

Optimisation of Omalizumab Dosage in Patients with Severe Persistent Allergic Asthma Using *recovery*ELISA

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Abstract

Background The objective of this study was to investigate the suitability of *recovery*ELISA as a method to monitor treatment with therapeutic antibodies using the example of omalizumab.

Methods The *recovery*ELISA is a newly developed immunoassay technology that measures three parameters in one test: the free level of antigen, the level of therapeutic antibody and the specific dose-response interaction which represents the actual activity of the drug. A retrospective and observational analysis was performed on 197 serum samples from 17 patients (13 ± 4 years of age) with severe persistent allergic asthma who received add-on treatment with omalizumab.

Results The mean omalizumab serum level during antibody therapy was 59 ± 45 µg/mL; the kit's upper detection limit of 140 µg/mL was exceeded in 27 samples. Antibody concentrations between 50 and 140 µg/mL were found in 64 samples. Independent of the omalizumab dosage, nearly all measurements were in a range of absolute saturation as regards the IgE binding rate. Almost complete binding of IgE with a recovery of added labelled IgE of <1 % was reached within a maximum of 11 days.

Conclusions The biochemical activity of therapeutic antibodies can be examined by *recovery*ELISA and their residual activity can be determined. Thus, further individualisation of therapy with biologics is possible using this

test which seems to be suitable to diminish side effects and reduce costs.

1 Introduction

Therapeutic antibodies are now a very effective and specific treatment in various diseases and are usually low in side effects. For instance, omalizumab, an antibody against immunoglobulin E (IgE), and also antibodies against tumour necrosis factor- α (TNF α), such as adalimumab and etanercept, have been introduced clinically and are widely used.

IgE-mediated diseases such as severe allergic asthma can be almost completely cured by treatment with the anti-IgE antibody omalizumab [1–3]. Omalizumab was recently the first biopharmaceutical to be approved for the treatment of chronic idiopathic urticaria after it was shown to markedly alleviate the clinical symptoms [4].

Various TNF α inhibitors have proven effective in rheumatological conditions where the pathophysiological role of TNF α has been confirmed. Major consequences of the rheumatic disorder can thus be treated effectively. It is striking that these antibodies are increasingly being used successfully off-label for pathophysiologically related conditions such as sarcoidosis, Crohn's disease and psoriasis, but usually without individual dose adjustment [5].

Other targeted uses of therapeutic antibodies are in malignant disease, where specific tumour factors important in pathogenesis are inhibited. These are usually small molecule conjugates which involve binding therapeutic antibodies or proteins to small molecules. The accuracy of therapeutic antibodies is thereby combined with the efficacy of low molecular weight substances, and side effects that can otherwise occur with classic low molecular weight

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drugs are reduced. It is currently possible to treat breast cancer with confirmed overexpression of Her2 (human epidermal growth factor receptor2) with Herceptin® (trastuzumab, MAb against metastatic breast cancer) and other tumours with an angiogenesis inhibitor like bevacizumab if it is administered in the tumour vascularisation phase [6, 7].

Despite these advances, the aims of drug development to date have not included monitoring these innovative treatment options by the achieved serum or activity levels. The dosage is uniformly based on previous measurement of the baseline situation so the dosage of omalizumab, for example, is obtained from a dosage table that considers the total IgE in the serum and the patient's weight before the start of treatment [8, 9]. IgE production is diminished on the medication [10, 11]. Nevertheless, in the omalizumab manufacturer's information for professionals it is stated that total IgE serum levels may increase during therapy and that a further IgE measurement after discontinuation of therapy is useful only 1 year thereafter [8]. The aim of omalizumab treatment is to reduce free IgE in serum to below a threshold of 50 ng/mL, when significant effects in the allergic reaction cascade can probably no longer be expected [12]. The largely schematic administration leaves little room for individualised therapy. On the contrary, the dosage of such highly effective medication is almost 'blind'. The initial dosage schema included only patients with serum IgE levels below 1500 IU/mL and maximal body weight of 50 kg, and only for dosage intervals of 4 weeks. The drug is still not licensed for use in patients with high body weight and high IgE levels. Correlations of serum levels and activity are not given [13].

