

Successful desensitization to alemtuzumab, with flow cytometric analysis of peripheral blood cells

Costa Carvalho J¹, Macário MC², Batista S², Alen Coutinho I¹, Laranjeira P^{3,4,5,6}, Loureiro C¹, Paiva A^{3,4,5,7}, Todo Bom A^{1,8}

¹Allergy and Clinical Immunology Department, Centro Hospitalar e Universitário de Coimbra EPE, Coimbra, Portugal

²Neurology Department, Centro Hospitalar e Universitário de Coimbra EPE, Coimbra, Portugal

³Flow Cytometry Unit, Clinical Pathology Department, Centro Hospitalar e Universitário de Coimbra EPE, Coimbra, Portugal

⁴Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

⁵Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal

⁶Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal

⁷Instituto Politécnico de Coimbra, ESTESC-Coimbra Health School, Ciências Biomédicas Laboratoriais, Coimbra, Portugal

⁸Institute of Pathophysiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Corresponding author:

Jóni Costa Carvalho

Serviço de Imunoalergologia, Centro Hospitalar e Universitário de Coimbra, Praceta Prof. Mota Pinto, 3000-075 Coimbra

E-mail: joniccarrvalho@gmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0795

Key words: Alemtuzumab. Desensitization. Flow Cytometry. Hypersensitivity. Multiple Sclerosis.

Palabras clave: Alemtuzumab. Desensibilización. Citometría de flujo. Hipersensibilidad. Esclerosis múltiple.

Alemtuzumab is a monoclonal antibody (mAbs) anti-CD52 indicated for patients with highly active multiple sclerosis [1]. It leads to rapid cytolysis and depletion of the CD52 lymphocytes, resulting in the repopulation and immunomodulation of the cells involved in the immunopathogenesis of multiple sclerosis [2].

Infusion-related reactions to alemtuzumab are common, mostly due to cytokines released from lysed cells, consisting clinically of pyrexia, headache, nausea, pruritus and cutaneous rash [1,2]. Occasionally, severe reactions occur, such as non-IgE-mediated anaphylaxis (anaphylactoid reactions) [1,2]. These reactions mimic IgE-mediated reactions, which are rarely reported and present more frequently with severe manifestations [1,2]. Infusion-related reactions are managed primarily by slowing infusion rates and additional premedication, whereas IgE-mediated reactions require desensitization protocols for a safe readministration [1,3]. Desensitization by the incremental small doses subthreshold for anaphylaxis drives mast cells and basophils into inhibitory pathways, inducing a temporary state of tolerance [3]. Peak drug concentration may differ from standard protocols and so efficacy may be different from clinical trials [4]. Several studies confirmed the efficacy of mAbs delivered through desensitization protocols however, there are no studies for alemtuzumab [3,4]. As alemtuzumab depletes CD52 cells, it is possible to monitor cell depletion by flow cytometry of peripheral blood

cells. To the best of our knowledge, one case of alemtuzumab desensitization is described in the literature, in a patient with confirmed IgE-mediated reaction and a hematologic malignancy [1]. We present a case of a hypersensitivity reaction to alemtuzumab and a 12-step desensitisation protocol in a patient with multiple sclerosis.

A 31-year-old Caucasian woman diagnosed with relapsing-remitting multiple sclerosis initiated treatment with glatiramer acetate followed by natalizumab, which was discontinued due to cough, dyspnea and erythematous rash during the second course, and replaced by fingolimod. Upon increasing relapses, alemtuzumab was proposed at the age of 40-year-old.

