

## Original article

## The importance of IgE antibody levels in anti-IgE treatment

**Background:** Dosages of anti-immunoglobulin (Ig)E in treatment of allergic asthma is based on total IgE body pool assuming that IgE antibodies responsible for the disease are evenly distributed among patients. This assumption was evaluated.

**Methods:** Median and quartile concentrations of IgE and IgE antibodies to cat and mite in 6461 sera submitted to an allergy laboratory during 2003–2005 were calculated and expressed in groups of different IgE levels.

**Results:** Of 3872 (60%) samples from adults with a serum IgE level of 30–700 kU/l, 22.2% had IgE antibodies ( $\geq 0.35$  kU<sub>A</sub>/l) to mite, 36.0% to cat and 8.1% to both. The relative concentration of IgE antibody of IgE decreased with increasing IgE, indicating a more specific response in patients with slightly elevated serum IgE. At a hypothetical serum IgE level of 10 kU/l, the threshold recommended for anti-IgE treatment, 25% of the originally mite- and/or cat-positive population in the < 75 kU/l IgE group still would have detectable IgE antibodies.

**Conclusions:** Sera from patients sensitized to mite and cat with moderate serum IgE levels have a high proportion of IgE antibodies; in the 30–74 kU/l group, as much as 10% of the IgE could be specific to one allergen. An increase of the anti-IgE dosage given to such patients should be considered, especially because IgE antibodies with different, relevant specificities have an additive effect in triggering inflammatory cells.

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Treatment of immunoglobulin (Ig)E-mediated, persistent, allergic asthma with anti-IgE, e.g. omalizumab (Xolair, Novartis AG, Basel, Switzerland) is a new therapeutic approach (1). The treatment can be prescribed to adults with a serum level of IgE, often referred to as 'total IgE', between 30 and 700 kU/l. However, no recommendations are given to the concentrations of IgE antibodies to the offending allergens, although an allergic inflammation is mediated by allergen-specific IgE antibody molecules (2), sometimes called 'specific IgE'.

The majority of asthma is allergic in nature (3). Not only mast cells are involved, but also basophils (4) and at least in the latter case the relative concentration of IgE antibody to IgE seems to be most relevant for sensitization (5).

In a recent report (6, 7) evaluating the efficacy omalizumab was found to be less effective in patients with low IgE levels, defined as  $\leq 75$  kU/l. In order to analyse the distribution of IgE and IgE antibody in a representative allergic population a survey of IgE and IgE antibodies to cat and house dust mite allergens in serum samples submitted to an allergy laboratory was performed. A significant percentage of patients with normal IgE levels, i.e.  $\leq 75$  kU/l, were found to have high concentrations of IgE antibodies in serum, a finding that

deserves reconsideration of responders and dosing of anti-IgE.

## Material and methods

### Data

Results from the Allergy Laboratory, Karolinska University Hospital, Stockholm of samples analysed for IgE and IgE antibodies to cat dander and house dust mite during 2003–2005, together with the patients' year of birth, were collected from existing files.

### IgE and IgE antibodies

The concentration in serum of IgE (kU/l) was determined by ImmunoCAP<sup>TM</sup> Total IgE (Phadia AB, Uppsala, Sweden) and that of IgE antibodies (kU<sub>A</sub>/l) to cat dander allergen (e1) and house dust mite *Dermatophagoides pteronyssinus* (d1) by ImmunoCAP<sup>TM</sup>-Specific IgE (Phadia AB) according to the manufacturer's instructions. The relative IgE antibody concentration was expressed for each sample as absolute value in percentage of 'total-IgE'.

### Statistical analyses

The distribution measures (median, quartiles and percentiles) were calculated for IgE, IgE antibody and the relative concentrations of IgE antibody with STATISTICA 6.0.

## Results

During the 3-year period 3872 of 6461 (60%) samples were found to have a serum IgE level between 30 and 700 kU/l and come from a patient 12 years or older, i.e. would have fulfilled the criteria for receiving omalizumab, results presented refer to this group. Of those, 860 (22.2%) had significant concentrations ( $\geq 0.35$  kU<sub>A</sub>/l) of IgE antibodies to mite, 1393 (36%) to cat and 313 (8.1%) to both.

