

Variability of total and free IgE levels and IgE receptor expression in allergic subjects in and out of pollen season

Mats Carlsson^{1,*}, Lisa Thorell², Anders Sjölander², Susanne Larsson-Faria^{1,+}

¹Astra Zeneca R&D Mölndal, Sweden,

²Thermo Fisher Scientific, Uppsala, Sweden

⁺ Author employed at AstraZeneca at the time of this work

Running title: Seasonal variation of biomarkers in SAR patients

Scientific category: Clinical Immunology

*Corresponding author:

Mats Carlsson

AstraZeneca R&D Mölndal

SE-431 83 Mölndal, Sweden

Phone: +46 31 7065359

mats.carlsson@astrazeneca.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: 10.1111/sji.12270

This article is protected by copyright. All rights reserved.

Abstract

The inter- and intra-individual variability and seasonal variation of IgE, and high (FcεRI) and low affinity (CD23) IgE receptor expression in blood of seasonal allergic rhinitis (SAR) subjects, is not well studied. Thirty-two otherwise healthy subjects with a history of SAR to birch pollen and a positive skin prick test to birch pollen were sampled three times out of the pollen season and three times during the pollen season. FcεRI and CD23 expression were analysed using flow cytometry. Total IgE was analysed using ImmunoCAP® and free IgE was analysed with a novel customised research assay using an IgG-FcεRI-chimera protein coupled to ImmunoCAP as capture reagent, ImmunoCAP specific IgE conjugate and ImmunoCAP IgE calibrators. The performance of the free IgE assay compared well with the reference ImmunoCAP Total IgE assay. The working range of the assay was 0.35-200 kU/l IgE. FcεRI expression on basophils and CD23 expression on B cells showed low intra-subject variability both in and out of the pollen season (< 10 % CV). There was a small seasonal difference with lower total IgE levels (120 vs 128 kU/L; $p=0.004$) and FcεRI expression (283 vs 325 mean fluorescence intensity (MFI); $p<0.001$) during the pollen season. IgE, FcεRI expression and CD23 expression fulfilled biomarker and assay requirements of variability and allergen exposure affected the biomarkers only to a minor degree. The free IgE assay may be used for measurement of free IgE levels in patients after anti-IgE antibody treatment.

Introduction

Bronchial asthma is a chronic inflammatory disease in the airways characterised by bronchial hyperreactivity and reversible limitation of airflow that causes wheezing, shortness of breath, cough and dyspnoea. The International treatment guidelines Global Initiative for Asthma (GINA 2010) [1] recommends that the treatment of asthma should be adjusted stepwise

according to disease severity. However, there are a number of asthmatics who are not controlled in their disease despite the use of medium-or high-dose of inhaled glucocorticosteroids combined with long-acting inhaled β 2-agonist and add-on treatments with leukotriene modifiers or sustained-release theophylline (GINA step 4). In patients that are severely uncontrolled in their disease the addition of oral glucocorticosteroids or anti-IgE treatment may be efficient (GINA step 5).

Treatment with an anti-IgE humanized monoclonal antibody (omalizumab) has been tested in clinical trials in allergic asthma, allergic rhinitis and peanut allergy. Omalizumab may be beneficial in other allergic diseases as well, like food allergy, chronic urticaria and atopic dermatitis, and may be supplementary in specific immunotherapy. However, omalizumab has only been approved to treat uncontrolled moderate-to severe asthmatics as described above [2, 3], but was recently for Xolair approved in US and EU for the treatment of chronic idiopathic urticaria (CIU) and for the treatment of chronic spontaneous urticaria (CSU), respectively.

In allergic diseases, like rhinitis and asthma, sensitized individuals are producing allergen-specific IgE antibodies. The binding of allergen to allergen-specific IgE antibodies present on mast cells and basophils leads to cross-linking and activation of high affinity IgE receptors ($Fc\epsilon RI$) and, a subsequent mediator release responsible for the allergic inflammation [4]. $Fc\epsilon RI$ may also be found on dendritic cells, monocytes and eosinophils which are regarded as other important cell types for the development of allergic inflammation and disease. Evidence from *in vitro* experiments and human clinical studies suggests that circulating IgE levels may regulate the expression of $Fc\epsilon RI$ and the receptor occupancy on basophils [5-7]. Further evidence comes from clinical studies with anti-IgE treatment that shows a reduction in $Fc\epsilon RI$ density correlating to the reduction in free IgE [8, 9] and also a reduction of occupied receptors [8]. This correlated with decreased symptoms.

Another important receptor for binding IgE is the low affinity receptor, FcεRII or CD23 although its function differs from that of FcεRI. CD23 is expressed on B cells and monocytes and both CD23 expression and soluble CD23 levels are increased in allergic disorders. It is speculated that CD23 has a dual role in regulating IgE production. During antigen presentation it is thought that CD23 increases IgE production by aiding presentation of antigen/allergen by B cells to T cells. However, the presence of excess IgE and antigen/allergen CD23 may be a negative regulator of IgE production [4]. In allergic subjects it has been shown that CD23 expression is increased, and that *in vitro* monocyte CD23 expression can be upregulated by IL-4 and IL-13. Immunotherapy has been shown to reduce CD23 expression on B cells in relation to changes in disease severity and symptom relief in allergic subjects [10]. Data from clinical studies with Lumixilimab, an anti-CD23 Ab, indicate a decrease in mean B cell counts in addition to decreased IgE [11]. In summary, these data suggest that CD23 has a role in IgE mediated allergic disease.

