Hypersensitivity Reactions to Biologicals: an EAACI position paper"

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ABSTRACT

Because of their selectivity, biologicals are crucial therapeutic agents in oncological, immunological, and inflammatory diseases and their use in clinical practice is broadening. Biologicals are among the most common drugs that can cause hypersensitivity reactions (HSRs), and this is primarily attributed to an explosion in new treatment options that has developed through personalized and precision medicine. Patients can develop HSRs to these agents during the first lifetime exposure or after repeated exposure. Despite its relatively high prevalence, the underlying mechanisms and optimal management of HSRs to biologicals remain incompletely explained. In this position paper, the authors provided evidence-based recommendations for the diagnosis and management of HSRs to biologicals. Additionally, the document defines unmet needs, which should be topics of future studies.

Key words: biologicals, hypersensitivity reactions, allergy, monoclonal Abs, interferon, interleukins, drug hypersensitivity, desensitization,

BOX 1 Definitions and abbreviations

HSRs: hypersensitivity reactions

mAbs: monoclonal Ab

- omab for murine (0% human) mAbs
- *ximab:* chimeric mAb
- *zumab:* humanized mAb
- *mumab: fully* human mAb

...cept: The names of the fusion proteins end in

IRR**:** infusion related reaction

IFNs: interferons

IL: interleukins

CRR: cytokine release reaction

INTRODUCTION

Biologicals are, in general, large molecular weight therapeutics that are synthesized by living organisms, and bind a specific determinant, such as a cytokine or receptor (1) Biologicals with reported rate of any type of HSR higher than %1 and/or anaphylaxis on individual indication are presented on the Table-1 (2)
Because of this selectivity, biologicals are crucial therapeutic agents in oncological, immunological, and inflammatory diseases and their use in clinical practice is broadening (3*).*
Biologicals are among the most common drugs that can cause hypersensitivity reactions (HSRs) that have rapidly increased over the course of the twenty-first century, and this is primarily attributed to an explosion in new treatment options that has developed through personalized and precision medicine. Patients with numerous diseases and pathologies can develop HSRs that occur during the first lifetime exposure or after repeated exposure to biologicals (4)
Despite its relatively high prevalence, the underlying mechanisms and optimal management of HSRs to biologicals remain incompletely explained. In this position paper, the authors provide evidence-based recommendations for the diagnosis and management of HSRs to biologicals. A panel of experts was called by the EAACI Drug Allergy Section and Biological WG.

METHODS – SEARCH STRATEGY

This Position Paper (PP) was commissioned by the European Academy of Allergy and Clinical Immunology (EAACI). The task force group (TF) performed an intensive electronic literature search in MEDLINE, PubMed, Web of Science, and Google Scholar databases of scientific societies, by using the primary key words: biologicals, hypersensitivity reactions, allergy, monoclonal Abs, interferon, interleukins, drug hypersensitivity, desensitization. Besides, each TF member used extra key words as appropriate for each specific section. The search included in vivo and in vitro trials in English language. During the development of the PP, the TF group consulted and discussed the process in meetings organized in June 2017in Helsinki, in November 2017 in Verona, in May 2018 in Munich, in November 2018 in Porto, in June 2019 Lisbon, and in October 2019 in Rimini/Italy. Statement, recommendation and unmet needs were carefully reviewed and the quality of evidence was graded by the TF members, using the SIGN criteria as Grade A, B, C, D. (Supplementary file, table 1) (5,6)

CLASSIFICATION

Adverse reactions to biologicals cannot be classified according to traditional classification because of their different properties from chemical drugs (7). Thus, first Pichler proposed a classification with five types of adverse side-effects of biologicals based on pathomechanisms (8) (Supplementary file, Table 2). However, recently a new classification was proposed considering phenotypes, endotypes and biomarkers indicating underlying endotype (9, 10). Based on this new classification, immediate HSRs to biologicals are further classified into infusion-related (IRR), cytokine release, type 1 (IgE/non IgE) and mixed reaction (Table 2).

Infusion-related reactions

Patients mostly suffer from common IRR at first 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 cytokines such as IL-6 and tumor necrosis factor-α .

Cytokine release reactions
Clinical symptoms and signs are usually due to the cytokine release that is characterized by elevated serum TNF-αlpha and IL-6 levels at the time of the reaction compared with their normal baseline. The difference between IRR and cytokine release reactions is the self-limiting nature of IRR on repeated exposure and the response to premedication. Infusion related reaction with Cetuximab is an exceptional IgE-mediated reaction occurring at the first exposure due to preformed in the environment preformed IgE antibodies (11)

Type I reactions (IgE/non-IgE)
Reactions are associated IgE or non-IgE mediated mast cell/basophil degranulation leading to massive histamine leukotrienes, and prostaglandins release. These reactions occur at least one administration without reaction and their symptomatology is similar to IgE-mediated reactions. The distinctive point between IgE and non-IgE mediated reactions is skin prick test which is negative for non-IgE mediated reactions.

Mixed reactions
Mixed reactions are combination of cytokine release and IgE-mediated reactions. Skin test positivity and/or specific IgE to implicated biologicals as well as increased levels of tryptase, IL-1, IL-6 and TNF-αlpha can occur.