When the median and low/high, i.e. 25%/75%, quartiles for IgE and IgE antibodies to mite and cat (Table 1) were calculated for the patients grouped according to IgE levels, the IgE antibody concentrations were found to be stable or increase slightly with increasing IgE levels. In contrast, the relative IgE antibody concentration, calculated as kU<sub>A</sub>/l of IgE antibody in percentage of IgE in kU/l, decreased with increasing IgE levels (Table 2 and Fig. 1). In patients sensitized to both cat and mite the higher quartile was as high as 26% in the lowest IgE

Table 1. The distribution of immunoglobulin (Ig)E antibodies (kU<sub>A</sub>/l) to cat, mite or both in serum samples from patients 12 years or older with IgE between 30 and 700 kU/l, divided into groups according to the IgE level

| kU <sub>A</sub> /l                       | 'Total IgE' (kU/l) |       |        |         |         |
|------------------------------------------|--------------------|-------|--------|---------|---------|
|                                          | 30–700             | 30–74 | 75–149 | 150–299 | 300–700 |
| Mite-positive ( <i>n</i> = 860)          |                    |       |        |         |         |
| Median                                   | 1.3                | 1.5   | 1.4    | 1.2     | 1.3     |
| Low quartile                             | 0.6                | 0.6   | 0.7    | 0.6     | 0.6     |
| High quartile                            | 4.3                | 4.7   | 5.5    | 3.6     | 3.4     |
| Cat-positive ( <i>n</i> = 1393)          |                    |       |        |         |         |
| Median                                   | 2.6                | 1.8   | 2.2    | 2.8     | 4.8     |
| Low quartile                             | 0.9                | 0.8   | 0.9    | 0.8     | 1.5     |
| High quartile                            | 7.2                | 4.3   | 5.8    | 9.2     | 14.0    |
| Cat- and mite-positive ( <i>n</i> = 313) |                    |       |        |         |         |
| Median                                   | 2.7                | 2.2   | 2.5    | 2.4     | 3.9     |
| Low quartile                             | 1.1                | 0.9   | 1.0    | 1.0     | 1.4     |
| High quartile                            | 9.4                | 5.3   | 7.7    | 7.2     | 12.0    |

The samples were submitted to an allergy laboratory for investigation during 2003–2005.

Table 2. The distribution of the relative immunoglobulin (Ig)E antibody concentrations (percentage IgE antibodies of IgE; for details, see legend to Table 1)

| IgE antibodies (%)                       | 'Total IgE' (kU/l) |       |        |         |         |
|------------------------------------------|--------------------|-------|--------|---------|---------|
|                                          | 30–700             | 30–74 | 75–149 | 150–299 | 300–700 |
| Mite-positive ( <i>n</i> = 860)          |                    |       |        |         |         |
| Median                                   | 0.9                | 3.2   | 1.3    | 0.9     | 0.3     |
| Low quartile                             | 0.3                | 1.1   | 0.7    | 0.3     | 0.1     |
| High quartile                            | 3.1                | 10.0  | 5.5    | 1.7     | 0.8     |
| Cat-positive ( <i>n</i> = 1393)          |                    |       |        |         |         |
| Median                                   | 2.0                | 3.7   | 2.2    | 1.3     | 1.0     |
| Low quartile                             | 0.7                | 1.6   | 0.9    | 0.4     | 0.3     |
| High quartile                            | 5.5                | 8.6   | 6.1    | 4.5     | 3.1     |
| Cat- and mite-positive ( <i>n</i> = 313) |                    |       |        |         |         |
| Median                                   | 3.1                | 11.0  | 5.5    | 2.5     | 2.1     |
| Low quartile                             | 1.2                | 3.7   | 2.0    | 1.1     | 0.8     |
| High quartile                            | 7.2                | 26.0  | 11.0   | 5.7     | 4.2     |

group, 30–74 kU/l, and 11% in the next group, 75–149 kU/l.

