Cruz, N. (2012). [Stevens-Johnson syndrome and toxic epidermal necrolysis in children]. *Gac. Med. Mex.* 148 (3), 265–275.

Strittmatter, G. E., Garstkiewicz, M., Sand, J., Grossi, S., and Beer, H. D. (2016). Human primary keratinocytes as a tool for the analysis of caspase-1-dependent unconventional protein secretion. *Methods Mol. Biol.* 1459, 135–147. doi:10.1007/978-1-4939-3804-9_9

Stutman, H. R. (1987). Stevens-Johnson syndrome and *Mycoplasma pneumoniae*: evidence for cutaneous infection. *J. Pediatr.* 111 (6 Pt 1), 845–847. doi:10.1016/s0022-3476(87)80200-7

Szczeklik, A. (1988). Aspirin-induced asthma as a viral disease. *Clin. Allergy* 18 (1), 15–20. doi:10.1111/j.1365-2222.1988.tb02838.x

Takahashi, R., Kano, Y., Yamazaki, Y., Kimishima, M., Mizukawa, Y., and Shiohara, T. (2009). Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. *J. Immunol.* 182 (12), 8071–8079. doi:10.4049/jimmunol.0804002

Tan, S. C., and Chan, G. Y. (2016). Relapsing drug-induced hypersensitivity syndrome. *Curr. Opin. Allergy Clin. Immunol.* 16 (4), 333–338. doi:10.1097/ACI.0000000000000288

Temesgen, Z., and Beri, G. (2004). HIV and drug allergy. *Immunol. Allergy Clin.* 24 (3), 521–531. doi:10.1016/j.iac.2004.03.006

Teraki, Y., Shibuya, M., and Izaki, S. (2010). Withdrawal of the culprit drug induces reactivation of human herpesvirus 6 in drug-induced hypersensitivity syndrome. *Int. J. Dermatol.* 49 (12), 1413–1415. doi:10.1111/j.1365-4632.2009.04410.x

Thompson, D. F., and Ramos, C. L. (2017). Antibiotic-induced rash in patients with infectious mononucleosis. *Ann. Pharmacother.* 51 (2), 154–162. doi:10.1177/1060028016669525

Thong, B. Y., and Tan, T. C. (2011). Epidemiology and risk factors for drug allergy. *Br. J. Clin. Pharmacol.* 71 (5), 684–700. doi:10.1111/j.1365-2125.2010.03774.x

Todd, G. (2006). Adverse cutaneous drug eruptions and HIV: a clinician's global perspective. *Dermatol. Clin.* 24 (4), 459–472. doi:10.1016/j.det.2006.06.008

Tohyama, M., and Hashimoto, K. (2011). New aspects of drug-induced hypersensitivity syndrome. *J. Dermatol.* 38 (3), 222–228. doi:10.1111/j.1346-8138.2010.01176.x

Tohyama, M., Hashimoto, K., Yasukawa, M., Kimura, H., Horikawa, T., Nakajima, K., et al. (2007). Association of human herpesvirus 6 reactivation with the flaring and severity of drug-induced hypersensitivity syndrome. *Br. J. Dermatol.* 157 (5), 934–940. doi:10.1111/j.1365-2133.2007.08167.x

Ushigome, Y., Kano, Y., Hirahara, K., and Shiohara, T. (2012). Human herpesvirus 6 reactivation in drug-induced hypersensitivity syndrome and DRESS validation score. *Am. J. Med.* 125 (7), e9–e10. doi:10.1016/j.amjmed.2011.10.027

van Dijk, S. M., Gardarsdottir, H., Wassenberg, M. W. M., Jelrik Oosterheert, J., de Groot, M. C. H., and Rockmann, H. (2016). The high impact of penicillin allergy registration in hospitalized patients. *J. Allergy Clin. Immunol. Pract.* 4 (5), 926–931. doi:10.1016/j.jaip.2016.03.009

Vega Alonso, T., Gil Costa, M., Rodríguez Recio, M. J., de la Serna Higuera, P., and de Médicos Centinelas de Castilla y León, R. (2003). [Incidence and clinical characteristics of maculopapular exanthemas of viral aetiology]. *Aten. Primaria* 32 (9), 517–523. doi:10.1016/s0212-6567(03)70781-1

Virgin, H. W., Wherry, E. J., and Ahmed, R. (2009). Redefining chronic viral infection. *Cell* 138 (1), 30–50. doi:10.1016/j.cell.2009.06.036

Watanabe, H. (2018). Recent advances in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms. *J. Immunol. Res.* 2018, 5163129. doi:10.1155/2018/5163129

Werblowsky-Constantini, N., Livshin, R., Burstein, M., Zeligowski, A., and Tur-Kaspa, R. (1989). Toxic epidermal necrolysis associated with acute cholestatic viral hepatitis A. *J. Clin. Gastroenterol.* 11 (6), 691–693. doi:10.1097/00004836-198912000-00020

Wetter, D. A., and Camilleri, M. J. (2010). Clinical, etiologic, and histopathologic features of Stevens-Johnson syndrome during an 8-year period at Mayo Clinic. *Mayo Clin. Proc.* 85 (2), 131–138. doi:10.4065/mcp.2009.0379

White, K. D., Chung, W. H., Hung, S. I., Mallal, S., and Phillips, E. J. (2015). Evolving models of the immunopathogenesis of T cell-mediated drug allergy: the role of host, pathogens, and drug response. *J. Allergy Clin. Immunol.* 136 (2), 219–235. doi:10.1016/j.jaci.2015.05.050

Woessner, K. M., Simon, R. A., and Stevenson, D. D. (2002). The safety of celecoxib in patients with aspirin-sensitive asthma. *Arthritis Rheum.* 46 (8), 2201–2206. doi:10.1002/art.10426

Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A., and Chen, I. S. (1990). HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* 61 (2), 213–222. doi:10.1016/0092-8674(90)90802-l

Zimmermann, P., and Curtis, N. (2017). Antimicrobial effects of antipyretics. *Antimicrob. Agents Chemother.* 61 (4), e02268–e02316. doi:10.1128/AAC.02268-16

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