EPIDEMIOLOGY AND RISK FACTORS

All biologicals have the potential to cause HSRs and due to increased use of these agents, HSRs have become more frequently reported. Reactions to mAbs vary by agent. The rates of immediate-type HSRs to specific biologicals have been reported as 5–10% for rituximab, 2–3% for infliximab, 3–22% for cetuximab, and 0.6–5% for trastuzumab (12-14).

Risk factors for HSR to biologicals include both patient's characteristics, such as the underlying disease to be treated, the patient's immune status, other drugs taken concomitantly and drug-related factors, such as degree of humanization, glycosylation pattern, type of cells from which it was obtained, dosing interval, and excipients with allergenic potential (7). Patients with anti-drug antibodies as IgG or IgE developed during treatment with biologicals or preexisting are more likely to have increased risk of immediate HSRs to biologicals (13, 15). An association between positive skin test and greater severity of initial reaction was reported (9). Interestingly, women are more prone to drug allergy in particular to chemotherapy medications and new biologicals and monoclonal antibodies (16).

Breakthrough reactions during desensitization appear in approximately 13.5% to 23% of patients with 2.3-2.6% multiple reactors (9, 17, 18).

A correlation between breakthrough reactions and positivity of skin tests has been controversial due to a recent paper which reported that the main predictor for breakthrough reactions is a positive skin test result (17); although recent papers have not supported this finding (19, 20).

CLINICAL PRESENTATION

Hypersensitivity reactions can occur on the first exposure or repeated exposures and may limit the use of biologicals, leading to therapy interruption and impaired quality of life (3, 21). Hypersensitivity reactions to biologicals include local injection site reactions and systemic infusion reactions (4). The clinical presentation of systemic immediate HSRs to biologicals ranges from mild cutaneous manifestations to life-threatening reactions (3).

Injection site reactions

These are the most common adverse reactions to subcutaneous biologicals and usually occur within 24-48 hours but may also occur immediately after injection. They are characterized by erythema, edema, itching or sometimes infiltrated plaques at the injection site and mild to moderate in severity. These reactions generally last 1-5 days and do no lead to cessation of the therapy (3, 4, 22, 23), however, exanthematous dissemination has been reported in rare cases (24). Some patients may develop recall reactions (local reactions at the site of previous reaction) (25).

Infusion reactions

Immediate HSRs occur during or within a few hours (particularly with subcutaneous route) from either a first or subsequent infusion (26, 27). Immediate reactions are more frequently systemic while delayed reactions are more frequently local after subcutaneous administration of biologicals (8).

The clinical manifestations of immediate HSRs vary considerably, ranging from mild to severe and even life-threatening. Muco-cutaneous symptoms are the most common, followed by respiratory and cardiac symptoms (Table 3) (9, 28-31).

This novel classification can help clinicians to describe the clinical presentation of the different phenotypes. Phenotypes classify HSRs according to the onset of symptoms, such as immediate versus delayed HSRs and according to the severity of symptoms, defined as Grade I, II and III for type I reactions (32).

Delayed/nonimmediate IRRs occur within the 14 days after the infusion. Typical symptoms are fever, malaise, arthralgia-arthritis, jaw pain, erythematous sometimes urticarial lesions, purpura and conjunctival erythema, consistent with a serum sickness-like reaction (SSLR). Sometimes patchy lung infiltrates, lymphadenopathy, splenomegaly, gastrointestinal symptoms and extremity weakness may also be accompanied. Delayed type IV reactions are mostly presented with maculopapular rash but more severe reactions such as symmetrical drug-related intertriginous and flexural exanthema (SDRIFE), Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) have also been reported. In the vast majority of cases, there is at least one administration without reaction (27). 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 and differentiate them from PNP, direct and indirect immunofluorescence could be used. There's a general agreement about avoiding rituximab that has caused type IV HSR such as SDRIFE, SJS, TEN, as well as SSLR

PATHOGENESIS OF IMMEDIATE AND DELAYED REACTIONS

The pathogenesis of HRs to biologicals represents a complex picture that has not been fully clarified. One of the main features of biologicals is immunogenicity, that is the capability of these agents to induce an immune response against the drug itself, leading to the development of anti-drug antibodies (ADA) (33). For this reason, both immediate and delayed reactions may be divided into ADA-mediated and non-ADA-mediated reactions (Table 4). Even if this classification is probably an over-simplification, it is a good "starting point" to set up a diagnostic work-up.

ADA-mediated reactions: immediate and delayed

Although few data are available about T cell response to biologicals so far, it is clear that ADA development is the result of a complete adaptive immune response (34) that starts with the uptake of biologicals by dendritic cells and its presentation to T cells that help B cells in antibodies production. ADAs are heterogeneous in their composition (isotype, affinity, specificity) (35) and this finding may explain why the clinical impact of immunogenicity may differ. ADAs may belong to the IgE isotype and IgE-mediated [type I] hypersensitivity reactions to biologicals may not be as rare as previously thought (12, 36). Of note is the fact that the IgE development is the result of a Th2-skewed cellular immune response against biologicals, as clearly demonstrated with rituximab and infliximab (36, 37). Sometimes IgE mediated reactions may occur as a first dose event, as in the case of cetuximab-induced reactions, and sustained by pre-existing cross-reacting IgE against "foreign" glycan structures that are present in the therapeutic antibody (11). Some authors identified the additive present in the drug formulation as the culprit factor of IgE-mediated reactions in biologicals-exposed patients. This event has been described for polysorbate in omalizumab-, erythropoietin- and darbepoetin-treated patients (11). Sensitization might derive from previous contact with the same excipients used in the formulation of vaccines and/or cosmetics (Fig 1).

