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Basophil Activation Test for the In Vitro Diagnosis of Tocilizumab Hypersensitivity

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To the Editor,

Tocilizumab is a humanised anti-human interleukin-6 receptor monoclonal antibody widely used to treat various inflammatory conditions, including rheumatoid arthritis, giant cell arteritis and cytokine release syndrome. Despite its efficacy, some patients develop hypersensitivity reactions (HSRs). HSRs to biological drugs are categorised into infusion reactions, cytokine release reactions, type I reactions (IgE-mediated), mixed reactions that are usually acute and immediate and type III and type IV reactions, which are characteristically delayed [1]. Most HSRs to tocilizumab present with mild cutaneous symptoms, though anaphylaxis has also been reported [1]. The diagnosis of tocilizumab hypersensitivity often poses a clinical challenge since the aetiology of the disease can be diverse, and symptoms can overlap with disease activity. Skin tests might be a simple and sensitive tool for diagnosing HSR to tocilizumab; however, their results may not always be conclusive, and re-exposure to the drug can be risky [2]. In vitro tests for identifying the culprit drug in immediate HSRs rely on an IgE-mediated mechanism [1]. Detection of specific IgE to tocilizumab is rare in patients with tocilizumab HSR, and commercially available testing options are limited [3]. The basophil activation test (BAT) is an in vitro cellular diagnostic test that could represent a useful tool to detect IgE-mediated reactions. The main advantage of BAT compared to specific IgE measurements is that it is a functional test that enables assessment of the actual allergenic activity of IgE antibodies and not only allergen sensitisation. BAT has proved to be helpful in diagnosing anaphylaxis to several biological drugs, such as rituximab, cetuximab and pertuzumab [4–6].

Our study aimed to assess the utility of BAT for diagnosing HSR to tocilizumab. Between 2019 and 2024, three patients with rheumatoid arthritis (RA) and suspected HSR to tocilizumab were examined at the University Clinic for Pulmonary and Allergic Diseases Golnik. Patients were managed, and samples were collected following standard diagnostic procedures, so no additional ethical approval was required for this study. A comprehensive clinical evaluation, skin prick tests and intradermal skin tests were performed on all patients, followed by a drug provocation test in patients with negative skin tests [2]. The severity of HSR was graded by the modified Brown classification [7]. Based on the results of clinical evaluation, skin tests and drug provocation test, patients were diagnosed as tocilizumab-allergic (TCZ-A). The control group included four patients (3 with RA and 1 with Behcet disease) who had been exposed to tocilizumab but were considered non-allergic (TCZ-C). Additionally, five healthy controls (HC) with no known allergic diseases were included for comparison. BAT was performed in all studied subjects as previously described [5]. Briefly, heparinised blood samples were incubated with tocilizumab at concentrations ranging from 0.1 to 500 μg/mL. The stimulation buffer alone served as an unstimulated control, and anti-FcεRI mAb served as an IgE-mediated positive control. Basophils were identified as CD123-positive and HLA-DR-negative cells, while CD63 was used as a basophil activation marker. A cut-off of 15% CD63+ basophils was considered a positive BAT result [4].

In TCZ-A patients, all three experienced grade 1 reactions, clinically manifesting as urticaria and generalised pruritus. The median age of TCZ-A was 62 years (range, 35–71), and all 3 were women. The median age of TCZ-C was 58 years (range, 40–62), and all 4 were women. Skin prick tests (SPT) with tocilizumab at 20 mg/mL were negative in all TCZ-A patients and TCZ-C. However, intradermal tests (IDT) were positive in 2 out of 3 TCZ-A patients at the highest concentration of 2 mg/mL, but were negative in all 4 TCZ-C patients. Basal serum

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Summary

- Basophil activation test (BAT) is a useful approach for diagnosing hypersensitivity reactions to tocilizumab.
- BAT offers insight into IgE-allergenic activity beyond sensitisation, complementing skin tests and IgE measurements.