These are usually expensive therapies with biopharmaceutical agents that are given long-term in high doses.

Our concern should be to integrate antibody therapies in individualised treatment plans as regards dosage, duration of use and evidence of effectiveness. The possibility of monitoring therapeutic antibodies with measurement of the pharmacologically effective concentration by *recovery*ELISA is presented using the example of omalizumab.

2 Method

The assay is based on the modified principle of a sandwich ELISA. Interactions between the therapeutic antibody (TA) omalizumab and free IgE present in the patient's serum and their effects on the test system were identified in traditional test systems. These interactions of substrates and test components led to incorrect measurements and ultimately to the manufacturer's recommendation not to measure IgE during antibody therapy and for up to one year thereafter [8].

The *recovery*ELISA is a combination of two assays, an IgE and an omalizumab assay. The main feature of the test is that the TA is present in the sample as a scavenger with unknown concentration. Standard addition of known levels of antigen is reduced by the scavenger antibody in the sample (Fig. 1). A newly developed calibration system includes both parameters in a two-dimensional calibration curve. Standard curves were developed from standardised serum samples to which defined quantities of IgE and omalizumab were added. Reference functions were derived for samples with and without omalizumab. An antibody that binds to the same epitope as the TA or the labelled therapeutic antibody itself acts as signal antibody. The labelled antibody and the unlabelled TA compete in the test for the same binding epitope. If a high level of active TA is present in the patient's serum, the number of measured signal antibodies will be reduced. If only little active TA is present in the sample, binding of the labelled signal antibodies is greater. Thus, there is a correlation between the systematic reduction of recovery of the signal antibody and the concentration of active TA. By means of mathematical functions and precise knowledge of the final concentration of the signal antibody, the concentration of TA (i.e. omalizumab) and free IgE can be calculated. A logarithmic function was used to describe the relationship between the concentration of TA and the binding (i.e. inactivation) of the antigen [14]. Multiple measurements of at least three of each sample with different standard additions ensure the reliability of the test.

It was shown that at a certain antibody concentration, recovery reaches close to zero. It can be read from the curve that above an antibody concentration of about 4–6 µg/mL no further increase in effectivity can be expected (Fig. 2). The measurements with this *recovery*ELISA now include the serum concentration of the antibody, the concentration of free IgE and the recovery of an added quantity of IgE in a percentage [15].

It must be mentioned that the measurements can be performed only in diluted serum as otherwise there is saturation of binding behaviour in the original serum. Because of the different binding kinetics of the two mixed kits for IgE and the TA, together with the very high concentration of TA often present in serum samples, the illustrated recovery curves suggest a 1:20 dilution of the serum. This dilution factor must be kept in mind while reading the recovery curve.

To investigate whether a similar relationship can be demonstrated in real samples, serum from 17 patients aged 13 ± 4 years (11 female and 6 male) with severe allergic asthma who were treated with omalizumab in a dosage of 422 ± 150 mg/month (range 225–750 mg) over a 3-year period were analysed. The inclusion criterion was evidence of sensitisation to a perennial aeroallergen and clinical

Fig. 1 Schematic description of the *recovery*ELISA. In contrast with the traditional ELISA (upper half), the sample contains the therapeutic antibody (lower half) as a second analyte. The recovery of labelled analyte I is reduced by the competing reaction between signal antibody and therapeutic antibody. *HRP* horseradish peroxidase