Among delayed reactions the development of ADAs has been more frequently associated with SSLR and thrombosis, whereas disseminated skin reactions seem to be less associated with ADA (38).

Non-ADA-mediated reactions: immediate and delayed

Among non-ADA-mediated reactions, the best characterized condition is represented by the Cytokine Release Reaction; it occurs when a large number of cells are activated through different mechanisms, leading to the release of very high levels of pro-inflammatory cytokines. CRS is clinically heterogeneous with symptoms occurring within minutes (immediate reactions) to hours/days after the treatment start (delayed reactions*)* (38). (Fig 2)

The complement (C') activation represents the second non-ADA-mediated mechanism involved in the pathogenesis of infusion reactions to BAs and it may be involved in both immediate and delayed reactions. The C' cascade may be directly activated by the drug (for aggregates or additives such as lipid excipients) or indirectly by circulating or tissue immune-complexes (ICs) formed between drug and ADA. During immediate events, C'activation leads to the release of anaphylatoxins (C3a and C5a) with subsequent mast cell activation, while delayed reactions may be mediated by deposition of ICs containing the drug that activate C' thus resulting in tissue damage and recruitment/activation of inflammatory cells (e.g. skin vasculitis, glomerular disease) (38). On the other hand, C' activation may occur regardless of ADA development and causes a unique adverse immune phenomenon, a C'

activation-related pseudoallergy (CARPA) (39), leading to an immediate HSR.

Beyond the role of T cells in ADA development, specific cellular immune response against the biological agent may also be involved in the pathogenic mechanism of delayed disseminated skin reactions. However, data confirming this hypothesis have not been extensively reported until now and only few cases have been analysed (40, 41). Of note, some delayed disseminated skin reactions result from the biologicals' inherent effects on the immune system as a direct molecular target-dependent event. This is the case of cutaneous adverse events induced by EGFR antagonists (42) or of exacerbation of psoriasis during TNF αlpha antagonist (43). Additionally, a role for T-lymphocytes-mediated delayed-type hypersensitivity reactions cannot be ruled out in some local injection site reactions, where an inflammatory infiltrate composed of lymphoid cells may be present (44).

Statements and recommendations

- ADA and non-ADA-mediated reactions may be clinically indistinguishable (Grade D)
- IgE-mediated reactions are responsible for some immediate type HRS, even if the majority of immediate type reactions are mediated by IgG ADA (Grade D)
- CRS and C'activation are responsible for some immediate type HRS (Grade C)
- T cells play a role in the development of ADA involved in immediate HRS (grade B)

- **Unmet needs**
- The definition of neutrophils and macrophages involvement in non-IgE-mediated reactions
- The association between ADA development and delayed systemic hypersensitivity reactions
- Definition of the role of T cells in both delayed disseminated skin reactions and ISR

DIAGNOSTIC APPROACH

Skin tests

Positive skin testing has been reported for patients with previous HSR towards rituximab, anti-TNF agents and trastuzumab (12). In some small case series, positive skin testing with biologicals has confirmed the *in vitro* detection of serum drug-specific IgE (36), thus showing the biological activity of BA-specific IgE in mast cells activation. Although it is essential to perform IDT (at immediate reading), as prick tests are usually negative, data from literature displays a very high concordance between the detection of serum IgE (performed by ImmunoCAP) and skin testing positivity for biologicals (30) to suggest that skin testing, being the most readily available diagnostic testing, may be useful in replacing *in vitro* test for the diagnosis of immediate IgE-mediated HSR to biologicals. One of the main limitations of skin testing for biologicals is represented by the lack of standardized procedures including those for drug concentrations (45). Specifically, for most biologicals there is insufficient evidence to date to recommend appropriate drug dilutions for skin prick test (SPT) and intradermal test (IDT). For anti-TNF alfa agents, we could take into consideration the experience of some groups referring infliximab (10 mg/ml) and adalimumab (40 mg/ml) 1:10 and 1:1 dilution as the non-irritating concentration for IDT and SPT, respectively (18, 46-49). **(**Table 5**).** In addition, Lieberman et al. have evaluated non-irritating test concentrations for omalizumab (50). However, multicentre studies designed to establish and validate drug skin test

concentrations using standard protocols are still lacking. The low availability of test solutions may be an additional limitation of skin testing, that reduce the diffusion and application of the diagnostic approach especially in small centres.

The time between the reaction and the evaluation is a crucial point, due to temporary unresponsiveness of the skin mast cells following the reaction and due to the rapid decrease displayed by IgE specific for biologicals. No negativization rate of skin testing for biologicals is available. For this reason, there is an insufficient evidence to recommend the timing to perform skin testing for biologicals up to now. Concerning safety, there were no unexpected adverse reactions to the *in vivo* procedures with biologicals even in patients with severe reactions (36, 51).

With regard to the *in vivo* allergy tests for delayed HSRs, not many data are available. *In vivo* tests have been carried out mainly in patients who developed Interferon (IFN)-related generalized skin reactions. Specifically, IDT at delayed reading (average of 72 h) seems to be useful in the management of generalized reactions to IFNs (52). For cutaneous delayed reactions to the other biologicals, the role of both IDT at delayed reading and patch test has never been investigated. Overall, *in vivo* tests for delayed reactions remain experimental, thus it is not possible to make any specific recommendations.