To simulate the effect of anti-IgE treatment on IgE, the IgE antibody values were recalculated to a theoretical serum IgE level of 10 kU/l, which is regarded as a successful reduction of available serum IgE. The IgE antibody concentrations that would have remained exceeded, in some cases, the level indicating significant IgE sensitization,  $\geq 0.35$  kU<sub>A</sub>/l (Table 3). In the 30–

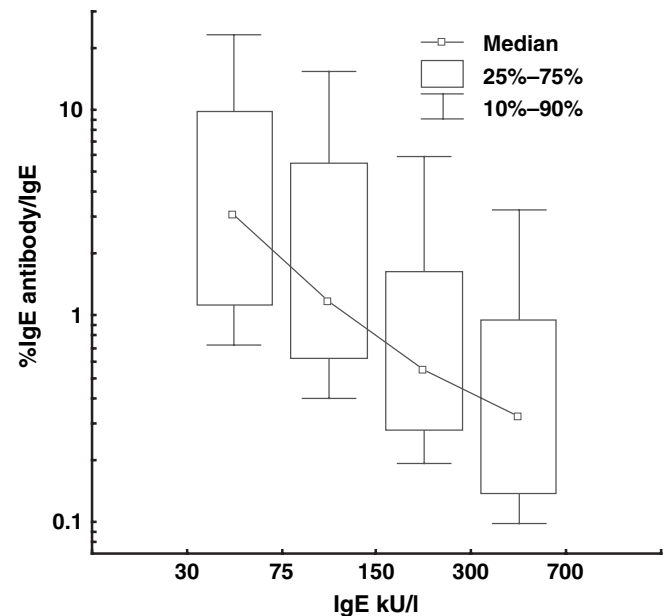


Figure 1. Median (□), low and high quartile (boxes) and 10% and 90% percentiles for immunoglobulin (Ig)E antibodies to mite in percentage of 'total IgE' in patients with a positive ( $\geq 0.35$  kU<sub>A</sub>/l) test for IgE antibodies to mite (for details see legend to Table 2).

Table 3. The distribution of immunoglobulin (Ig)E antibodies (kU<sub>A</sub>/l), calculated from hypothetical IgE levels of 10 kU/l and thus simulating an effective reduction of available serum IgE using recommended dosages of omalizumab (for details, see legend to Table 1)

| <10 kU/l               | 'Total IgE' (kU/l) |       |        |         |         |
|------------------------|--------------------|-------|--------|---------|---------|
|                        | 30–700             | 30–74 | 75–149 | 150–299 | 300–700 |
| Mite-positive          |                    |       |        |         |         |
| Median                 | 0.07               | 0.27  | 0.14   | 0.05    | 0.03    |
| Low quartile           | 0.07               | 0.14  | 0.08   | 0.03    | 0.02    |
| High quartile          | 0.13               | 0.70  | 0.46   | 0.14    | 0.06    |
| Cat-positive           |                    |       |        |         |         |
| Median                 | 0.14               | 0.35  | 0.22   | 0.13    | 0.11    |
| Low quartile           | 0.12               | 0.20  | 0.10   | 0.05    | 0.04    |
| High quartile          | 0.26               | 0.68  | 0.48   | 0.37    | 0.26    |
| Cat- and mite-positive |                    |       |        |         |         |
| Median                 | 0.10               | 0.36  | 0.24   | 0.11    | 0.08    |
| Low quartile           | 0.09               | 0.20  | 0.11   | 0.06    | 0.04    |
| High quartile          | 0.23               | 0.77  | 0.64   | 0.28    | 0.27    |

74 kU/l group, the higher quartiles for IgE antibodies to cat and mite were in the range of 0.68–0.77 kU<sub>A</sub>/l.

## Discussion

Knowledge about IgE (8) and its role in allergic inflammation (3) has increased our understanding of allergic diseases (9) and how they should be diagnosed and treated (10). Some years ago it was proposed (1) that by straightforward elimination of the IgE molecules in plasma exposure to relevant allergens would no longer be capable of initiating inflammation and thus disease. Extensive trials with a humanized monoclonal anti-IgE, omalizumab, showed clinical benefits and the drug was registered in the USA by FDA in 2003 and in Europe by EMEA in 2005 under the trade name Xolair (7).