Levels of IgE and IgE receptor expression and occupancy may serve as biomarkers during development of new anti-IgE monoclonal antibodies (mAbs). However, the variation of these biomarkers between and within subjects and in response to allergen challenge during pollen season is not well studied. The objective of this study was to assess the inter- and intra-individual variability and seasonal variation of the level of IgE, high and low affinity receptor expression and receptor occupation in blood from seasonal allergic rhinitis (SAR) subjects, both before and during a birch pollen season.

Materials and methods

Study design. This study was an open, non-randomized, single centre study including 32 otherwise healthy subjects with a history of SAR to birch pollen and a positive skin prick test to birch pollen. The subjects had a normal physical examination, 12-lead ECG, blood

pressure, pulse, and safety laboratory analysis (clinical chemistry, haematology and urinalyses) as judged by the investigator. They also had negative serology for HIV, Hepatitis B and C viruses. Table 1 shows the anti-allergy medication used during the study.

Subjects were excluded if tested positive for other seasonal allergy than to birch pollen and positive for house dust mite in or out of season. Subjects with a diagnosis of persistent asthma, clinically significant disease or disorder other than SAR (past or present), recent or ongoing upper respiratory tract infection at inclusion, acute illness or surgical procedures within 8 weeks before start of the study, participation in a drug-intervention study within 3 months before inclusion, use of any regular medication, herbal remedies or therapy within 2 weeks or vaccinations within 4 weeks of the first blood sampling, past or present alcohol or drug abuse, donation of blood within 3 months and plasma within 14 days prior to inclusion, planned surgery, dental procedure (only examination and X-ray allowed) and/or hospitalization during the study, habitual smoking or pregnancy, were excluded.

Blood samples were collected at three visits out of the birch pollen season (October to January) and three visits during the birch pollen season (mid April to mid May). The three visits out of and during the pollen season respectively were scheduled within 10 days, and separated by at least 2 days. Due to the time between the out of season and the during season sampling a second sampling for laboratory assessment (clinical chemistry, haematology, urinalyses and pregnancy test if applicable), pulse and blood pressure measurements, and an eligibility check was made just prior to the first sampling during the season.

The subjects were asked to abstain from intake of anti-histamines 5 days before the skin prick test and 24 hours before blood sampling, abstain from drinking alcohol 48 hours before blood sampling and abstain from painkillers and analgesic agents other than paracetamol 48 hours before blood sampling.

All subjects signed the subject information and consent form and the study was approved by the Independent Ethics Committee in Lund, Sweden.

Statistical analysis. The statistical analysis was done by using Gauss from AptechSystems Inc. and the Riemann Library. Gauss kernel revision: 6.0.48, Riemann Library version: 2.4.1. The full analysis set consisted of all included patients with data collected from at least two visits.

Main analysis of IgE data was made using linear mixed effect models with study period, cohort and the interaction between study period and cohort as fixed factors, and patients within cohort as a random factor. Analyses were done on the log scale due to the skew distribution of data. This means that estimates and standard deviations were computed on log transformed data. When estimates were exponentiated back to the linear scale, treatment differences appeared as ratios of geometric means. Similarly standard deviations were recalculated as coefficients of variation (CV). Further variance components were computed for each cohort separately (adjusting for study period) and for each study period separately. For method comparison between the free and total IgE assays, data were presented graphically according to the suggestion of Altman-Bland. In the first type of graph, data from the two methods were plotted against each other with the line of identity drawn. In the second kind of graph the average value of the two assays were plotted versus the difference with the bias and the limits of agreement drawn. The bias is the mean difference between the two assays. The limit of agreement is a 95% confidence interval for a single difference. Due to the skew distribution of data, graphs and all computations were made on the log scale. When these measures were exponentiated back, the bias became a geometric mean ratio for the two assays, and the limits of agreement became a multiplicative constant denoting the width of the confidence interval in relation to the bias. As repeatability measures the coefficient of

repeatability (CoR) and the intraclass correlation coefficient (ICC) were computed for each method. CoR is the width of a 95% confidence interval for a single measurement. ICC is the ratio (in percent) between the between-patient variance and the total variance. Correlations between free IgE and FcεRI expression on basophils, free IgE levels and CD23 expression on B-cells were illustrated with plots. Due to the skew distribution of free IgE this was indicated on the log scale, while the other parameters were kept on the linear scale. Relations between the variables were modelled in two ways; first assuming independency of all observations (overall relation), then assuming patient as the cluster (relation within patient). Overall relations could be non-linear and were fitted mostly to describe data. Relations within patient were assumed linear and the test of the slope then became a test of the linear relation between the two methods. All hypothesis testing was done using two-sided alternative hypotheses. P-values less than 5 % were considered statistically significant. There were no data available on similar studies under similar conditions to enable a sample size determination based on statistical considerations. The number of patients was therefore chosen based on practical aspects for these kinds of studies. Thirty (30) patients to complete the study was considered sufficient to evaluate the inter- and intra-individual variability of IgE and the other variables, both before and during the pollen season.