In vitro tests

The *in vitro* diagnostic approach of immediate HSRs towards biologicals is aimed at verifying the development of an immune response characterized by ADA. A number of analytical formats including radioimmunoassay (RIA) or radioimmunoprecipitation (RIP) assay, surface plasmon resonance, and electrochemiluminescence are available, however, bridging ELISA is the most frequently used assay to evaluate ADA in treated patients (53). ELISA in this format is a drug-sensitive assay because the presence of circulating drug in the serum may interfere with ADA detection, thereby leading to false negative results (54). In addition, false positive results may occur in the bridging ELISA format due to cross-binding of IgG by rheumatoid factors or anti-hinge antibodies. For this reason, all ADA-positive samples at the initial screening assay have to be further evaluated in a confirmatory test (54). Confirmed positive samples may be submitted to further characterization to define the IgE isotype, using the ImmunoCAP platform (not commercially available) and other home-made immunoassays. ImmunoCAP sensitivity depends on the BA involved. In fact, ImmunoCAP sensitivity to cetuximab ranges from 68% to 92% and specificity from 90% to 92% depending on HR severity (55), whereas anti-infliximab IgE in ImmunoCAP has a sensitivity of 26% and a specificity of 90% (30). This depends on the fact that not all HSRs to biologicals are IgE-mediated (although ADA-mediated).
Because of the weak association of ADA development and the onset of delayed reactions, ADA measurement could be suggested only in the evaluation of skin vasculitis, thromboembolic events and SSLR.

There are commercially available tests for the assay of non-isotype-specific ADA (CE marked), although few laboratories practice this assay routinely (30, 57). Furthermore, the number of tests available for different biologicals is expanding. However, it would be useful if the company that creates and produces a new biologic, or its biosimilar, also makes available the specific test for ADA. The lack of commercially available tests for IgE ADA detection represents a crucial unmet need in the diagnostic work up of immediate HSRs to biologicals.
In vitro test for the detection of IgE and non-IgE ADA could be used in a preventive manner.

ADA+ positive patients are at risk of developing HSR (56), and the re-exposure of the patient to a second cycle of treatment after drug interruption increases the risk of HSRs (30, 57). Specifically, high ADA levels, early ADA onset or drug disappearance is predictive of HSR, at least for infliximab-treated patients. In addition, these patients with high and early ADA response more frequently develop anti-drug IgE (57). Overall, these data suggest that drug levels and ADA should be closely monitored during the first year of treatment or after the first infusion of the second cycle after interruption. Detection of IgE ADA is advisable when high and early ADAs are detected.

For reactions sustained by non-ADA-mediated mechanisms special laboratory parameters such as cytokines (IL-6, IL-8, IL-10, TNF-a, IFN-g) and complement factors (C5a, C3a, CH50) might be evaluated in an early phase of the reaction to understand the pathophysiology of the event, even if the clinical relevance of these parameters has to be validated. Serial serum tryptase determinations (between 30 minutes and 2 hours after the onset of symptoms) should be included in the diagnostic algorithm of immediate hypersensitivity reactions clinically defining as anaphylaxis. Tryptase is the most studied marker in anaphylaxis and it is a rather specific mast cell mediator. Tryptase is the most important biomarker in anaphylaxis so far, but is still far from being the ideal biomarker for this; in fact, there is a need to identify new potential useful biomarkers. Serial measurements of tryptase, although are laborious in daily clinical practice, may more likely identify the peak of tryptase, thus increasing the sensitivity of the test. However, it is important to note that also CARPA is associated with an increase of tryptase and that basophils also contain and release this mediator (58). Some papers describing the use of basophil activation test (BAT) for the diagnosis of HSR to biologicals can be found, particularly in rituximab-related infusion reactions (59). However, studies in larger group of patients are needed to confirm the findings and to establish BAT as a diagnostic tool. Although circulating biological-specific T cells have been described in treated patients (34), at the moment the exact position of T cell assay as diagnostic tool in the evaluation of patients with delayed HSR has to be defined.

Statement and recommendations

- Skin testing is the most readily available diagnostic test (Grade D)
- Skin testing may be useful to replace *in vitro* test for the diagnosis of immediate IgE-mediated HR to biologicals (Grade C)
- Intradermal test should be performed, as prick test is usually negative (Grade D)
- Skin testing for biologicals is a safe procedure even in patients with severe immediate reactions (Grade D)
- Cross-reactivity between similar medications could to be assessed either by available skin testing, specific IgE, or BAT testing
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Unmet needs:

- Standardization of drug concentrations for skin testing with biologicals
- Evaluation of the negativization rate for skin testing with biologicals
- Commercial availability of *in vitro* assay for the evaluation of IgE ADA
- Definition of the role of IDT and/or patch test for delayed reactions
  Definition of the role of T cell assays for the evaluation of drug sensitization to biologicals

Drug Provocation Test

Even if this technique was considered already in 2009 (51)**,** it was not until 2015 when specific data on drug provocation testing (DPT) with biologicals were reported by the Ramon y Cajal University Hospital (RCUH), Madrid, Spain (60). This same group recently published a mega study on DPT including a large cohort of 95 patients reacting to biologicals (17*)*. DPT is a diagnostic technique that involves the controlled administration of a drug for the study of DHRs (61,62) and should be distinguished from re-challenge techniques with their different aims (3, 27, 28, 63).

