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TITLE: Hypersensitivity Reactions to Biologicals: an EAACI position paper

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ABSTRACT

Biologicals are crucial targeted therapeutic agents in oncological, immunological, and inflammatory diseases, and their use in clinical practice is broadening. In recent years, the spread of Personalized Precision Medicine has facilitated a proliferation of new treatment options, especially biologicals. Consequently, biologicals are now among the drugs that most frequently cause hypersensitivity reactions (HSRs). Patients can develop HSRs to these agents during the first-lifetime exposure or after repeated exposure, and these HSRs can be potentially life-threatening or limit therapeutic options. Despite the relatively high prevalence, the underlying mechanisms of these HSRs remain obscure, and the optimal management pathways are still a matter of discussion. In this Position Paper, the authors will provide evidence-based recommendations for diagnosing and managing HSRs to biologicals. Additionally, the document defines unmet needs as an opportunity to shape future research.

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
PP: Position Paper
TF: Task Force
IRR**:** infusion related reaction
IFNs: interferons
IL: interleukins
GM-CSF: granulocyte macrophage colony stimulating factor
TNF-α: Tumor necrosis factor-α

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CRR: cytokine release reaction
SSLR: Serum Sickness Like Reaction
SDRIFE: symmetrical drug-related intertriginous and flexural exanthema
SJS: Stevens-Johnson Syndrome
TEN: Toxic epidermal necrolysis
PNP: Paraneoplastic pemphigus
ADA: Anti-drug antibodies
DPT: Drug provocation testing
ICs: Immune-complexes
CARPA: C' activation-related pseudoallergy
SPT: skin prick test
IDT: Intradermal test
BAT: Basophil activation test
RIA: Radioimmunoassay
RIP: radioimmunoprecipitation RDD: *R*apid drug desensitization
EGFR: Epidermal growth factor receptor

INTRODUCTION

Biologicals are a diverse group of medicines that include growth factors, immune modulators, monoclonal antibodies (mAbs), and vaccines. Biologicals are, in general, sizeable molecular weight therapeutics that are synthesized by living organisms and interact with the immune system by binding specific determinants. Some examples of biologicals include namely, cytokine receptors [such as etanercept (soluble cytokine receptors), and abatacept (CTLA4-IgG1Soluble cellular ligands)], soluble mediators [cytokines (such as GM-CSF), and interferons (INFs)] or mAbs to specific antigens or mediators (1, 2).

Because of their selectivity, biologicals are crucial therapeutic agents in oncological, immunological, and inflammatory diseases, and their use in clinical practice is broadening (3*).* Hypersensitivity reactions (HSRs) to biologicals have steadily increased in frequency over the 21st century, likely linked to countless new treatment options that have spurred from the spread of Personalized Precision Medicine. Currently, biologicals are among the drugs that most frequently

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cause hypersensitivity reactions. Patients with numerous diseases and pathologies can develop HSRs during their first-lifetime exposure or after repeated exposures to biologicals (4). Table 1 shows data on biologicals with a reported rate of any type of HSR higher than 1% and/or anaphylaxis on individual indication (2).

Despite its relatively high prevalence, most underlying mechanisms remain unclear, and optimal management is still a matter of discussion. The European Academy of Allergy and Clinical Immunology (EAACI) Drug Allergy Section and Biological WG created a Task Force to address this issue and appointed experts in the field to produce a Position Paper. In this Position Paper, the authors will provide evidence-based recommendations for diagnosing and managing HSRs to biologicals by mainly focusing on mAbs.

METHODS – SEARCH STRATEGY

The EAACI commissioned this Position Paper (PP). The appointed 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 keywords: biologicals, hypersensitivity reactions, allergy, mAbs, interferon, interleukins, drug hypersensitivity, desensitization. Besides, each TF member used extra keywords as appropriate for each specific section. The search included in vivo and in vitro trials in the 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. The TF members carefully reviewed the statements, recommendations and unmet needs, including grading of the quality of evidence using the SIGN criteria as Grade A, B, C, D. (Supplementary file, table 1) (5,6)

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CLASSIFICATION

Adverse reactions to biologicals resist traditional classification because of their different properties from chemical drugs (4, 7). Thus, first, Pichler proposed a classification involving five types of adverse side-effects of biologicals based on pathomechanisms (8) (Supplementary file, Table 2). Recently, researchers have proposed a new classification merging traditional one with new findings on phenotypes, endotypes and biomarkers (9, 10). This new classification divides HSRs to biologicals into infusion-related reactions (IRRs), cytokine release reactions (CRRs), type I (IgE/non-IgE) and mixed reactions, which are usually immediate, as well as Type III and IV that are characteristically non-immediate (Table 2).

Infusion-related reactions

Patients predominantly suffer from IRR at first infusion. Although the pathogenesis of these reactions is not very clear, it's usually affected by the infusion rate, pointing out the possibility of a non-immunologic mechanism and the role of the inflammatory cytokines such as IL-6 and tumor necrosis factor-α (TNF-α).

Cytokine release reactions

Clinical signs and symptoms are usually due to cytokine release. Tests can show elevated serum TNF-α and IL-6 levels at the time of the reaction when compared with their normal baseline. The main difference between IRRs and CRRs is the self-limiting nature of IRRs on repeated exposure and the response to premedication.