Principle of Sandwich-ELISA



Principle of *recovery*ELISA

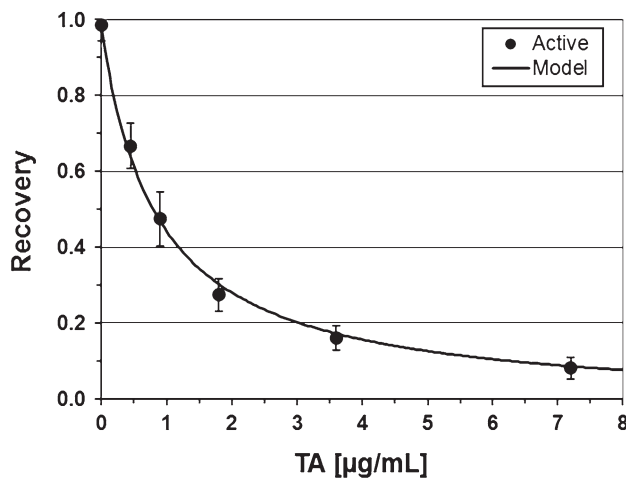
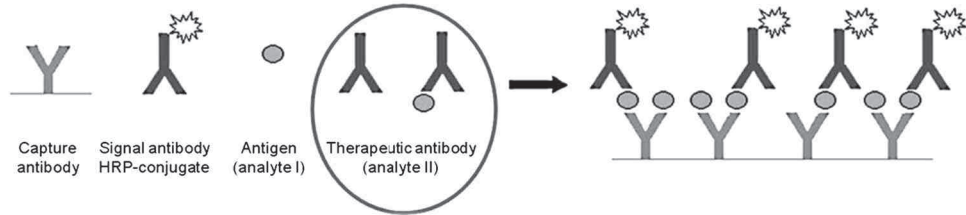


Fig. 2 Recovery curve of the immunoassay for omalizumab [11]. *Active* mean with standard deviation, *Model* curve according to equation, *TA* therapeutic antibodies

binding rate for added IgE were determined. Fifty-seven of these measured samples were further classified into eight groups according to the administered omalizumab dose and the time elapsed between the injection and the blood collection. A precondition was that constant doses had been given at fixed intervals in the preceding treatment cycles.

The following groups were established: no therapy ($n = 9$), 450 mg/2 weeks ($n = 7$), 450 mg/3 weeks ($n = 4$), 300 mg/2 weeks ($n = 10$), 300 mg/4 weeks ($n = 11$), 300 mg/5 weeks ($n = 7$), 150 mg/4 weeks ($n = 5$) and 150 mg/5 weeks ($n = 4$). Mean values of the obtained recovery values per group were calculated for the recovery diagram.

3 Results

The measured mean omalizumab concentrations were far above the antibody concentrations of up to 8 µg/mL shown in the recovery curve, independent of the administered dose. The mean omalizumab serum level in the measured samples during antibody therapy was 59 ± 45 µg/mL; the kit's upper detection limit of 140 µg/mL was exceeded in 27 samples. Antibody concentrations between 50 and 140 µg/mL were found in 64 samples.

When the omalizumab serum concentrations of the divided groups were correlated with the recovery of the added IgE, the results revealed an almost identical course compared to the calculated model (Fig. 3).

No further decline in recovery was observed with a mean antigen concentration of more than 10 µg/mL. Measurements with antibody concentrations below 10 µg/mL constituted the steeply decreasing part of the recovery curve.

disease refractory to treatment despite high-dose inhaled steroid therapy in combination with long-acting β_2 -agonists or leukotriene receptor antagonist and/or systemic steroid therapy in the previous 3 months. All-day symptoms, frequent asthma exacerbations and hospitalisations, repeated use of relieving medication and a marked impairment of quality of life were assessed as treatment refractory. The dosage and dosing frequency are determined on the basis of the patient's total IgE in serum and body weight before the start of the treatment. A total IgE outside the dosage table for omalizumab did not lead to exclusion.