Indications and Contraindications

DPT might be performed prior to rapid drug desensitization (RDD) as a way to prevent non-hypersensitive patients from unnecessary RDD procedures for different drugs (17, 60, 64) In the RCUH study (17) 30% of all referred patients with an unequivocal clinical history of a DHR to biologicals showed negative DPT and therefore could avoid RDD. (Supplementary Table-3). DPT is also necessary to prevent a wrong diagnosis when more than one drug is involved in the initial reaction (17, 65). Contraindications for DPT should be the same as the general contraindications for DPT **(61)** including the lack of access to adequate installations and/or to drug allergy expert personnel and/or to specific resources that ensure appropriate risk-management plans (17, 60). Additionally, we should take into account the specific characteristics of these drugs and avoid DPT in patients who do not need any further treatment with the culprit drug or who are going to change to an alternative (and equally effective) treatment **(**17, 60). Additionally, DPT may help validating diagnostic tools, and further understand the phenotypes, endotypes, and mechanisms of DHRs, which will be useful for many future patients. However, only expert drug allergy centers with specific research objectives and specific approval by institutional ethic boards should include this indication (17, 60-62).

Drug provocation test in practice

In the RCUH studies 67-69% (17, 60) of all performed DPTs with biologicals were negative**.** The initial reactions for these patients were, according to Brown (32), mainly moderate to severe (63-67%), and the patients presented with different symptoms, namely cutaneous (77-87% of patients), respiratory (50-60%), cardiovascular (33-40%), fever/chills (27-57%), gastrointestinal (23-37%) and neuromuscular (13-27%) (17, 60) (Supplementary table **Table 3).**

DPT implementation and patient selection may vary locally. We are aware that the range of possibilities for adequate indication, optimal protocols, and safe location for DPT must be locally flexible **(**60**)**. Each center should be responsible for deciding what the real local possibilities are to perform DPT following RCUH protocol. (**Table -6)**

Statements and Recommendations:

- DPT is the diagnostic Gold Standard (Grade B).
- DPT prevents a significant number of patients from unnecessary drug desensitization. (Grade B).
- DPT has a good safety profile when performed in specialist centers (Grade C).
- DPT is a high-risk technique and benefits from dedicated spaces and expert personnel (Grade C).

Unmet needs:

- Standardization of protocols and selection of candidates, whilst acknowledging valid local variations.
- Multicenter studies and identification of differences in populations.

THERAPEUTICAL APPROACH

The primary objective of an allergist approaching HSRs to biologicals should regard the patient and ensure the administration of the reactive first-choice treatments under safety-first policies. Allergists rarely witness initial reactions. Thus, the fundamental role of the allergist in the prevention and management of initial reactions is to lead an institutional effort, including:

· The presence of a specific and well-organized multidisciplinary team led by allergists for the diagnosis and management of these reactions, including the option of desensitization.

· Specific institutional protocols for optimal treatment of initial reactions and for rapidly classifying and diagnosing the patient (referral to multidisciplinary team).

· Patient empowerment: Patients need to be reassured, informed of their referral to the multidisciplinary team, and later empowered by this team to make informed decisions on their conditions based on two fundamental pillars, namely, (i) indication of treatment by their responsible physicians and (ii) risk assessment by the Allergist.

· Risk assessment strategies. They might vary locally, but they must be based on a "safety-first policy" and founded on three fundamental pillars: (i) access to appropriate facilities and specific resources; (ii) locally designed risk management strategies open to tailored plans based on individual assessment, phenotyping and endotyping; and, (iii) access to expert personnel capable of appropriate patient selection and management provided the two previous pillars are met.

Desensitization Programs

Desensitization is a therapeutic approach and safely administers the needed medication and provides a temporary tolerance to drugs to which patients have presented immediate reactions. Recommendations for desensitization include the use of first line therapies, which cannot be substituted and that either increase the quality of life of patients or their life span in evidence-based studies. Prior to recommending desensitization switching to alternate products with equal efficacy should be evaluated. Cross-reactivity between similar medications needs to be assessed either by available skin testing, specific IgE, or BAT testing.

Specific institutional programs for drug desensitization are known to be a successful approach to biologicals hypersensitivity, and many original articles show excellent results on the progressively outstanding performance of desensitization programs and their achievements in local applications and improvements (17, 18, 29, 51, 65-69). Once a reaction has been defined as type I (IgE/non IgE) or type IV the potential for desensitization needs to be assessed if the drug is used for first line therapy. The new classification of HSRs to biologicals can help clinicians to decide treatment plans including desensitization (9). To optimize desensitization, it is important to identify the phenotypes, endotypes and biomarkers that can be desensitized and contraindications in every candidate for desensitization (Table-7).

Upon the occurrence of a HSR to a biological, skin test should be conducted 4-6 weeks after the HSR if available and BAT should also be evaluated if skin test is negative and BAT is available (51, 69). Prior desensitization, a premedication protocol consisting of H1 blockers (such as cetirizine -10 mg orally) and H2 blockers (such as famotidine -20 to 40 mg164 orally or intravenously) is administered. Additional premedication such as ASA and montelukast can be administered if flushing and bronchospasm occur during the initial reaction, respectively (29, 66, 67).