Serum collection. Venous blood was collected into Venoject tubes for serum sampling or Venojects tubes containing sodium heparin for plasma sampling. Serum tubes were left to clot for 30 min at room temperature, centrifuged at $1500 \times g$ at room temperature, for 15 minutes. The clear phase was collected immediately, aliquoted and stored at -70°C until analyses. For plasma, blood tubes were gently mixed and centrifuged within 30 minutes at $1500 \times g$, at room temperature for 15 minutes. The upper plasma phase was collected immediately, aliquoted and stored at -70°C until analyses. Blood for receptor expression and

occupancy measurements was transported to the laboratories within 1h and were rolled at room temperature for at least 15 minutes upon receipt. Blood sampling was done between 8 and 10 am, and at the same time for each subject at all visits.

Measurement of total IgE in serum. Total IgE was analysed in serum using the ImmunoCAP[®] Total IgE (Phadia AB, Uppsala, Sweden).

Measurement of free IgE. Free IgE was analysed in serum using a novel customised research assay. Briefly, the assay was based on the ImmunoCAP platform using an IgG-FcεRI-chimera protein coupled to ImmunoCAP as capture reagent, the ImmunoCAP Specific IgE conjugate for detection, and ImmunoCAP IgE calibrators. The assay had been verified for discriminatory ability, precision, recovery of spiked IgE and dilution linearity. In addition, the accuracy of the assay by comparing a WHO standard (WHO reference preparation 75/502) and an accelerated stability study of the IgG-FcεRI-chimera protein ImmunoCAP had been performed. The working range of the assay was 0.35-200 kU/l. Precision (CV), recovery (difference), and dilution linearity (difference) fulfilled the prespecified target of <20% CV (data not shown). The accelerated stability testing indicated a stability of the IgG-FcεRI-chimera protein ImmunoCAP up to 2 years when stored at 2-8°C. The assay was also tested to measure free IgE in serum from donors with different baseline IgE levels after the addition of varying concentrations of human anti-IgE mAbs [12, 13].

Measurement of FcεRI expression and IgE receptor occupancy. For measurements of FcεRI expression and IgE receptor occupancy whole blood was incubated with anti-CD45-PC7 (Beckman Coulter), anti-CD203c-APC (Miltenyi Biotec) and anti-FcεRIα-PE (eBioscience) or anti-IgE-FITC (VWR) antibodies with PE mouse IgG2b isotype control

(eBioscience) or FITC-goat IgG isotype control (Beckman Coulter), respectively. Samples were incubated at 37°C for 15 minutes, fixed and lysed using FACS-Lyse solution (BD FACS™ Lysing solution, BD Biosciences) for 15 minutes at room temperature, washed with stain buffer (BD Biosciences), re-suspended in Isoton II buffer (Beckman Coulter) containing 1% Cyto-Chex (Alpha Laboratories) and analysed on a flow-cytometer (FC500 MPL, Beckman Coulter). Prior to the study the method had been validated with respect to specificity, intra-assay variation and sample stability pre-and post analysis. Data was analysed using CXP software (Beckman Coulter). Mean fluorescence intensity (MFI) was determined after gating on the basophil population.

Measurement of CD23 expression. For measurements of CD23 expression, whole blood was incubated with anti-CD19-FITC, anti-CD14-PerCP and anti-CD23-PE antibodies with IgG₁-PE Isotype control (all from BD Biosciences). Samples were incubated at 37°C for 15 minutes, fixed and lysed using FACS-Lyse solution (BD FACS™ Lysing solution, BD Biosciences) for 15 minutes at room temperature, washed with stain buffer (BD Biosciences), re-suspended in stain buffer and analysed on a flow-cytometer (FACS Calibur, BD Biosciences). Prior to the study the method had been validated with respect to specificity, intra-assay variation and sample stability pre-and post analysis Data was analysed using Cellquest software (BD Biosciences) and Flow-Jo software (Tree Star, San Jose, CA, US). Mean fluorescence intensity (MFI) was determined after gating on the B cell or monocyte populations.

Results

Patients

Of the 33 patients allocated to the study, 16 (48.5%) were males and 17 (51.5%) were females. Their average age was 31.8 years (range: 19-54) and their average body mass index

(BMI) was 23.5 kg/m² (range: 19-30). All but one was Caucasians (one Black/White). One subject dropped out after the first blood sampling so 32 subjects were used for analysis. During the birch pollen season 3 more subjects dropped out so full data sets were available from 29 subjects.

Comparison of total and free IgE assays

Free and total IgE were measured by different assays in order to validate the new assay for measurement of free IgE. The assays compared well with each other (Fig. 1). Relative differences tended to be somewhat larger in samples with lower concentrations of IgE. The mean bias indicated a 30% higher level in average when assessed as total IgE (Fig. 1). Coefficient of repeatability and intraclass correlation coefficient was comparable between the two assays (data not shown).