Type I reactions (IgE/non-IgE)

Reactions are associated with IgE or non-IgE mediated mast cell/basophil degranulation, leading to the release of preformed mediators. Typically, IgE-mediated reactions need several exposures before occurring, although there are exceptions such as cetuximab, which can cause IgE-mediated reactions on first exposure due to preformed IgE antibodies (11). Skin testing positive results might differentiate IgE and non-IgE mediated reactions, but the lack of standardization with skin tests to biologicals and the possibility of false positives and false negatives should be kept in mind. The other methods for detecting IgE may be supportive.

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Mixed reactions

Mixed reactions are a combination of cytokine release and IgE-mediated reactions. We can find skin testing positive results and/or positive specific IgE to the implicated biological, as well as increased levels of tryptase, IL-1, IL-6 and TNF-α.

Serum Sickness Reactions

Type III reactions are rare. 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 biologicals.

Type IV Reactions

Delayed type IV HSRs to mAbs, may range from non-severe maculopapular rash to severe cutaneous reactions. T-cells seem to be the main protagonist in these reactions, but other mechanisms may be involved.

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EPIDEMIOLOGY AND RISK FACTORS

All biologicals can cause HSRs, and due to the increased use of these agents, HSRs have become more frequently reported. Reactions to mAbs vary by agent. For example, the rates of immediate-type HSRs to specific biologicals range from 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 concomitant drugs, and drug-related factors, such as degree of humanization, glycosylation pattern, type of sourcing cells, dosing interval, and excipients with allergenic potential (4,7). Patients with anti-drug antibodies as IgG or IgE developed during treatment with biologicals, or preexisting, are more likely to have an increased risk of immediate HSRs to biologicals (13, 15). Some authors find an association between positive skin test results and greater severity of initial reactions (9). Interestingly, women are more prone to drug allergy, particularly chemotherapy medications and new biologicals and mAbs (16).

Breakthrough reactions during desensitization appear in approximately 13.5% to 23% of patients with 2.3-2.6% multiple reactors (9, 18, 20,21). A correlation between breakthrough reactions and the positivity of skin tests has been controversial due to a recent paper that reported that the main predictor for breakthrough reactions is a positive skin test result (17), although recent articles have not supported this finding (18, 19, 20).

CLINICAL PRESENTATION

HSRs may limit the use of biologicals, leading to therapy interruption and impaired quality of life (3, 4, 21-24). Immediate HSRs occur during or within a few hours (particularly with subcutaneous route) from either a first or subsequent infusion. Delayed/non-immediate HSRs occur within the 14 days after the infusion (25-27). Immediate reactions are more frequently systemic, while delayed reactions are more frequently local after subcutaneous administration of biologicals (3, 4, 8, 25, 26).

The clinical manifestations of immediate HSRs vary considerably, ranging from mild to severe

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and even life-threatening. Mucocutaneous symptoms are the most common, followed by respiratory and cardiac symptoms (Table 3) (3, 9, 28-34).

Infusion-related Reactions

Clinical presentations resemble Type I or CRR, but they are mild to moderate in severity and subside gradually with the following infusion.

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.

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.

Mixed Reactions

Clinical presentations are characterized by wheezing, flushing, urticaria, pruritus, with fever/chills, nausea, pain, headache, and rigor.

Delayed/non-immediate HSRs are presented as serum sickness reaction and Type IV reactions,

Serum Sickness Reactions

Typical symptoms are fever, malaise, arthralgia-arthritis, jaw pain, erythematous, and sometimes urticarial lesions, purpura, and conjunctival erythema, consistent with a serum sickness like reaction (SSLR). Occasionally, these reactions can feature patchy lung infiltrates, lymphadenopathy, splenomegaly, gastrointestinal symptoms, and limb weakness. 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. Correct diagnosis of serum sickness reaction remains an unmet need (35).

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Type IV reactions

These reactions are mainly demonstrated 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. ((2, 3, 8, 24, 27) In the vast majority of cases, there is at least one administration without reaction 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 (36).

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 severity. These reactions generally last 1-5 days and do not lead to interrupting therapy (2, 3, 22, 23). However, exanthematous dissemination has been reported in rare cases (24). Some patients may develop recall reactions (local reactions at the site of the previous reaction) (22, 25).

PATHOGENESIS OF HYPERSENSITIVITY REACTIONS

The pathogenesis of HSRs to biologicals represents a complex picture that has not been fully clarified. One of the main features of biologicals is immunogenicity, which is the capability of these agents to induce an immune response against the drug itself, leading to 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 workup.

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

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production. ADAs are heterogeneous in their composition (isotype, affinity, specificity) (37), 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, 38). 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 (38, 39). Sometimes IgE mediated reactions may occur as a first dose event, as in the case of cetuximab-induced reactions, and sustained by preexisting 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 patients exposed to biologicals. This has been described for polysorbate in omalizumab-, erythropoietin- and darbepoetin-treated patients (12). Sensitization might derive from the previous contact with the same excipients used, for instance, in vaccines 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 (40).

Non-ADA-mediated reactions: immediate and delayed

Among non-ADA-mediated reactions, the best-characterized condition is represented by the CRR; 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. CRR is clinically heterogeneous, with symptoms occurring within minutes (immediate reactions) to hours/days after the treatment start (delayed reactions*)* (40) (Fig 2).

The complement (C') activation represents the second non-ADA-mediated mechanism involved in the pathogenesis of infusion reactions to biologicals, 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 the 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 activates C' thus resulting in tissue

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damage and recruitment/activation of inflammatory cells (e.g. skin vasculitis, glomerular disease) (40). 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) (41), 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 a few cases have been analyzed (42, 43). 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 (44) or of exacerbation of psoriasis during TNF αlpha antagonist (45). 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 (46).