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 results 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, a tryptase level should be drawn and 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 (4, 9, 21). (**Figure 3).**

Statement and Recommendations:

- Type I, cytokine-release syndrome, mixed reactions are candidates for desensitization (Grade B).
- Rapid drug desensitization to biologicals is safe and effective (Grade A).
- Breakthrough reactions are less severe that initial HSR (Grade C).
- Type IV HSR, excluding SCARs is candidates for desensitization ((Grade C).

Unmet needs:

- The long-term impact on drug efficacy is unknown because RDD protocols differ.
- What is the difference between different desensitization protocols?
- Overall cost of desensitization is similar to standard administration.

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Table-1. Frequently used biologicals, their targets, and implicated mechanisms to hypersensitivity reactions

Biologicals	Target molecule	Target Diseases	Implicated mechanisms to HSRs
Infliximab	TNF-alpha inhibitor	Inflammatory diseases	Type I, III, IV
Etanercept	TNF-alpha-IgG fusion protein	Inflammatory diseases	Type I, IV
Adalimumab	TNF-alpha inhibitor	Inflammatory diseases	Type I, III, IV
Golimumab	TNF-alpha inhibitor	Inflammatory diseases	NR
Certolizumab	TNF-alpha inhibitor	Inflammatory diseases	NR
Tocilizumab	IL-6	Inflammatory diseases	Type I, Type IV
Efalizumab	CD11a	Inflammatory diseases	Type I
Secukinumab	IL-17A	Inflammatory diseases	Type I
Anakinra	IL-1R	Inflammatory diseases	Type 1
Belimumab	BAFF	Inflammatory diseases	Type 1, CRR
Ustekinumab	IL-12 and IL-23	Inflammatory diseases	NR
Rituximab	CD20	Tumors	Type I, III, CRR mixed
Ofatumumab	CD20	Tumors	Type I, CRR
Obinutuzumab	CD20	Tumors	CRR
Brentuximab	CD30	Tumors	Type I
Trastuzumab	HER-2	Tumors	Type I, CRR
Pertuzumab	HER-2	Tumors	Type I
Cetuximab	EGFR	Tumors	Type I
Mogamulizumab	CCR4	Tumors	Type IV
Bevacizumab	VEGF	Tumors	Type I, Type IV
Pembrolizumab	PD-1	Tumors	Type I,
Omalizumab	IgE	Allergic diseases	Type I, Type III
Benralizumab	IL-5R alfa	Asthma	Type I
Reslizumab	IL-5	Asthma	Type I
Mepolizumab	IL-5	Asthma	Type I
Dupilumab	IL-4 R alfa	Asthma, atopic dermatitis, nasal polyps	Type I

Abbreviations: IBD, inflammatory bowel disease; RA, rheumatoid arthritis; Ps, psoriasis;
HER-2, human epidermal receptor-2; EGFR: human epidermal growth factor receptor, PD-L1 Programmed death ligand 1, CRR: Cytokine Release Reaction, VEGF: vascular endothelial growth factor-A; CCR4, CC chemokine Receptor-4, NR: not reported

Table-2. Classification of Hypersensitivity Reactions to Biologicals

Infusion related reactions	➢ At first infusion ➢ Flushing, chills/rigor, fever, tachycardia, hypertension, dyspnea, nausea, vomiting and syncope ➢ Self - limiting nature
Cytokine release reactions	➢ At first infusion ➢ Flushing, chills/rigor, fever, headache, back pain tachycardia, hypertension, dyspnea, nausea, vomiting and syncope
Type I (lgE/non lgE) reactions	➢ At repeated infusion ➢ Flushing, pruritis, urticaria, dyspnea, hypertension and life-threatening anaphylaxis ➢ Release of mast cell and basophilic mediators
Mixed reactions	➢ lgE Mediated plus cytokine release features
Type III reactions	➢ Soluble antigen - antibody (lgG/M) deposit in tissues (local or systemically)
Delayed Type IV reactions	➢ 12 hours to several weeks after exposure BA ➢ Maculopapular rash to SJS/TEN ➢ Tcell mediated

Table-3. Clinical presentations of immediate hypersensitivity reactions to biologicals

Symptoms		Prevalence (%)			
	Patient characteristics	n= 24 Adult	n= 30 Adult	n= 67 Adult	n= 104 Adult
	Underlying diseases	NA	RA, SPA, VAS, BID	Lymphoma	Hematologic malignancies, CTD, other autoimmune diseases
	Culprit MoAb **(Ref no) * Retrospective data**	Rituximab (20)	Infliximab (30)	Rituximab (31)	16 different mAbs (9)
	History of atopy/allergic disease	31%	NA	19%	37%
	History of drug allergy	NA	NA	46%	27%
Constitutional symptoms		46			
Fever					5
Chills/cold				9	5
Diaphoresis				1	1
Rigors				6	7
Pain					
Back pain			3	6	7
Jaw, neck, arm/shoulder pain					5
Mucocutaneous		92			
Flushing/warmth/erythema			60	21	32
Pruritis			38	45	29
Urticaria			15	16	19
Other rash				9	1
Angioedema			5		5
Upper Airways		NA			
Nasal congestion					1
Itchiness, tickle, sore, hoarseness, lump in throat				25	8 4
Tongue swelling					
Respiratory		88			
Cough				4	3
Chest tightness					11
Dyspnea			50	10	21
Wheezing					3
O2 desaturation			20		7
Cardiovascular		67			
Bradycardia					2
Chest pain				9	6
Hypertension				3	2
Hypotension			5	1	11
Presyncope					3
Syncope			12		4