Omalizumab is prescribed to patients with allergic asthma having a serum IgE concentration of 30–700 kU/l. Interestingly, no specific recommendations are given concerning the concentrations of relevant allergen-specific IgE antibodies, 'specific IgE'. Recently, it was reported that omalizumab seemed to be less efficient in patients with serum IgE levels  $\leq 75$  IU/ml (6), i.e. in the range of 30–75 kU/l. It is well known that atopic patients can mount a very specific IgE antibody response; relative concentrations in the order of 50–75% have been found (S. G. O. Johansson, unpublished data). The aim of the treatment was to reduce serum IgE to 10 kU/l or below (11). It is possible that this does not result in low enough concentrations of IgE antibodies in patients with a high proportion of relevant IgE antibodies. These patients would most likely be found among those with normal to moderately high IgE levels.

The distribution of IgE and IgE antibodies to cat and mite, two allergens to which patients with persistent allergic asthma are often simultaneously sensitized and exposed, was studied in sera sent to an allergy laboratory for diagnosis of IgE-mediated allergy. During the period of study, 2003–2005, IgE antibodies to either of the two allergens were tested for in 6461 sera. Of those, 60% fulfilled the serological and 'clinical' criteria for receiving omalizumab.

Among those with a positive ( $\geq 0.35$  kU<sub>A</sub>/l) IgE antibody test to mite or cat there was a tendency to higher serum levels of IgE antibodies with increasing IgE levels. However, when the IgE antibody concentrations were expressed in percentage of the serum IgE level the tendency was the reverse: the groups with the lower IgE values had the highest proportions of IgE antibodies. For mite there was a 10-fold increase from the highest IgE group (IgE: 300–700 kU/l), to the lowest (IgE: 30–74 kU/l), group, for cat a close to fourfold and for those

sensitized to both allergens a fivefold increase. The high quartile for relative IgE antibody concentrations in patients simultaneously IgE-sensitized to cat and mite in the lowest group was as high as 26%. Thus, in 25% of patients with a positive IgE antibody test, despite a normal or slightly increased IgE concentration, at least one of four of the IgE molecules could be identified as antibody active.

In order to evaluate the possibility that patients treated with omalizumab according to the recommendations could still have significant levels of serum IgE antibodies, the observed IgE antibody values were recalculated to a hypothetical IgE level of 10 kU/l, the value aimed at by the treatment. In the two lowest IgE groups, i.e. 30–74 and 75–149 kU/l, the 75 percentiles for IgE antibodies both to cat and mite allergens were found to be clinically significant. Actually, in the lowest group the IgE antibody levels were twice as high as the cut-off value for a positive test.

This study clearly documents that IgE-sensitized patients with normal to moderately high serum IgE levels, i.e.  $< 150$  kU/l, can have surprisingly high concentrations of IgE antibodies. Thus, even if serum IgE is pushed down to levels of 10 kU/l or below there can be many patients, in this study about one quarter of the sensitized population, that have high enough concentrations of IgE antibodies to initiate a clinically relevant allergic inflammation. This patient fraction could be higher because the patient sample analysed most likely includes patients with mild allergy.

The downregulation of IgE receptors on mast cells in patients on omalizumab could theoretically decrease the triggering potential of low levels of IgE antibodies (12). However, as only approximately 1000 IgE receptor bridges is enough to trigger the cells even a significant decrease of the normal number of 500 000–1,000 000 receptors could have limited effect.

The findings are even more prominent when the relative concentration of IgE antibodies is considered where figures in the studied sample of up to 26% were found. There are studies indicating that the higher the relative concentration of IgE antibody of IgE is the more prone are the IgE antibodies to stimulate inflammatory cells to initiate an allergic inflammation (5). Special consideration should also be given to patients sensitized to more than one allergen to which they can be simultaneously exposed, because it has been shown that IgE antibodies of different allergen specificities have an additive effect both on symptoms (13) and basophil stimulation (14).

Our findings strongly suggest that prospective studies should be performed where the clinical efficacy of a dosage of omalizumab not only based on IgE body pool, but also adapted to the relative concentration of relevant IgE antibodies, is evaluated.

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