Variability of total and free IgE levels in patients in and out of pollen season

The intra-subject variation of free and total IgE was low (< 10% CV) both out of and during the pollen season and the inter-subject variation was high (Table 2). The individual variation of free and total IgE is shown in Figure 2. There were statistically significantly lower levels of both free (92.1 vs 99.5 kU/L) and total IgE (119.6 vs 128 kU/L) during the pollen season compared to out of the season ($p < 0.001$) (Table 2).

Flow cytometry analysis

For all three flow cytometry biomarkers (FcεRI expression, IgE occupancy, and CD23 expression) cell distribution and scatter patterns were good, confirming satisfactory preparation and transport of the samples. For all subjects, the isotype controls confirmed no unspecific binding of the labelling antibodies (results not shown).

FcεRI expression on basophils

FcεRI expression on basophils showed large inter-subject variability (34.5 and 38.7 % CV) and low intra-subject variability (7.0 and 5.9 % CV) both out of and during the pollen season (Table 2). For some subjects, the basophil population was difficult to gate out using the gate set as default gate. In total 5 different basophil gates have been used in this study. The expression of FcεRI was lower during the pollen season compared to out of the season ($p<0.001$) (Table 2).

IgE occupancy on basophils

The intra-subject variability of IgE occupancy on basophils was larger than for the other assays but was similar out of and during the pollen season (16.1 and 17.2 % CV) (Table 2). Around 50% of the subjects had a basophil population with a broad distribution of IgE-occupancy. For some of these subjects, the variation of IgE between the duplicate samples was too high to report any mean value. However, the data was still sufficient for statistical analysis. Table 2 shows that IgE occupancy was lower during the pollen season compared to out of the season ($p<0.001$).

CD23 expression on B cells and monocytes

Expression of CD23 on monocytes was very low and precision was insufficient (> 20 % CV), why statistical evaluation was not done (data not shown). Expression of CD23 on B cells was high, although the expression pattern was a gradual expression on the B cells population. The precision in the assay was high with a difference between duplicate samples of less than 10 % CV (data not shown). Intra-subject variability was very low, both outside and during the pollen season (9.6 and 7.9 % CV) and there was a considerable inter-subject variability in the CD23 expression (50 % CV) (Table 2). No effect of allergen exposure during the birch pollen

season compared with the out of season was observed ($p=0.441$).

Correlation of biomarkers and with free IgE levels

The data presented includes both study periods and all three cohorts. The variation in all biomarkers was much higher between patients (inter-subject) than within patients (intra-subject) why there could be a correlation within patient that vanished or changed when adding patients with values from different levels. To investigate this, linear models were fitted to each patient's data. Within a patient there was a statistically significant correlation for the relations between: free IgE levels and FcεRI expression on basophils; free IgE levels and CD23 expression on B cells; and between FcεRI expression on basophils and IgE occupancy of FcεRI on basophils (Table 3).

Discussion

In drug development it is beneficial having robust biomarkers. They can serve as an indicator in early clinical development of efficacy and increase the confidence in the programme for further development. Furthermore, robust biomarkers may be used in early clinical testing for dose selection and not at least as markers for patient stratification. The objective of this study was to assess the inter- and intra-individual variability and seasonal variation of biomarkers relevant to follow during anti-IgE treatment of severe allergic asthmatics SAR subjects were studied both before and during a birch pollen season. The levels of total and free IgE were measured in serum. In blood the expression and IgE occupancy of FcεRI on basophils, and CD23 expression on monocytes and B cells, were measured. In addition, the correlation of biomarkers with free IgE was investigated.

The blockade of IgE by omalizumab (Xolair[™]) has proven efficient in the treatment of individuals with allergic moderate-to-severe asthma and rhinitis [14, 15]. The mechanism of

action appears to be associated with the profound reduction of circulating IgE. Omalizumab rapidly reduces free IgE levels within 2 hours post-treatment, due to the formation of complexes between the mAb and the IgE not bound to cells [16]. The reduction of free IgE is associated with a later (within weeks) decrease in the expression of FcεRI on basophils and mast cells. The latter effect is believed to drive the onset of action and the clinical effect. In early clinical studies in asthma and rhinitis, testing omalizumab, free IgE levels correlated with clinical efficacy. It was noticed that most clinical benefit was obtained when free IgE levels were reduced below 40 ng/ml (16.7 IU/ml). Hochhaus *et al* [17] confirmed after analysing the early clinical studies and file data that most clinical effect was reached when free IgE levels were reduced to a level below 50 ng/ml. However, when later analyzing the relation between omalizumab pharmacokinetics, IgE pharmacodynamics and symptoms in more severe allergic patients this clinical target concentration was found to be lower [18].

The data in four independently conducted Phase III trials showed each consistently a correlation of free IgE levels with clinical outcomes. To obtain the maximum clinical effects, the target concentration was set to 14 ng/ml (5.8 IU/ml). Taken together, these data demonstrate that free IgE is an important pharmacodynamics biomarker in the development of an anti-IgE treatment. However, interesting observations have been made on the clinical efficacy and the relationship to total IgE levels and the relationship between total and allergen specific IgE. In a paper by Corren *et al* [19] they described the treatment of mild perennial allergic rhinitis subjects with omalizumab. The results showed that subjects with initially lower total IgE levels also had the relatively smallest reduction in free IgE and skin test reactivity. A possible explanation to this finding has been reported earlier that the relative level of specific IgE may be higher in subjects with a low level of total IgE [20]. Later good clinical efficacy was demonstrated in subjects having initial specific IgE level less than 1% of total IgE level but lower clinical efficacy was demonstrated in subjects having initial specific

IgE level that were more than 3-4% of the total IgE level [21].