Tachycardia		3	1	2
Gastrointestinal	29			
Abdominal pain			1	5
Bloating				1
Diarrhea				1
Nausea/vomiting		10	4	4
Neuromuscular	29			
Disorientation/hallucination				1
Headache				3
Numbness/weakness/tingling				3
Sense of impending doom				2

*Data are derived from the indicated references

CTD: Connective tissue diseases, RA: Rheumatoid arthritis, SPA: Spondyloarthritis, VAS. Vasculitis, BID: Bowel inflammatory diseases,

Table-4: Pathogenesis of hypersensitivity reactions to biologicals

Mechanisms	Responsible immune component	timing of onset/clinical presentation
ADA-mediated		
IgE dependent	- IgE-FcεRI interaction on mast cells and basophils	Immediate/urticaria, anaphylaxis
Non IgE dependent	- IgG-FcγRIII interaction on basophils, neutrophils, macrophages - Mast cell activation via C' system activated by ICC between ADA and drug	Immediate/ Urticaria, Anaphylaxis
	- ICC between ADA IgG and drug, tissue deposition and C' activation	Delayed/serum sickness-like disease, skin vasculitis
Non-ADA mediated		
CRR	Cytokine release through different mechanisms	Immediate to delayed (anaphylaxis-like; flu-like syndrome; cytokine storm with MOF)
CARPA	Lipids and/or aggregates activate C' system and direct mast cell activation by C3a, C5a	Immediate/Anaphylaxis-like
T cell mediated	Active T cells	Delayed/ Disseminated skin reactions and Injection site reaction

Table -5. Skin test concentrations for TNF- alfa inhibitors

Infliximab

Authors (year), Ref (.)	Drug concentration	Dilution for Prick test	Dilution for IDT	N° cases
Vultaggio (2010) (33)	10 mg/ml	1:1000-1:1	1:10000-1:10	11
Matucci (2013) (27)	10 mg/ml	1:1000-1:1	1:1000-1:1	23
Freling (2015) (46)	2 mg/ml	1:10-1:1	1:100-1:1	24
Brennan (2009) (48)	10 mg /ml	1:1	1:100-1:10	6
Bavbek (2016) (15)	10 mg/ml	1:1	1:1000-1:10	1

Adalimumab

Authors (year)	Drug concentration	Dilution for Prick test	Dilution for IDT	N° cases
Benucci (2008) (43)	50 mg/ml	1:1	1:10-1:1	2
Bavbek (2015) (44)	40 mg/ml	1:1000-1:1	1:1000-1:1	5

Etanercept

Authors (year)	Drug concentration	Dilution for Prick test	Dilution for IDT	N° cases
Bavbek (2015) (44)	50 mg/ml	1:1	1:1000-1:10	7
de la Varga Martinez (44)	25 mg/ml	1:1	1:100-1:10	2
Benucci (2008) (43)	25 mg/ml	1:1	1:5-1:250	2

Table--6. Details on drug provocation test with biologicals

Timing	The patient's next scheduled treatment should be used as DPT.	
Dosage and number of steps	**Standard approach**	Protocol as per Manufacturer's Instructions and Institutional Recommendations.
	Cautious approach	Starting at 1/4 or even 1/8 dose/minute of the standard Progressively increasing to 1/1 in a every 30 minutes might be a more cautious approach for severe initial reactions, very immediate rapid-onset reactions, or higher risk assessments.
Concomitant drugs	**Precautions**	Certain authors recommend caution with beta-blockers and ACE inhibitors **(17, 32, 60, 61).**
	Intensified pre-medications	Not recommended **[(17, 60, 61)**, as they can help to induce a false temporary tolerance
	Biologicals in chemotherapy regime	To keep standard regimes unaltered, additional required medications (other antineoplastics, leucovorin, etc.) should be also administered as prescribed by the referring physician.
	DPTs with concomitant drugs	Whenever needed, provocations with other non-biological drugs such as premedication, concomitant drugs possibly involved in the initial reactions were performed before DPT with the culprit-drug (**17, 60)**.
Results	Test was considered positive when it reproduced the original symptoms or showed an objective DHR (**17, 60)**.	
Restart protocol	In case of a positive DPT, once symptoms are controlled after adequate treatment and the patient is asymptomatic, the infusion may be immediately (approximately within 30 min after the DHR) restarted at 1/4 of the final infusion rate for 15 min, and then increased to 1/2 of the initial infusion rate until all the medication was administered ('restart protocol') **(60).** A phenomenon of temporary tolerance after the positive DPT reaction allows patients to safely receive the remaining treatment **(60)**	
Follow up	Patients with a negative DPT are eligible to continue with standard administrations. But, some may need to be retested after the first negative DPT, this might be true for patients with a short elapsed time from initial reaction to testing.	
"Uncontrolled" DPTs	Multidisciplinary institutional teams lead by allergists are the key for avoiding the risks of "uncontrolled DPTs" (i.e., administering a culprit-drug or a cross-reactive drug to a reactive patient lacking allergy/risk assessment, in inappropriate environments, by untrained and/or unaware personnel). .	
Location	Should ideally include 1:1 nurse: patient ratio, intensive surveillance by expert personnel (including bedside physical presence of an allergist), continuous monitoring access to crash cart, access to oxygen, readily available prefilled syringes with adrenaline, rapid access to Intensive Therapy Unit if necessary	