- **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 non-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

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- 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 (9, 12, 20). In some small case series, positive skin testing with biologicals has confirmed the *in vitro* detection of serum drug-specific IgE (38), thus showing the biological activity of biological-specific IgE in mast cells activation. Although it is essential to perform intradermal test (IDT) (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). This suggests that skin testing, being the most readily available diagnostic testing, may be useful in replacing *in vitro* tests to diagnose immediate IgE-mediated HSR to biologicals. One of the main limitations of skin testing for biologicals is the lack of standardized procedures, including ideal drug concentrations (47). There is insufficient evidence to date to recommend appropriate drug dilutions for skin prick test (SPT) and IDT for most biologicals (9, 18, 20). 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, 22, 23, 30, 38, 48-51) **(**Table 5**).** In addition, Lieberman et al. have evaluated non-irritating test concentrations for omalizumab (1.25 mcg/ml) (52). 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 reduces the diffusion and application of this diagnostic approach, especially in small centres. Of note, all necessary aids and rescue drugs must be readly available in the health setting where skin testing for biologicals are performed.

The time between the reaction and the evaluation is crucial due to the temporary unresponsiveness of the skin mast cells following the reaction and the rapid decrease displayed by IgE specific for biologicals. No negativization rate of skin testing for biologicals is available. For this reason,

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currently, there is insufficient evidence to recommend a specific ideal timing to perform skin testing for biologicals. Regarding safety, there were no unexpected adverse reactions to the *in vivo* procedures with biologicals, even in patients with severe reactions (38, 53).

Concerning the *in vivo* allergy tests for delayed HSRs, not much 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 managing generalized reactions to IFNs (54). For delayed cutaneous reactions to 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 aims to verify the development of an immune response characterized by ADA. Several 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 (55). ELISA in this format is a drug-sensitive assay because circulating drug in the serum may interfere with ADA detection, thereby leading to false-negative results (56). 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 (56). Confirmed positive samples may be submitted to characterize the IgE isotype, using the ImmunoCAP platform (not commercially available) and other homemade immunoassays. ImmunoCAP sensitivity depends on the biologicals involved. ImmunoCAP sensitivity to cetuximab ranges from 68% to 92% and specificity from 90% to 92% depending on HSR severity (57), 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.

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There are commercially available tests for the assay of non-isotype-specific ADA (CE marked), although few laboratories practice this assay routinely (30, 58, 59). Furthermore, the number of tests available for different biologicals is expanding. However, it would be helpful if the company that creates and produces a new biologic or 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 workup 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 (58), and the re-exposure of the patient to a second cycle of treatment after drug interruption increases the risk of HSRs (30, 60). 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 (60). 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.

Alfa-Gal specific IgE test may be useful not only to identify IgE to a-Gal in cetuximab- reactive patients to define the mechanism of HRS, but also in cetuximab-unexposed patients, to predict the risk of HRS at the first drug administration (61)

Tryptase is the most studied biomarker in anaphylaxis, although it is still far from being the ideal biomarker for this. 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 a rather specific mast cell mediator, and its elevation suggests an ADA-mediated mechanism of the reaction. However, it is noteworthy that CARPA is associated with an increase of tryptase and that basophils also contain and release this mediator (62). Serial measurements of tryptase, although laborious in daily clinical practice, may more likely identify the peak of tryptase, thus increasing the test's sensitivity.

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Basophil activation test (BAT) could be included in the allergy workup of immediate HSR when an ADA-mediated mechanism is suspected. Some papers describing the use of basophil activation test (BAT) to diagnose HSR to biologicals can be found, particularly in rituximab-related infusion reactions (63). However, studies in larger series of patients are needed to confirm the findings and establish BAT as a diagnostic tool.

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 (62, 64).

Although circulating biological-specific T cells have been described in treated patients, at the moment, the exact position of T cell assay as a diagnostic tool in the evaluation of patients with delayed HSR has to be defined (34).

- **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 tests should be performed, as prick tests are 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 be assessed either by available skin testing, specific IgE, or BAT testing
-
- **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 assessment of IgE ADA

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- Definition of the role of IDT and/or patch test for delayed reactionsDescription of the role of T cell assays for the evaluation of drug sensitization to biologicals

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Drug Provocation Test

Even if this technique was considered already in 2009 (53)**,** it was not until 2015 when Ramon y Cajal University Hospital (RCUH), Madrid, Spain, reported data specifically on drug provocation testing (DPT) with biologicals (59). This same group recently published an extensive 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 (65, 66) and should not be confused with other re-challenge techniques with different aims (3, 27, 31, 67).

Indications and Contraindications

DPT might be performed before rapid drug desensitization (RDD) as a way to prevent non-hypersensitive patients from unnecessary RDD procedures for different drugs (17, 59, 68). In the RCUH study (17), 30% of all referred patients with an unequivocal clinical history of a DHR to biologicals showed negative DPT and 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, 69). Contraindications for DPT should be the same as the general contraindications for DPT **(**65**),** 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, 59)

Additionally, we should consider 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, 59). Additionally, DPT may help validate diagnostic tools and further understand the phenotypes, endotypes, and mechanisms of DHRs, which will be helpful 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, 59, 65, 66).

Drug provocation test in practice

In the RCUH studies, 67-69% (17, 59) 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%). (Supplementary table Table -3 and 4).