A total of 197 serum samples were obtained from the patients prior to an omalizumab injection. Using the *recovery*ELISA (RIO, BioTeZ GmbH, Berlin, Germany), free IgE, omalizumab serum concentrations and the current

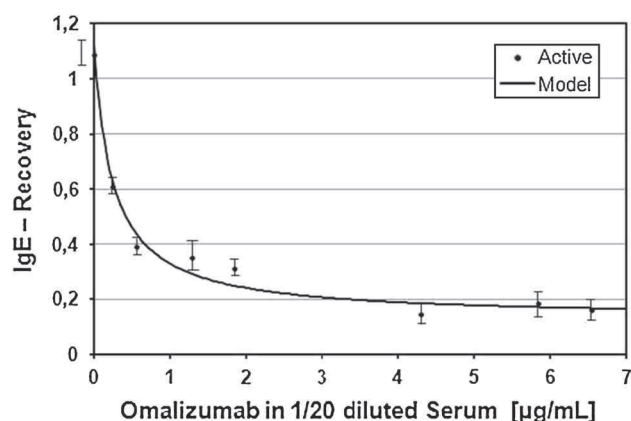


Fig. 3 Illustration of the relationship between omalizumab concentration in serum and recovery of added immunoglobulin E (IgE) in eight real samples at a dilution of 1:20

The almost complete binding of the IgE with a recovery of added labelled IgE of <1 % was reached within a maximum of 11 days, independent of the omalizumab dose applied.

Table 1 shows the means obtained for free IgE, omalizumab and the recovery, stating the extremes in each case.

On average, there was no significant trend in the serum concentration of free IgE 11 days after administration. The measured free IgE levels varied around a mean of about 25 IU/mL with maxima of up to 90 IU/mL and minima below the defined measurement range.

The results of the omalizumab concentration in the observation period did not reveal any decline over time and illustration of kinetics for calculation of the biological half-life was not possible.

4 Discussion

With the increasing importance of biologics in medicine, it will become all the more important to develop adequate methods for monitoring treatment.

A direct and/or indirect interaction appears to take place between administration of omalizumab and IgE production. Numerous studies confirmed that omalizumab not only reduces free IgE which activates the allergic cascade, but Fc ϵ RI receptors on basophils and dendritic cells are also down-regulated. Both processes lead to inhibition of IgE-mediated release of proinflammatory mediators [16, 17].

Using clinical samples, it was shown that the efficacy of omalizumab can be measured in the sense of the expected IgE binding. When the treatment recommendations were followed, serum levels far above the range included in the recovery curve were found with the great majority of the samples. The results suggest that patients are treated with excessively high doses of omalizumab during long-term therapy as has already been indicated in previous reports [10, 11, 18]. Katz et al. [19] showed that the clinical efficacy of omalizumab treatment is maintained when the dosing intervals are extended. Patients on long-term therapy tolerated a trial of omission of omalizumab without problems [20]. In a study by Nopp et al. [21, 22], most patients remained clinically stable or demonstrated improvement up to 3 years after discontinuation of omalizumab therapy. There are also reports on patients for whom the omalizumab dose had been reduced by half who still maintained normal total IgE levels and whose health status had not deteriorated even 1 year later [18]. Several years of treatment with omalizumab, based exclusively on body weight before the start of treatment and total IgE levels, seems to be an oversimplified approach. In the long term it does not do justice to the complex mechanism of action of omalizumab.

The viewpoint of Korn et al. [23] that monitoring of the free IgE concentration and also the total IgE in serum cannot be currently recommended for assessing the response to therapy or for monitoring omalizumab therapy was not confirmed. In the cited article, the measurements using *recovery*ELISA were not performed with the

Table 1 Mean concentrations of omalizumab, free IgE and the recovery of added labelled IgE

Dose [mg]	Days after dose	<i>n</i>	Omalizumab in serum [µg/mL] ^a	free IgE [IU/mL] ^a	Recovery [%]
0	0	9	0.0 ± 2.5 (0–3)	873.7 ± 827.0 (153–1850)	100.0
300	12	10	85.9 ± 80.3 (29–306)	23.6 ± 22.8 (6–86)	1.0 ± 86.0
450	14	7	116.7 ± 80.2 (54–275)	26.1 ± 5.8 (19–33)	1.2 ± 50.0
450	21	4	130.8 ± 96.0 (45–248)	31.8 ± 15.6 (22–55)	1.1 ± 77.0
150	27	5	11.2 ± 2.6 (8–15)	12.5 ± 4.2 (6–17)	3.3 ± 94.0
300	27	11	25.8 ± 11.9 (6–43)	45.9 ± 33.3 (6–90)	3.0 ± 1.7
150	33	4	5.0 ± 1.9 (1–48)	23.3 ± 25.1 (1–48)	7.9 ± 2.4
300	35	7	36.7 ± 10.5 (24–50)	9.9 ± 13.1 (0–33)	2.7 ± 76.0

Values are given as mean ± SD; range in parentheses

^a The stated results are rounded up from the measurement in diluted serum

IgE immunoglobulin E

necessary serum dilution but with the original serum and subsequent saturation of binding behaviour.