Table-7. Phenotypes, endotypes and contraindications for desensitization to biologicals

Indications Phenotypes	Indications Endotypes	Contraindications
Immediate HSR Grade 1, 2, 3	Type 1	SJS
Delayed HSR	Type IV HSR (excluding those with SCARs)	DRESS
	Cytokine Release Reaction	TEN
	Mixed reactions (Cytokine release + Type I)	AGEP
		Serum sickness

Figure 1: From pathogenesis to clinical features of ADA-mediated reactions

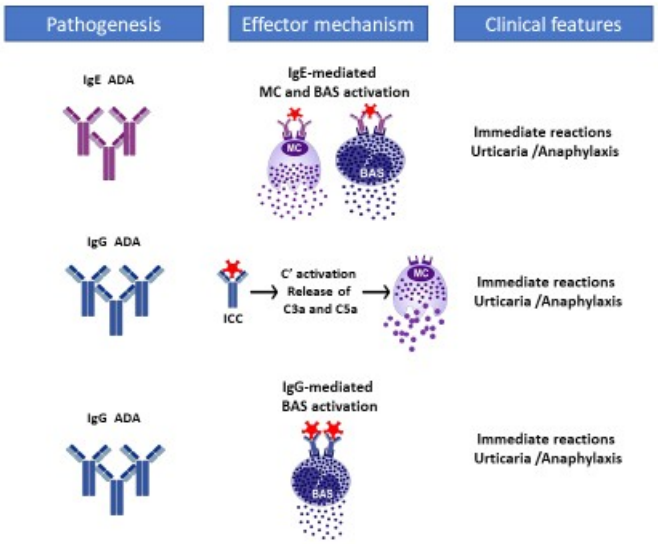

Figure 2: cytokine release syndrome: mechanisms

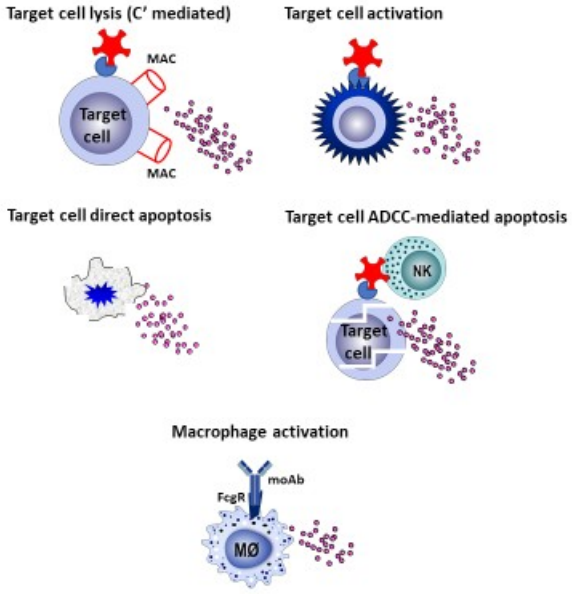

Figure -3

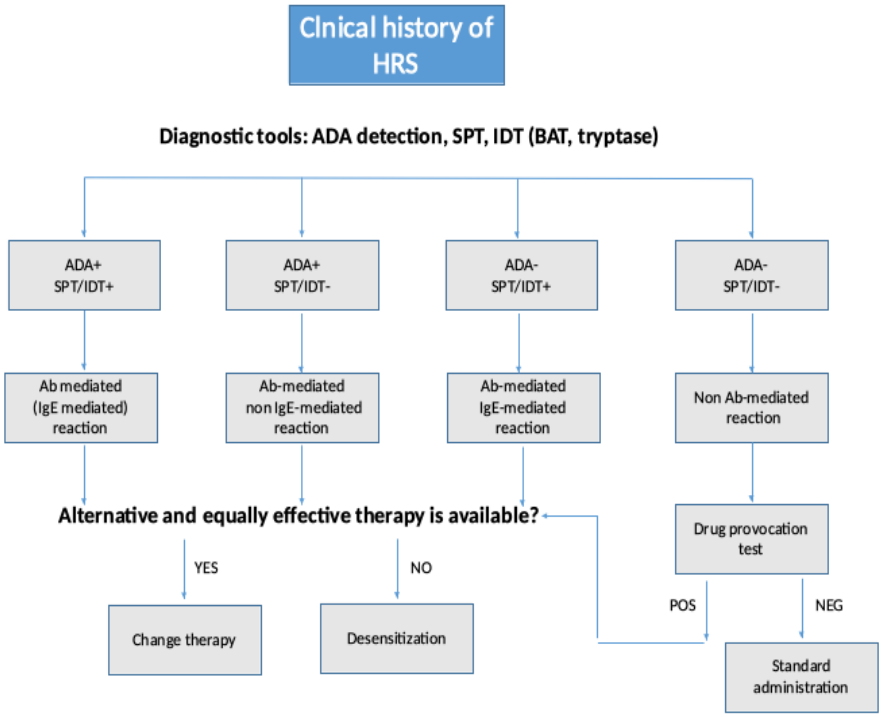

Figure Legends

Figure -1 legend: From pathogenesis to clinical presentations of ADA mediated reactions

Figure -2 legend: cytokine release syndrome: mechanisms

Figure -3 legend: Diagnostic algorithm for hypersensitivity reactions to biologicals