Several assay formats using therapeutic antibody or IgE receptor protein as capture have been reported [15, 16, 18, 22-24] and only recently one assay has become commercially available [25]. We developed a robust, customised research assay for free IgE with sensitivity to measure free IgE levels below the target concentration of 14 ng/ml by using the ImmunoCAP platform. The method was verified with results for discriminatory ability, precision, linearity and stability and compared well with the total IgE assay. The assay has also been used to demonstrate *ex vivo* the suppression of IgE by two anti-IgE mAbs, omalizumab and MEDI4212, in serum from individuals with a broad range of baseline IgE [12, 13].

In the present study, IgE was measured with two different methods: a novel assay for free IgE, intended for research use in patients treated with anti-IgE, and a total IgE assay. The free IgE assay will only measure uncomplexed IgE, not bound to therapeutic antibody, while in the absence of anti-IgE treatment the two assays should give similar results. In the present study the two methods compared well, and the free IgE assay can thus be considered fully validated.

The levels of total and free IgE showed large inter-subject variability as expected, while the intra-subject variability was very low (<10 % CV) both in and out of the pollen season. Lower IgE levels were found during the pollen season which might reflect a seasonal difference with a larger turnover of IgE. However, varying results on seasonal impact on IgE levels have previously been reported. Our study is in contrast to a similar study reporting increase of birch specific IgE during and after the birch pollen season [26]. Two other studies found no changes in the total IgE levels between in or out of pollen season [27, 28]. This may also be true for SAR patients allergic to other allergens [29, 30] but also increased levels of total IgE in season have been reported [30]. There may be several explanations to the

differences in outcome of studies on the variation of total IgE levels in and out of pollen season. It has been described seasonal fluctuation of total IgE levels where the lowest values are found in February and the highest values in September [31]. In our study we collected serum samples under pollen season (mid April to mid May) and out of the pollen season (October to January). This could explain the finding of lower total IgE levels during pollen season as the highest total IgE levels may be found 3-4 months after pollen season. A simple explanation is that production of total IgE is initiated during season, and after expansion of B cells and maturation into plasma cells, the highest levels of total IgE occurs several months after the initial trigger.

To minimize the influence of ongoing asthma medication, we studied the seasonal variation of biomarkers in SAR patients allergic to birch pollen. This patient population has a milder disease in the airways as compared to moderate to severe persistent asthmatics allergic to perennial aeroallergens, which is the approved patient population for omalizumab treatment. The level of inflammation in the two disease populations may have an impact on the levels of total and specific IgE. We describe in this paper lower total IgE levels during the pollen season. The difference in total IgE levels between pollen season and out of season was small but because of low intra-subject variability, a significant difference between seasons was found. As mentioned previously a similar study on birch pollen allergic SAR patients reported an increase of birch specific IgE during and after the birch pollen season [26]. It would have been interesting to also evaluate the variation between in and out of birch pollen season specific IgE levels, and the correlation with total IgE levels. However, this study remains to be done and whether the relatively small seasonal variation of total IgE, described in this study, would also have been seen in individuals with multiple seasonal allergies.

Expression of the high-affinity IgE receptor, FcεRI on basophils showed a large inter-subject variability, but intra-subject variability was low both in and out of the allergen season

(< 10% CV). There was a small seasonal difference with expression being lower during the season, similar to IgE levels. Seasonal variation of FcεRI expression on basophils has not been reported previously. However, our results with lower free and total IgE levels in serum and lower FcεRI expression on basophils during the pollen season is consistent with data suggesting strong correlation between serum IgE levels and FcεRI expression on basophils [5-7].

IgE occupancy, *i.e.* IgE bound to FcεRI on basophils, also demonstrated large inter-subject variability. The intra-subject variability was larger than for the other assays but was similar in and out of the pollen season. IgE bound on basophils correlated with increasing IgE levels and increasing receptor expression up to IgE levels of 10^2 kU/l. At higher IgE levels a bell-shaped correlation may reflect that reagents in the assay were limiting and that the assay needs more development work. Still, the correlation of FcεRI expression and IgE occupancy is in agreement with published data [5, 7] and suggests that IgE occupancy as a biomarker behaved as expected in our hands despite the limitation of reagents in the assay at high IgE levels.