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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 **(**59**)**. Each center should decide whether to include DPT in their pathways and how to do it, based on their local possibilities and expertise (Table -6**).**

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- **Statements and Recommendations**
- DPT is the diagnostic Gold Standard in allergy to biologicals (Grade B).
- DPT with biologicals prevents a significant number of patients from unnecessary drug desensitization. (Grade B).
- DPT with biologicals has a good safety profile when performed in specialist centers (Grade C).
- DPT with biologicals is a high-risk and high-complexity technique and requires dedicated spaces and expert personnel (Grade C).
-
- **Unmet needs**
- Standardization of DPT protocols for biologicals and refinement of candidate selection whilst acknowledging valid local variations.
- Multicenter studies and identification of differences in populations.

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THERAPEUTICAL APPROACH

The primary concern of an allergist approaching HSRs to biologicals should be the patient. In this context, the objective would be to ensure that the patient can safely continue with the necessary first-choice treatments. 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 diagnosing and managing these reactions, including the option of desensitization.

· Specific institutional protocols for optimal treatment of initial reactions and for rapidly classifying and diagnosing the patient (fast-track 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 (9, 17-19, 59).

Desensitization Programs

Rapid Drug Desensitization is a therapeutic approach to safely administer medication causing an HSR by providing a temporary immune tolerance to drugs to which patients have presented immediate reactions (70). RDD mechanisms have been studied *in* vitro, and several studies showed data on large series of patients undergoing RDD after reacting to biologics (9, 17-20, 29, 59, 71). RDD is considered in reactive patients when there is a need for first-line therapies with no equally effective alternative or when the said first-line treatment increases the quality of life of patients or their life span in evidence-based studies. Before recommending RDD, switching to alternate products with equal efficacy should be evaluated. Cross-reactivity between similar

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medications needs to be assessed either by available skin testing, specific IgE, or BAT testing.

Specific institutional programs for RDD are known to be a successful approach to biologicals hypersensitivity, and many original articles show excellent results on the progressively outstanding performance of RDD programs and their achievements in local applications and improvements (9, 17-20, 29, 59) (Supplementary Table 5, 6). The new classification of HSRs to biologicals can help clinicians decide treatment plans, including RDD (9). RDD is a high-risk and high-complexity technique that should only be performed by expert allergists in dedicated allergy-led spaces. RDD is a technique that needs personalization, and it is essential to precisely profile candidate patients (making use of the classifications explored in this manuscript, including endotypes, phenotypes, and biomarkers) and identify the potential contraindications in every candidate (Table-7).

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Upon the occurrence of an HSR to a biological, a skin test should be conducted 4-6 weeks after the HSR if available, and BAT should also be evaluated if the skin test is negative and BAT is available (53, 73). Before RDD, a premedication protocol consisting of H1 blockers (such as cetirizine -10 mg orally) and H2 blockers (such as famotidine -20 to 40 mg orally or intravenously) can be administered (bearing in mind that some biologicals already include these drugs as premedication in their product information) (9, 17-20, 29). Additional premedication such as ASA and montelukast can be issued if flushing and bronchospasm occur during the initial reaction, respectively (73).

RDD should always be performed on patients with positive in vivo/in vitro tests, regardless of the grade of the initial HSRs (32). If the test results are negative and the risk assessment is favorable (e.g., 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 following 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 Recommendation**
- Type I, cytokine-release syndrome, and mixed reactions are candidates for desensitization (Grade B).
- Rapid drug desensitization to biologicals is safe and effective (Grade A).
- Breakthrough reactions are less severe than initial HSR (Grade C).
- Type IV HSRs, excluding SCARs, are amenable 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?
- The overall cost of desensitization is similar to standard administration.
- The impact of RDD on quality of life.

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CONFLICT OF INTEREST

SB reports speaker fees from AstraZeneca, GSK and Novartis and she is also on the AstraZeneca, GSK and Novartis advisory board . All outside the present work.

MP reports he has no conflict of interest

EAC reports he has no conflict of interest

MC reports she has no conflict of interest

ABD is on the GSK and Novartis advisory board. All outside the present work

SH reports she has no conflict of interest

RMB reports he has no conflict of interest

SSS reports she has no conflict of interest

AV reports she has no conflict of interest

AUTHORS' CONTRIBUTION

MP and SB as chair and secretary led the task force, managing, the development and integration of the manuscript, seeking approval by the EAACI regulatory bodies, and making the final editorial decisions. SB authored 'Abstract, Introduction and Classification', ABD authored 'Clinical Presentation', AV authored Pathogenesis and Diagnostic Approach' EAC and RBM co-authored 'drug provocation tests' and 'therapeutical approach', MC, SH and SSSco-authored 'Therapeutical Approach'. Each author was responsible for their own section and has presented many drafts, revisions, and agreed on final versions. SB, EAC, RMB, and AV contributed to the final editing.

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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
Abatacept	Soluble cellular ligands: (CTLA4-IgG1)	Inflammatory diseases	Type I, III, 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

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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

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

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Table-2. Classification of Hypersensitivity Reactions to Biologicals

Immediate reactions	
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 (IgE/non IgE) reactions	*At repeated infusion *Flushing, pruritus, urticaria, hypertension, dyspnea, and life- threatening anaphylaxis *Release of mast cell and basophilic mediators
Mixed reactions	*IgE mediated plus cytokine release features
Delayed reactions	
Serum Sickness Reactions	*Soluble antigen-antibody (IgM/G) deposit in tissues (local or systemically)
Type IV reactions	*12 hours to several weeks after exposure to biologicals *Maculopapular rash to SJS/TEN *T cell mediated