tryptase was normal in both TCZ-A (median 5.2 μg/L, range 4.5–5.4) and in TCZ-C (median 4.8 μg/L, range 1.1–5.0) patients. In the HC group, the median age was 30 years (range, 20–74), with four out of five participants being women. The CD63 basophil response after stimulation with 500 μg/mL tocilizumab was 86.5%, 17.4% and 1.3%, respectively (median 17.4%, range 1.3–86.5) in TCZ-A versus 1.7%, 1.7%, 1.4% and 0.9% respectively (median 1.6% range 0.9–1.7) in TCZ-C and 2.0%, 1.7%, 1.5%, 0.7% and 0.0%, respectively (median 1.5%, range 0.0–2.0) in HC. The median CD63 basophil response after stimulation with 100 μg/mL tocilizumab was 28.6%, 1.1% and 1.6%, respectively (median 1.6%, range 1.1–28.6) in TCZ-A versus 2.0%, 0.5%, 2.3% and 0.9%, respectively (median 1.5%, range 0.5–2.3) in TCZ-C and 2.0%, 1.7%, 1.5%, 0.2% and 0.3%, respectively (median 1.5%, range 0.2–2.0) in HC (Figure 1). Additionally, since excipients, such as polysorbate 80 (PS 80), have been implicated in immediate HSRs