It is evident that CD23 has a central role in allergic disease by the capacity of enhancing IgE synthesis and also from its capacity of facilitating antigen presentation of allergens [4]. It has been reported that the expression of CD23 and release of sCD23 is strongly increased in allergic disorders [32]. We studied the CD23 expression on B cells from SAR patient in- and out of allergen season. The CD23 expression on B cells was high and reproducible using the tested assay. The intra-subject variability was low (< 10% CV) but there was quite a large inter-subject variability. We did not find any indication of a seasonal variation in CD23 expression in response to allergen exposure although others have reported increased number of CD23 positive B cells during season [33]. However, a seasonal increase in soluble CD23 has been reported in SAR [34, 35]. In a similar study to ours the investigators could not find

seasonal variation of other cytokines involved in allergic inflammation as GM-CSF, IL-5, IL-5, IL-8, IL-13 and eotaxin [36]. Although we do not see a reduced expression in membrane bound CD23 it does not exclude the possibility that the number of CD23 positive B cells are lower out of the pollen season and a large subset of B cells have essentially unchanged high CD23 expression. Finally, CD23 may have a limited value as a biomarker for monitoring anti-IgE treatment effect and there is no information available on CD23 expression on blood B cells and monocytes after anti-IgE treatment. It has, however, been shown that anti-IgE treatment had no effect on CD23 expression in bronchial biopsies after 16 weeks [37].

Finally, the correlation of biomarkers and with free IgE was investigated. As the variation of all biomarkers was much higher between patients than within patients, linear models were fitted to each patient data. Significant correlations were found between levels of free IgE with; FcεRI on basophils, with CD23 expression on B cells; but not with IgE occupancy of FcεRI on basophils. In contrast to the latter result significant correlation was found between FcεRI expression on basophils and IgE occupancy. Taken together our results and others, the biomarkers described above may be used to study efficacy in future clinical studies on anti-IgE treatment.

Acknowledgements

We would like to thank Lena Lilieblad at the Clinical Pharmacology Unit for recruiting the patients and organising the study logistics and sample collection, Ulf Hindorf and Lennart Jansson for clinical input to the study protocol (AstraZeneca R&D Lund Sweden).

MedImmune Cambridge, Cambridge, UK for providing the construct of IgG-FcεRI-chimera protein and AstraZeneca Biotech, Södertälje, Sweden for producing the IgG-FcεRI-chimera protein. Robert Movérare (Thermo Fisher Scientific, Uppsala, Sweden) for his valuable input to the manuscript.

References

- 1 Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. 2011. Available from: <http://www.ginasthma.org>.
- 2 Chang TW, Wu PC, Hsu CL, Hung AF. Anti-IgE antibodies for the treatment of IgE-mediated allergic diseases. *Adv Immunol* 2007;93:63-119.
- 3 Morjaria JB, Gnanakumaran G, Babu KS. Anti-IgE in allergic asthma and rhinitis: an update. *Expert Opin Biol Ther* 2007;7:1739-47.
- 4 Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18:693-704.
- 5 Malveaux FJ, Conroy MC, Adkinson NF, Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest* 1978;62:176-81.
- 6 Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: Relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997;99:699-706.
- 7 Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashan DW. The relationship between serum IgE and surface levels of FcεR on human leucocytes in various diseases: correlation of expression with FcεRI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol* 2000;106:514-20.
- 8 MacGlashan DW Jr, Bochner BS, Adelman DC *et al*. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-45.
- 9 Lin H, Boesel KM, Griffith DT *et al*. Omalizumab rapidly decreases nasal allergic response and FcεRI on basophils. *J Allergy Clin Immunol* 2004;113:297-302.
- 10 Jung CM, Prinz JC, Reiber EP and Ring J. A reduction in allergen-induced FcεR2/CD23

- expression on peripheral B cells correlates with successful hyposensitization in grass pollinosis. *J Allergy Clin Immunol* 1995;95:77-87.
- 11 Rosenwasser LJ, Busse WW, Lizambri RG, Olejnik TA, Totoritis MC. Allergic asthma and an anti-CD23 mAb (IDEC-152): Results of a phase1, single-dose, dose-escalating trial. *JACI* 2003;112:563-70.
 - 12 Cohen ES, Eriksson P-OF, Von Wachenfeldt K *et al.* In vitro properties of MEDI-4212, a human anti-IgE antibody for the treatment of allergic asthma. *Am J Respir Crit Care Med* 2009;179:A5726.
 - 13 Cohen ES, Dobson CL, Käck H *et al.* A novel IgE-neutralizing antibody for the treatment of severe uncontrolled asthma. *mAbs* 2014;6:1-9.
 - 14 Nowak D. Management of asthma with anti-immunoglobulin E: A review of clinical trials of omalizumab. *Respir Med* 2006;100:1907-17.
 - 15 Casale TB, Bernstein IL, Busse WW *et al.* Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;100:110-21.
 - 16 MacGlashan DW, Bochner BS, Adelman DC *et al.* Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-45.
 - 17 Hochhaus G, Brookman L, Fox H *et al.* Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin* 2003;19:491-9.
 - 18 Lowe PJ, Tannenbaum S, Gautier A, Jimenez P. Relationship between omalizumab pharmacokinetics, IgE pharmacodynamics and symptoms in patients with severe persistent allergic (IgE-mediated) asthma. *Br J Clin Pharmacol* 2009;68:61-76.
 - 19 Corren J, Shapiro G, Reimann J *et al.* Allergen skin test and free IgE levels during reduction and cessation of omalizumab treatment. *J Allergy Clin Immunol* 2008;121:506-