SJS: Stevens-Johnson Syndrome, TEN: toxic epidermal necrolysis

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

		Prevalence (%)			
Symptoms	**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 (29)	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					
	Fever	46			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

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Upper Airways	NA			
Nasal congestion				1
Itchiness, tickle, sore, hoarseness,			25	8
lump in throat				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

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

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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
	- ICs 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	Immediate/Anaphylaxis-like

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	cell activation by C3a, C5a	
T cell mediated	Active T cells	Delayed/ Disseminated skin reactions and Injection site reaction

ADA: anti- drug antibodies, CRR: cytokine release reaction, CAPRA: C' activation-related pseudoallergy, MOF: multi organ failure, C: complement, ICs: immune-complexes

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

Infliximab

Authors (year), Ref (.)	Drug concentration	Dilution for Prick test	Dilution for IDT	N° cases
Vultaggio (2010) (38)	10 mg/ml	1:1000-1:1	1:10000-1:10	11
Matucci (2013) (30)	10 mg/ml	1:1000-1:1	1:1000-1:1	23
Freling (2015) (51)	2 mg/ml	1:10-1:1	1:100-1:1	24
Brennan (2009) (53)	10 mg /ml	1:1	1:100-1:10	6
Bavbek (2016) (18)	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) (48)	50 mg/ml	1:1	1:10-1:1	2
Bavbek (2015) (49)	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) (49)	50 mg/ml	1:1	1:1000-1:10	7
de la Varga Martinez (50)	25 mg/ml	1:1	1:100-1:10	2
Benucci (2008) (48)	25 mg/ml	1:1	1:5-1:250	2

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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 and progressively increasing to 1/1 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**	Some authors recommend caution with beta-blockers and ACE inhibitors.
	Intensified premedications	Not recommended, 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, DPTs with other non-biological drugs such as premedication, concomitant drugs possibly involved in the initial reactions should be performed before DPT with the culprit drug
Results	Test was considered positive when it reproduced the original symptoms or showed an objective DHR	
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 HSR) 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'). A phenomenon of temporary tolerance after the positive DPT reaction allows patients to safely receive the remaining treatment	
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 the initial reaction to testing.	
"Uncontrolled"	Multidisciplinary institutional teams led by allergists are the key for avoiding the risks	

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DPTs	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 Care Unit.

DPT: drug provocation test, HSR: hypersensitivity reaction. Based on the experience of Ramon y Cajal university Hospital, Madrid, Spain **(ref. 17, 59, 69).**

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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

SCARs: Severe Adverse Cutaneous Reactions, SJS: Stevens-Johnson Syndrome, TEN:toxic epidermal necrolysis, AGEP: acute generalized exanthematous pustulosis, DRESS: drug rash with eosinophilia and systemic symptoms

Figure Legends

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

Different effector mechanisms involved in ADA-mediated reactions are illustrated. Mast cells and basophils can be activated by IgE ADA, upon cross linking of FcERI. ADA belonging to IgG isotype may participate in the formation of immunocomplexes, that activate C' cascade with the release of anaphylotoxins (C3a, C5a) that subsequently activate mast cells. Basophils may be also activated directly by IgG ADA.

ADA: anti-drug antibodies; C': complement; BAS: basophils; MC: mast cells

Figure 2 : Cytokine Release Syndrome: mechanisms

Different cells may be involved in the cytokine release syndrome (CRS), including target cells, macrophages and natural killer (NK) cells. Biological agents may induce direct activation or apoptosis of target cells. In addition, Complement (C') activation with MAC (Membrane Attack

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Complex) formation on the cell surface as well as Antibody-Dependent Cellular Cytotoxicity (ADCC) may result in cytokine release. Finally macrophage activation induced by biological agents via FcγR may be involved in CRS. A combination of these mechanisms may occur. Mϕ: macrophage

Figure 3: Diagnostic algorithm for hypersensitivity reactions to biologicals

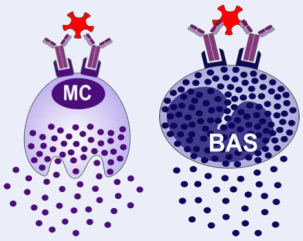
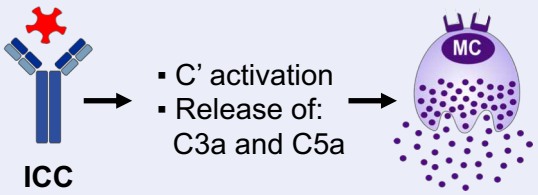
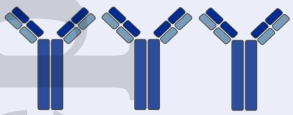
A clinical algorithm to be applied for the definition of mechanisms of hypersensitivity reactions to biologicals and for their management during the allergy workup is proposed.