Treatment schedule was an infusion of 12 mg/day (rate 25mL/h, 4 hours) for 5 consecutive days, followed by a second course 12 months later, for 3 consecutive days. The standard neurologist's protocol, adapted from European Medicines Agency recommendations, included premedication with methylprednisolone 1g, clemastine 2mg and paracetamol 1g each day, to prevent cytokine release syndrome (CRS). On day 2 of the baseline course, two hours after infusion, the patient developed generalized urticaria, facial angioedema, dyspnea, tachycardia and nausea. Symptoms resolved after interruption of the infusion and administration of clemastine, and a probable CRS was assumed. Treatment continued the next day, with rates reduced to half and addition of famotidine twice daily. A mild urticaria reappeared during the 3 remaining days, disappearing after completing the course.

The patient was referred to the Allergology consultation to assess the possibility of a second course with alemtuzumab. Diagnostic workup included skin testing using alemtuzumab (Lemtrada®; 10 mg/mL, prick 1:1, intradermal (ID) 1:100), polysorbate 80 (PS80) (containing eye drops, Refresh advanced®; 5 mg/mL, prick 1:1, ID 1:10), latex

extract (ALK®; 500 mcg/ml) and controls with histamine (Roxall®; 10 mg/ml) and saline [1,5]. Natalizumab was unavailable for testing, moreover it wasn't considered a future treatment option. Skin tests were performed following European Academy of Allergy and Clinical Immunology recommendations, without complications [6]. Tests were positive to alemtuzumab ID 1:100 dilution. Same dilutions were tested in healthy controls with negative results [1].

Risk assessment classified patient as high-risk, according to the Ramon y Cajal University Hospital severity classification [7]. Drug provocation testing wasn't considered and given the absence of alternatives with similar efficacy, it was agreed for the patient to receive alemtuzumab through a desensitisation protocol. Protocol was based on the standard flexible rapid drug desensitisation (RDD) designed by Brigham & Women's Hospital (BWH) [3]. A 12-step protocol was formulated, including 3 solutions with progressively higher concentrations, for a 2-2.5 fold escalation between doses (table EI) [3]. Premedication included the standard as per neurologist's protocol, without adding medication as suggested by several authors [7].

The RDD involved multidisciplinary work between allergologists, neurologists and pharmaceuticals. It was carried out in a dedicated space, equipped with resources for treating anaphylaxis, intensive care access, and a nurse and allergist at the bedside.

The temporary state of tolerance induced by desensitization seems to depend on drug's half-life [1,3]. Alemtuzumab's mean half-life is 4-5 days however, for the first administered 30mg is 2-32h [1]. There have been reported successful RDD with short half-life drugs, performed on the first day and no need for further RDD the following consecutive days of treatment [8]. However, due to lack of data for alemtuzumab, the

RDD was performed on the 3 consecutive days of treatment. Desensitization was successfully tolerated, without reactions.

The pharmacokinetics, the possible different peak drug concentration and potential alemtuzumab immunogenicity could compromise efficacy [9]. Alemtuzumab frequently generates anti-drug antibodies, which are associated with loss of efficacy and risk of hypersensitivity reactions [9]. To determine efficacy, a flow cytometry of peripheral blood cells was performed on each day of RDD and 3 months after treatment. The patterns of depletion in the pretended cells were similar to studies of alemtuzumab efficacy on multiple sclerosis (Figure I, Table E2 and E3) [2]. Moreover, after one year of follow-up, the patient had no relapses.

This case reports a successful RDD to alemtuzumab in a patient with a confirmed IgE-mediated reaction. The index reaction on day 2 was atypical. Anaphylaxis on first exposure to mAbs have been described, although patients were pre-sensitized (for example, reactions to cetuximab related with α -gal sensitization) [3,10]. We believe a pre-sensitization and 'boost' by first infusion might explain the reaction. A relation between natalizumab and alemtuzumab reaction, such as their common excipient PS80, could explain a pre-sensitization. However, skin tests were negative and sensitization to PS80 appears rare and of limited evidence [5]. Alternatively, the intrinsic anaphylactogenic characteristics of PS80 might be relevant [5]. Another hypothesis, is a mixed reaction, involving both IgE and non-IgE mechanisms, such as IgG anti-drug antibodies or cytokine release, with a further sensitization to alemtuzumab. Anti-drug antibodies are frequent in highly immunogenic mAbs and can be responsible for immune complex, complement activation or anaphylatoxins synthesis [3]. Nevertheless, RDD is indicated in these mixed reactions [3].