- 11.
- 20 Johansson SGO, Öman H, Nopp A, Pettersson S. The importance of IgE antibody levels in anti-IgE treatment. *Allergy* 2006;61:1216-9.
- 21 Johansson SGO, Nopp A, Öman H *et al.* The size of the disease relevant IgE antibody fraction in relation to 'total-IgE' predicts the efficacy of anti-IgE (Xolair®) treatment. *Allergy* 2009;64:1472-7.
- 22 Busse W, Corren JC, Lanier BQ *et al.* Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;108:184-90.
- 23 Hamilton RG, Marcotte GV, Saini SS. Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving omalizumab (Xolair) therapy. *J Immunol Methods* 2005;303:81-91.
- 24 Ädelroth E, Rak S, Haahtela T *et al.* Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2000;106:253-9.
- 25 Roberts S, Collwell M, Wells H *et al.* An immunoassay to measure free IgE in individuals receiving anti-IgE therapy. *J Allergy Clin Immunol* 2011;127:AB210
- 26 Linden M, Larsson S, Greiff L *et al.* Circulating eosinophil numbers as surrogate biomarker of symptomatic disease in seasonal allergic asthma and rhinitis. *J Allergy Clin Immunol* 2000;105:S293.
- 27 Winther L, Reimert C, Skov P *et al.* Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis. *Allergy* 1999;54:436-45.
- 28 Di Gioacchino M, Cavallucci E, Di Stefano F *et al.* Influence of total IgE and seasonal increase of eosinophil cationic protein on bronchial hyperreactivity in asthmatic grass-

- sensitized farmers. *Allergy* 2000;55:1030-4.
- 29 Castillo JG, Oehling A, Sanz ML, Gamboa PM. Seasonal influence on serum and receptor-bound IgE in pollinosis. *Ann Allergy* 1990;64:542-6.
- 30 Di Lorenzo G, Mansueto P, Melluso M *et al.* Non-specific airway hyperresponsiveness in mono-sensitive Sicilian patients with allergic rhinitis. Its relationship to total serum IgE levels and blood eosinophils during and out of the pollen season. *Clin Exp Allergy* 1997;27:1052-9.
- 31 Salkie ML, Weimer N. The influence of season and of sex on the serum level of total IgE and on the distribution of allergen-specific IgE. *Clin Biochem* 1984;17:362-6.
- 32 Gagro A, Rabati S. Allergen-induced CD23 on CD4+ T lymphocytes and CD21 on B lymphocytes in patients with allergic asthma: Evidence and regulation. *Eur J Immunol* 1994;24:1109-14.
- 33 Håkansson L, Heinrich C, Rak S, Venge P. Activation of B-lymphocytes during pollen season. Effect of immunotherapy. *Clin Exp Allergy* 1998;28:791-8.
- 34 Ohashi Y, Nakai Y, Kakinoki Y *et al.* Immunotherapy affects the seasonal increase in specific IgE and interleukin-4 in serum of patients with seasonal allergic rhinitis. *Scand J Immunol* 1997;46:67-77.
- 35 Ohashi Y, Nakai Y, Tanaka A *et al.* Immunotherapy decreases seasonal rise in serum-soluble CD23 in seasonal allergic rhinitis. *Laryngoscope* 1998;108:706-11.
- 36 Larsson S, Greiff L, Hansson L *et al.* One-year study of plasma cytokines in seasonal allergic asthmatics and rhinitics compared with healthy volunteers. *J Allergy Clin Immunol* 2000;105:S150.
- Djukanović R, Wilson SJ, Kraft M *et al.* Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004;170:583-93.

Table 1

Prescribed anti-allergic medication during the study.

Medication group	ATC code	Number of patients All n=33
Other antihistamines for systemic use	R06AX	10 (30%)
Corticosteroids	R01AD	6 (18%)
Piperazine derivates	R06AE	6 (18%)
Other anti-allergics	S01GX	6 (18%)
Anti-allergics excluding corticosteroids	R01AC	2 (6%)
H2-receptor antagonists	A02AB	1 (3%)
Inhaled combinations of LABA+GCS	R03AK	1 (3%)
Glucocorticoids	H02AB	1 (3%)

Table 2

Inter- and intra-subject variation of biomarkers out of and during the birch pollen season.

	% CV				Estimates		Period comparison		
	Within patient		Between patient		Out of season	In season	ratio	95% C.I.	p- value
	Out of season	In season	Out of season	In season					
Total IgE	7.0	6.5	120.5	122.4	128.0	119.6	1.07	(1.02- 1.12)	0.004
Free IgE	7.7	8.7	147.1	146.6	99.5	92.1	1.08	(1.02- 1.14)	0.006
FcεRI expression on basophils	7.0	5.9	34.5	38.7	325.1	283.3	1.15	(1.12- 1.17)	<0.001
IgE occupancy of FcεRI on basophils	16.1	17.2	29.7	25.5	137.1	115.4	1.19	(1.12- 1.26)	<0.001
CD23 expression on B cells	9.6	7.9	50.0	49.5	110.5	109.1	1.01	(0.98- 1.05)	0.441

Table 3

Estimated regression parameter for the relations between biomarkers and free IgE in serum.