ADA: anti-drug antibodies, SPT: skin prick test; IDT: intradermal test; Ab: antibody;

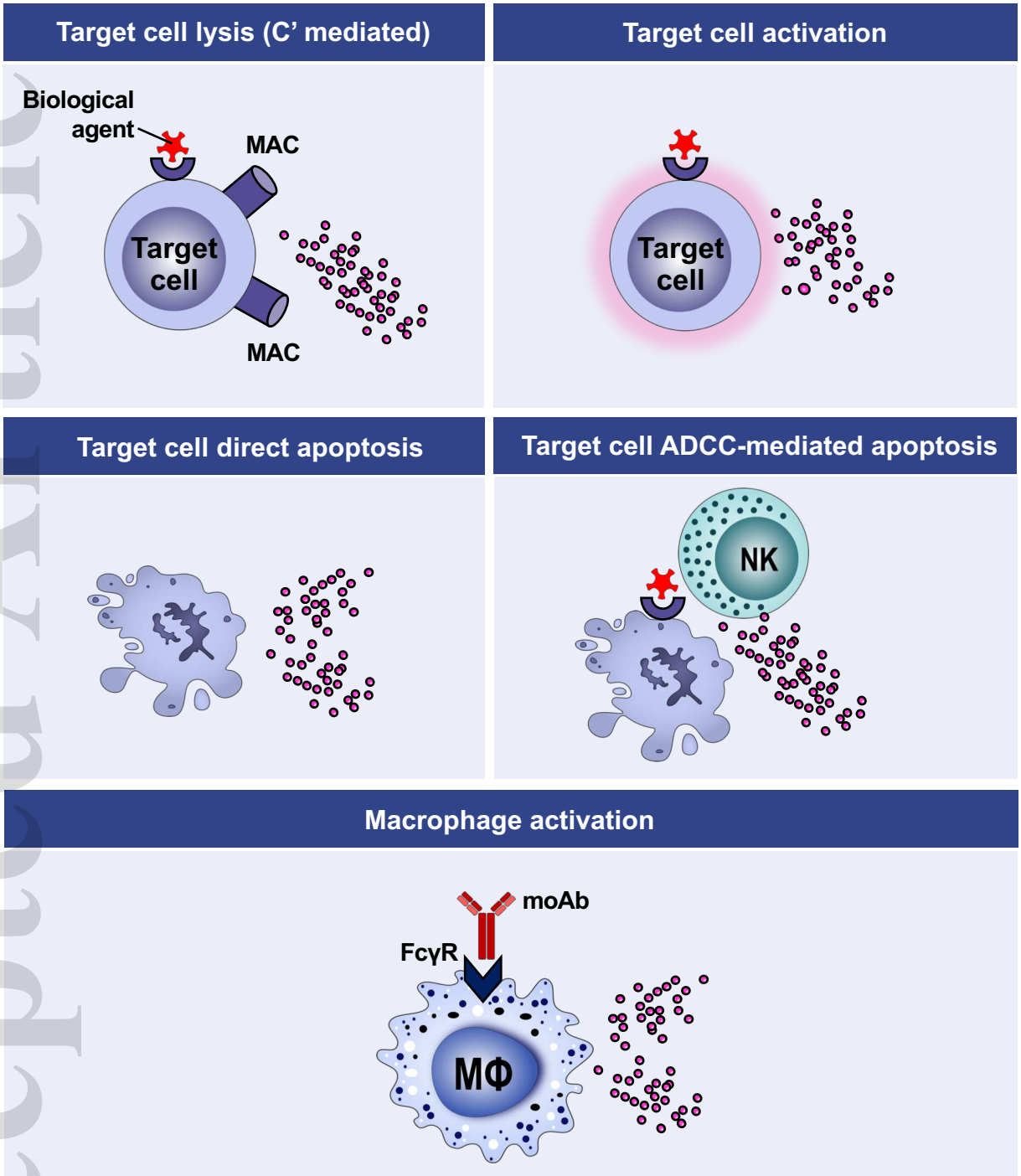
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Pathogenesis	Effector mechanism	Clinical features
IgE ADA 	IgE-mediated MC and BAS activation 	▪ Immediate reactions ▪ Urticaria /anaphylaxis
IgG ADA 		▪ Immediate reactions ▪ Urticaria /anaphylaxis
IgG ADA 	IgG-mediated BAS activation 	▪ Immediate reactions ▪ Urticaria /anaphylaxis