In our study we found a mean omalizumab serum level of about 60 µg/mL; in 27 samples, however, the level exceeded the upper detection limit of the kit of 140 µg/mL. From our recovery curves and considering the biological half-life of omalizumab of 26 days [13], it must be concluded that any further increase in the serum concentration of omalizumab above 10 µg/mL would not lead to a greater effect. Taking this as a basis for calculation of remaining activity, we can estimate that a serum level of about 100 µg/mL may result in sufficient activity of the drug for about 3 months.

There are still no established laboratory methods of confirming the activity of other therapeutic antibodies that have already been clinically introduced. A concentration–efficacy relationship was recorded in the approval documentation only for adalimumab [24]. In these studies, the adalimumab concentration in the serum was correlated with the American College of Rheumatology ACR20 rheumatoid arthritis score in 15 patients, revealing the maximum effect to be reached at about 8–10 µg/mL adalimumab. This concentration corresponds to the antibody level that should be achieved during prolonged therapy [5]. However, no pharmacological data are available as to how this curve correlates with binding of the released TNFα and whether there is a correlation between TNFα binding and the described side effects [25].

The benefit of these expensive therapies also appears to be confirmed from the medicosocial aspect. It was shown that TNFα antibodies, despite their high treatment costs, provide savings compared with traditional treatment regimens with regard to other care expenditure for patients, disability, other therapies and unfitness for work [26]. The overall healthcare costs due to TNFα-mediated diseases can therefore be ultimately diminished even by extremely expensive antibody therapies.

The presence of the therapeutic antibody in the sample interferes with conventional immunoassays and the antigen against which the antibody is directed is measured incorrectly. By contrast, with the *recovery*ELISA, the serum concentrations of therapeutic antibodies, the freely available antigen and the antibody's currently available binding capacity are measured simultaneously. This test is also suitable for identifying residual antibody activity and a possible renewed rise in the antigen after cessation of therapy. This allows safety monitoring and promotion of individualised dose optimisation. Overdosing is avoided, risks are diminished and costs are reduced. The *recovery*ELISA can fill a diagnostic gap in treatments with monoclonal antibodies. The possibility of treatment monitoring presented here can markedly improve personalised medicine in the area of antibody therapy.

It would be welcome if new therapeutic antibodies were immediately complemented with a tool that enables objective pharmacodynamic and pharmacokinetic monitoring of efficacy as well as the rather more subjective monitoring of efficacy, especially as the cause for many treatment failures or side effects has so far remained unexplained.

From the economic point of view, the development of adequate treatment monitoring to optimise the therapy is urgently needed for sustainable use of resources in order to ensure long-term success of treatment in all age groups. Careful dosing of such medication reduces the costs and might facilitate an extension of the range of indications [14].

5 Conclusions

Therapeutic antibodies have revolutionised medical treatment options in recent years. However, these are often very costly treatments involving high dosages and often long-term medication. To promote individualised dose optimisation, development of methods for routine treatment monitoring is urgently required. Using *recovery*ELISA as a method to optimise omalizumab posology in patients with severe persistent allergic asthma has been demonstrated in our study. The *recovery*ELISA has the potential to greatly improve personalised medicine in the area of therapeutic antibodies.

Acknowledgments and Disclosures J-O. Steiß has received lecture fees from Novartis. G. Becher acted as an external independent reviewer for approval of immunoassays for IVD from BioTez GmbH, Berlin, Germany. No other potential conflict of interest relevant to this article was reported. No funding was provided for this investigation and this article.

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