Variable	Parameter	Estimate	s.e.	95% C.I.	p-value
Free IgE – FcRεRI expression	Intercept	263.1	15.0	(233.7-292.5)	
	Slope	0383	0.057	(0.272-0.494)	0.000
Free IgE – IgE occupancy	Intercept	141.6	8.4	(125.2-158.0)	
	Slope	-0.057	0.036	(-0.127-0.0013)	0.110
Free IgE – CD23 expression	Intercept	102.3	13.1	(76.7-127.9)	
	Slope	0.135	0.039	(0.057-0.212)	0.001
FcRεRI expression – IgE occupancy	Intercept	23.7	16.0	(-7.6-54.9)	
	Slope	0.339	0.044	(0.252-0.426)	0.000

Legend to Figures

Figure 1

Alltman-Bland plots demonstrate the comparison of IgE levels in serum samples measured by the free IgE assay and a standard total IgE assay. The average value of the two assays are plotted vs the difference with the bias. Computations were done on the log scale due to a skew distribution.

Figure 2

Free and total IgE is shown by patient and cohort. Visit 3-5 shows the IgE levels out of pollen season and visits 9-11 shows IgE levels in pollen season.

Figure 1

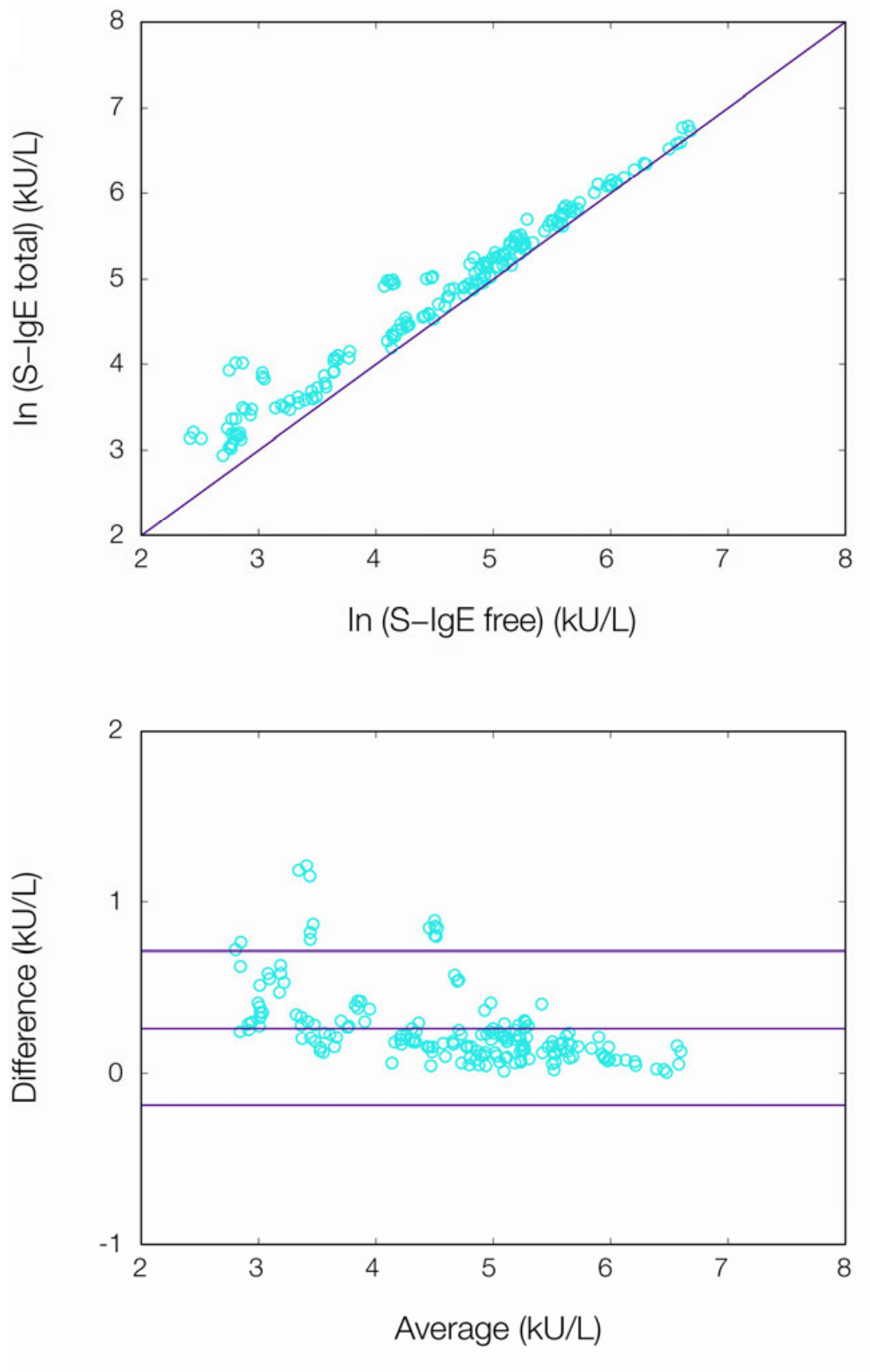


Figure 2