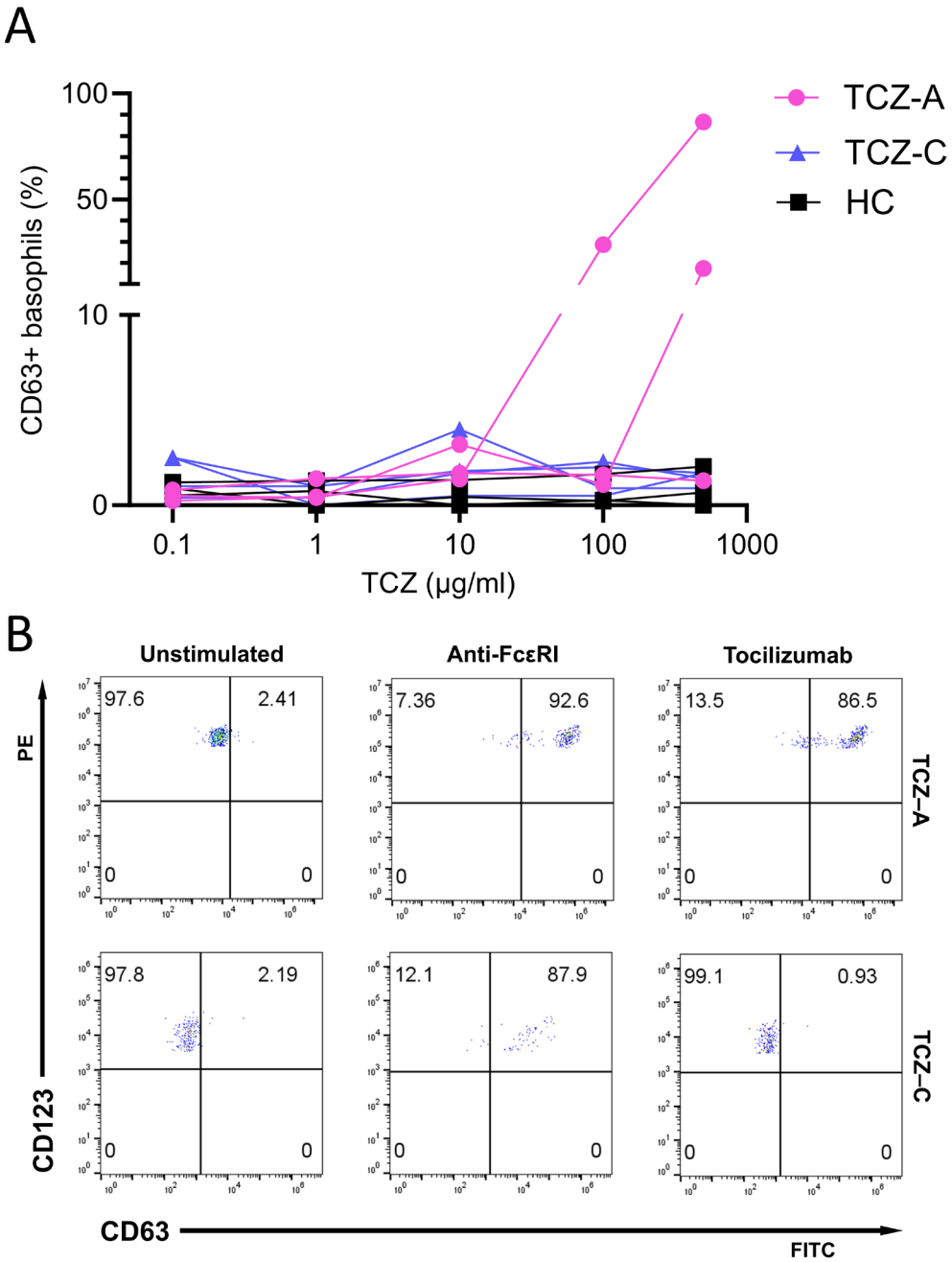

FIGURE 1 | (A) A basophil activation test (BAT) in response to stimulation with tocilizumab in tocilizumab-allergic patients (TCZ-A), TCZ-exposed non-allergic controls (TCZ-C) and in healthy controls (HC). (B) Representative flow cytometric plots of basophil activation in TCZ-A patients with positive BAT results and in TCZ-C. For controls, stimulation buffer alone (unstimulated control) and anti-FcεRI mAbs (positive control) were used.

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[8], we performed BAT using PS 80 at concentrations ranging from 0.0001 to 1.0 mg/mL in two TCZ-A patients and in three HC. All results were negative, with CD63 basophil responses after PS 80 stimulation as follows: at 1 mg/mL, TCZ-A showed 4.8% and 1.0%, while HC showed 4.3%, 4.9% and 3.8%; at 0.1 mg/mL, TCZ-A had 3.6% and 3.0%, and HC had 3.5%, 1.5% and 0.0%.

Recent data show that HSR to monoclonal antibodies are the second most commonly reported cause of drug-associated anaphylaxis, following antibiotics [9]. Our findings suggest that BAT may be a valuable tool for diagnosing HSR to tocilizumab. BAT was positive in two TCZ-A patients with positive skin test results with tocilizumab. However, it was negative in one TCZ-A patient with a negative skin test but a positive drug provocation test, suggesting an infusion reaction rather than an IgE-mediated mechanism. Given the high concentrations of tocilizumab required for a positive BAT response in our study, we considered an excipient-related reaction. However, BAT with PS 80, even at high concentrations, was negative. Although other formulation components cannot be fully excluded, our findings suggest that the anti-IL-6 antibody itself may be responsible for the positive BAT result to tocilizumab.

To our knowledge, this is the first study assessing BAT's effectiveness in diagnosing tocilizumab-related HSR [4]. Our results suggest that BAT could be a safe and complementary in vitro tool, providing quantitative data on IgE-allergenic activity rather than just sensitisation. Additionally, BAT may play an important role in assessing drug desensitisation risk assessment, thus contributing to the personalised management of patients with HSR to monoclonal antibodies.

Author Contributions

Ana Koren, Peter Kopač and Peter Korošec designed the study. Peter Kopač treated patients, collected patient samples and provided clinical data. Luka Dejanović performed the experiments. Ana Koren and Luka Dejanović performed the analysis. Ana Koren wrote the manuscript. Ana Koren, Luka Dejanović, Peter Korošec and Peter Kopač critically reviewed the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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