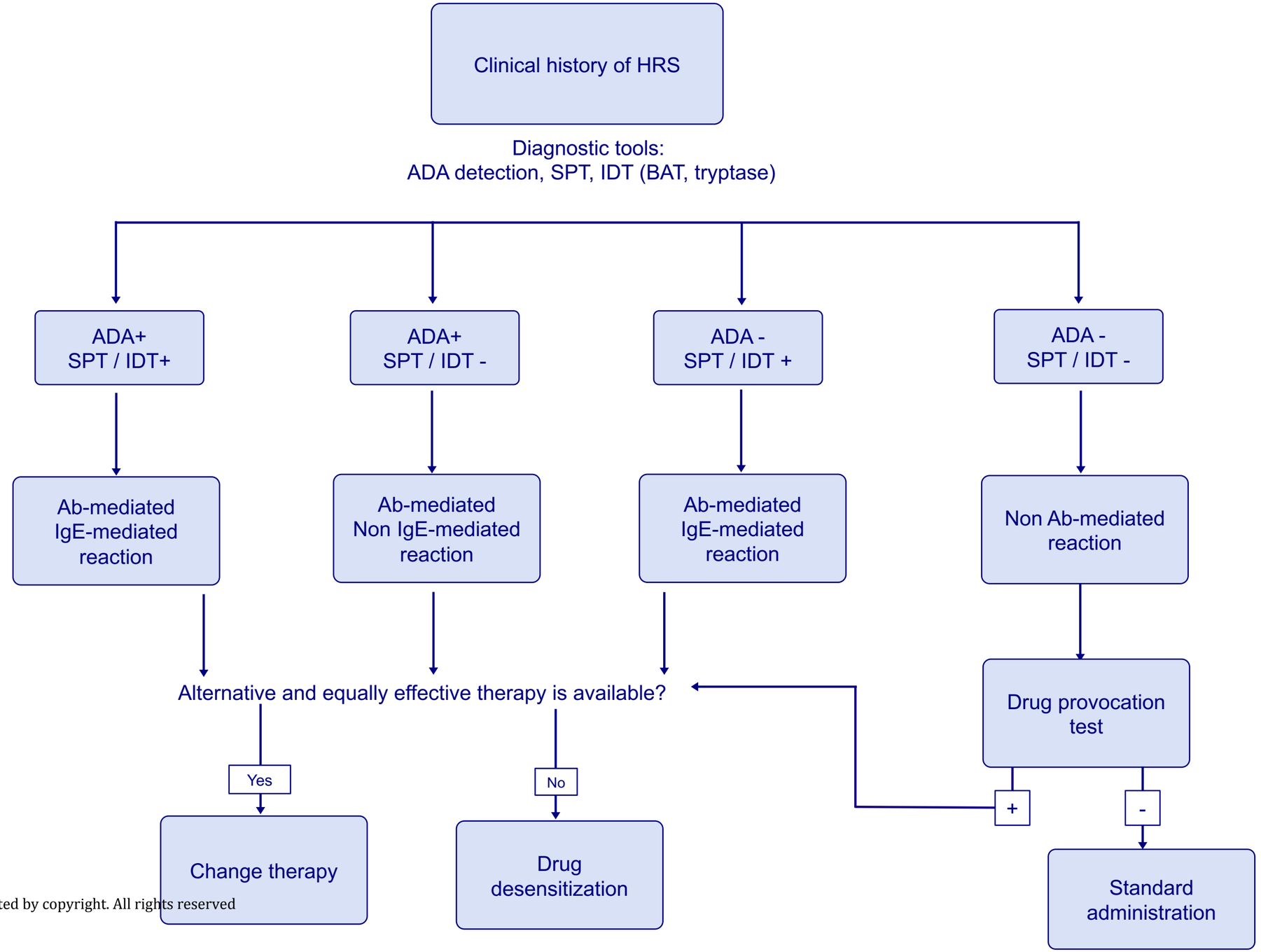
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Supplementary files

SIGN Quidelines

A: represent at least one meta-analysis, systematic review, or randomized controlled trial (RCT) rated as 1++, and directly applicable to the target population; or A body of evidence consisting principally of studies rated as 1+, directly applicable to the target population, and demonstrating overall consistency of results
B. A body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 1++ or 1+
C. A body of evidence including studies rated as 2+, directly applicable to the target population and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 2++
D. Evidence level 3 or 4; or Extrapolated evidence from studies rated as 2+

Table 1. Levels of evidence

1++ High quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+ Well conducted meta-analyses, systematic reviews, or RCTs with a low risk of bias
1− Meta-analyses, systematic reviews, or RCTs with a high risk of bias
2++ High quality systematic reviews of case-control or cohort studies
2+ High quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relationship is causal
2− Case-control or cohort studies with a high risk of confounding or bias and a significant risk that the relationship is not causal
3 Non-analytic studies eg., case reports and case series
4 Expert opinion

RCTs, randomized controlled trials.

Table-2. Classification of adverse side-effects of biologicals

Type α (overstimulation)		Reactions related to cytokine and cytokine released syndrome
Type β (hypersensitivity)		Reactions include both immediate and delayed hypersensitivity reactions
Type γ (cytokine or immune imbalance)	Immunodeficiency	Increased risk of tuberculosis (anti-TNF agents), hypogammaglobulinemia (rituximab)
	Autoimmunity	SLE or vasculitis (IFN-γ)
	Atopic disorders	Atopic dermatitis (anti-TNF agents)
Type δ (cross-reactivity)		Cross-reactions related to the expression of the same antigen on different tissue Acneiform lesions (cetuximab)
Type ε (nonimmunologic side-effects)		Neuropsychiatric side effects including confusion or depression (IFN-γ)

Table-3. Drug provocation testing with biologicals

DPTs	**Patients n = 30 (%)** **Ref no: (59)**	**Patients n = 95 (%),** **Ref no: (17)**
Negative	9/30 (30%)	47/95 (49%)
Positive	4/30 (13%)	13/95 (14%)
Not undergone	17/30 (57%)	35/95 (37%)
	SAFETY OF DPT (N = 4)	**SAFETY OF DPT** (n = 13)
Severity of the reaction (in DPT positive cases)	n = 4	n = 13 (%)
Grade 1	3/4	5/13 (38)
Grade 2	2/4	7/13 (54)
Grade 3	0/4	1/13 (8)

Table 4. A drug provocation test or drug challenge with rituximab as per the RCUH standard protocol based on the officially available product information by the European Medicines Agency (EMA) (a total dose of 500 mg of rituximab).

Total dose	500 mg		Solution concentration		Drug	
Solution A	500 ml		1 mg/ml		Rituximab	
Step	Solution	Rate (ml/h)	Administered volume (ml)	Time (min)	Administered dose (mg)	Cumulative dose infused (mg)
1	A	50	25	30	25	25
2	A	100	50	30	50	75
3	A	150	75	30	75	150
4	A	200	100	30	100	250
5	A	250	125	30	125	375
6	A	300	125	25	125	500

Total infusion time: 2 hours 55 minutes.