Alemtuzumab administered via RDD seemed to deplete the pretended cells. Thus, there is no reason to believe that RDD is affecting efficacy. Embedding the alemtuzumab pharmacokinetics while planning the RDD and involving capable multidisciplinary teams was most certainly useful to prevent unnecessary risks and to ensure the high standards of care during RDD.

Conflicts of interest

The authors declare that they have no funding, conflict of interest or competing interests. The data have been presented in poster form at the 40th annual meeting of the Portuguese Society of Allergy and Clinical Immunology.

Ethics approval

The study was in accordance with the ethical standards of the Ethics Committee of Centro Hospitalar e Universitário de Coimbra EPE and was performed in accordance with the ethical standards as laid down in Declaration of Helsinki. Informed consent was obtained from the patient for study and publication.

References

1. Gutiérrez-Fernández D, Saldaña-Valderas M, de la Varga-Martínez R, Foncubierta-Fernández A, Fernández-Anguita MJ, Fernández-Valle MDC, et al. Hypersensitivity to alemtuzumab. A safe and effective desensitization protocol: A case report. *J Oncol Pharm Pract.* 2019;25(4):1016-20.
2. Thomas K, Eisele J, Rodriguez-Leal FA, Hainke U, Ziemssen T. Acute effects of alemtuzumab infusion in patients with active relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflamm.* 2016; 29;3(3):e228.
3. Castells Guitart MC. Rapid drug desensitization for hypersensitivity reactions to chemotherapy and monoclonal antibodies in the 21st century. *J Investig Allergol Clin Immunol.* 2014;24(2):72-9.
4. Sloane D, Govindarajulu U, Harrow-Mortelliti J, Barry W, Hsu FI, Hong D. Safety, Costs, and Efficacy of Rapid Drug Desensitizations to Chemotherapy and Monoclonal Antibodies. *J Allergy Clin Immunol Pract.* 2016; 4(3), 497–504.
5. Banerji A, Wickner PG, Saff R, Stone CA Jr, Robinson LB, Long AA, et al. mRNA Vaccines to Prevent COVID-19 Disease and Reported Allergic Reactions: Current Evidence and Suggested Approach. *J Allergy Clin Immunol Pract.* 2021;9(4):1423-37.
6. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, et al. Skin test concentrations for systemically administered drugs - an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy.* 2013 Jun;68(6):702-12.
7. Martí-Garrido J, Vázquez-Revuelta P, Leonart-Bellfill R, Molina-Mata K, Muñoz-Sánchez C, Madrigal-Burgaleta R. Pilot Experience Using Drug Provocation Testing for the Study of Hypersensitivity to Chemotherapy and Biological Agents. *J Investig Allergol Clin Immunol.* 2021;31(2):166-8.

8. Divekar R, Butterfield J, Maddox D. Successful rapid desensitization to temozolomide: A case series. *J Allergy Clin Immunol Pract.* 2016;4(3):545-6.
9. Baker D, Ali L, Saxena G, Pryce G, Jones M, Schmierer K, et al. The Irony of Humanization: Alemtuzumab, the First, But One of the Most Immunogenic, Humanized Monoclonal Antibodies. *Front Immunol.* 2020;11:124.
10. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al: Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med.* 2008; 358: 1109-17.

Figure I. Identification, quantification and characterization of peripheral blood cells by flow cytometry, at baseline and 2 hours after the complete course (day 3), using desensitization protocols. Yellow events correspond to neutrophils, eosinophils are represented in orange, monocytes in green, T cells in blue, NK cells in dark blue, and B cells correspond to pink events.