LEGEND:The recommended initial rate for the first infusion of rituximab, as per EMA product information leaflets, is 50 mg/h (beware this is not ml/h, and needs conversion); after the first 30 minutes, it can be escalated in 50 mg/h increments every 30 minutes, to a maximum of 400 mg/h. Premedication consisting of an anti-pyretic and an antihistaminic, e.g. paracetamol and diphenhydramine, should always be given before each administration. Always check product information leaflets and local protocols for specific administration recommendations in specific populations. Product information leaflets are freely available for all products at www.ema.europa.eu. Specifically, the product information leaflet for rituximab can be found at:www.ema.europa.eu/en/documents/product-information/mabthera-epar-product-information_en.pdf (accessed 7 August 2020).

Table 5. Example of intravenous Pembrolizumab desensitization protocol

Solution 1	300	cc of	0.017	mg/ml	5.000
Solution 2	250	cc of	0.200	mg/ml	50.000
Solution 3	250	cc of	1.984	mg/ml	496.096

Step	**Solution**	**Rate (cc/h)**	**Time (min)**	**Administered dose (mg)**	**Cumulative dose (mg)**
1	1	2	15	0.0083	0.0083
2	1	5	15	0.0208	0.0292
3	1	10	15	0.0417	0.0708
4	1	20	15	0.0833	0.1542
5	2	5	15	0.2500	0.4042
6	2	10	15	0.5000	0.9042
7	2	20	15	1.0000	1.9042
8	2	40	15	2.0000	3.9042
9	3	10	15	4.9610	8.8651
10	3	20	15	9.9219	18.7870
1	3	40	15	19.8438	38.6309
12	3	75	186	461.3691	500.0000

			--		
	Total time =		351	minutes	

In ***Table***, a standard 3 bags 12 step pembrolizumab DS protocol is presented. In this protocol, each bag has a 250 mL volume. As shown in the table, concentration of the first bag increases from 1/1000, 1/100 in 2nd bag eventually reaching the full dose in 3rd bag. The cumulative dose achieved at the end of step 12 is the target dose. At each step, sectioned into 15-minute intervals, the dose doubles, starting at .0069 mg and reaches a suboptimal dose of 10.95 mg before the last step, where the remaining dose is administered. As demonstrated in the table, the rate is also doubled, starting at 2.5 mL/hr, reaching a final rate of 80 mL/hr, with a total time of 5.66 hours.

Table 6. Example of an RCUH standard RDD protocol adaptation (total dose of 600 mg of rituximab diluted in a volume of 500 ml of saline solution 0.9%).

Total dose	600 mg		Solution concentration		Total dose in each solution (mg)		Drug
Solution A	500 ml		0.024 mg/ml		12		Rituximab
Solution B	500 ml		0.24 mg/ml		120		Rituximab
Solution C	500 ml		1.2 mg/ml		600		Rituximab
Step	Solution	Rate (ml/h)	Administered volume (ml)	Time (min)	Administered dose (mg)	Fold increase per step (mg/min)	Approximative cumulative dose infused (mg)
1	A	88	22	15	0.0	NA	0.0
2	A	100	25	15	0.6	NA	0.6
3	A	200	50	15	1.2	x2	1.8
4	A	400	100	15	2.4	x2	4.2
5	B	88	22	15	0.0	NA	4.2
6	B	100	25	15	6.0	x2.5	10.2
7	B	200	50	15	12.0	x2	22.2
8	B	400	100	15	24.0	x2	46.2
9	C	88	22	15	0.0	NA	46.2
10	C	150	37.5	15	45.0	x1.9	91.2
10'	C	212	424	120	508.8	x1.4	600.0
Total infusion time: 270 min (4.5 h)							
Premedication: Ideally, premedication should comply with the manufacturer's instructions, EMA product information, and institutional protocols for standard rituximab infusion. The RCUH does not recommend systematic additional premedication beyond product information. However, there are cases in which tailored premedication may be added at the physician's discretion.							
Total dose calculation and discarded volume: Not all the volume in solutions A, B, or C is infused. The protocol starts with a solution A containing a 1/50 dilution, then a solution B with a 1/5 dilution, and ends with a final solution C containing a full concentration of the culprit drug. The total dose to be infused during solution C is calculated by subtracting the cumulative dose administered in steps 1-8 from the total desired dose.							
Adjustments to the volume of the bags: The standard volume in the solution bags for the RCUH RDD protocol is 250 ml**.** In some cases, bag volume might need adjustment depending on manufacturer's instructions, product information, institutional protocols, or physician's preference (for example, in the current example, the bag volume is 500 ml).							
Additional bags for high risk patients: Whenever needed in high risk patients, additional solutions with lower concentrations than solution A may be added previous to solution A to ensure a more cautious starting dose, and, whenever possible, as determined on the basis of an endpoint titration according to local protocols (in case of positive skin tests).							
Flushing steps: Each solution uses an individual infusion line previously primed with 22 ml of the dilutor substance. Steps 1, 5 and 9 are considered "line flushing steps" (in which, in this case, 22 ml of the dilutor substance are administered). The line flushing steps volume might vary depending on local infusion sets.							
Adjustments to final infusion rate: Step 10 may be adapted to the desired final infusion rate according to the standard regimes indicated by the referred oncologist (additional steps may be added in order to reach higher infusion rates while maintaining a maximum dose increasing by 2-fold to 2.5-fold with each step).							
Avoiding human errors: Infusion pumps with automatic multi-step infusion options (Alaris® SE double channel) were used to avoid human errors associated with manually changing infusion rates every 15 minutes**.** We used 22 ml infusion systems for these pumps (Alaris® SE I pump smartsite infusion set), but if other infusion systems were used, the "line flushing steps" might need adjustment.							

RCUH, Ramon y Cajal University Hospital; RDD, Rapid Drug Desensitization; NA, not applicable.